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Risk factors and interventions for primary prevention of gallbladder stones: Cochrane systematic review and meta-analysis of randomised controlled trials

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Αφιερωμένο στην οικογένειά μου

Table of Contents

List of Figures

List of Tables

Abbreviations

ABC Transporters	ATP-Binding Cassette Canalicular Transporters
ABCB4	ATP-binding cassette transporter, subfamily B, member 4
ABCB11	ATP-binding cassette transporter, subfamily B, member 11
ABCG5	ATP-binding cassette transporter, subfamily G, member 5
ABCG8	ATP-binding cassette transporter, subfamily G, member 8
ATP	Adenosine Triphosphate
CHBG	Cochrane Hepato-Biliary Group
CI	Confidence Interval
CSI	Cholesterol Saturation Index
ITT	Intention-To-Treat
HCV	Hepatitis C Virus
HDL-C	High Density Lipoprotein Cholesterol
HRT	Hormonal Replacement Therapy
LCD	Low-Calorie Diet
LDL-C	Low Density Lipoprotein Cholesterol
OR	Odds Ratio
NNT	Number Needed To Treat
NSAID	Non-steroidal anti-inflammatory drug
RR	Risk Ratio
SCI	Spinal Cord Injury
TG	Triglycerides
UDCA	Ursodeoxycholic Acid
VLCD	Very Low-Calorie Diet

1. Abstract

Background: Cholecystectomy is used for the management and prevention of gallbladder stones and poses a major burden on healthcare resources. Obesity and rapid weight loss are known risk factors for gallstones, the prevalence of which is rising given the obesity epidemic. We conducted a Cochrane systematic review and meta-analysis of randomised controlled trials to evaluate the efficacy of using non-surgical interventions for the primary prevention of gallbladder stones in high risk adults.

Methods: Electronic searches (Cochrane Library, CENTRAL, Medline, Embase, and Science Citation Index Expanded) and manual searches (up to July 2013) were carried out with no restrictions on publication status or language. The intervention comparisons included pharmacological (ursodeoxycholic acid, UDCA) or non-pharmacological (high-fat weight loss diet) versus control interventions (placebo, no intervention, fibre supplement or low-fat weight loss diet). Random-effects meta-analyses were performed and intertrial heterogeneity and bias was evaluated with subgroup, sensitivity, regression, and sequential analysis.

Results: Overall, 14 trials comprising a total of 1,942 participants undergoing weight loss through dieting, or after bariatric surgery (13 trials); or participants following cardiac surgery (one trial) were included. UDCA reduced the risk of ultrasonically verified gallstones compared with control interventions (risk ratio (RR) 0.32, 95% confidence interval (CI) 0.19 to 0.55, I^2=60%). UDCA was more beneficial in participants undergoing weight loss through diet alone (RR 0.17, 95% CI 0.11 to 0.25, I^2=0%) than after bariatric surgery (RR 0.42, 95% CI 0.21 to 0.83, I^2=64%). Two trials found that high dietary fat content reduced the formation of gallstones during weight loss achieved through low calorie diets (RR 0.09, 95% CI 0.01 to 0.61, I^2=0%). Regression analysis showed no evidence of small study effects. No additional beneficial or harmful effects on remaining outcomes were identified.

Conclusions: These results suggest that during weight loss UDCA and high dietary fat content may prevent formation of gallstones. Beneficial mechanistic effects may include enhanced gallbladder motility. The above prevention strategies could represent cost-effective alternatives to cholecystectomy.

Hintergrund: Für die Prävention und Behandlung von Gallensteinen wird die Cholezystektomie eingesetzt. Sie stellt eine erhebliche Kostenbelastung für das Gesundheitswesen dar. Adipositas und schnelle Gewichtsabnahme sind Risikofaktoren für Gallensteine, und die Prävalenz von Gallensteinen steigt durch die Adipositas-Epidemie. Ziel dieser Arbeit war es, ein systematisches Cochrane Review und eine Meta-Analyse von randomisierten kontrollierten Studien durchzuführen, um die Wirksamkeit von nicht-chirurgischen Eingriffen zur primären Prävention von Gallensteinen bei Patientengruppen mit hohem Risiko zu bewerten.

Methode: Elektronische Recherchen (Cochrane Library, CENTRAL, MEDLINE, EMBASE und Science Citation Index Expanded) und manuelle Literaturstudien (bis einschließlich Juli 2013) wurden ohne Einschränkungen bezüglich Publikationsstatus oder Sprache durchgeführt. Diese Interventionen setzten sich aus pharmakologischen (Ursodeoxycholsäure, UDCA) und nicht-pharmakologischen (fettsupplementierte Diäten zur Gewichtsabnahme) versus Kontroll-Interventionen (Plazebo, keine Intervention, Ballaststoff-Ergänzung oder fettarme Diäten zur Gewichtsabnahme) zusammen. Random-Effects-Meta-Analysen wurden durchgeführt und Intertrial-Heterogenität und Bias wurden mit Subgruppen-, Sensitivitäts-, Regressions- und Sequenz-Analysen ausgewertet.

Ergebnisse: Es wurden insgesamt 14 Studien mit 1.942 Teilnehmer eingeschlossen. Die Teilnehmer erzielten die Gewichtsabnahme durch Diät oder bariatrische Chirurgie (13 Studien) oder unterzogen sich einer kardiochirurgischen Operation (1 Studie). Die UDCA-Intervention reduzierte das Risiko von Gallensteinen im Vergleich zur Kontrollgruppe (Risiko-Verhältnis (RR) 0,32, 95%-Konfidenzintervall (CI) 0,19 - 0,55, I^2=60%). UDCA war effektiver, wenn der Gewichtsverlust durch Diät allein erreicht wurde (RR 0,17, 95%-CI 0,11 - 0,25, I^2=0%) als nach Adipositaschirurgie (RR 0,42, 95%-CI 0,21 bis 0,83, I^2=64%). Zwei Studien legen nahe, dass fettreiche, kalorienarme Diäten zur Gewichtsabnahme die Inzidenz von Gallensteinen reduzieren (RR 0,09, 95%-CI 0,01-0,61, I^2=0%). Es wurden keine weitere vorteilhaften Effekte oder unerwünschte Wirkungen identifiziert.

Fazit: Die Ergebnisse der Meta-Analyse legen nahe, dass UDCA sowie fettsupplementierte kalorienarme Diäten das Risiko von Gallensteinen, während einer Gewichtsreduktion reduzieren. Diese Präventionsstrategien stellen kostengünstige Alternativen zur Cholezystektomie beim Gallensteinleiden dar.

2. Introduction

The prevalence of gallstones is currently between 10% and 20% in Western adults, with a projected rise given the increasing ageing population and obesity epidemic.[55] [98] [243] Over 30% of Americans are now obese,[76] but the United States is not alone in their rising obesity rates, since recent statistics also report 67 and 53% of men and women in Germany are overweight and 23% and 24% are obese, respectively.[66] Additionally, 65% of men and 58% of women in England are overweight or obese, and 31% and 28% of boys and girls, respectively.[82] A longitudinal study comprising 90,302 women reported a sevenfold risk of gallstones in morbidly obese compared to normal weight populations.[202] Moreover, a recent study in 77,679 Danish individuals reported a causal association between elevated BMI and increased risk of symptomatic gallstone disease.[203] A recent multi-ethnic population based cross-sectional study in the United States[106] reported 766 children between the ages of 10 to 19 to have gallstones. The risk was substantially higher for the extreme obese.

Gallstones are predominantly asymptomatic, however, an estimated 25% of stone carriers develop symptoms and complications such as cholecystitis, cholangitis, and pancreatitis.[63] Patients with symptomatic gallbladder stones frequently require hospital admission and laparoscopic cholecystectomy. Non-surgical options for preventing gallstones are currently underused, and over 50,000 cholecystectomies are performed each year in the United Kingdom, of which over 90% are carried out in the National Health Service (NHS).[21] [194] Germany and the United States surpass this rate with approximately 170,000 and 700,000 cholecystectomies per year, respectively.[53] [117] This corresponds to a substantial burden on healthcare resources.

This burden may be further compounded by some negative outcomes that have been linked to cholecystectomy. In particular, cholecystectomy is associated with a 1.6% risk of damage to the bile ducts and a 0.5% mortality risk.[1] In addition obese patients undergoing gastric bypass surgery with concomitant cholecystectomy often require longer hospital stays and have a risk of post-operative complications.[73] Prophylactic cholecystectomy is proposed for these individuals due to the high risk of developing symptomatic gallstones following rapid weight loss,[129] although the risk of developing symptoms might actually be moderate.[90] Prophylactic cholecystectomy is

contraindicated in certain situations, such as post-cardiac surgery patients who have an increased risk of gallstones.[15] [100] A Cochrane review reported symptom recurrence in up to 40% of post-cholecystectomised patients.[97] In fact, post-cholecystectomy syndrome is reported in 5 to 47% of patients[31] [88] and includes a wide range of symptoms (biliary and extra-biliary) and is often characterised by pre-surgery symptom recurrence.

Once a person has been cholecystectomised, there is a continual flow of bile to the duodenum which consequently increases the production of secondary bile acids (deoxycholic and lithocholic acid). This occurs as a result of an increased enterohepatic circulation and subsequent degradation by intestinal gut bacteria of the primary bile acids. It has been suspected, that secondary bile acids elicit carcinogenic effects. Lagergren et al.[113] reported an increased risk of intestinal cancer following cholecystectomy. More recently, the same authors reported a weak association between oesophageal adenocarcinoma and patients with cholecystectomy.[114] Though confounders, such as obesity were not controlled for in the study, this observed association could be attributed to increased concentrations of bile in gastric fluid, which consequently may come into contact with the oesophagus during gastro-oesophageal reflux. The study also found an association between hepatocellular carcinoma and cholecystectomy.[115] In contrast, other studies do not report an increased cancer risk after cholecystectomy, as illustrated in a meta-analysis by Zhao et al.[260] Therefore, the potential carcinogenic effects have yet to be fully substantiated.

2.1 Pathobiology

Gallstones are essentially made up of cholesterol crystals, mucin, calcium, bilirubinate and proteins which precipitate to form biliary sludge and subsequently gallstones. The ratio of these components determines which of three categories the stones belong to. Cholesterol gallstones comprise > 90% of cases and consist primarily of cholesterol monohydrate crystals, whereas the main component of black and brown pigment stones is calcium bilirubinate (Table 1).[182] The prevalence of cholesterol stones is much higher than either black or brown pigment stones.

The formation of gallstones involves the liver, gallbladder and intestine and the detailed pathobiology of gallstones has been summarized in one of our previous reviews.[209] There are three fundamental steps that lead to gallstone formation:

1. Excess cholesterol and/or bilirubin cannot be solubilized by mixed micelles and lead to the supersaturation of bile,
2. gallbladder hypomotility,
3. destabilisation of bile through proteins offsetting the crystallization sequences.

Table 1. Classification of gallstones

	Cholesterol stones	Black pigment stones	Brown pigment stones
Main Composition	Cholesterol monohydrate	Bilirubin polymers + Calcium	Calcium bilirubinate
Location	Gallbladder	Gallbladder	Infected bile ducts
Prevalence	> 90%	~2%	~10%

Adapted from Stokes et al.[208]

The pathobiological process is as follows: adenosine triphosphate (ATP)-dependent transport proteins, referred to as ATP-binding cassette canalicular transporters (ABC transporters) secrete biliary lipids into bile; unilamellar vesicles (composed of phosphatidylcholine and cholesterol) and simple micelles (composed of bile salts and cholesterol) form. Whilst they are channeled through the biliary tract and into the gallbladder, they are converted into mixed micelles. If these mixed micelles are challenged with more cholesterol in bile than they can solubilise (indicated by a cholesterol saturation index (CSI) > one), then cholesterol-rich multilamellar vesicles (liquid crystals) form. The aggregation of these multilamellar vesicles precedes the formation of solid cholesterol crystals.

The physical-chemical composition of bile plays a fundamental role in the formation of gallstones and the balance of biliary lipid concentrations (bile salts, bilirubin,

cholesterol and phospholipids) determines the extent of solubility of biliary cholesterol and bilirubin. When bile becomes supersaturated with cholesterol, this is on account of an increased ratio of cholesterol and/or bilirubin to bile salts or phospholipids. It is usually due to cholesterol hypersecretion or the hyposecretion of bile salts and/or phospholipids.[137] Intestinal hypomotility increases the bacterial colonic formation of the secondary bile salt, deoxycholate, which leads to more lithogenic bile thus causing gallstones. In fact, slow intestinal transit and increased deoxycholate are commonly reported in patients with cholesterol gallstones.[170] Figure 1 depicts the aforementioned pathophysiological events in cholesterol gallstone formation.

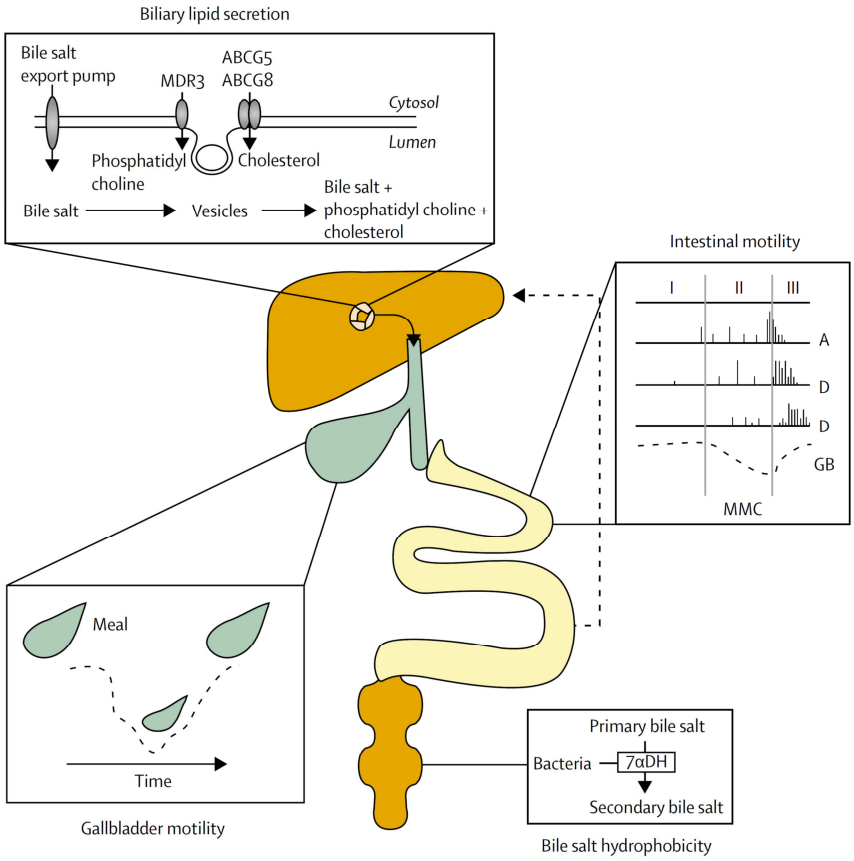


Figure 1. Pathophysiology of cholesterol gallstone formation. Reproduced with permission from Portincasa et al.[171] Copyright © 2006 Elsevier Ltd

2.2 Environmental and genetic risk factors

2.2.1 Genetic factors

The disparity in gallstones amongst different ethnic groups points to a strong genetic propensity to form gallstones.[110] In fact, gallstone susceptibility 'thrifty' genes are suggested to have evolved during the Great Ice Age.[38] Genetic factors account for 25% of the risk in Europeans.[95] Moreover, gallstones form more frequently (i.e. the risk is five-fold) in those with a family history of gallstones.[13] Genetic risk is dependent on the type of mutations, for example in monogenic mutations (such as *ABCB4*) gallstones are a result of a strong genetic component,[108] however a stronger environmental influence is observed in carriers of common risk variants, hence gallstones are often an end result of higher order interactions between multiple genetic and lifestyle determinants.[208]

Numerous candidate Lithogenic *(LITH)* genes such as the common *ABCG8* mutation p.D19H, which increases hepatobiliary cholesterol efflux, confer an increased risk of gallstones.[109] This genetic risk is amplified with certain environmental factors. Genetic variants such as the ABCG5/8 variants do not fully explain this increased genetic risk, however the transport activity of these proteins (cholesterol hemitransporter) may be amplified, thus increasing the risk of gallstones. Twin studies actually show genetic risk factors to account for 25% of total risk and environmental factors for 75%.[96]

The increased prevalence of gallstones has been illustrated in geographically and ethnically disparate locations in which stark differences in dietary intake are apparent.[206] The composition of gallstones in Native Americans, post-war European countries and East Asian countries differs, and cholesterol gallstones are more prevalent in regions that have adopted 'Westernized' dietary habits, thus illustrating a strong dietary influence.[159] Dietary and other environmental risk factors (summarised in Table 2) are discussed in more detail below, and are based on our previous publication reviewing risk factors for gallstones.[208]

Table 2. Summary of major risk factors for gallbladder stones

Risk Factors	Cholesterol stones	Black pigment stones
Family history (genetics) / ethnicity	✓	
Increasing age	✓	
Female gender, parity	✓	
Obesity, especially central adiposity	✓	
Rapid weight loss / bariatric surgery	✓	
Physical inactivity	✓	
Diet High calorie / carbohydrate / glycaemic load Low fibre	✓	
MS Dyslipidaemia (↑ TG,↓ HDL-C) Insulin resistance, diabetes	✓	
Vitamin B12 and folic acid deficiency		✓
Prolonged total parenteral nutrition	✓	
Drugs Estrogen therapy (HRT) Somatostatin analogue-octreotide Calcineurin inhibitors Fibrates	✓	
SCI	✓	
Crohn's disease	✓	✓
Cystic fibrosis		✓
Surgery Gastrectomy	✓	✓
Ileal resections		✓
Anaemia (haemolytic, sickle cell)		✓
Liver cirrhosis	✓	✓
Chronic Hepatitis C Virus infection	✓	
Enterohepatic bacteria (*Helicobacter spp.*)	✓	

Adapted from Stokes et al.[208]

HDL-C, High Density Lipoprotein Cholesterol; HRT, Hormone Replacement Therapy;

MS, Metabolic Syndrome; SCI, Spinal Cord Injury; TG, Triglycerides

2.2.2 Diet and other lifestyle factors

2.2.2.1 Macronutrients

A 'Westernized' diet usually comprises a high-caloric, high-carbohydrate, low fibre and generally a nutrient-depleted diet. Of note, large epidemiological studies from the US, Europe, Japan, and China report these dietary aspects as risk factors for cholesterol gallstones.[223 224 226-228 234] A high carbohydrate diet and a concomitant high glycaemic load increased symptomatic gallstone risk, as well as cholecystectomy in two large US prospective epidemiological association studies using cohorts from the Health Professionals Follow-up Study (men) and the Nurses' Health Study (women).[226 227] In particular, two cohorts also reported a specific increased risk of gallstones with refined sugar consumption, a finding commonly reported by others.[8 143 145 186] Interestingly, fibre consumption, particularly long-term intake has been linked to a reduced risk of gallstones, and conversely low fibre intake is purported to be a risk factor for stone formation.[14 93 132 143 192 223]

Given the clear association between a typical Western diet and gallstones, the question of how nutrients encourage cholelithiasis begs asking. High caloric consumption may indirectly increase this risk through instigating obesogenic effects. Of note, obesity is an established risk factor and is discussed below. Moreover, a fibre-depleted, calorie rich diet increases biliary cholesterol secretion and slows intestinal transit, which has been reported in patients with gallstones.[74] A high-fibre diet therefore, may accelerate intestinal transit, and exert its protective effects as this reduces not only constipation, but also deoxycholate formation (known to increase the cholesterol saturation of bile).[133] Animal studies have demonstrated these beneficial effects of fibre. Specifically, Schwesinger et al.[183] partially suppressed cholesterol gallstone formation in a prairie dog model by adding soluble fibre (as psyllium) to a diet consisting of 1.2% cholesterol. A high carbohydrate, high glycaemic load diet may trigger gallstone development indirectly through lipid-related alternations, such as raised plasma triglycerides (TG) and reduced high density lipoprotein cholesterol (HDL-C). It may also lead to exacerbations of insulin resistance and of other conditions of the metabolic syndrome, also linked to gallstone incidence. Elevated hepatic cholesterol synthesis and increased bile salt

malabsorption via hepatic insulin resistance may follow,[28] all of which are risk factors for gallstones.[116]

The role of lipids in gallstone formation is to date inconclusive.[45] In broad terms, saturated and trans fatty acids have been associated with an increased risk in prospective follow-up studies.[225] [231] In contrast, poly- and monounsaturated fatty acids have been linked to a decreased risk.[222] Dietary cholesterol is of particular interest, given the inherent role of cholesterol in gallstone formation. Experimental as well as human studies (of observational and interventional nature) report mixed results when evaluating the association between high cholesterol diets and gallstones risk.[120] [158] These discrepancies may be attributed to innate differences in lipid metabolism - a concept that is further supported by studies reporting a genetic influence on lipid metabolism, such as the *ABCG8* genes that determine dietary cholesterol absorption.[25]

2.2.2.2 Micronutrients

A decreased prevalence of ultrasonographically identified gallstones was detected in a population-based observation study with regular vitamin C supplementation (Odds Ratio [OR] 0.34, 95% CI 0.14 to 0.81, $p<0.01$).[244] This finding has been documented in other studies and appears to be more prominent in women as opposed to men.[89] [193] This protective influence may occur through the mediative effects of cholesterol 7α-hydroxylase activity (the rate-limiting enzyme in bile acid synthesis), which is reduced by ascorbic acid, as is cholesterol catabolism in bile, accordingly.

Moreover, a protective association was also observed with dietary magnesium intake in a large prospective cohort with the relationship being independent, and dose-responsive (Relative Risk [RR] 0.68, 95% CI 0.57 to 0.82, $p<0.001$).[230] A magnesium deficiency has not only been related to insulin resistance and diabetes but also to raised plasma TG, low density lipoprotein cholesterol (LDL-C) and lower HDL-C levels.[104] Reduced magnesium levels have also been linked to the metabolic syndrome;[54] correspondingly, magnesium may improve insulin sensitivity and lipid profile and hence have a positive effect on gallstone risk. Additionally, magnesium may exert its protective effects through its influence on gallbladder contractions via

cholecystokinin (CCK) stimulation; however precise mechanistic effects remain to be established. Finally, vitamin B12 and folic acid may influence gallstone formation, and these vitamins are discussed in another section.

2.2.2.3 Alcohol

The influence of alcohol consumption on gallstone risk remains controversial, as many studies have reported an inverse association between the two,[14 123 126 245] however, others have failed to observe any correlation.[19 107 168 180] Völzke et al.[243] reported an OR of 0.77 (95% CI 0.54 to 1.09, p=0.14) in men consuming 0 - 20 g alcohol daily. These odds were further reduced when the daily intake was between 20 - 60 g alcohol (OR 0.72, 95% CI 0.51 to 1.03, p=0.07) and were significantly reduced with > 60 g alcohol per day (OR 0.42, 95% CI 0.23 to 0.78, p<0.05). Alcohol consumption may reduce the CSI and increase HDL-C, thus inhibiting cholesterol stone formation.[219] On the contrary, severe alcohol abuse will raise the risk of (pigment) gallstones by means of liver damage and reduced synthesis of bile salts. This has been illustrated in studies showing alcohol-related cirrhosis to be a strong independent risk factor for gallbladder stones.[61 172]

2.2.2.4 Coffee

As with alcohol consumption, large epidemiological studies assessing coffee intake and risk of gallstones report diverse results. Indeed, both significant and non-significant inverse associations have been reported between coffee intake and incidence of gallstones.[49 92 125 142 161] In contrast, there are also documented reports of no association between the two,[112 180 245] or even an increased risk of gallstones with coffee consumption.[19 87 243] One cannot be sure whether these discrepancies result from ethnic and/or complex gene-environment interactions, however caffeine and perhaps additional components in coffee might alter serum lipids and impact on enterohepatic bile salt circulation, thus reducing gallstone risk.

2.2.2.5 Physical inactivity

Physical activity has been associated with a reduced risk of gallstones, in particular the symptomatic variety and hence with cholecystectomy. These associations have been illustrated in two large US cohorts,[122] [124] but also in a European cohort (European Prospective Investigation of Cancer (EPIC) cohort.[18] Of note, findings from the European cohort suggest a 70% risk reduction of symptomatic gallstones could be achieved after five years in those who are physically active. In addition, 34% of symptomatic gallstones in men could possibly be prevented with 30 minutes of endurance exercise (i.e. running, cycling) if carried out five times per week.[122] Altered hepatobiliary function could in theory be responsible for these beneficial effects of physical activity on gallstones. Moreover, physical activity not only enhances gut motility and increases bile acid excretion,[165] [249] but it can also raise HDL-C [50] via its influence on plasma TG and insulin release,[102] [220] all of which lower biliary cholesterol saturation. As such, a sedentary lifestyle will increase the risk not only of gallstones, but of the complications that can ensue.

2.2.2.6 Metabolic syndrome

The cluster of conditions belonging to the metabolic syndrome including dyslipidaemia (particularly hypertriglyceridaemia and low HDL-C), diabetes and insulin resistance/hyperinsulinaemia are all often co-morbid with gallstone disease, as such, they are suggested to be risk factors.[188] [232] [243] In fact, a two-to-threefold higher prevalence of gallstones in insulin-resistant individuals and in those with type 2 diabetes has been observed.[179] A mouse model illustrated a molecular link between the metabolic syndrome and cholesterol gallstones via hepatic insulin resistance, and consequently increased biliary cholesterol secretion.[28] Of note, increased hepatic cholesterol secretion, gallbladder dysmotility and supersaturated bile are all aggravated by the metabolic syndrome and this in turn may set the stage for subsequent gallstones formation.[6] Interestingly, in societies that have adopted a 'Westernized' diet, alarmingly high rates of the metabolic syndrome are also observed, as are gallstones.[2] To this end, mutual common dietary risk factors are likely to increase the risk of both the metabolic syndrome and of gallstones.

2.2.2.7 Obesity

Obesity could very well be the common denominator between diet and gallstone risk as it is part of the spectrum of the metabolic syndrome and it is a well-established risk factor for gallstones.[243] Obesity promotes insulin resistance and biliary hypersecretion of cholesterol, leading to lithogenic bile.[24 28 144] Interestingly, the type of adiposity is also reported to influence gallstone formation. Prospective cohort studies have reported a stronger association with central adiposity relative to limb or lower extremity adiposity, therefore regional fat distribution may further intensify stone risk.[72] In fact, abdominal adiposity was associated with an increased risk of symptomatic gallstones and with cholecystectomy, independently of BMI in two US cohorts.[224 228]

2.2.2.8 Rapid weight loss and/or surgery for obesity

Paradoxically, not only is obesity linked to an increased risk of gallstones, but so is rapid weight loss, (i.e. > 1.5 kg per week), and/or weight loss greater than 25% body weight.[127 191] These findings have been illustrated in studies where patients follow very low calorie diets and/or undergo bariatric surgery (e.g. Roux-en-Y gastric bypass).[81 94 127 128 251 258] Specifically, a 13% gallstone incidence was observed after 17 weeks in obese participants following a combined liquid and solid foods low-calorie diet (925 kcal).[201] Liddle et al.[128] reported a 25% increased risk of gallstones when obese subjects followed a 2100 kJ (500 kcal) diet for eight weeks, and Li et al.[127] found a ~30% increased risk post bariatric surgery. Of note, gallstones formation during rapid weight loss has been referred to as a possible adverse event, as such clinicians are obliged to inform patients of the risks.[73 166]

Slower weight loss will reduce the risk of gallstones forming, however this weight loss must be sustained because weight cycling (i.e. weight that is lost and regained) also increases the risk of gallstones and this risk appears to be independent of body weight.[229] Weight cycling not only correlates with gallstones, but also with its complications and might trigger symptomatic gallstones as data from a large cohort observed an increased risk of cholecystectomy in such subjects.[214] Interestingly, this cohort illustrated a risk of 31% in women with at least one moderate weight cycle (4.5

- 8.5 kg), however this increased to 68% in subjects with severe weight fluctuations (≥ 9 kg). The reason for this observed risk is multifactorial and includes impaired gallbladder motility, and the consequent bile stasis and biliary sludge which follow. However, an altered ratio of cholesterol to bile salts in the gallbladder also results from rapid weight loss and weight cycling. This is due to the amplified cholesterol mobilisation from peripheral tissue, and the concomitant increased hepatic cholesterol synthesis.[67 131 175]

2.2.3 Environmental factors

2.2.3.1 Treatments / medication

Prolonged total parenteral nutrition (TPN) can weaken gastrointestinal stimulation, thus impairing gallbladder emptying. This can cause bile stasis which predisposes to biliary sludge and eventually gallstones. The incidence of gallstones however – which reportedly can range from 20% to 75% – can be reduced with concomitant oral intake.[10 70] There are several other conditions, such as spinal cord injury (SCI), that can lead to bile stasis.[11 16] Of note, 30% of patients with SCI are reported to have gallstones, or to have undergone cholecystectomy.[148]

Certain medications such as octreotide (a somatostatin analogue) can trigger bile stasis. In fact, reduced intestinal transit was observed in those taking octreotide, and this led to lithogenic bile salt pool formation and subsequent gallstone development.[29 84] Calcineurin inhibitors, namely ciclosporin and tacrolimus, can also cause gallstones via the inhibition of the hepatocanalicular bile salt export pump.[139 205] Furthermore, fibrates (cholesterol-lowering drugs) raise biliary cholesterol saturation and reduce bile salt synthesis, thus also increasing the risk of gallstones.[196] In contrast however, some other types of cholesterol lowering drugs, (i.e. statins) decrease hepatic cholesterol biosynthesis and consequently reduce cholesterol concentrations in bile, and so might reduce the risk of gallstones as demonstrated in prospective follow-up and case-control studies.[20 233] Interestingly, ezetimibe was shown in mouse models to exert its protective effect through the reduced absorption of intestinal cholesterol coupled with an increase in bile flow.[246 262]

Estrogens such as oral contraceptives and postmenopausal hormonal replacement therapy (HRT) significantly increase the risk of cholesterol gallstones and cholecystectomy.[39 56 243] Innate estrogen levels are also aetiologically linked to gallstones, as women compared to men appear to have a heighted gallstone risk, in particular multiparous women. This may be because estrogen enhances hepatic lipoprotein uptake and increases hepatic cholesterol synthesis.[56 247] Progesterone, another steroid hormone, impairs gallbladder contraction and reduces the rate of emptying, hence might also lead to stone formation.[174]

2.2.3.2 Infections, metabolic diseases and surgical procedures

Bile duct infections such as those from parasites (e.g. Clonorchis sinensis, Opisthorchis viverrini or Ascaris lumbricoides) can trigger brown pigment stone formation by means of bacterial β-glucuronidase which reverses soluble conjugated bilirubin back to its insoluble unconjugated form.[188] Thus, brown pigment stones result due to the precipitation of bilirubin into calcium salts of long-chain fatty acids.[118] Moreover, cholesterol gallstones may result from increased colonic bacterial formation of deoxycholate which causes increased secretion and saturation of biliary cholesterol.[27 153] Of note, the presence of enterohepatic *Helicobacter* species have been detected in bile and might possibly play a causal role in gallstone pathogenesis.[137 197] This was illustrated in an experimental study, where a very high prevalence of gallstones (80%) was found in C57L/J mice after infection with enterohepatic *Helicobacter* strains.[136] Likewise, increased quantities of Gram-positive anaerobes and increased 7α-dehydroxylating activity were observed in the caecum of patients with gallstones compared to controls.[217]

Infections such as the chronic hepatitis C virus (HCV) are also correlated with the presence of gallstones. A US cohort (NHANES III) comprising 13,465 participants observed an increased risk of gallstones (OR 3.2, 95% CI 1.08 to 9.45) and cholecystectomy (OR 4.57, 95% CI 1.57 to 13.27) in men with HCV compared to men without the virus.[30] This risk does not appear to be gender specific, as other studies also report a heighted risk of gallstones with presence of HCV in both men and

women.[3] [212] Insulin resistance has been suggested as the causal link between the two conditions, because it is able to increase bile cholesterol saturation.[3] Insulin resistance could emerge from the development of central obesity and liver steatosis.[3] [44] [52]

In addition to HCV, liver cirrhosis is a well-documented risk factor for gallstones, particularly for black pigment stones.[42] [206] Abnormal gallbladder motility, bile salt malabsorption, coupled with a decreased synthesis of bile salts can initiate increased enterohepatic cycling of unconjugated bilirubin and eventually stone formation in the cirrhotic patient.[241] This exact mechanistic effect is suggested as causal for black pigment stones in cystic fibrosis,[241] and in patients with Crohn's disease, particularly if accompanied by extended ileal resection.[33] [118] [160] In fact, a vitamin B12 deficiency often occurs in ileal resections (as it is actively absorbed in the terminal ileum) and a deficiency of vitamin B12 (and folic acid) – both of which are required for generating red blood cells – may increase stone risk by exacerbating anaemia.[51] Black pigment stones have been reported in people with blood disorders such as hemolytic and sickle cell anaemia.[118] Other surgical procedures, such as total gastrectomy can increase gallstone formation, particularly when total gastrectomy is accompanied by lymph node dissection.[105] Fukagawa et al.[64] identified gallstones in 26% of 672 patients following gastrectomy with lymph-node dissection. This increased risk may result from damage to the hepatic branch of the vagal nerves which diminishes gallbladder contractility.

2.3 Lifestyle interventions purported to reduce the risk of gallstones

Evidence exists in support of 'Westernised' lifestyle habits such as physical inactivity and high-calorie, high-carbohydrate and saturated fat diets, as well as low fibre intake to confer an increased risk of cholelithiasis and to promote *LITH* gene penetrance.[206] Rapid weight loss and weight cycling also increase formation of gallstones primarily due to gallbladder stasis and reduced biliary bile salt secretion.[229] [250] Lifestyle interventions such as dietary fat manipulation during low calorie dieting or physical activity have been investigated for gallstone prevention. Beneficial effects may result from increased intestinal motility.[18] Moreover, clinical studies using bile acids,

especially the secondary bile acid ursodeoxycholic acid (UDCA) have been assessed during rapid weight loss, given their ability to enhance cholesterol solubility. A meta-analysis of five randomised trials on UDCA for gallstones prevention post bariatric surgery reported a protective effect against gallstones.[237] UDCA is able to decrease the lithogenicity of bile by reducing the intestinal absorption and biliary secretion of cholesterol as well as through shifting the phase separation of bile towards solubilisation in micelles and vesicles.[35 146 181] Given its reported efficacy, UDCA may have great potential as a preventive agent against gallstones.

2.4 Why is it important to meta-analyse non-surgical interventions?

The American Medical Association has just declared obesity to be a disease requiring medical treatment and prevention.[9] Childhood obesity is currently ~ 20% and there is a strong likelihood that obese children are likely to be obese in later life or will attempt weight loss at some stage.[82] In fact, a rise in adolescent bariatric surgery has recently been reported.[83] Given the burden associated with gallstones and the rise in both adult and childhood obesity, we anticipate an increase in individuals at risk of gallbladder stones and consequently an increase in healthcare costs. Successful non-surgical preventions may need greater consideration. However, most randomised controlled trials with non-pharmacological and pharmacological interventions include small sample sizes and their combined effect is unclear. We therefore conducted a Cochrane systematic review and meta-analysis of randomised controlled trials to investigate the efficacy of non-surgical preventive options for gallbladder stones in adults.

2.5 Meta-analysis for evidence based medicine and the Cochrane Collaboration

A meta-analysis is defined as the statistical synthesis of data from separate but similar studies to obtain a quantitative summary of the results from these independent studies. The goal of a meta-analysis is ultimately to estimate the true treatment effect with high accuracy, and allows one to assess for consistency across

individual studies. A systematic review that includes a meta-analysis of high quality (i.e. well conducted) homogenous randomised controlled trials is considered the highest quality evidence in evidence based medicine. A systematic review facilitates the process by identifying, appraising and synthesising the research and presenting it in a systematic yet meaningful manner. It serves to inform researchers, consumers, healthcare providers and policy makers.

The Cochrane Collaboration is an international highly reputable organisation dedicated to evidence based medicine. It was founded in 1993 and named in honour of Archie Cochrane, a British medical researcher whose work in the 1970s greatly influenced the conversion of epidemiology into a scientific discipline.[242] In the 1980s Archie Cochrane was the first to refer to a systematic review of randomised controlled trials and encouraged the scientific discipline to endorse such a technique for delineating scientific evidence (http://www.cochrane.org/about-us/history). In 1992 the first Cochrane centre was established in Oxford, UK which subsequently led to the creation of the Cochrane Collaboration.[40]

To date there are 53 different Cochrane Review Groups, each with a specific disease focus. The aim of these review groups is to support the production of high quality systematic reviews and meta-analyses, which they do through established protocols and regulations. The Cochrane Handbook of Systematic Reviews[79] guides the authors of systematic reviews. Authors wishing to perform a systematic review are first required to submit a title registration form to the respective review group with an expression of interest on the review they would like to conduct. In our case, our review group is the Cochrane Hepato-Biliary Group (http://hbg.cochrane.org/). Once this is approved, authors are required to submit a full protocol which is subject to peer-review. Upon acceptance of the final version of the protocol, this is then published and authors are permitted to conduct the full review. Our title registration form and our published protocol can be found at the end of this thesis.

3. Methods

This systematic review and meta-analyses were performed according to a published protocol[207] and followed the instructions in the *Cochrane Handbook for Systematic Reviews of Interventions.*[68 79] The main objective was to evaluate the non-surgical primary prevention of gallbladder stones in adults.

3.1 Types of studies

We included randomised clinical trials irrespective of blinding, language, sample size, or publications status. Quasi-randomised trials and observational studies were only eligible for inclusion in the analyses of adverse events.

3.2 Types of participants

Adults (at least 18 years of age) of either gender were included irrespective of ethnicity. Participants were eligible for inclusion if they did not have gallbladder stones at baseline. Trials conducted in children where appraised in the search for data on harm.

3.3 Types of interventions

Trials were considered for inclusion when at least one study group was allocated to receive a non-pharmacological intervention or a pharmacological intervention. Orally administered non-pharmacological and pharmacological interventions were included irrespective of the dose or class of drug. The control groups were allocated to placebo, no intervention, or to non-pharmacological or pharmacological, interventions. The threshold for duration of therapy was set to a minimum of four weeks, as studies report gallstones to typically form after this time frame.[67 128]

3.4 Types of outcome measures

All outcome measures were assessed, where possible, at the maximum duration of follow-up.

3.4.1 Primary outcomes

Based on the specifications provided in the Cochrane Hepato-Biliary Group module Gluud,[68] the following primary outcome measures were included:

1. Mortality (all-cause).
2. Morbidity (formation of ultrasonically verified gallbladder stones and symptomatic gallbladder stones).
3. Number and types of adverse events (using definitions specified by authors of included trials or based on The International Conference on Harmonisation Expert Working Group International).[86]

3.4.2 Secondary outcomes

1. Quality of life.
2. Cholecystectomy.
3. Bile lithogenicity (defined as changes in physiological parameters of bile composition indicative of an increased risk of gallstones, e.g. cholesterol saturation index (CSI),[37] nucleation time for cholesterol crystal formation,[80] or presence of cholesterol crystals).
4. Weight loss (reduction in body weight assessed in kg or using the body mass index (BMI)).

3.5 Search strategy for identification of trials

Eligible trials were identified through electronic and manual searches. Randomised clinical trials were included. Male and female adults were included irrespective of ethnicity. Participants were eligible for inclusion if they did not have gallbladder stones at baseline verified by ultrasonography. We searched the Cochrane Hepato-Biliary Group Controlled Trials Register,[68] the Cochrane Central Register of Controlled Trials (CENTRAL) in *The Cochrane Library*, MEDLINE, EMBASE, and Science Citation Index Expanded. The last search update was performed in July 2013 (Appendix 1).

Trial registries were scanned in two search portals: the US National Institutes of Health (www.clinicaltrials.gov) and the WHO International Clinical Trial Registry Platform (www.who.int/ictrp/search/en/). We originally planned to include unpublished trials, but no such trial was identified. The manual search comprised scanning reference lists of relevant papers.

All references identified in the searches were reviewed and potentially eligible trials were listed and compared against the inclusion criteria. Excluded trials were listed with the reason for exclusion. The data was extracted using standardised data extraction forms (Appendix 2) from the Cochrane Center. These forms were slightly modified to suit this systematic review/meta-analysis. Authors of individual trials were contacted for any unclear or missing information. Two trials were translated into English before the data extraction.

3.6 Assessment of bias

Trials were assessed using the Cochrane Collaboration risk of bias tool.[79] The following information was extracted for each trial by at least two authors and risk of bias for each domain was rated as low (unlikely to significantly influence the results), high (likely to significantly influence the results), or unclear:

3.6.1 Allocation sequence generation

- Low risk of bias: sequence generation was achieved using computer random number generation or a random number table. Drawing lots, tossing a coin, shuffling cards, and throwing dice are adequate if performed by an independent research assistant not otherwise involved in the trial.
- Uncertain risk of bias: the method of sequence generation was not specified.
- High risk of bias: the sequence generation method was not random.

3.6.2 Allocation concealment

- Low risk of bias: the participant allocations could not have been foreseen in advance of, or during, enrolment. Allocation was controlled by a central and independent randomisation unit. The allocation sequence was unknown to the investigators (for example, if the allocation sequence was hidden in sequentially numbered, opaque, and sealed envelopes).
- Uncertain risk of bias: the method used to conceal the allocation was not described so that intervention allocations may have been foreseen in advance of, or during, enrolment.
- High risk of bias: the allocation sequence was likely to be known to the investigators who assigned the participants.

3.6.3 Blinding of participants, personnel, and outcome assessors

- Low risk of bias: blinding was performed adequately, or the assessment of outcomes was not likely to be influenced by lack of blinding.
- Uncertain risk of bias: there was insufficient information to assess whether blinding was likely to induce bias on the results.
- High risk of bias: no blinding or incomplete blinding, and the assessment of outcomes were likely to be influenced by lack of blinding.

3.6.4 Incomplete outcome data

- Low risk of bias: missing data were unlikely to make treatment effects depart from plausible values. Sufficient methods, such as multiple imputations, have been employed to handle missing data.
- Uncertain risk of bias: there was insufficient information to assess whether missing data in combination with the method used to handle missing data were likely to induce bias on the results.
- High risk of bias: the results were likely to be biased due to missing data.

3.6.5 Selective outcome reporting

- Low risk of bias: all outcomes were pre-defined (for example, in a published protocol) and reported, or all clinically relevant and reasonably expected outcomes were reported.
- Uncertain risk of bias: it is unclear whether all pre-defined and clinically relevant and reasonably expected outcomes were reported.
- High risk of bias: one or more clinically relevant and reasonably expected outcomes were not reported, and data on these outcomes were likely to have been recorded.

3.6.6 Other biases

- Low risk of bias: The study appears to be free of other sources of bias.
- High risk of bias: There is at least one important risk of bias. For example, the study: had a potential source of bias related to the specific study design used; or has been claimed to have been fraudulent; or had some other problem.
- Unclear risk' of bias: There may be a risk of bias, but there is either: insufficient information to assess whether an important risk of bias exists; or insufficient rationale or evidence that an identified problem will introduce bias.
- Inappropriate influence of funders (or, more generally, of people with a vested interest in the results) is often regarded as an important risk of bias: information

about vested interests was collected and presented in the 'Characteristics of included studies' table. The 'Risk of bias' table was used to assess specific aspects of methodology that might have been influenced by vested interests and which may lead directly to a risk of bias.

3.7 Statistical analysis

The data was analysed using the Cochrane Review software, Review Manager 5, STATA 12 (Stata Corp. Texas, USA) and Trial Sequential Analysis (Copenhagen Trial Unit, Copenhagen, Denmark). The primary meta-analyses were performed using random effects models due to expected clinical heterogeneity. Different interventions were analysed separately. Fixed effect models were used to evaluate the robustness of the results but were only reported if they differed from that of the random effects models. The measures of treatment effect were expressed as risk ratios for dichotomous data and weighted mean differences for continuous outcomes, both with 95% confidence intervals and with I^2 as markers of heterogeneity. Based on the I^2 values, heterogeneity was classed as not important (< 40%), moderate (40% to 60%), substantial (> 60% to 75%) or considerable (> 75%). The number needed to treat (NNT) was computed for dichotomous data when the confidence interval did not cross one. When trials included more than two intervention groups, multiple groups were combined to create a single pair-wise comparison.[79] Data on all participants randomised were sought to allow intention-to-treat analyses including all participants, irrespective of compliance or follow-up.

3.7.1 Subgroup analysis and investigation of heterogeneity

The risk of small study effects was analysed through regression analyses (Egger's test). We performed the following subgroup analyses to evaluate the influence of: participant type (type of weight loss method); treatment dose (medium-to-high or low dose UDCA, i.e. 1000 - 1200 mg or 500 - 750 mg, respectively); and risk of bias (low versus high or unclear risk of bias).

3.7.2 Sensitivity analysis

Sensitivity analyses evaluated the importance of losses to follow-up (good outcome analyses assuming that losses to follow-up were treatment successes and poor outcome analyses assuming that losses to follow-up were treatment failures). We also repeated the analyses using the 0.5 continuity correction. This provides imputed data for analysis in trials reporting zero events in both arms, which comprised two trials in our meta-analyses.[134 140] The results of these analyses are only reported if the conclusions differed from the primary analyses.

3.7.3 Trial sequential analysis

Cumulative meta-analyses are at risk of producing random errors due to sparse data and multiple testing.[17 34 218 252] Therefore, sequential analysis was performed to assess the robustness of the data.[43] The required information size was defined as the number of participants needed to detect or reject an intervention effect, and was estimated based on the event proportion in the control group, the observed relative risk reduction, and the diversity of the meta-analysis.[252 253] The alpha was set to 5% and the power to 80%. On the basis of the required information size, trial sequential monitoring boundaries were constructed. Firm evidence was defined as being established if the sequential monitoring boundary was crossed before reaching the required information size. If the boundary was not crossed, the evidence was not conclusive.

4. Results

Overall, we identified 3,044 references through our electronic searches and 17 references through manual searches (Figure 2). No unpublished trials were identified through correspondence with pharmaceutical companies (Aptalis, Astellas, Bristol-Myers Squibb, Consolidated Chemicals Ltd., Eli Lilly, Falk Pharma, Hoechst Marion Roussel, Johnson and Johnson, Lunbeck, Norgine Pharmaceuticals, Novartis, Pfizer, and Sanofi-Aventis).

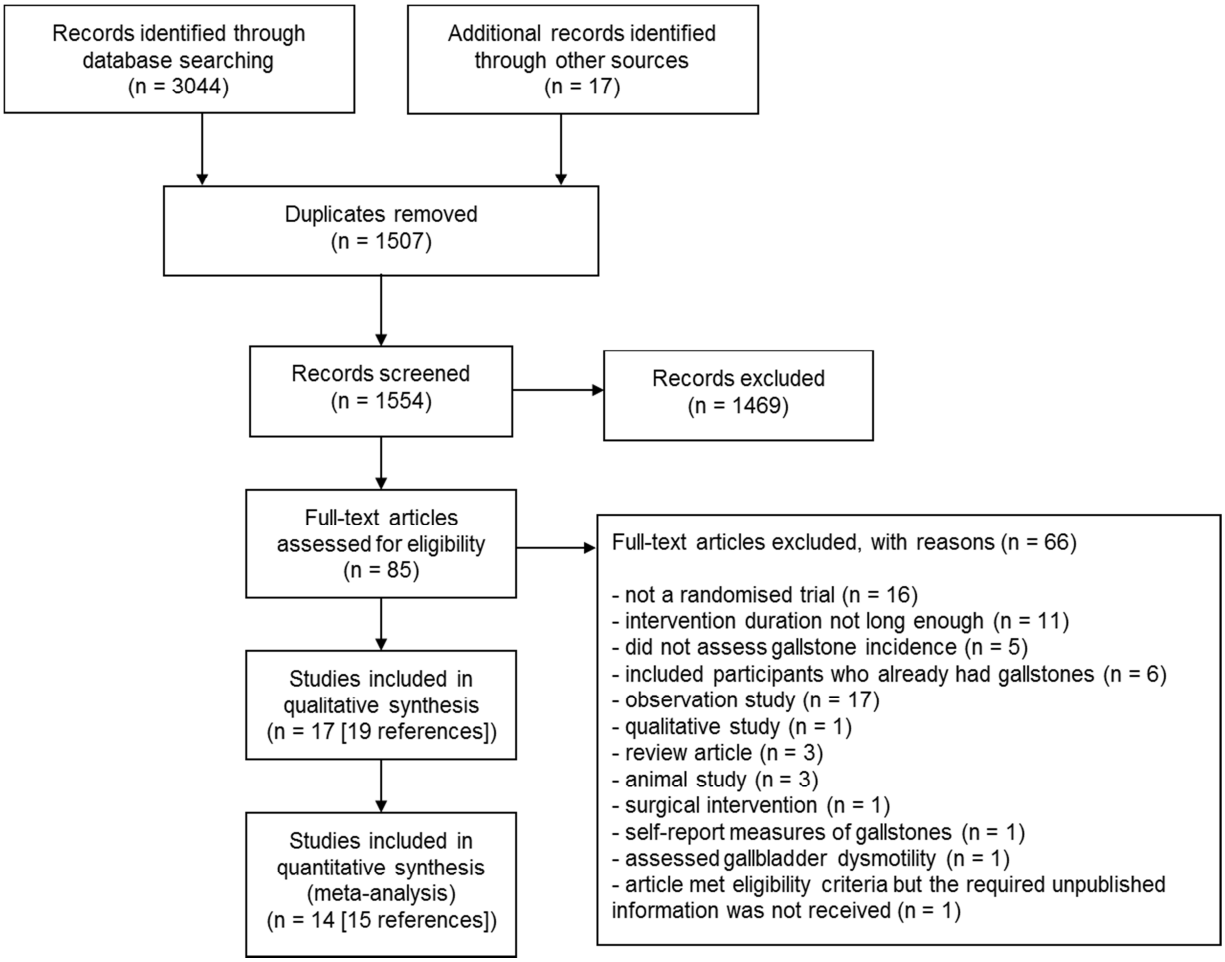


Figure 2. Flow chart for the identification and selection of included randomised trials

Of note, one trial had to be excluded from our quantitative analysis because we were unable to extract data on the outcome measures.[81] Therefore, after excluding this trial, the duplicates and references that did not refer to trials that fulfilled our inclusion criteria, 19 references (corresponding to 17 trials) were eligible for the qualitative data synthesis. Fifteen references (corresponding to 14 trials) fulfilled our inclusion criteria for the meta-analyses. Appendix 3 summarises the excluded trials, together with the reason for exclusion.

4.1 Included studies

4.1.1 Characteristics

Two trials were multicentre in design,[191 213] and the remaining were single centred. The trials were all published as full paper articles from 1988 to 2003. One trial was published in Italian[47] and another trial in Spanish,[149] which also included a short publication in English.[150] The remaining trials were English language papers. Gallstones were diagnosed by ultrasonography. One trial used additional cholecystography[254] and another trial used abdominal computerised tomography scans.[141]

4.1.2 Participants

Thirteen trials investigated obese participants (defined as BMI > 30 kg/m^2) about to embark on weight loss. The majority of participants were female (range 42 - 100%). Eight of these trials used caloric restriction (details summarised in Table 3) based on a low-calorie (LCD: 900 - 1679 kcal/day) or very low-calorie diet (VLCD: < 800 kcal/day) for weight loss. The remaining five trials assessed weight loss post bariatric surgery. Finally, one trial evaluated participants after major cardiac surgery.[5]

Table 3. Dietary composition of weight loss diets

Trial		kcal	Protein (g)	Carbohydrates (g)	Fat (g)	Fibre (g)	Cholesterol (mg)
Broomfield[35]		520	55	79	1		
De Filippo[47]		1000-1200	60-70	100-170	20-43	35-40	165-220
Festi[57]	*Intervention*	577	55	61.7	12.2		
	Control	535.2	44.4	82.2	3		
Gebhard[67]	*Intervention*	900	90	68	30		90
	Control	520	50	76	< 2		30
Marks[134]		520	NG	NG	NG		
Mendez-Sanchez[140]		1200	60	180	27		
Moran[149 150]		1679*	67	248	48	20	
Shiffman[191]		520	50	79	1-3		

Abbreviations: NG, not given

*Each patient had to reduce their total energy intake by 500 kcal and was instructed to follow a diet with 15% protein, 60% carbohydrate, 25% fat, as specified above.

4.1.3 Interventions

Two of these 14 trials compared 12 weeks of a high-fat modification weight reducing diet versus low-fat modification weight reducing diet.[57 67] The diet in the intervention and control groups included 12.2 g versus 3.0 g fat[57] or 30 g versus 2 g fat per day.[67] Twelve trials assessed 300 to 1200 mg/day UDCA (median 750 mg/day). The duration of treatment ranged between six weeks to 18 months, and the duration of follow-up ranged from six weeks to 60 months. Two trials included three different doses of UDCA: 300/600/1200 mg.[191 213] Four trials included a third allocation arm in which participants received 1300 mg/day of acetylsalicylic acid (aspirin),[35] 1600 and 600 mg/day of ibuprofen, respectively,[134 257] or 11.3 g/day of omega-3 fatty acids.[140] Table 4 summarises the main characteristics of the included studies and the full details extracted for each study are in appendix 4.

Table 4 Characteristics of randomised controlled trials of non-surgical interventions for primary gallbladder stone prevention

Trial	Country	Patients (n)	Intervention (dose/day)	Intervention duration (wks)	Follow-up (wks)	Baseline weight (kg)	Mean weight lost (kg)	Percentage weight lost	Drop outs (excl withdrawals)	Main inclusion criteria
Ai[5]	Japan	52 54	600 mg UDCA; control	24	240	-	-		15 14	Non-obese (post cardiac surgery)
Broomfield[35]	USA	23 22 23	VLCD + 1200 mg UDCA; VLCD + 1300 mg aspirin; VLCD placebo	16	19	106 98 106	21 25 21	20 26 20	5 8 4	Obese
De Filippo[47]	Italy	20 20	LCD + 600 mg UDCA; LCD + placebo	16	16	105 101	10 8	10 8	0 0	Obese
Festi[57]	Italy	16 16	VLCD + high-fat; VLCD + low-fat	12	12*	115 110	20 19	17 17	5 5	Obese
Gebhard[67]	USA	7 6	LCD + high-fat; VLCD + low-fat	12	12*	114 105	25 23	22 22	0 0	Obese
Marks[134]	USA	16 15 16	VLCD + 1200 mg UDCA; VLCD + 1600 mg ibuprofen; VLCD + placebo	12	12	100 110 114	10[$] 11[$] 11[$]	10 10 10	20[§]	Obese
Mendez - Sanchez[140]	Mexico	14 14 14	LCD + 1200 mg UDCA; LCD + 11.3 g omega-3 fatty acids; LCD + placebo	6	6	80 84 82	6 7 6	8 8 7	4[§]	Obese
Miller[141]	Austria	76 76	500 mg UDCA; placebo	24	96	136 136	50 51	37 38	12 16	Obese (post bariatric surgery)
Moran[149 150]	Mexico	18 18	LCD + 750 mg UDCA; LCD + 15g fibre	8	8	90 86	6 6	7 7	0 0	Obese
Shiffman[191]	USA	742 255	VLCD + 300/600/1200 mg UDCA; VLCD + placebo	16	16	128 129	25 24	20 19	255[§]	Obese
Sugerman[213]	USA	231 74	300/600/1200 mg UDCA; placebo	24	24[†]	137 144	40 38	29 26	72[§]	Obese (post bariatric surgery)
Williams[254]	Canada	44 42	10 mg/kg UDCA; placebo	Up to 72	Up to 72	-	40 43	-	6 0	Obese (post bariatric surgery)
Worobetz[256]	Canada	13 16	1000 mg UDCA; placebo	12	12	147 143	25 29	17 20	3 2	Obese (post bariatric surgery)
Wudel[257]	USA	20 20 20	600 mg UDCA; 600 mg ibuprofen; placebo	24	48	159	48	28	5 5 9	Obese (post bariatric surgery)

Abbreviations: LCD, low calorie diet; UDCA, ursodeoxycholic acid; VLCD, very low calorie diet

*The entire study duration was 24 weeks, however only the first 12 weeks were included in this systematic review as this was the weight loss phase.

[†]54 patients were followed up for 48 weeks but only data from the 24-week time point is included.

[$] Weight loss reported only for the six-week time point

[§]Reported no significant difference between groups.

4.2 Risk of bias in included studies

Overall the aggregate selection, performance and detection bias was relatively low in comparison to attrition, reporting and other biases, as illustrated in figure 3 below.

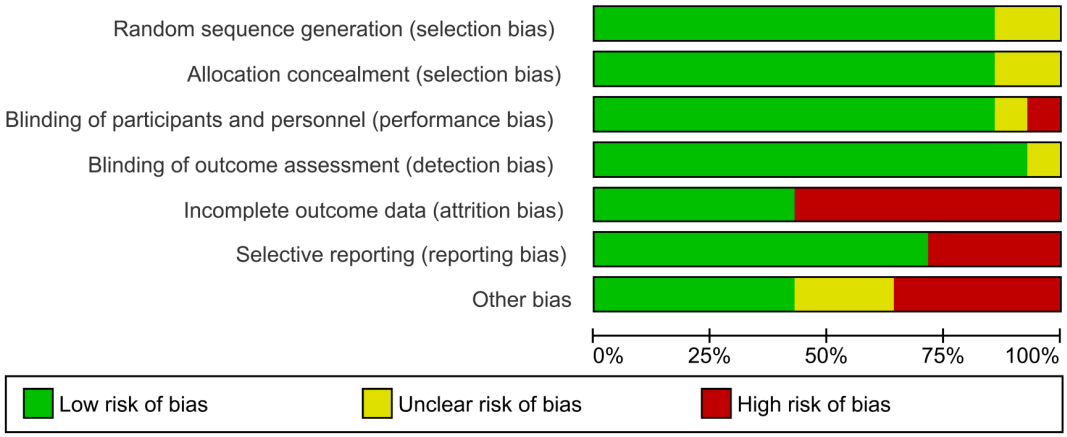


Figure 3. Aggregate risk of bias for all included trials

Specifically, none of the trials were classed as having a high risk of bias based on the allocation methods (see Figure 4). Two trials did not mention the allocation sequence generation, and two trials did not describe the allocation concealment. Two trials were classed as having a high or unclear risk of performance and detection bias. The remaining trials were double-blind and included blinding of outcome assessors as well as participants. Seven trials were classed as having a high risk of attrition bias because data on patients who were lost to follow up were incomplete. This was considered the main source of bias in these trials. For three trials[134 191 213] the allocation group was not specified for participants with missing outcome data. Four trials did not report the clinically important outcomes at all the measured time points. These included one trial from the dietary fat modification interventions[67] and three from the intervention trials with UDCA.[35 134 254] In the other trials included in the meta-analyses, outcomes measures were explicitly defined and reported.

Four trials were funded by pharmaceutical companies either fully[191 213] or partially.[254 257] The industry funding did not obviously affect the overall trial design or analysis, as the dose and duration of the interventions assessed did not differ from remaining trials. Two trials were terminated early due to high attrition and slow recruitment[134] or

because the incidence of symptomatic gallstones was considered too high in the control group.[67] Five trials reported power calculations,[67 141 149 191 213] and one of these trials did not achieve the expected power.[67]

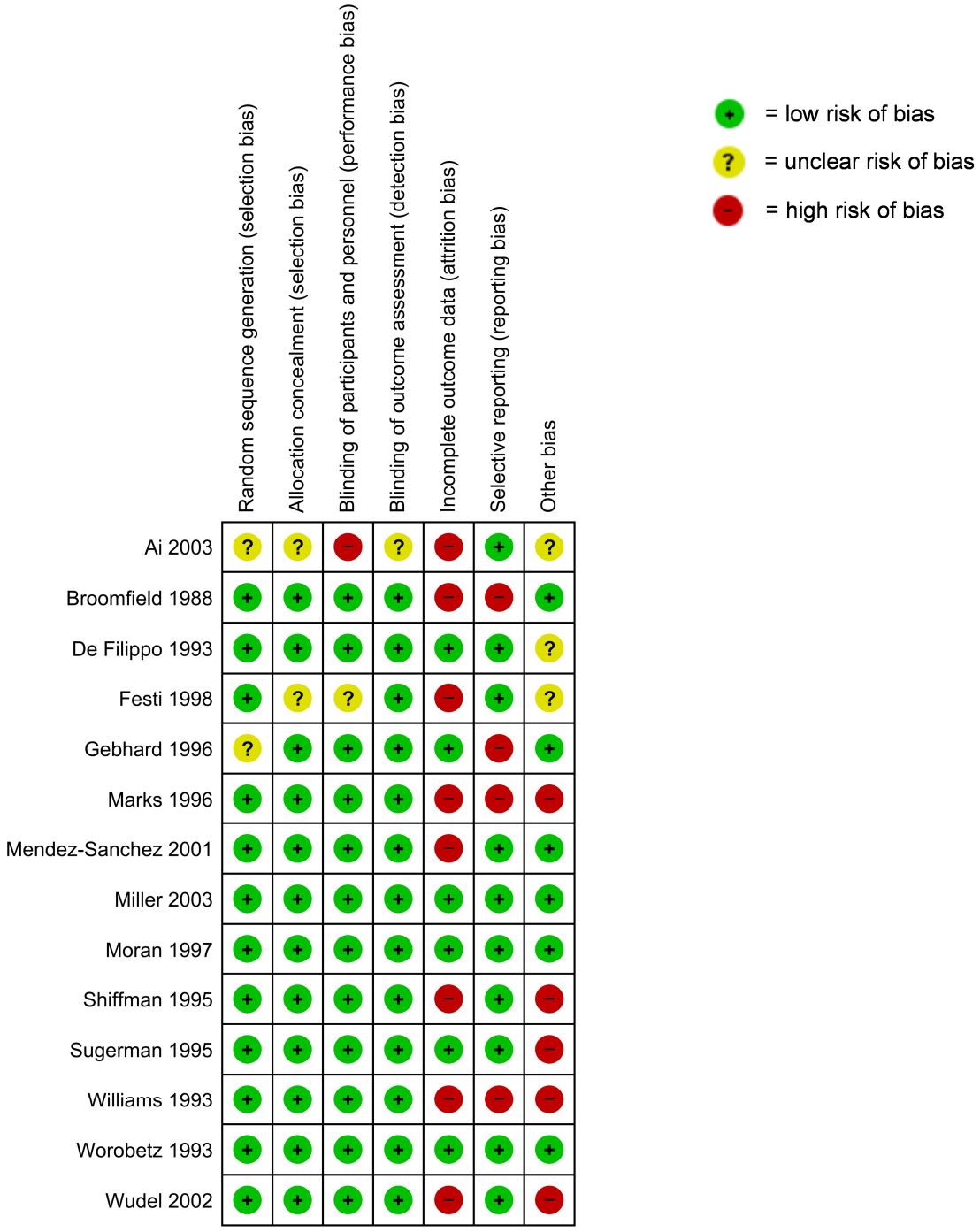


Figure 4. Individual trial risk of bias for each domain

4.3 UDCA interventions

As shown in Figure 5 below, from the 12 trials on UDCA versus control interventions, 66 of 1,269 participants (5%) in the intervention group and 145 of 628 participants (23%) in the control group developed gallstones, which corresponds to a risk ratio of 0.32 (0.19 to 0.55, I^2=60%). The corresponding NNT was 13 patients. No deaths were reported.

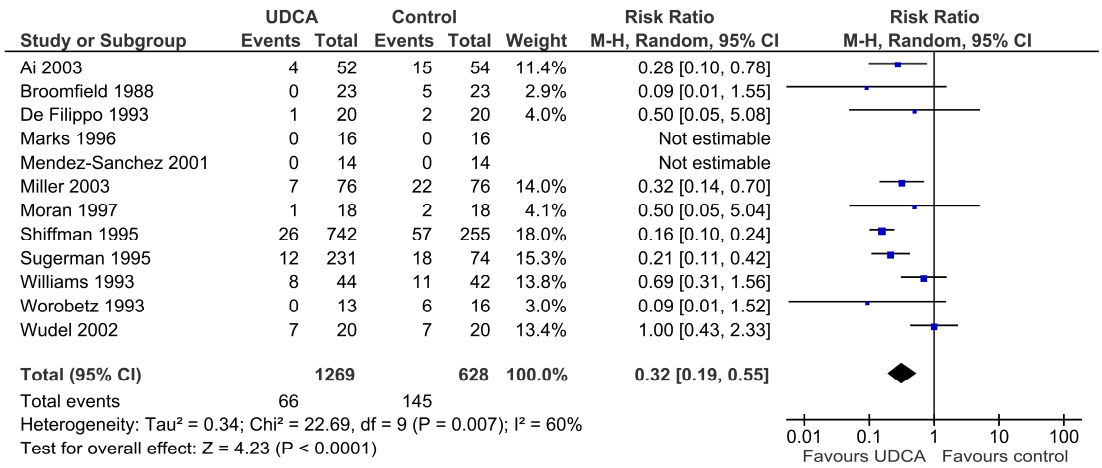


Figure 5. Meta-analysis of gallstone formation in patients receiving UDCA versus control interventions

Six trials reported the number of participants who underwent cholecystectomy due to symptomatic gallstones.[5 35 141 213 256 257] Two of these trials however, did not report the allocation group for these participants.[35 257] A meta-analysis of the weight loss trials showed that UDCA reduced the risk of cholecystectomy for symptomatic stones, with a risk ratio of 0.19 (0.07 to 0.49, I^2=0%, Figure 6).

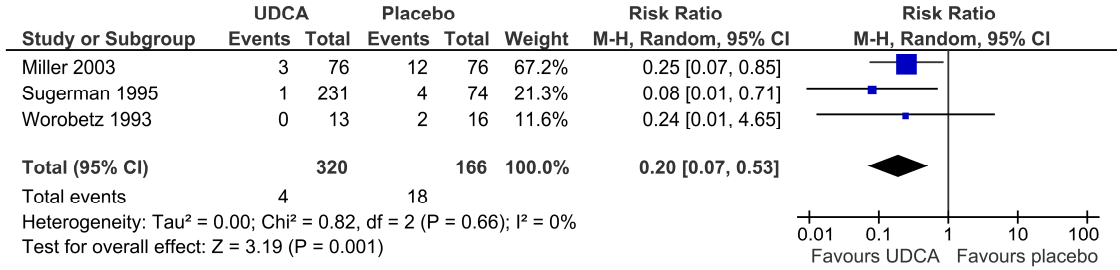


Figure 6. Meta-analysis of cholecystectomy in obese patients receiving UDCA versus control interventions during weight loss

Due to differences in the assessment of bile lithogenicity, we were unable to perform meta-analyses on this physical-chemical outcome. Two[35 134] of three diet alone trials found a significantly beneficial effect of UDCA versus placebo on CSI and the third trial reported a decreased trend for the UDCA group and an increased trend for the placebo group.[191] A fourth trial noted that the CSI did not significantly change during the trial in any of the groups, though a decreased trend was observed and cholesterol nucleation time decreased significantly in both UDCA and placebo groups.[140] Finally, one trial[35] also reported a significant increase in CSI at week four (from baseline) in those who developed gallstones and was significantly higher than those who did not develop gallstones (who had no significant change from baseline).

Weight loss was equal in the UDCA and placebo groups in all trials (range 6 - 51 kg).[35 47 134 140 141 149 191 213 254 256 257] We were able to include data from four trials in the meta-analysis below (Figure 7), confirming the finding of equal weight loss, with a weighted mean difference of -0.01 (-1.07 to 1.06, I^2=0%).[35 140 149 191]

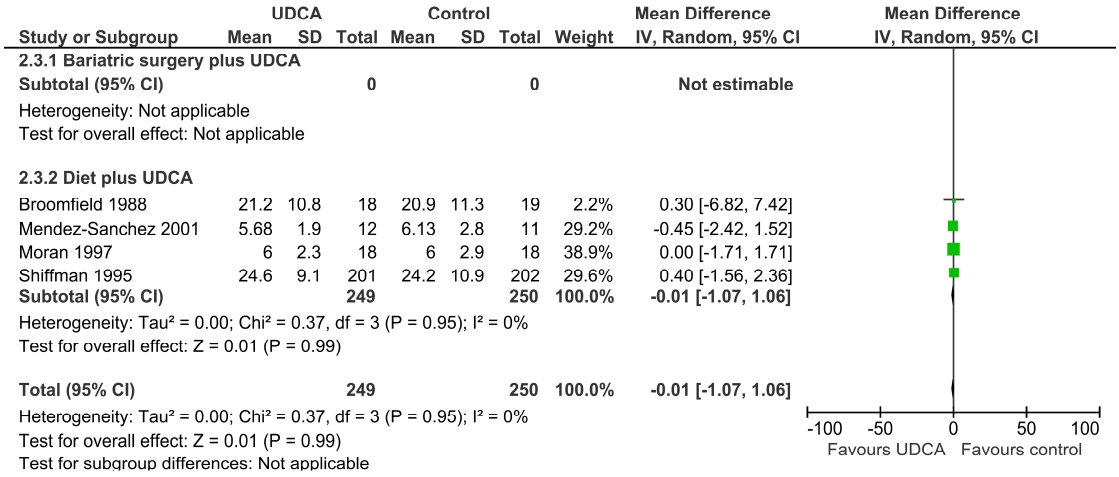


Figure 7. Meta-analysis of weight loss in obese patients receiving UDCA versus control interventions during weight loss

4.3.1 Subgroup and sensitivity analyses

Amongst the weight loss trials, Figure 8 illustrates that UDCA was more beneficial when only caloric restriction was used for weight loss as compared to the trials that also included bariatric surgery (P=0.03). This finding was corroborated when using an available case analysis (test for subgroup differences P=0.02).

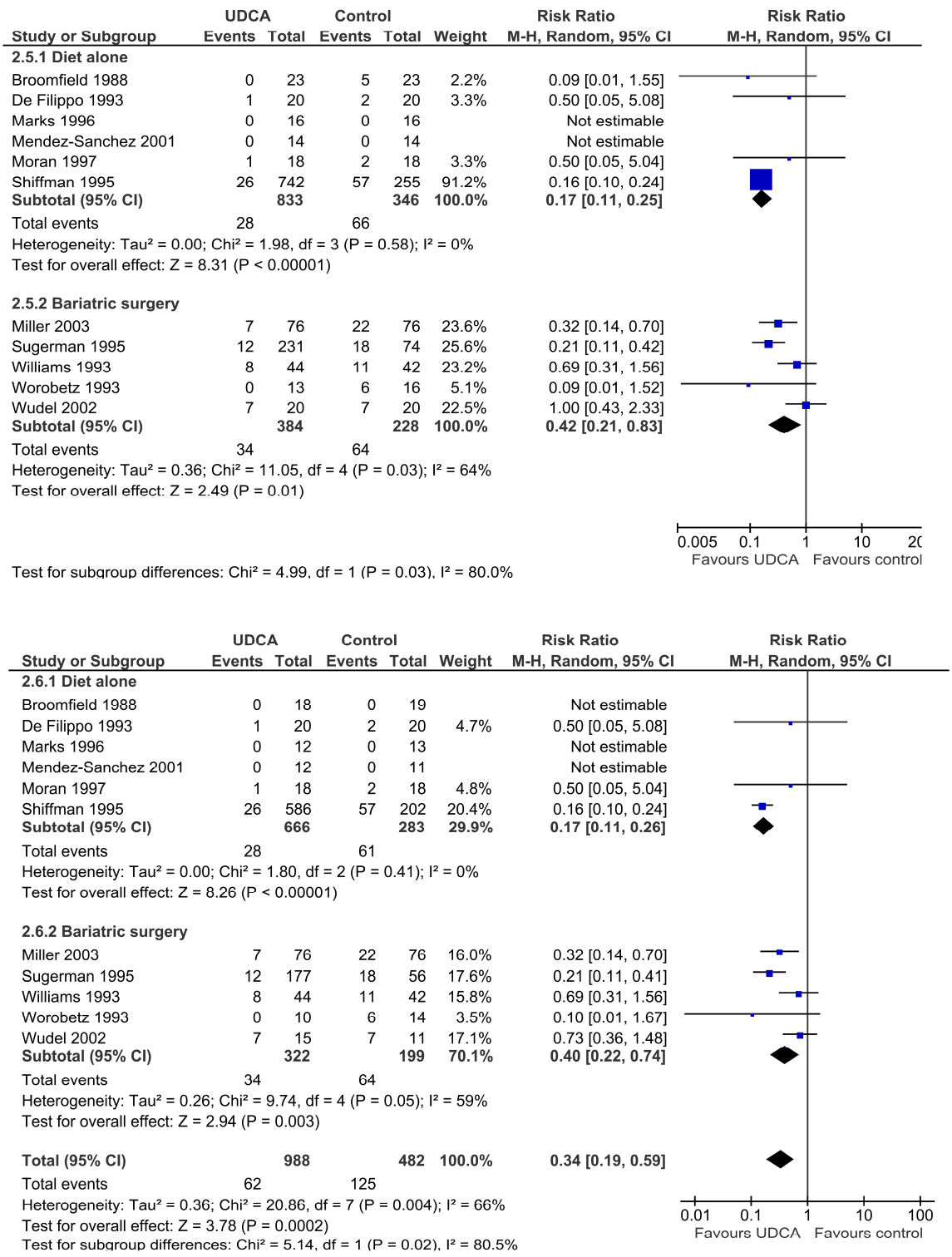


Figure 8. Meta-analyses of gallstone formation in obese patients receiving UDCA versus control interventions during weight loss with diet alone or after bariatric surgery based on ITT (top) and per protocol (bottom) analysis

The effect of UDCA did not differ between trials on weight loss or cardiac surgery (test for subgroup differences P=0.76) (see Figure 9).

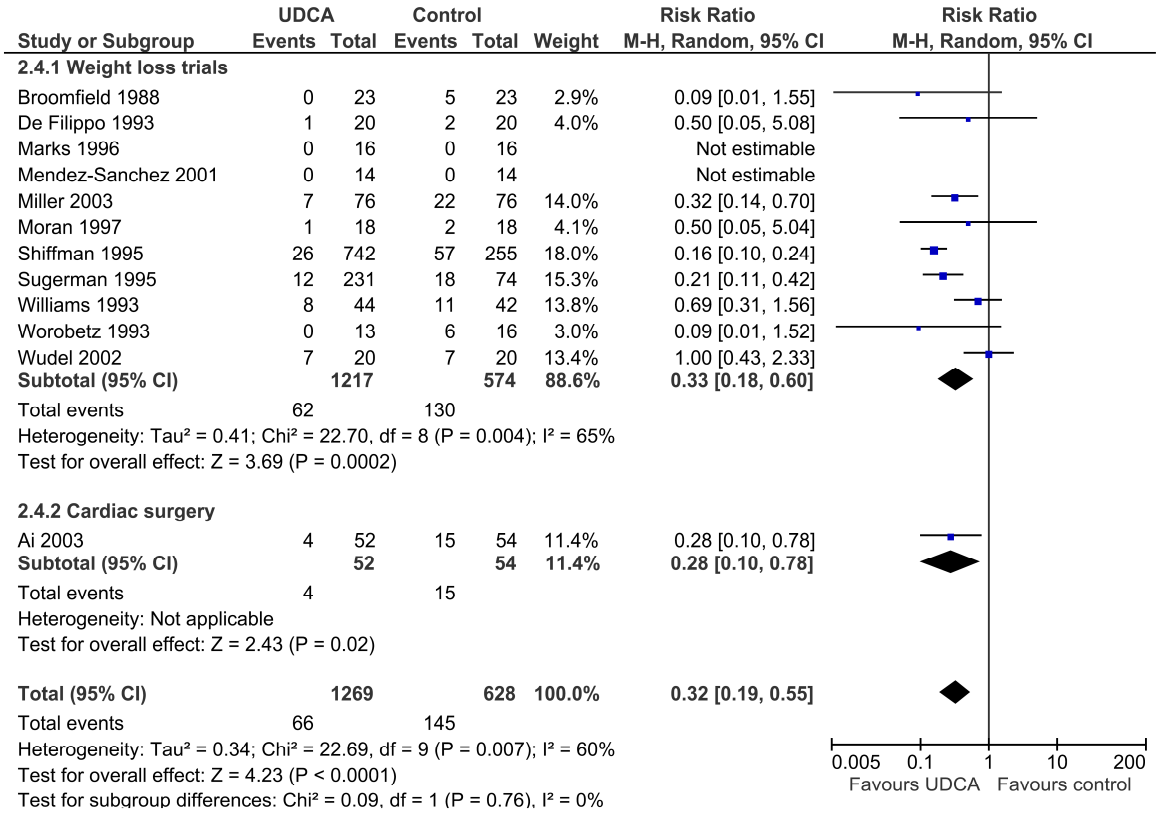
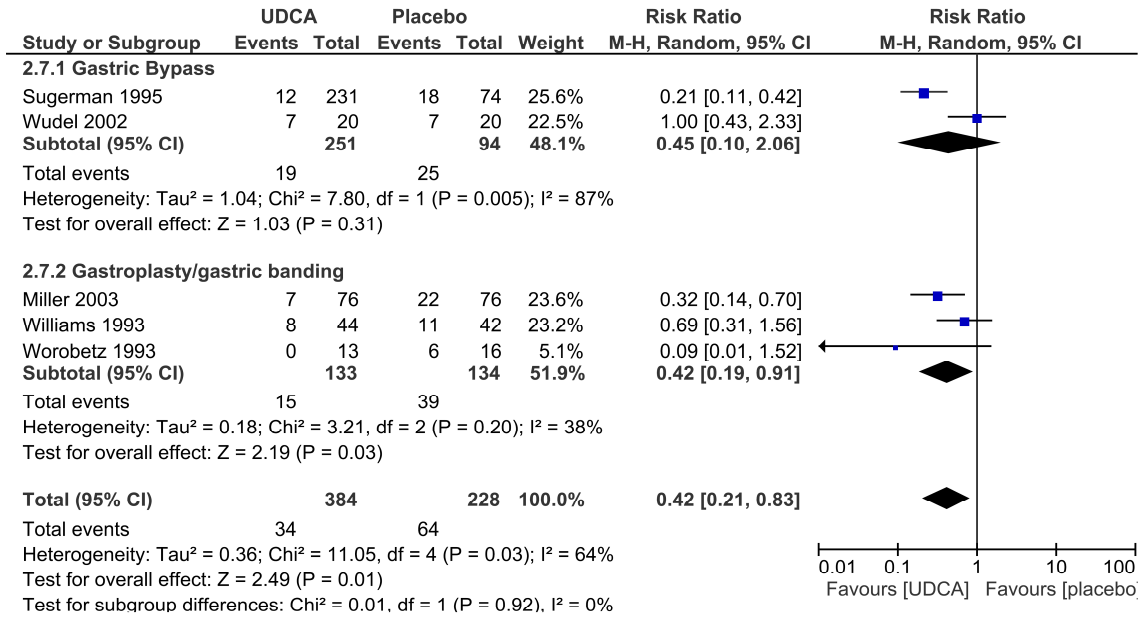


Figure 9. Meta-analysis of gallstone formation in patients receiving UDCA versus control interventions based on patient type (i.e. obese patients during weight loss versus non-obese patients post cardiac surgery)

The type of bariatric surgery (Figure 10, top) did not influence the effect of UDCA (test for subgroup differences P=0.92). Likewise, no difference was seen between trials which administered the lower or higher dose of UDCA (test for subgroup differences P=0.12) (see Figure 10, bottom).



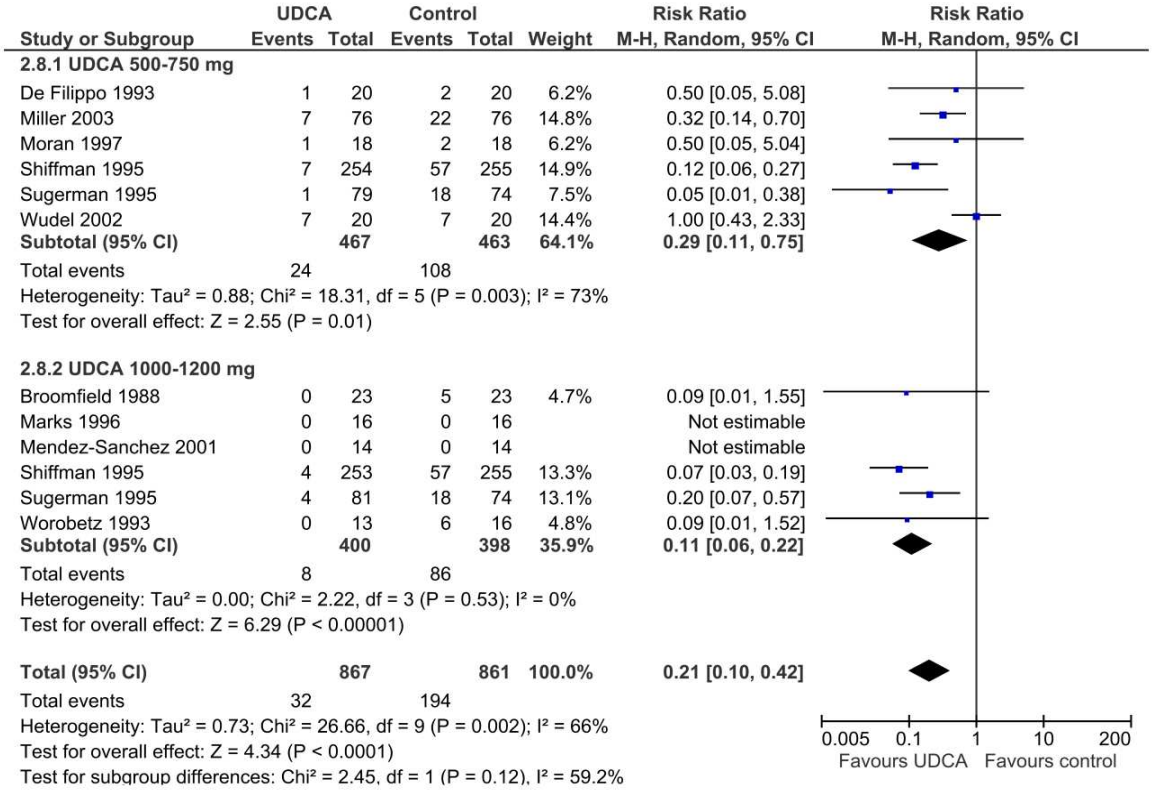


Figure 10. Meta-analyses of gallstone formation in patients receiving UDCA versus control interventions based on type of bariatric surgery (top) and dose of UDCA (bottom)

There were no available data to assess quality of life. There was no difference between trials with a low compared to a high or unclear risk of bias based on the allocation or blinding methods (test for subgroup differences P=0.76 for both analyses), or in subgroups of trials stratified by attrition bias (test for subgroup differences P=0.54), reporting of outcomes (test for subgroup differences P=0.80), or other biases (test for subgroup differences P=0.65). The effect of UDCA was confirmed when the analyses were repeated using good or poor outcome analysis (P=0.00002 and P<0.00001, respectively). All the figures for the above bias analyses can be found in appendix 5.

4.3.1 Regression and trial sequential analyses

No evidence of small study effects was identified (Egger's test P=0.53) as illustrated in the funnel plot below (Figure 11).

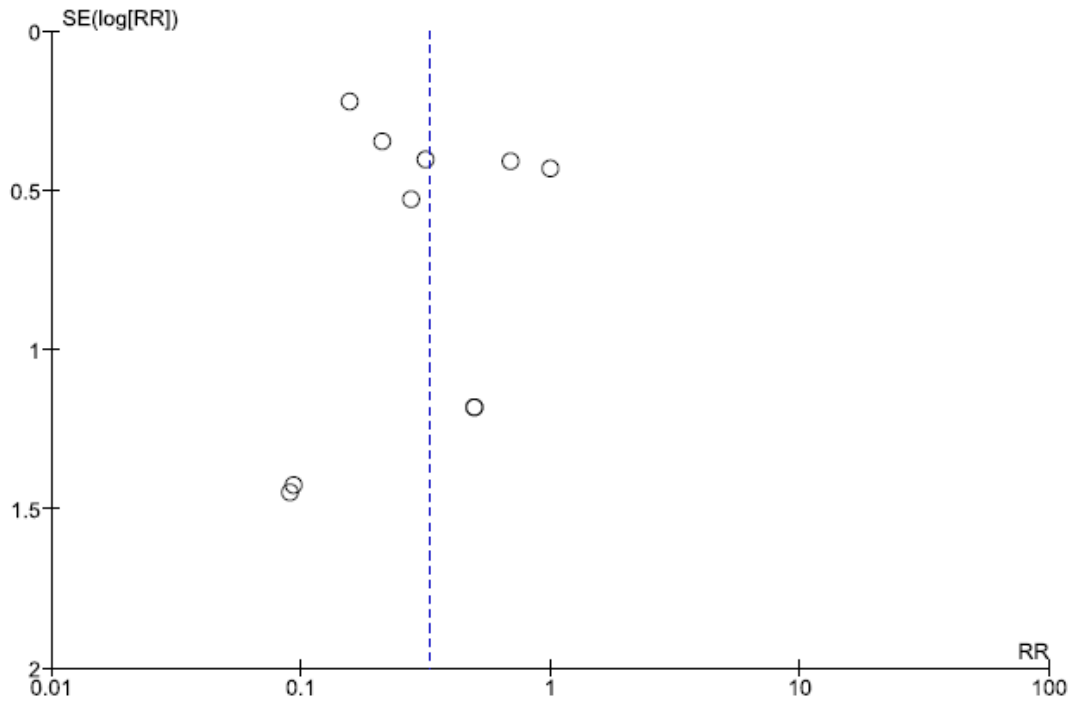


Figure 11. Funnel plot of trials using UDCA versus control interventions

A sequential analysis was performed using relative risks (random effects) and with alpha set to 5% and power to 80%. The relative risk reduction was set to 77%, the incidence in the control group to 20% and the heterogeneity to 80%. The graph (Figure 12) did not confirm the overall result of the meta-analysis, since the trial sequential monitoring boundary (inward sloping red line) was not crossed before reaching the required information size.

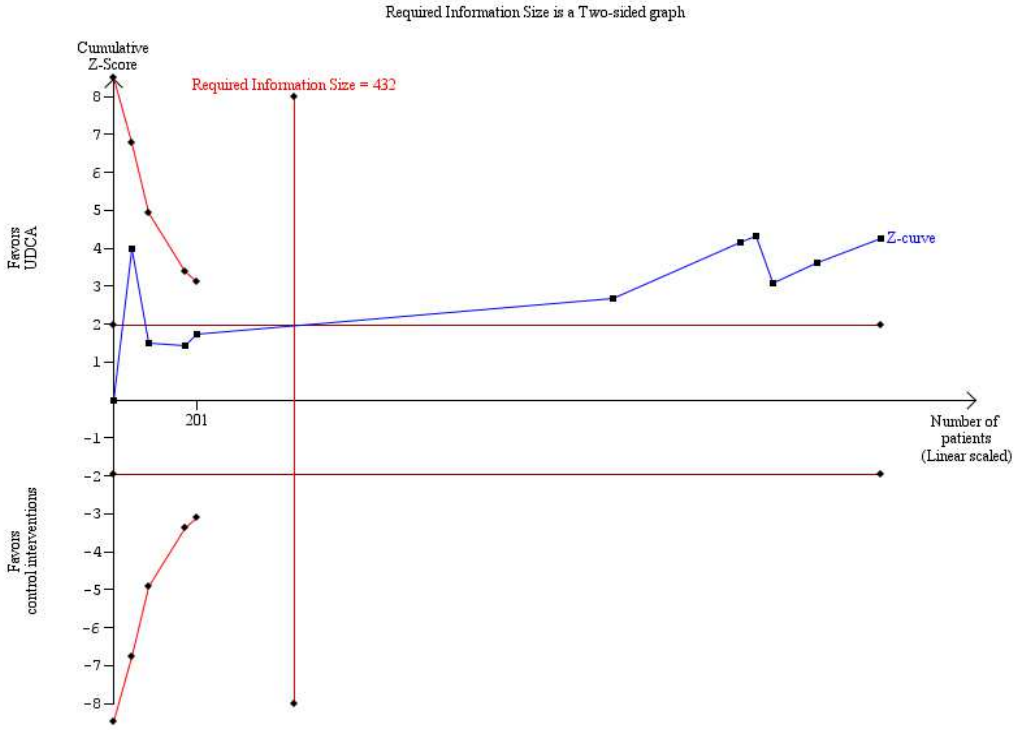


Figure 12. Trial sequential analysis of UDCA versus control interventions in all trials

The required information size was calculated to 432 participants based upon a control group gallstone incidence of 23%; a relative risk reduction of 77%; an alpha of 5%; a beta of 20% (80% power). The blue cumulative Z curve crosses the conventional alpha of P = 0.05, but does not touch the trial sequential alpha spending monitoring boundaries (inward sloping red lines).

We also repeated the regression and sequential analyses for trials on weight loss through diet alone or post bariatric surgery. No small study effects were seen when analysing trials on diet alone (Egger's test P=0.284) or trials on bariatric surgery (P=0.989). The sequential analyses did confirm the results of the subgroup analyses of trials on weight loss through diet alone as it crossed the sequential monitoring boundary (Figure 13) but sequential analysis did not confirm the results of the meta-analysis for the trials that included participants post bariatric surgery (Figure 14).

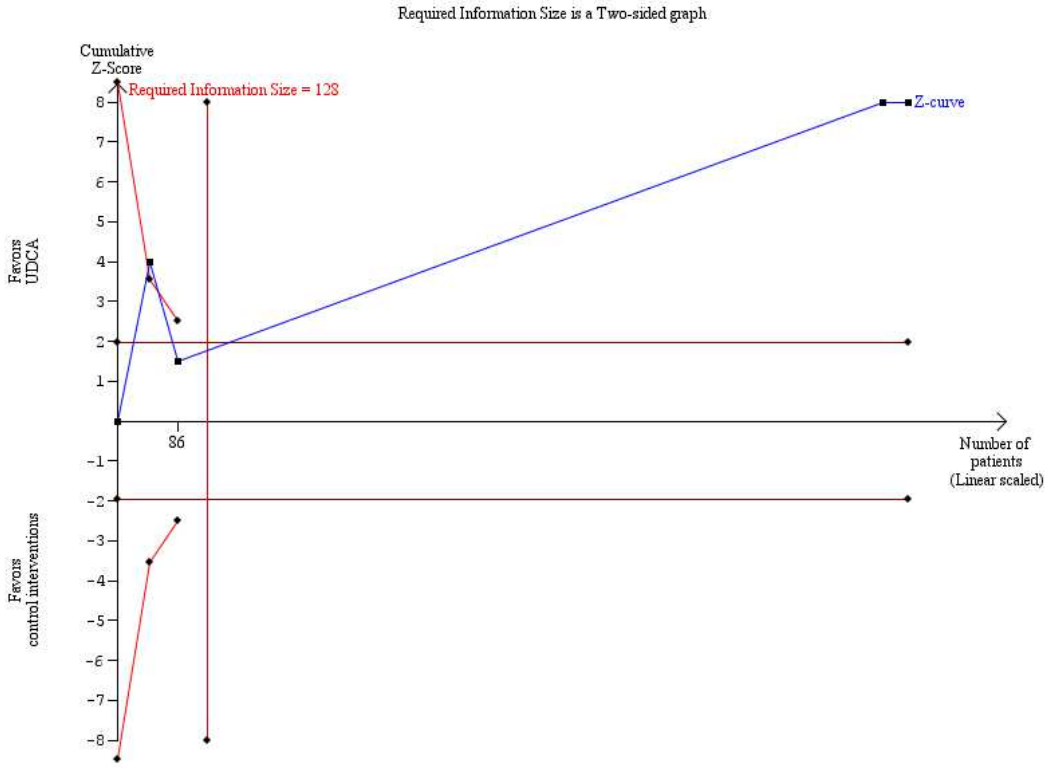


Figure 13. Trial sequential analysis of UDCA versus control interventions on diet alone

The required information size was calculated to 128 participants based upon a control group gallstone incidence of 19%; a relative risk reduction of 82%; an alpha of 5%; and a beta of 20% (80% power). The blue cumulative Z curve crosses the conventional alpha of P=0.05 twice, and the trial sequential alpha spending monitoring boundaries (inward sloping red lines).

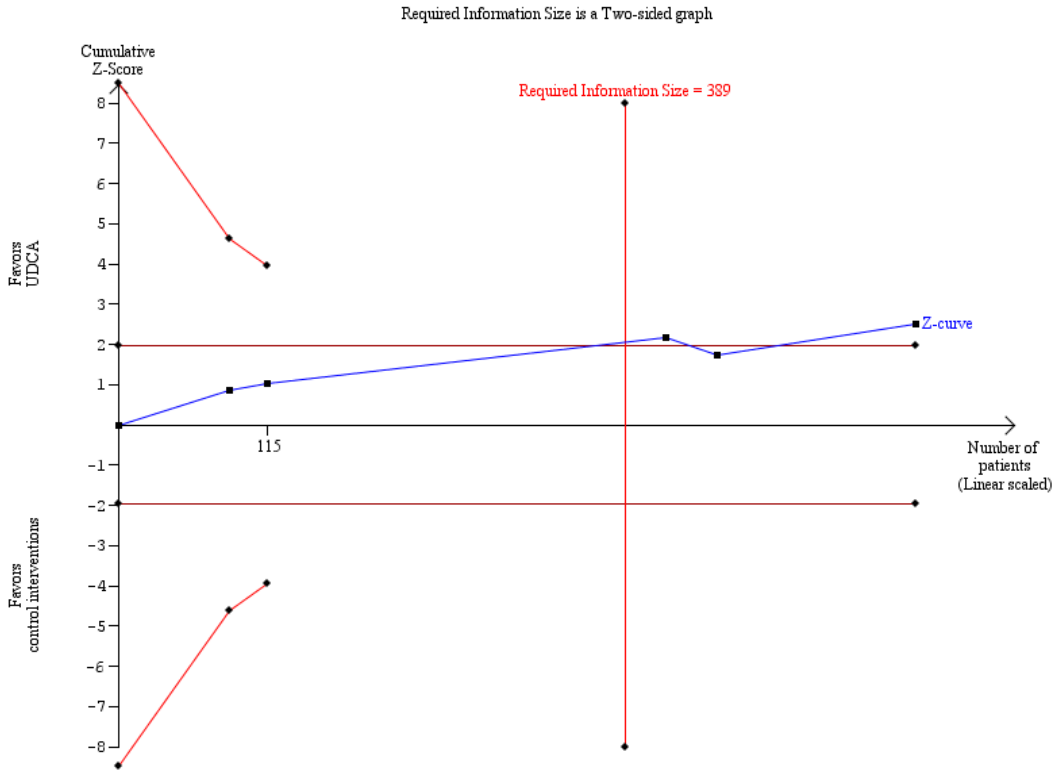


Figure 14. Trial sequential analysis of UDCA versus control interventions post bariatric surgery

The required information size was calculated to 389 participants based upon a control group gallstone incidence of 29%; a relative risk reduction of 68%; an alpha of 5%; and a beta of 20% (80% power). The blue cumulative Z curve crosses the conventional alpha of P=0.05, but not the trial sequential alpha spending monitoring boundaries (inward sloping red lines).

4.4 Interventions with dietary fat modification

The non-pharmacological interventions assessed included high versus low-fat diets during weight loss (two trials). The weight loss in the intervention and control groups ranged from 19 to 25 kg.[57 67] None of the 23 participants in the intervention group and 10 of 22 (45%) participants in the control groups developed gallbladder stones. Random effects meta-analysis (Figure 15) showed that high dietary fat modification during weight loss reduced the risk of gallstones (risk ratio, 0.09, 0.01 to 0.61, I^2=0%). No deaths were reported. Quality of life was not assessed. Both trials reported a similar pattern in bile lithogenicity in both treatment groups, but did not report data that allowed meta-analyses. The trials described an initial increase in lithogenicity following the diet and subsequently a decrease to values lower than those at baseline during follow-up. We were unable to analyse the outcomes cholecystectomy or weight loss due to differing reporting methods.

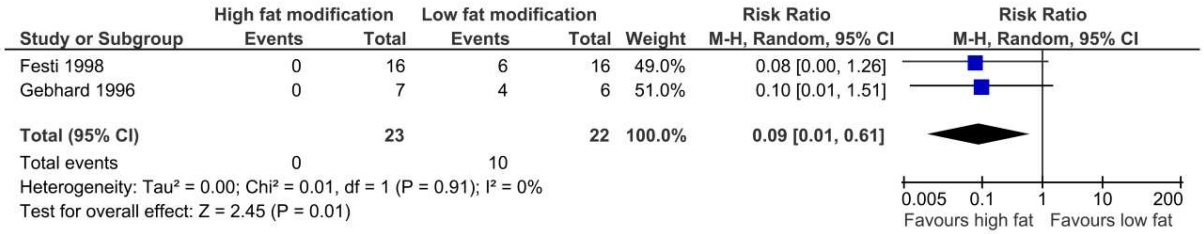


Figure 15. Meta-analysis of gallstone formation in patients receiving a high-fat versus a low-fat weight loss diet

4.5 Other pharmacological/non-pharmacological interventions

The number of trials and participants assessing aspirin, ibuprofen and omega-3 fatty acids was small (Figure 16). One trial[257] found that patients receiving ibuprofen formed gallstones at a higher rate than the placebo or UDCA groups. This was a trial with high attrition. Adverse events were not clearly reported. None of the remaining interventions demonstrated beneficial or detrimental effects on gallstones. These results and those of the subgroup analyses above are presented in table 5.

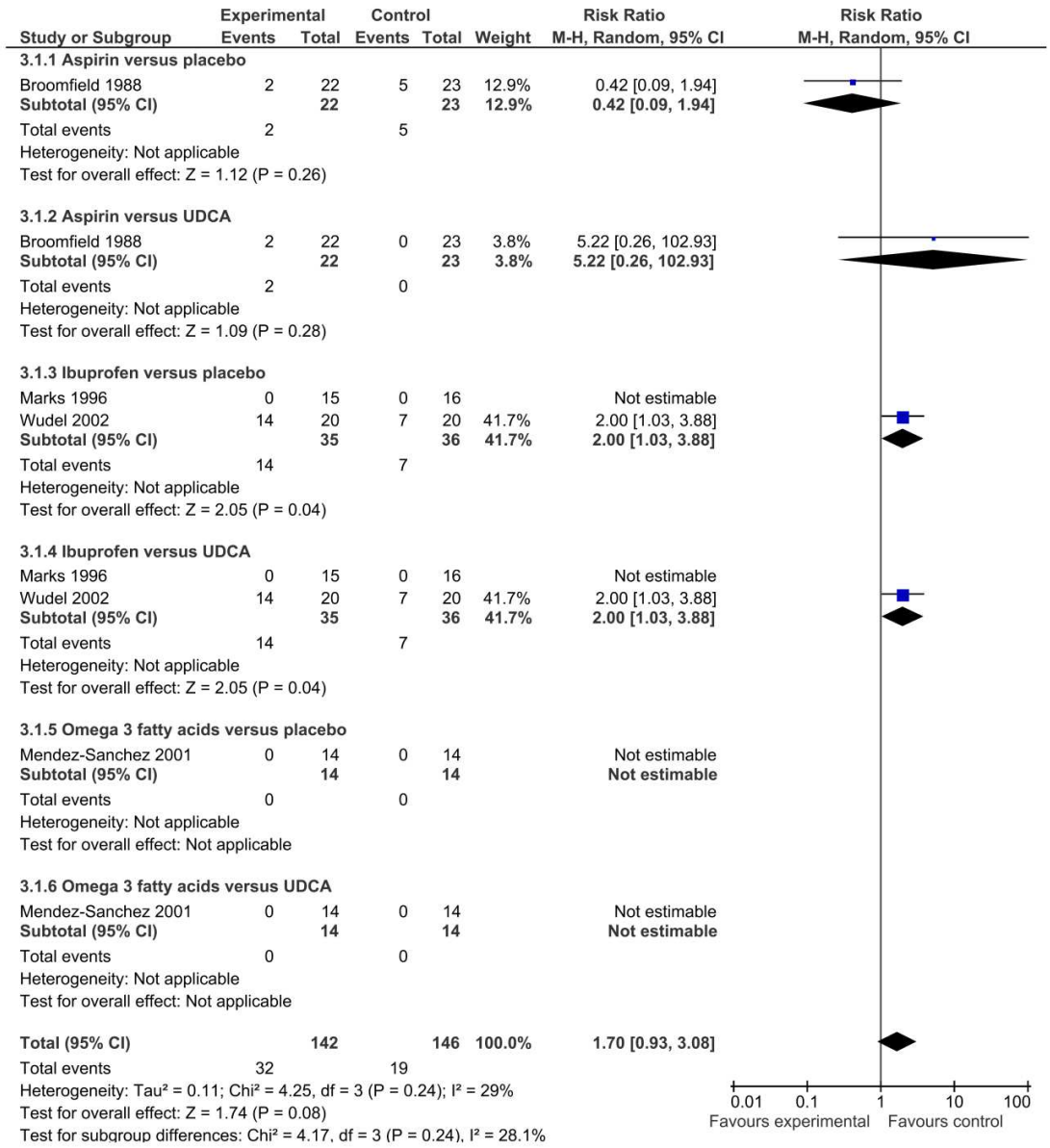


Figure 16. Meta-analyses of gallstone formation in patients receiving various interventions

Table 5. Summary of subgroup random effects meta-analyses

Outcome or Subgroup	Studies	N	Effect Estimate RR [95% CI]	Heterogeneity I^2 %
Gallstone formation in trials on diet alone or bariatric surgery using available case analysis	11	1470	0.34 [0.19, 0.59]	66
Weight loss diet alone	6	949	0.17 [0.11, 0.26]	0
Bariatric surgery	5	521	0.40 [0.22, 0.74]	59
Gallstone formation in different types of bariatric surgery	5	612	0.42 [0.21, 0.83]	64
Gastric bypass	2	345	0.45 [0.10, 2.06]	87
Gastroplasty/gastric banding	3	267	0.42 [0.19, 0.91]	38
Gallstone formation in relation to dose of UDCA	10	1728	0.21 [0.10, 0.42]	66
UDCA 500-750 mg	6	930	0.29 [0.11, 0.75]	73
UDCA 1000-1200 mg	6	798	0.11 [0.06, 0.22]	0
Gallstone formation in relation to allocation methods	12	1897	0.32 [0.19, 0.55]	60
Low risk of bias	11	1791	0.33 [0.18, 0.60]	65
Unclear risk of bias	1	106	0.28 [0.10, 0.78]	NA
Gallstone formation in relation to blinding	12	1897	0.32 [0.19, 0.55]	60
Low risk of bias	11	1791	0.33 [0.18, 0.60]	65
High risk of bias	1	106	0.28 [0.10, 0.78]	NA
Gallstone formation in relation to attrition bias	12	1897	0.32 [0.19, 0.55]	60
Low risk of bias	5	562	0.26 [0.16, 0.42]	0
High risk of bias	7	1335	0.36 [0.14, 0.90]	81
Gallstone formation in relation to selective reporting	12	1897	0.32 [0.19, 0.55]	60
Low risk of bias	9	1733	0.30 [0.17, 0.51]	57
High risk of bias	3	164	0.38 [0.06, 2.61]	51
Gallstone formation in relation to other bias	12	1897	0.32 [0.19, 0.55]	60
Low risk of bias	5	291	0.28 [0.14, 0.57]	0
High or unclear risk of bias	7	1606	0.36 [0.18, 0.72]	76
Gallstone formation good outcome analysis	11	1791	0.39 [0.25, 0.60]	37
Gallstone formation poor outcome analysis	11	1791	0.59 [0.51, 0.68]	0
Aspirin versus placebo	1	45	0.42 [0.09, 1.94]	NA
Aspirin versus UDCA	1	45	5.22 [0.26, 102.93]	NA
Ibuprofen versus placebo	2	71	2.00 [1.03, 3.88]	NA
Ibuprofen versus UDCA	2	71	2.00 [1.03, 3.88]	NA
Omega 3 fatty acids versus placebo	1	28	NE	NA
Omega 3 fatty acids versus UDCA	1	28	NE	NA

Abbreviations: NA, not applicable; NE, not estimable

4.6 Adverse events

UDCA did not increase the risk of adverse events (Table 6). Overall, few serious events were reported. The most common adverse events were gastrointestinal-related complaints. Only one[185] of the three[138 146 185] trials included qualitatively in this review reported adverse events with UDCA supplementation. No adverse events were described in the dietary fat modification trials.

Table 6. Reported adverse events with UDCA administration

Trial	UDCA mg (per day)	No. adverse events in treatment group % (n)	Type of adverse events in treatment group (n or %)	Type of adverse events in control group (n or %)
Ai[5]	600	Did not report on adverse events at all	-	-
Broomfield[35]	1200	Did not report on adverse events at all	-	-
De Filippo[47]	600	0	-	-
Marks[134]	1200	0	-	-
Mendez-Sanchez[140]	1200	13 (2)	Abdominal bloating and constipation (n=2)	Abdominal bloating and constipation (n=2) †
Miller[141]	500	8 (6)	Nausea, constipation (n=6)*	Nausea, constipation (n=2)*
Moran[149 150]	750	0	-	-
Scott[185]	600	25 (17)	Nausea (n=9) Diarrhoea (n=5) Dry skin/pruritus (n=3)	Not reported
Shiffman[191]	300/600/1200	Not reported	Common complaints were*: Constipation (27%) Headache (27%) Diarrhoea (23%) Dizziness (17%) Upper respiratory infections (16%) 13 patients withdrew due to adverse events	Common complaints were*: Constipation (26%) Headache (30%) Diarrhoea (24%) Dizziness (16%) Upper respiratory infections (13%) 5 patients withdrew due to adverse events
Sugerman[213]	300/600/1200	Not reported	Vomiting or skin rashes*	Vomiting or skin rashes*
Williams[254]	10 (mg/kg)	9 (20)	Medication intolerance (n=9)*	Medication intolerance (n=7)*
Worobetz[256]	1000	8 (1)	Epigastric burning upon medication ingestion and was withdrawn (n=1)	
Wudel[257]	600	Did not report on adverse events at all	-	-

*no differences in adverse events between the placebo and intervention groups.

†These adverse events were reported for the group receiving omega-3 fatty acids.

5. Discussion

5.1 Summary of main results

This systematic review suggests that UDCA and high-fat weight loss diets may be considered in the primary prevention of gallstones during weight loss. Our meta-analyses mainly include obese adults, who belong to a high risk group with regards to cholesterol gallstone formation. No effect on mortality was established and no major adverse effects were reported. The effects on bile lithogenicity could not be meta-analysed, but some trials found improvements with UDCA administration. This is consistent with the reduced cholesterol supersaturation of bile, the physical-chemical prerequisite for lowering gallstone risk.

5.1.1 Efficacy of UDCA for gallstone prevention

The number of patients who developed gallstones in the UDCA and control groups was 5 versus 23%, respectively. Of note, none of the trials reported significant differences in weight loss between the intervention and the control groups. The NNT to prevent one patient from forming gallbladder stones will depend on the baseline weight of the included patients. Our results suggest that about 13 patients have to be treated with UDCA to prevent one patient from developing gallstones. We have no data to allow an assessment of the NNT to prevent one patient from developing symptomatic stones, but the expected incidence suggests that the number will be considerably higher.

The one trial comprising patients post cardiac surgery found a reduced incidence of gallstones in those receiving UDCA compared to controls. Incidentally, most of the gallstones in the controls were black pigment stones. The relevance of this outcome with UDCA is exemplified by the recently published study investigating outcomes of cholecystectomy in 1,687 US heart transplant recipients.[100] Mortality in this cohort was 2.2% and predictors included open cholecystectomy and gallstone disease. The authors urge that consideration should be given before prophylactic cholecystectomy is performed.

The observed effect in our meta-analysis on obese subjects undergoing weight loss seemed to depend on the weight loss method, with patients on bariatric surgery having a smaller benefit than patients on diets alone. Gallstone incidence in the UDCA and control groups was 3 and 19% (diet alone trials), compared to 9 and 28% (post bariatric surgery trials). Our data do not allow an assessment of the reason for the difference, however, a weight loss greater than 25% body weight is reported to increase the risk of gallstones forming.[127] However, the influence of absolute weight loss on gallstones has yielded conflicting findings, as several prospective observational studies in morbidly obese participants presenting for bariatric surgery have not observed a linear relationship between body weight and the prevalence of gallstones, and absolute amount of weight loss presented no increased risk in these participants.[189 190] Despite these findings, a curvilinear relationship between the rate of weight loss in obese participants and the incidence of gallstones has indeed been demonstrated,[251] with a maximum of 1.5 kg per week being assessed as optimal to limit the risk.

Moreover, differences in intestinal and/or gallbladder motility as well as baseline patient characteristics may have contributed to these findings. A non-randomised trial excluded from this review found rapid weight loss post laparoscopic gastric banding to impair gallbladder emptying.[7] Parenteral nutrition studies highlight the role of gallbladder hypomotility in the development of gallstones, which can prolong the residence time of excess cholesterol in the gallbladder and promote biliary sludge formation.[195] Gallbladder motility may be modulated by a high-fat diet and UDCA in different ways,[99 190] with some of the included trials that assessed gallbladder function reporting reduced gallbladder contraction in the placebo compared to the UDCA groups,[35 134] or significantly faster gallbladder emptying after a three-month intervention with UDCA compared to placebo.[257]

5.1.2 When to initiate UDCA therapy

An important consideration is when to begin prophylactic UDCA therapy. Non-surgical trials commenced UDCA therapy immediately upon caloric restriction, whereas bariatric surgery trials initiated UDCA within days,[141 213 256] or weeks of

surgery.[254] Since gallstones take approximately four weeks to develop, preventive treatment should theoretically begin immediately. Observational studies[189 190] report the incidence of ultrasonically verified gallstones to approximate 36% within six months after Roux-en-Y gastric bypass, and the incidence of gallstones stabilises from thereon, when assessed at 12 and 18 months. Most of the UDCA interventions included in these meta-analyses lasted between three and six months, coinciding with when the majority of weight loss occurs. This could indicate a 'critical period' for initiating interventions to prevent gallstones in these patients.

5.1.3 Effect of low-calorie diets on gallstones

The risk of gallstones appears to be significant in both LCD and VLCDs.[201 258 259] Most recently, a study of 8,361 individuals reported the risk of symptomatic gallstones including cholecystectomy to be three times higher in those treated with a VLCD as compared to a LCD.[90] This study did not assess the presence of gallstones at baseline with ultrasonography. One would expect however, that VLCDs as compared to LCDs further increase the risk of gallstones, particularly as weight loss is more rapid with the former. The authors speculate that the dietary fat content (which was between 7 g and 9 g) might have played an influential role.[90] This was not as low as that of the low-fat diets in the trials included in this meta-analysis (1 - 3 g), but it was also not as high as that of the high-fat diets (12 - 30 g) of the trials herein.

5.1.4 Influence of dietary fat content on gallstones

We compared the rate of gallstone formation in the placebo groups of the trials that provided participants with a VLCD (~500 kcal) and between 1 g to 3 g fat. The incidence of gallstones ranged from 0% to 66.7%, with a median of 22.3%.[35 57 67 134 191] In this review, a weight reducing diet higher in fat (19 - 30%) appears to reduce the incidence of gallstones compared to a weight reducing diet lower in fat (3 - 5%). No adverse events were reported, but the trials were not free from bias; our analysis only included two trials with small sample sizes. Although this finding must be interpreted with caution, a mechanistic rationale exists, since a diet higher in fat

stimulates gallbladder contractility.[67 210] Conversely, a low-fat diet is associated with gallbladder stasis and may increase the risk of gallstones – a finding that has also been reported in other non-randomised studies using VLCD and 1 g fat, not included in this systematic review.[128 258]

Both the studies included in the meta-analysis reported greater gallbladder emptying when subjects received a high fat test meal compared to a low fat test meal. Specifically, Gebhard et al.[67] compared two liquid diets with different caloric and fat contents (520 kcal + < 2 g fat versus 900 kcal + 30 g fat distributed between three meals). Gallbladder emptying was compared between the low-fat meal (< 2 g) versus the high-fat (10 g) meals, and poor gallbladder emptying was reported with the former. Festi et al.[57] compared two isocaloric VLCD with different fat content (3 g versus 12.2 g/day) and found gallbladder emptying was significantly lower in the low-fat diet group. Finally, these two studies also noted a similar pattern in CSI in both treatment groups. This included an initial increase in CSI after six[57] and eight weeks,[67] followed by a decrease to values lower than those at baseline after three[57] and six months.[67] This is synonymous with reports of increased cholesterol mobilisation during weight loss, which gradually tapers off. Correspondingly, the incidence of gallstones was higher in the low-fat diet groups in both studies.

5.2 Possibility of new prevention modalities

Given the findings of these meta-analyses, there may even be the potential to ameliorate gallstone risk in patients with a combination of non-pharmacological and pharmacological means, whereby a weight reducing diet with a relatively high fat content is provided alongside UDCA therapy. The higher risk of gallstones during weight loss indicates reduced gallbladder motility due to reduced gallbladder stimulation and the presence of increased bile lithogenicity, most likely due to increased reverse cholesterol transport and reduced biliary bile salt secretion.[24 71] Interestingly, the pathogenesis of cholesterol gallstones suggests that the lithogenic state may be irreversible as a result of cholesterol absorption by the gallbladder wall with subsequent disruption of smooth muscle function.[119] Research on gallstone incidence and on cholesterol and fat intake is controversial and may depend on lipid

composition. For example, an increased gallstone incidence was reported with a higher saturated and *trans* fat intake in some prospective follow-up studies,[225 231] but reduced with a high polyunsaturated and monounsaturated fat intake.[222]

5.3 Consideration of non-modifiable risk factors

Non-modifiable risk factors, such as genetics, should also be taken into account because they are reported to account for about 25% of gallstone risk.[95] In particular, mutations in genes encoding hepatocanalicular transporters are reported to cause cholelithogenesis given their aptitude in modifying bile composition and causing retention of substances normally secreted in bile, thus influencing the bile formation process. The influence of the *ABCG5/G8* gene variants (two cholesterol hemi-transporters) on intestinal absorption and biliary secretion of cholesterol may play a significant role.[25] In fact, they function together as a heterodimer influencing cholesterol excretion so intensely, that related loss-of-function mutations are shown to cause sitosterolemia (a rare genetic disorder of lipid metabolism, characterized by excess concentrations of cholesterol and phytosterols) in serum).[59]

Moreover, the *ABCG8* mutation p.D19H variant has been identified in independent cohorts worldwide as a common susceptibility factor for cholesterol gallstone disease.[36 69] ABC transporters control biliary lipid secretions across the canalicular hepatocyte membranes and therefore play a crucial role in regulating the physical-chemistry of bile. Given the inherent predisposition to gallstones, genetic screening for high risk individuals might help in the precise identification of candidates for primary stone prevention with UDCA and/or high-fat diets, drugs inhibiting cholesterol synthesis and/or intestinal absorption, or modulators of nuclear receptors involved in cholesterol and bile acid homeostasis.[12 41 109] However, RCTs would need to be conducted before this approach can be fully endorsed. Figure 17 depicts the sequence of considerations that could be followed for the prevention of gallstones in obese patients wishing to lose weight.

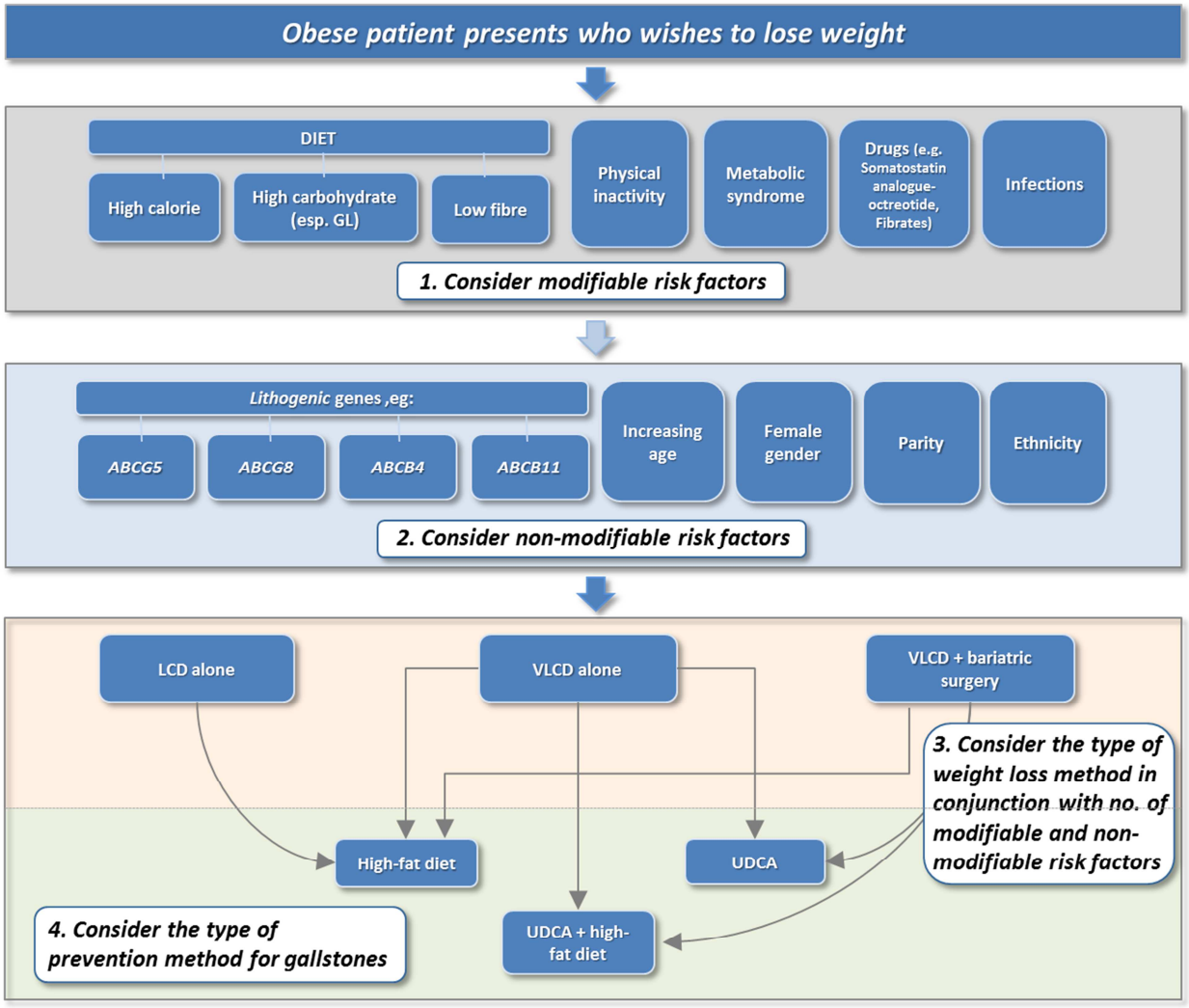


Figure 17. Flow chart for preventive options against gallstones during weight loss

Abbreviations: ABCB4, ATP-binding cassette transporter, subfamily B, member 4; ABCB11, ATP-binding cassette transporter, subfamily B, member 11; ABCG5, ATP-binding cassette transporter, subfamily G, member 5; ABCG8, ATP-binding cassette transporter, subfamily G, member 8; GL, glycaemic index; LCD, low-calorie diet; UDCA, ursodeoxycholic acid; VLDC, very low-calorie diet

5.4 Summary of secondary findings

5.4.1 Acetylsalicylic acid in patients following a VLCD

Only one study assessed acetylsalicyclic acid (aspirin) against UDCA therapy or placebo for gallstone prevention. Broomfield et al.[35] included obese patients undergoing weight loss only by using a VLCD (i.e. without bariatric surgery). After 16 weeks of treatment, they observed the highest incidence of gallstones to occur in patients in the placebo group, followed by those in the aspirin group and no gallstones were found in the UDCA group. This is suggestive, yet inconclusive of a beneficial effect of aspirin on gallstone prevention.

The evidence surrounding the role of aspirin in gallstone prevention is relatively scarce. Because aspirin is a prostaglandin inhibitor, its beneficial mechanistic effects are believed to result from the inhibition of mucin glycoprotein secretion in the gallbladder.[77] Such a finding was reported in prairie dogs by LaMont and coworkers, [121] whereby oral aspirin intake prevented cholesterol gallstones formation. Interestingly, in the trial by Broomfield et al.[35] increased glycoprotein concentrations in bile were observed in patients in the placebo group (regardless of whether they formed gallstones or not), but not in the non-stone formers of the UDCA or the aspirin group. However, in the two patients in the aspirin group who formed gallstones (and also in one patient who had crystals), there was an increased glycoprotein concentration, as compared to the non-stone formers. Moreover, Broomfield et al.[35] also noted decreased prostaglandin concentrations in the group receiving aspirin, whereas both the UDCA and the placebo groups displayed increased concentrations. One limitation to the above findings in this study is that only two patients in the aspirin group formed gallstones, and both these patients had very low concentrations of aspirin in serum, which is suggestive of non-compliance with the study regime.

A lack of a protective effect of aspirin was subsequently reported by Kurata et al.[111] who assessed whether aspirin taken orally reduced the need for gallstones-related hospitalization, rather than for prophylaxis against gallstones per se. This study included 4,524 subjects from the AMIS study (The Aspirin Myocardial Infarction

Study) of whom 2,267 were randomized to receive 500 mg aspirin twice daily and 2,257 placebo. All subjects were followed up for three years. No baseline differences were detected between the two groups. The follow-up revealed that only 11 patients were hospitalised for gallstones during this time period but that aspirin did not necessarily reduce hospitalisation for gallbladder disease.

5.4.2 Ibuprofen in patients following a VLCD

Two of the included trials assessed ibuprofen for the prevention of gallstones during weight loss and the findings are inconclusive with regard the efficacy of ibuprofen for gallstone prevention during weight loss. In one of the trials,[134] obese patients followed a VLCD for 12 weeks and received either 12 mg UDCA or 1600 mg ibuprofen or a placebo daily. None of the patients in this study developed gallstones. In the second trial,[257] obese patients received 600 mg UDCA or 600 mg ibuprofen or placebo daily for 24 weeks post bariatric surgery. In each of the UDCA and placebo groups 7/20 patients developed gallstones compared to 14/20 patients in the ibuprofen group respectively. This study however, was biased because of an excessively high attrition rate. In fact, a per protocol analysis (including only the patients who finished the study) showed gallstones developed in 7/15 and 7/11 patients in the UDCA and placebo groups, respectively. In the ibuprofen group, 14/15 patients formed gallstones. Therefore incidence of gallstones was lowest in the UDCA group but surprisingly was highest in the ibuprofen group. Further analyses in this study noted a higher percentage of gallbladder emptying in the UDCA group three months post surgery; this was reduced in comparison in the ibuprofen group.

One reason for this unexpected finding could be that stone formers in this study lost more weight compared to the non-stone formers and that many of these patients happened to be allocated to the ibuprofen group. In addition, not only was attrition high in this study, but compliance was assessed with self report measures via telephone interviews. Complete compliance was achieved in only 28% of patients. The authors report no differences in self report assessment of compliance between groups or between stone and non-stone formers, but there may generally have been bias in the self recall process.

There is a clear discrepancy in stone formation in those taking ibuprofen between the two trails mentioned above. This difference may be attributable to the dosage given, i.e. 1600 mg in the group in which no stones were observed[134] versus 600 mg in the group in which most subjects formed stones.[257] This could provide indications on what could theoretically be deemed an adequate therapeutic dose. More trials however, would be needed to substantiate this. Moreover, ibuprofen should reduce the risk of stone formation via prostaglandin inhibition and reduction of nucleation and growth of cholesterol crystals, as reported with aspirin.[204] One could hypothesise that the observed increased risk of gallstones with ibuprofen may be related to the fact that these patients had undergone bariatric surgery, compared to the other trial in which only a VLCD as a weight loss method was used. However, another trial studied the influence of long term use of non-steroidal anti-inflammatory drugs (NSAIDs) (i.e. < two months) in 230 morbidly obese patients immediately post gastric bypass and found NSAID use to be associated with reduced mucin concentrations and lower cholesterol/phospholipid ratios in gallbladder bile.[204] The increased risk may therefore be attributable to confounding factors not controlled or clearly accounted for in this one trial.[257]

5.4.3 Omega-3 fatty acids in patients following a LCD

Only one trial investigated the effects of omega-3 fatty acid for prevention of gallbladder stones during weight loss and did not observe any incidence of gallstones in 15 obese women when taking 11.3 g/day of omega-3 fatty acids.[140] Likewise none of the participants in the placebo group or those receiving UDCA developed gallstones. However, all participants followed a LCD as co-intervention and the entire study duration was six weeks, therefore this may not have been long enough to see an effect since the weight loss would not be as rapid with a LCD.

A low prevalence of cholesterol gallstones has been reported in populations consuming omega-3 fatty acids (such as in Alaskan natives),[32] however this may be attributable to synergistic effects of the non-Westernised diet (i.e. low in refined carbohydrates) being consumed. Nevertheless, a study in patients with existing gallstone disease reported a decrease in biliary cholesterol saturation, following

intake of 3.75 g omega-3 fatty acids daily.[26] The above study included in this review also assessed CSI and did not find a difference between any of the groups during the intervention duration. Cholesterol nucleation time decreased in the placebo and in the UDCA group but did not change in the omega-3 fatty acid group. Since this finding is unexpected for the UDCA group, the authors speculate that this might be due to the short intervention duration.

In addition, experimental studies studied dietary fish oil supplementation in African green monkeys and found a reduced gallstone incidence and a lower CSI in those fed a diet with fish oil (22%) compared with 67% in those fed a diet with lard.[184] More recently, omega-3 fatty acids are reported to attenuate cholesterol gallstones in mice through suppressing mucin production.[101] In summary, further research, particularly in the form of clinical trials is warranted to evaluate whether omega-3 fatty acids could play a role in gallstone prevention.

5.5 Strength and limitations of the findings

This is the first review on several non-surgical interventions for the prevention of gallstones. UDCA reduced the risk of gallstones compared with control interventions and was more beneficial in participants undergoing weight loss through diet alone than after bariatric surgery. A high-fat low calorie diet also reduced the formation of gallstones during weight loss. We were unable to comment on the development of symptomatic gallstones in all included trials but a recent meta-analysis of 6,048 obese patients concluded that prophylactic cholecystectomy during laparoscopic gastric bypass should be avoided in patients without gallstones due to the low necessity of subsequent cholecystectomy (< 6.8%).[248] These findings may have important implications as the question of whether to perform cholecystectomy to prevent gallstones, particularly in obese patients undergoing weight loss, is debatable. A cost-effectiveness study of prophylactic cholecystectomy stated that the primary factor influencing the cost-effectiveness model is the incidence of gallbladder-related symptoms post-surgery.[23] A review of the evidence in 2010 reported a relatively low incidence of gallstones (5 - 10%) post gastric bypass in patients who did not take UDCA and that the majority of gallstone cases were

asymptomatic.[173] A similar finding was reported in a study comprising 13,443 participants post bariatric surgery who were followed up for 22 years.[169] Although the postoperative rate of obese participants requiring cholecystectomy is higher than the general population, the actual risk remains low. Hence, a conventional approach (i.e. using non-surgical means for primary stone prevention) may be preferred.

The small number of identified trials and correspondingly low sample sizes for some of the meta-analyses is the main limitation of this review, particularly for the non-pharmacological interventions. Several clinically relevant outcomes were also not addressed in the identified trials, in particular quality of life measures. Moreover, a high risk of attrition bias was identified as several trials reported high drop-out rates, and this was reflected in the meta-analyses. The complexity with these trials is that participants were following a weight loss diet (as co-intervention) which, by default, yields high attrition.[78] It is possible that the poor compliance reflects the difficulty in following the weight loss diets, rather than with the interventions for primary stone prevention. In support of this, many trials did not find significant differences in attrition or in adverse events between the treatment and control groups.

6. Conclusions and clinical implications

The obesity epidemic in both adults and children contributes to the increased prevalence of gallstones. Given that obese adults and children are also attempting to lose weight, we may consequently be faced with an ever-growing incidence of gallstones, thereby further compounding the current burden on the healthcare system. Though mortality is rare in cholelithiasis, morbidity is high. Therefore, early intervention is crucial, particularly as it has been documented as an adverse event in those undergoing rapid weight loss. Non-surgical options for the prevention of gallstones currently remain underused in clinical practice. The meta-analyses herein suggest that UDCA and a diet higher in dietary fat may prevent gallbladder stones forming during weight loss. We need to call more attention to the potential benefits of therapies such as the specific nutritional composition of weight loss diets and/or UDCA. Evidence-based guidelines are needed to identify interventions that could be feasible to use in practice, particularly in individuals at highest (genetic) risk. Further research encompassing genetic screening in obese patients undergoing weight loss would also help elucidate whether the risk of gallstones can be genetically quantified in such patients, risk of stones accurately predicted and subsequently avoided with the aforementioned interventions. Furthermore, responsible dietary advice for obese patients is paramount regardless of whether weight loss is attempted through lifestyle changes or bariatric surgery.

7. References

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Appendix 1. Full electronic search

Database	Period of Search	Search Strategy
Cochrane Hepato-Biliary Group Controlled Trials Register	Up until July 2013	(ultrasonograph* OR ultrasound* OR ecograph* OR 'ursodeoxycholic acid*' OR ursodiol OR UDCA OR ('non-steroid* anti-inflammatory' AND (drug* OR agent*)) OR ibuprofen OR aspirin OR obesity OR 'bariatric surger*' OR 'weight loss' OR 'diet therap*' OR 'caloric restriction' OR 'low calorie diet*' OR 'liquid diet*' OR fat* OR protein* OR carbohydrate* OR fibre OR micronutrient* OR 'physical activit*' OR exercise*) AND (cholelithiasis OR gallstone* OR 'gall* stone*' OR 'black pigment stone*')
Cochrane Central Register of Controlled Trials in *The Cochrane Library*	Latest issue	#1 MeSH descriptor Ultrasonography explode all trees #2 ultrasonograph* OR ultrasound* OR ecograph* #3 (#1 OR #2) #4 MeSH descriptor Ursodeoxycholic Acid explode all trees #5 ursodeoxycholic acid* OR ursodiol OR UDCA #6 (#4 OR #5) #7 MeSH descriptor Anti-Inflammatory Agents, Non-Steroidal explode all trees #8 (non-steroid* anti-inflammatory AND (drug* OR agent*)) OR ibuprofen OR aspirin #9 (#7 OR #8) #10 MeSH descriptor Obesity explode all trees #11 obesity #12 (#10 OR #11) #13 MeSH descriptor Bariatric Surgery explode all trees #14 bariatric surger* #15 (#13 OR #14) #16 MeSH descriptor Weight Loss explode all trees #17 weight loss* #18 (#16 OR #17) #19 MeSH descriptor Diet Therapy explode all trees #20 diet therap* OR caloric restriction OR low calorie diet* OR liquid diet* OR fat* OR protein* OR carbohydrate* OR fibre #21 (#19 OR #20) #22 MeSH descriptor Micronutrients explode all trees #23 micronutrient* #24 (#22 OR #23) #25 MeSH descriptor Exercise explode all trees #26 physical activit* OR exercise* #27 (#25 OR #26) #28 (#3 OR #6 OR #9 OR #12 OR #15 OR #18 OR #21 OR #24 OR #27) #29 MeSH descriptor Cholelithiasis explode all trees #30 cholelithiasis OR gallstone* OR gall* stone* OR 'black pigment stone*' #31 (#29 OR #30) #32 (#28 AND #31)
MEDLINE (Ovid SP)	1970 to July 2013	1. exp Ultrasonography/ 2. (ultrasonograph* or ultrasound* or ecograph*).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 3. 1 or 2 4. exp Ursodeoxycholic Acid/ 5. (ursodeoxycholic acid* or ursodiol or UDCA).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 6. 4 or 5 7. exp Anti-Inflammatory Agents, Non-Steroidal/ 8. ((non-steroid* anti-inflammatory and (drug* or agent*)) or ibuprofen or aspirin).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]

		9. 7 or 8 10. exp Obesity/ 11. obesity.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 12. 10 or 11 13. exp Bariatric Surgery/ 14. bariatric surger*.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 15. 13 or 14 16. exp Weight Loss/ 17. weight loss*.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 18. 16 or 17 19. exp Diet Therapy/ 20. (diet therap* or caloric restriction or low calorie diet* or liquid diet* or fat* or protein* or carbohydrate* or fibre).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 21. 19 or 20 22. exp Micronutrients/ 23. micronutrient*.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 24. 22 or 23 25. exp Exercise/ 26. (physical activit* or exercise*).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 27. 25 or 26 28. 3 or 6 or 9 or 12 or 15 or 18 or 21 or 24 or 27 29. exp Cholelithiasis/ 30. (cholelithiasis or gallstone* or gall* stone* or 'black pigment stone*').mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 31. 29 or 30 32. 28 and 31 33. (random* or blind* or placebo* or meta-analysis).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 34. 32 and 33
EMBASE (Ovid SP)	1980 to July 2013	1. exp echography/ 2. (ultrasonograph* or ultrasound* or ecograph*).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 3. 1 or 2 4. exp ursodeoxycholic acid/ 5. (ursodeoxycholic acid* or ursodiol or UDCA).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 6. 4 or 5 7. exp nonsteroid antiinflammatory agent/ 8. ((non-steroid* anti-inflammatory and (drug* or agent*)) or ibuprofen or aspirin).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 9. 7 or 8 10. exp OBESITY/ 11. obesity.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 12. 10 or 11 13. exp bariatric surgery/ 14. bariatric surger*.mp. [mp=title, abstract, subject headings, heading word,

		drug trade name, original title, device manufacturer, drug manufacturer] 15. 13 or 14 16. exp weight reduction/ 17. weight loss*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 18. 16 or 17 19. exp diet therapy/ 20. (diet therap* or caloric restriction or low calorie diet* or liquid diet* or fat* or protein* or carbohydrate* or fibre).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 21. 19 or 20 22. exp trace element/ 23. micronutrient*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 24. 22 or 23 25. exp EXERCISE/ 26. (physical activit* or exercise*).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 27. 25 or 26 28. 3 or 6 or 9 or 12 or 15 or 18 or 21 or 24 or 27 29. exp CHOLELITHIASIS/ 30. (cholelithiasis or gallstone* or gall* stone* or 'black pigment stone*').mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 31. 29 or 30 32. 28 and 31 33. (random* or blind* or placebo* or meta-analysis).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 34. 32 and 33
Science Citation Index Expanded	1970 to July 2013	# 4 #3 AND #2 AND #1 # 3 TS=(random* or blind* or placebo* or meta-analysis) # 2 TS=(cholelithiasis OR gallstone* OR gall* stone* OR black pigment stone*) # 1 TS=(ultrasonograph* OR ultrasound* OR ecograph* OR ursodeoxycholic acid* OR ursodiol OR UDCA OR (non-steroid* anti-inflammatory AND (drug* OR agent*)) OR ibuprofen OR aspirin OR obesity OR bariatric surger* OR weight loss OR diet therap* OR caloric restriction OR low calorie diet* OR liquid diet* OR fat* OR protein* OR carbohydrate* OR fibre OR micronutrient* OR physical activit* OR exercise*)

Appendix 2. Data collection form (template)

Data collection form

Intervention review – RCTs and non-RCTs

Notes on using a data extraction form:

- Be consistent in the order and style you use to describe the information for each report.
- Record any missing information as unclear or not described, to make it clear that the information was not found in the study report(s), not that you forgot to extract it.
- Include any instructions and decision rules on the data collection form, or in an accompanying document. It is important to practice using the form and give training to any other authors using the form.

Review title or ID

Study ID *(surname of first author and year first full report of study was published e.g. Smith 2001)*

Report ID *(if different to Study ID)*	**Report IDs of other reports of this study** *(e.g. duplicate publications, follow-up studies)*

Notes:

General Information

Date form completed *(dd/mm/yyyy)*	
Name/ID of person extracting data	
Reference citation	
Study author contact details	
Publication type *(e.g. full report, abstract, letter)*	
Notes:	

Study eligibility

Study Characteristics	**Eligibility criteria** *(Insert inclusion criteria for each characteristic as defined in the Protocol)*	**Eligibility criteria met?** Yes	No	Unclear	**Location in text or source** *(pg & ¶/fig/table/other)*
Type of study	Randomised Controlled Trial	☐	☐	☐	
	Quasi-randomised Controlled Trial	☐	☐	☐	
	Controlled Before and After Study • Contemporaneous data collection • Comparable control site • At least 2 x intervention and 2 x control clusters	☐	☐	☐	
	Interrupted Time Series • At least 3 time points before and 3 after the intervention • Clearly defined intervention point	☐	☐	☐	
	Other design (specify):	☐	☐	☐	
Participants		☐	☐	☐	
Types of intervention		☐	☐	☐	
Types of outcome measures		☐	☐	☐	
INCLUDE ☐ **EXCLUDE** ☐					
Reason for exclusion					
Notes:					

DO NOT PROCEED IF STUDY EXCLUDED FROM REVIEW

Characteristics of included studies

Methods

	Descriptions as stated in report/paper		Location in text or source *(pg & ¶/fig/table/other)*
Aim of study *(e.g. efficacy, equivalence, pragmatic)*			
Design*(e.g. parallel, crossover, non-RCT)*			
Unit of allocation *(by individuals, cluster/ groups or body parts)*			
Start date			
End date			
Duration of participation *(from recruitment to last follow-up)*			
Ethical approval needed/ obtained for study	☐ Yes ☐ No ☐ Unclear		
Notes:			

Participants

	Description *Include comparative information for each intervention or comparison group if available*		**Location in text or source** *(pg & ¶/fig/table/other)*
Population description *(from which study participants are drawn)*			
Setting *(including location and social context)* ***plus country***			
Inclusion criteria			
Exclusion criteria			
Method of recruitment of participants *(e.g. phone, mail, clinic patients)*			
Informed consent obtained	☐ Yes ☐ No ☐ Unclear		
Total no. randomised and how were they randomised? *(or total pop. at start of study for NRCTs)*			

Post randomization drop outs **Reason:**		
Clusters *(if applicable, no., type, no. people per cluster)*		
Baseline imbalances		
Withdrawals and exclusions *(if not provided below by outcome)*		
Age		
Sex		
Race/Ethnicity		
Body weight (kg)		
BMI (kg/m^2)		
Severity of illness		
Co-morbidities		
Other relevant sociodemographics		
Subgroups measured	• non pharma Tx • Pharma Tx • Pharma vs non Pharma Tx • Pts receiving Bariatric Surgery • Pts trying to lose weight • SGS alone • SGS and asymptomatic GS	
Subgroups reported		
Assessment of Gallstones		
Other details related to groups (ie. details of any other group or any other intervention related to patients)		
Notes:		

Intervention groups

Copy and paste table for each intervention and comparison group

Total No. of Intervention Groups □

Intervention Group 1

	Description as stated in report/paper	**Location in text or source** *(pg & ¶/fig/table/other)*
Group name		
No. randomised to group *(specify whether no. people or clusters)*		
Theoretical basis *(include key references)*		
Description *(include sufficient detail for replication, e.g. content, dose, components)*		
Duration of treatment period		
Timing *(e.g. frequency, duration of each episode)*		
Delivery *(e.g. mechanism, medium, intensity, fidelity)*		
Providers *(e.g. no., profession, training, ethnicity etc. if relevant)*		
Co-interventions		
Economic variables *(i.e. intervention cost, changes in other costs as result of intervention)*		
Resource requirements *(e.g. staff numbers, cold chain, equipment)*		
Integrity of delivery (ie intervention itself)		
Compliance		
Notes:		

Intervention Group 2

	Description as stated in report/paper	**Location in text or source** *(pg & ¶/fig/table/other)*
Group name		
No. randomised to group *(specify whether no. people or clusters)*		
Theoretical basis *(include key references)*		
Description *(include sufficient detail for replication, e.g. content, dose, components)*		
Duration of treatment period		
Timing *(e.g. frequency, duration of each episode)*		
Delivery *(e.g. mechanism, medium, intensity, fidelity)*		
Providers *(e.g. no., profession, training, ethnicity etc. if relevant)*		
Co-interventions		
Economic variables *(i.e. intervention cost, changes in other costs as result of intervention)*		
Resource requirements *(e.g. staff numbers, cold chain, equipment)*		
Integrity of delivery (ie intervention itself)		
Compliance		
Notes:		

Intervention Group 3

	Description as stated in report/paper	**Location in text or source** *(pg & ¶/fig/table/other)*
Group name		
No. randomised to group *(specify whether no. people or clusters)*		
Theoretical basis *(include key references)*		
Description *(include sufficient detail for replication, e.g. content, dose, components)*		
Duration of treatment period		
Timing *(e.g. frequency, duration of each episode)*		
Delivery *(e.g. mechanism, medium, intensity, fidelity)*		
Providers *(e.g. no., profession, training, ethnicity etc. if relevant)*		
Co-interventions		
Economic variables *(i.e. intervention cost, changes in other costs as result of intervention)*		
Resource requirements *(e.g. staff numbers, cold chain, equipment)*		
Integrity of delivery (ie intervention itself)		
Compliance		
Notes:		

Outcomes

Copy and paste table for each outcome.

No. of outcomes collected □

No. of outcomes reported on □

Outcome 1

	Description as stated in report/paper		**Location in text or source** *(pg & ¶/fig/table/other)*
Outcome name			
Time points measured *(specify whether from start or end of intervention)*			
Time points reported			
Outcome definition *(with diagnostic criteria if relevant)*			
Person measuring/ reporting			
Unit of measurement *(if relevant)*			
Scales: upper and lower limits *(indicate whether high or low score is good)*			
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear		
Imputation of missing data *(e.g. assumptions made for ITT analysis)*			
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*			
Power			
Notes:			

Outcome 2

	Description as stated in report/paper		Location in text or source *(pg & ¶/fig/table/other)*
Outcome name			
Time points measured *(specify whether from start or end of intervention)*			
Time points reported			
Outcome definition *(with diagnostic criteria if relevant)*			
Person measuring/ reporting			
Unit of measurement *(if relevant)*			
Scales: upper and lower limits *(indicate whether high or low score is good)*			
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear		
Imputation of missing data *(e.g. assumptions made for ITT analysis)*			
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*			
Power			
Notes:			

Outcome 3

	Description as stated in report/paper		**Location in text or source** *(pg & ¶/fig/table/other)*
Outcome name			
Time points measured *(specify whether from start or end of intervention)*			
Time points reported			
Outcome definition *(with diagnostic criteria if relevant)*			
Person measuring/ reporting			
Unit of measurement *(if relevant)*			
Scales: upper and lower limits *(indicate whether high or low score is good)*			
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear		
Imputation of missing data *(e.g. assumptions made for ITT analysis)*			
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*			
Power			
Notes:			

Outcome 4

	Description as stated in report/paper		Location in text or source *(pg & ¶/fig/table/other)*
Outcome name			
Time points measured *(specify whether from start or end of intervention)*			
Time points reported			
Outcome definition *(with diagnostic criteria if relevant)*			
Person measuring/ reporting			
Unit of measurement *(if relevant)*			
Scales: upper and lower limits *(indicate whether high or low score is good)*			
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear		
Imputation of missing data *(e.g. assumptions made for ITT analysis)*			
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*			
Power			
Notes:			

Outcome 5

	Description as stated in report/paper	Location in text or source *(pg & ¶/fig/table/other)*
Outcome name		
Time points measured *(specify whether from start or end of intervention)*		
Time points reported		
Outcome definition *(with diagnostic criteria if relevant)*		
Person measuring/ reporting		
Unit of measurement *(if relevant)*		
Scales: upper and lower limits *(indicate whether high or low score is good)*		
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear	
Imputation of missing data *(e.g. assumptions made for ITT analysis)*		
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*		
Power		
Notes:		

Other

Study funding sources *(including role of funders)*		
Possible conflicts of interest *(for study authors)*		
Notes:		

Risk of Bias assessment

See Chapter 8 of the Cochrane Handbook. Additional domains may be added for non-randomised studies.

Domain	**Risk of bias** Low risk ☐ High risk ☐ Unclear ☐	**Support for judgement** *(include direct quotes where available with explanatory comments)*	**Location in text or source** *(pg & ¶/fig/table/other)*
Random sequence generation *(selection bias)*	☐ ☐ ☐		
Allocation concealment *(selection bias)*	☐ ☐ ☐		
Blinding of participants and personnel *(performance bias)*	☐ ☐ ☐	**Outcome group: All/**	
(if separate judgement by outcome(s) required)	☐ ☐ ☐	**Outcome group:**	
Blinding of outcome assessment *(detection bias)*	☐ ☐ ☐	**Outcome group: All/**	
(if separate judgement by outcome(s) required)	☐ ☐ ☐	**Outcome group:**	
Incomplete outcome data *(attrition bias)*	☐ ☐ ☐	**Outcome group: All/**	
(if separate judgement by outcome(s) required)	☐ ☐ ☐	**Outcome group:**	
Selective outcome reporting? *(reporting bias)*	☐ ☐ ☐		
Other bias	☐ ☐ ☐		
Notes:			

Data and analysis

Copy and paste the appropriate table for each outcome, including additional tables for each time point and subgroup as required.

For RCT/CCT
Binary (Dichotomous) outcome

1. ***All cause mortality at max follow-up***
2. ***Proportion of pts with GS-related complications***
3. ***Proportion of pts with Tx-related complications***
4. ***Proportion of pts with GS***
5. ***Porportion of pts Tx by cholecystectomy***
6. ***Changes in biliary lithogenicity***
7. ***Tx stoppage or withdrawal***

1.

	Description as stated in report/paper				**Location in text or source** *(pg & ¶/fig/table/other)*
Comparison					
Outcome					
Subgroup (analyses)					
Time point *(specify from start or end of intervention)*					
Results (ie. 2 x 2 table)	**Intervention**		**Comparison**		
	No. with event	Total in group	No. with event	Total in group	
Any other results reported *(e.g. odds ratio, risk difference, CI or P value)*					
No. missing participants					
Reasons missing					
No. participants moved from other group					
Reasons moved					
Unit of analysis *(by individuals, cluster/groups or body parts)*					
Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*					
Reanalysis required? *(specify, e.g. correlation adjustment)*	☐ Yes ☐ No ☐ Unclear				
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear				
Reanalysed results					
Notes:					

2.

	Description as stated in report/paper				**Location in text or source** *(pg & ¶/fig/table/other)*
Comparison					
Outcome					
Subgroup (analyses)					
Time point *(specify from start or end of intervention)*					
Results (ie. 2 x 2 table)	**Intervention**		**Comparison**		
	No. with event	Total in group	No. with event	Total in group	
Any other results reported *(e.g. odds ratio, risk difference, CI or P value)*					
No. missing participants					
Reasons missing					
No. participants moved from other group					
Reasons moved					
Unit of analysis *(by individuals, cluster/groups or body parts)*					
Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*					
Reanalysis required? *(specify, e.g. correlation adjustment)*	☐ Yes ☐ No ☐ Unclear				
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear				
Reanalysed results					
Notes:					

3.

	Description as stated in report/paper				**Location in text or source** *(pg & ¶/fig/table/other)*
Comparison					
Outcome					
Subgroup (analyses)					
Time point *(specify from start or end of intervention)*					
Results (ie. 2 x 2 table)	**Intervention**		**Comparison**		
	No. with event	Total in group	No. with event	Total in group	
Any other results reported *(e.g. odds ratio, risk difference, CI or P value)*					
No. missing participants					
Reasons missing					
No. participants moved from other group					
Reasons moved					
Unit of analysis *(by individuals, cluster/groups or body parts)*					
Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*					
Reanalysis required? *(specify, e.g. correlation adjustment)*	☐ Yes ☐ No ☐ Unclear				
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear				
Reanalysed results					
Notes:					

4.

	Description as stated in report/paper				**Location in text or source** *(pg & ¶/fig/table/other)*
Comparison					
Outcome					
Subgroup (analyses)					
Time point *(specify from start or end of intervention)*					
Results (ie. 2 x 2 table)	**Intervention**		**Comparison**		
	No. with event	Total in group	No. with event	Total in group	
Any other results reported *(e.g. odds ratio, risk difference, CI or P value)*					
No. missing participants					
Reasons missing					
No. participants moved from other group					
Reasons moved					
Unit of analysis *(by individuals, cluster/groups or body parts)*					
Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*					
Reanalysis required? *(specify, e.g. correlation adjustment)*	☐ Yes ☐ No ☐ Unclear				
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear				
Reanalysed results					
Notes:					

For RCT/CCT
Continuous outcome

1. **Quality of life**
2. **Weight lost in Kg**
3. **% weight lost**
4. **Change in BMI**

1.

	Description as stated in report/paper						**Location in text or source** *(pg & ¶/fig/table/other)*
Comparison							
Outcome							
Subgroup (analyses)							
Time point *(specify from start or end of intervention)*							
Post-intervention or change from baseline?							
Results	**Intervention**			**Comparison**			
	Mean	SD *(or other variance, specify)*	No. participants	Mean	SD *(or other variance, specify)*	No. participants	
Any other results reported *(e.g. mean difference, CI, P value)*							
No. missing participants							
Reasons missing							
No. participants moved from other group							
Reasons moved							
Unit of analysis *(individuals, cluster/ groups or body parts)*							
Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*							
Reanalysis required? *(specify)*	☐ Yes ☐ No ☐ Unclear						
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear						
Reanalysed results							
Notes:							

2.

	Description as stated in report/paper						**Location in text or source** *(pg & ¶/fig/table/other)*
Comparison							
Outcome							
Subgroup (analyses)							
Time point *(specify from start or end of intervention)*							
Post-intervention or change from baseline?							
Results	**Intervention**			**Comparison**			
	Mean	SD *(or other variance, specify)*	No. participants	Mean	SD *(or other variance, specify)*	No. participants	
Any other results reported *(e.g. mean difference, CI, P value)*							
No. missing participants							
Reasons missing							
No. participants moved from other group							
Reasons moved							
Unit of analysis *(individuals, cluster/ groups or body parts)*							
Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*							
Reanalysis required? *(specify)*	☐ Yes ☐ No ☐ Unclear						
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear						
Reanalysed results							
Notes:							

For RCT/CCT
Other outcome ie Count data outcome
1. No. of GS related adverse events

	Description as stated in report/paper				**Location in text or source** *(pg & ¶/fig/table/other)*
Comparison					
Outcome					
Subgroup (analyses)					
Time point *(specify from start or end of intervention)*					
No. participant	Intervention		Control		
Results ie no. of events	Intervention result	SD (or other variance)	Control result	SD (or other variance)	
	Overall results		SE (or other variance)		
Any other results reported					
No. missing participants					
Reasons missing					
No. participants moved from other group					
Reasons moved					
Unit of analysis *(by individuals, cluster/groups or body parts)*					
Statistical methods used and appropriateness of these					
Reanalysis required? *(specify)*	☐ Yes ☐ No ☐ Unclear				
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear				
Reanalysed results					
Notes:					

Other outcome ie Count data outcome
2. No. of Tx related adverse events

	Description as stated in report/paper				**Location in text or source** *(pg & ¶/fig/table/other)*
Comparison					
Outcome					
Subgroup (analyses)					
Time point *(specify from start or end of intervention)*					
No. participant	Intervention		Control		
Results ie no. of events	Intervention result	SD (or other variance)	Control result	SD (or other variance)	
	Overall results		SE (or other variance)		
Any other results reported					
No. missing participants					
Reasons missing					
No. participants moved from other group					
Reasons moved					
Unit of analysis *(by individuals, cluster/groups or body parts)*					
Statistical methods used and appropriateness of these					
Reanalysis required? *(specify)*	☐ Yes ☐ No ☐ Unclear				
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear				
Reanalysed results					
Notes:					

Other information

	Description as stated in report/paper	Location in text or source *(pg & ¶/fig/table/other)*
Key conclusions of study authors		
References to other relevant studies		
Correspondence required for further study information *(from whom, what and when)*		
Funding source		
Miscellaneous comments from study authors		
Notes:		

Appendix 3. Excluded studies with reason

Study	Reason
Acalovschi [4]	Longitudinal observation study.
Al-Jiffry [7]	not a randomised controlled trial.
Arffmann [12]	did not assess incidence of gallstones; intervention duration too short (2 wks).
Baudet [20]	looked at TPN and intervention duration between 2-3 wks.
Bell [22]	review article
Cometta [41]	intervention duration too short for most participants (mean 2 wks).
Davidson [46]	did not assess incidence of gallstones, or any hepato-biliary components.
De Oliveira [85]	retrospective observation study.
Desbeaux [48]	review article.
Einarsson [53]	intervention duration too short (3 wks); did not assess incidence of gallstones.
Fischer [58]	assessed gallstone dissolution with a short intervention lasting 10-12 days.
Fobi [60]	observation study.
Frenkiel [62]	randomised intervention trial for gallstones dissolution.
Fuller [65]	observation study.
Hamad [73]	not a randomised controlled trial.
Heim-Duthoy [75]	assessed gallstone incidence but intervention duration too short (14 days).
Henriksson [76]	intervention trial in participants with existing gallstones and cholecystectomy.
Heshka [77]	not a randomised controlled trial.
Hoy [81]	met inclusion criteria but did not receive required information from authors.
Jonkers [91]	Crossover design assessing gallbladder dysmotility. Only presented data at end of crossover period.
Kamrath [94]	not a randomised controlled trial.
Kiewiet [99]	retrospective observation study.
Klass [103]	intervention duration too short (3 wks).
Kurata [111]	retrospective analysis of symptomatic gallstones requiring hospitalisation.
Lee [121]	animal study.
Liddle [128]	not a randomised controlled trial.
Lustig [130]	some participants had undergone a cholecystectomy.
Mason [135]	qualitative study.
Mazzella [138]	did not assess interventions for primary prevention of gallstones. However, information on adverse events included in this review.
Mok [146,147]	did not assess interventions for primary prevention of gallstones. However, information on adverse events included in this review.
Nagem [151]	longitudinal observation study.
Nagem [152]	prospective observation study.
Neitlich [154]	not a randomised controlled trial.
Nougou [155]	prospective observation study.
O'Donnell [156]	intervention duration too short (1 wk).

Study	Reason
Östlund [169]	longitudinal observation study.
Oria [157]	retrospective observation study.
Pausawasdi [162]	animal study.
Pavel [163]	case-control study of gallstone risk with sun exposure.
Pazzi [164]	case-control study of gallstone risk with nonsteroidal anti-inflammatory drug use.
Pitt [167]	not a randomised controlled trial.
Rhodes [176]	intervention study in participants with gallstones.
Rubin [177]	intervention duration too short (48 hours).
Rudnicki [178]	nested case-control study.
Scott [185]	not a randomised controlled trial. However, information on adverse events included in this review.
Sengupta [187]	intervention duration too short (8 hour treatments on 3 occasions).
Shiffman [189]	prospective observation study.
Shiffman [190]	prospective observation study.
Sitzmann [195]	intervention duration too short; did not report on gallstone incidence.
Spirt [201]	not a randomised controlled trial.
Sterling [204]	retrospective observation study.
Storti [211]	used self-report.
Sorensen [198,199,200]	surgical interventions.
Tarantino [215]	prospective observation study.
Tazuma [216]	not a randomised controlled trial.
Trouillot [221]	did not assess interventions for primary prevention of gallstones.
Utter [235]	intervention duration too short (several hours).
Utter [236]	did not assess interventions for primary prevention of gallstones.
Venneman [238]	review article.
Vezina [239]	not a randomised controlled trial.
Villegas [240]	not a randomised controlled trial.
Wang [246]	not a randomised controlled trial.
Wilund [255]	animal study.
Yang [258]	not a randomised controlled trial.
Zapata [259]	not a randomised controlled trial.
Zoli [261]	not a randomised controlled trial; intervention duration too short.

Appendix 4. Characteristics of included studies

Ai 2003

Methods	Randomised comparison of UCDA versus control.
Participants	• Mean age: 55.7 years (UDCA), 56 years (control). • Females: 42% (UDCA), 44% (control). • Mean weight: not reported. • Mean BMI 21.1 kg/m^2 (UDCA), 21.1 kg/m^2 (control).
Interventions	• Intervention: 600 mg UDCA/day for 24 weeks. • Control: did not receive anything during these 24 weeks.
Outcomes	Formation of gallstones, values of hemolysis markers (haptoglobin, hemoglobin, lactate dehydrogenase, reticulocyte, total bilirubin), gallbladder contractility, blood transfusion volume, heart-lung machine running time.
Duration of follow up	60 months.
Collateral interventions	None.
Notes	• No additional information received from the primary investigators. • Country: Japan.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	unclear.
Allocation concealment (selection bias)	Unclear risk	unclear.
Blinding of participants and personnel (performance bias)	High risk	not placebo controlled.
Blinding of outcome assessment (detection bias)	Unclear risk	unclear.
Incomplete outcome data (attrition bias)	High risk	Participants lost to follow-up are not clearly included in the analyses.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	Unclear risk	No information on funding provided.

Broomfield 1988

Methods	Randomised comparison of UCDA versus placebo.
Participants	• Mean age: 39.8 years (UDCA), 35.7 years (placebo). • Females: 74% (UDCA), 78% (placebo). • Mean weight: 105.7 kg (UDCA), 106.2 kg (placebo). • Mean BMI (not reported).
Interventions	• Intervention: 1200 mg UDCA/day for 16 weeks. • Control: identical placebo for 16 weeks.
Outcomes	Bile cholesterol saturation, biliary glycoprotein and biliary prostaglandin E_2 concentrations, formation of gallstones (or crystals or microstones) and weight loss.
Duration of follow up	19 weeks.
Collateral interventions	Very low calorie low fat diet (520 kcal, 55 g protein, 79 g carbohydrate, 1 g fat, plus supplemental vitamins, trace elements, minerals) and 2 litres non-caloric liquid daily.
Notes	• The trial includes a third intervention group (1300 mg aspirin/day) that was excluded from the main meta-analyses. • No additional information received from the primary investigators. • Country: USA.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	High risk	Participants lost to follow-up were not clearly described or accounted for.
Selective reporting (reporting bias)	High risk	Clinically relevant outcomes not clearly reported at the end of follow-up.
Other bias	Low risk	Funding source: National Institutes of Health (grant).

De Filippo 1993

Methods	Randomised comparison of UCDA versus placebo.
Participants	• Mean age: 38 years (UCDA and placebo). • Females: 78% (UCDA and placebo). • Mean weight: 105.2 kg (UDCA), 100.8 kg (placebo). • Mean BMI: 39.0 kg/m^2 (UDCA), 38.3 kg/m^2 (placebo).
Interventions	• Intervention: 600 mg to 900 mg UDCA/day for 16 weeks. • Control: identical placebo for 16 weeks.
Outcomes	Formation of gallstones (or sludge or microlithiasis), weight loss, blood pressure, blood parameters (blood urea nitrogen, glucose, total protein, total and direct bilirubin, triglycerides, cholesterol, transaminase, alkaline phosphatase, gamma-glutamyl transferase) and blood erythrocyte, haemoglobin, hematocrit, leukocytes and platelets.
Duration of follow up	16 weeks.
Collateral interventions	Low calorie high fat diet (1000 to 1200 kcal, 60 to 70 g protein, 100 to 170 g carbohydrate, 20 to 43 g fat [6% saturated], 35 to 40 g fibre, 165 to 220 mg cholesterol).
Notes	• Additional information received from the primary investigators. • Country: Italy.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	Low risk	All participants completed the trial and were reported on.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	Unclear risk	Funding source: not described.

Festi 1998

Methods	Randomised comparison of very low calorie high fat diet versus very low calorie low fat diet.
Participants	• Mean age: 40.5 years (intervention and control group). • Females: 63% (intervention and control group). • Mean weight: not reported. • Mean BMI 41.6 kg/m^2 (intervention and control group).
Interventions	• Intervention: very low calorie high fat diet (577 kcal, 55.0 g protein, 61.7 g carbohydrate, 12.2 g fat, plus vitamins, trace elements and mineral supplements); at least 2 litres of non-caloric liquids per day for 12 weeks • Control: very low calorie low fat diet (535.2 kcal, 44.4 g protein, 82.2 g carbohydrate, 3.0 g fat, plus vitamins, trace elements and mineral supplements); at least 2 litres of non-caloric liquids per day for 12 weeks.
Outcomes	Formation of gallstones (or cholesterol crystals), gallbladder motility (gallbladder emptying, fasting volume), biliary lipid composition and cholesterol saturation index (bile acid and phospholipid molar percentages), weight loss and compliance.
Duration of follow up	12 weeks.
Collateral interventions	None.
Notes	• The trial includes a follow-up phase in which all participants followed a low calorie high fat diet for 12 weeks. The data from this follow up period are not included in this review. • Additional information received from the primary investigators. • Country: Italy.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random numbers.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding of participants and personnel (performance bias)	Unclear risk	Blinding of participants and personnel not described.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	High risk	Participants lost to follow-up were not clearly accounted for.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	Unclear risk	Unclear.

Gebhard 1996

Methods	Randomised comparison of high fat diet versus very low fat low calorie diet.
Participants	• Mean age: 40 years (intervention), 40 years (control). • Females: 71% (intervention), 83% (control). • Mean weight: 114 kg (intervention), 105 kg (control). • Mean BMI: 36 kg/m^2 (intervention), 37 kg/m^2 (control).
Interventions	• Intervention: low calorie high fat diet (900 kcal, 90 g protein, 67 g carbohydrate, 30 g fat and 90 mg cholesterol) for 12 weeks. • Control: very low calorie low fat diet (520 kcal, 50 g protein, 79 g carbohydrate, less than 2 g fat and 30 mg cholesterol) for 12 weeks.
Outcomes	Formation of gallstones (or cholesterol crystals), gallbladder motility (gallbladder emptying, fasting volume), bile saturation index (bile phospholipid molar ratio), blood lipids, weight loss and compliance.
Duration of follow up	24 weeks.
Collateral interventions	Oral supplements with the recommended daily allowances for vitamins/minerals.
Notes	• No additional information received from the primary investigators. • Conventional foods were resumed after the first 12 weeks and patients followed up for another 12 weeks. Data on gallstones from the first 12 weeks is included. • Country: USA. • Human Subjects Subcommittee encouraged cessation of enrolment because two of the participants with gallstones were symptomatic.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or control.
Blinding of participants and personnel (performance bias)	Low risk	Administration of blinded identically appearing packets containing the intervention or control.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	Low risk	All participants completed the trial and were reported on.
Selective reporting (reporting bias)	High risk	Clinically relevant outcomes are not reported at the end of follow-up.
Other bias	Low risk	Funding source: Department of Veterans Affairs Research Program (Sandoz Nutrition, Minneapolis provided the diets).

Marks 1996

Methods	Randomised comparison of UCDA versus placebo.
Participants	• Mean age: 41.4 years (UDCA), 39.4 years (placebo). • Females: 75% (UDCA), 63% (placebo). • Mean weight: 99.5 kg (UDCA), 114.2 kg (placebo). • Mean BMI: 34.8 kg/m^2 (UDCA), 37.0 kg/m^2 (placebo).
Interventions	• Intervention: 1200 mg UDCA/day for 12 weeks. • Control: identical placebo for 12 weeks.
Outcomes	Bile saturation index, biliary glycoprotein and biliary prostaglandin E_2 concentrations, biliary lipids, gallbladder contraction, formation of gallstones (or crystals), weight loss and compliance.
Duration of follow up	12 weeks.
Collateral interventions	Both groups followed a very low calorie diet (520 kcal; macronutrients unspecified).
Notes	• The trial includes a third intervention group (1600 mg ibuprofen/day) that was excluded from the main meta-analyses. • No additional information received from the primary investigators. • Country: USA • Recruitment was terminated early in this trial due to slow recruitment and larger than expected drop out rates.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	High risk	Losses to follow-up not clearly described for each group or accounted for in the analyses.
Selective reporting (reporting bias)	High risk	Clinically relevant outcomes not reported at the end of follow-up.
Other bias	High risk	Funding source: not reported.

Mendez-Sanchez 2001

Methods	Randomised comparison of UCDA versus placebo.
Participants	• Mean age: 37.8 years (UDCA), 39.7 years (placebo). • Females: 100% (UCDA and placebo). • Mean weight: 79.8 kg (UDCA), 81.9 kg (placebo). • Mean BMI: 34.2 kg/m^2 (UDCA), 33.4 kg/m^2 (placebo).
Interventions	• Intervention: 1200 mg UDCA/day for 6 weeks. • Control: identical placebo for 6 weeks.
Outcomes	Formation of gallstones, bile saturation index, nucleation time, weight loss, compliance.
Duration of follow up	6 weeks.
Collateral interventions	Low calorie diet (1200 kcal, 60 g protein [20 %], 180 g carbohydrate [60 %] , 27 g fat [20 %]) plus 1 litre water daily.
Notes	• The trial includes a third intervention group, (11.3 g omega-3 fatty acids/day) that was excluded from the main meta-analyses. • No additional information received from the primary investigators. • Country: Mexico.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	High risk	Participants lost to follow up were not clearly described for each group or accounted for in the analyses.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	Low risk	Funding source: partially funded by National Council on Science and Technology in Mexico (CONACyT).

Miller 2003

Methods	Randomised comparison of UCDA versus placebo.
Participants	• Mean age: 34.1 years (UDCA), 36.3 years (placebo). • Females: 81% (UDCA), 85% (placebo). • Mean weight: 137 kg (UDCA), 136 kg (placebo). • Mean BMI: 43.7 kg/m^2 (UDCA), 44.3 kg/m^2 (placebo).
Interventions	• Intervention: 500 mg UDCA/day for 24 weeks. • Control: identical placebo for 24 weeks.
Outcomes	Formation of gallstones, weight loss, compliance.
Duration of follow up	24 months.
Collateral interventions	None, however 50% received vertical banded gastroplasty and 50% received adjustable gastric banding within three days of intervention initiation.
Notes	• Additional information received from the primary investigators. • Country: Austria.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	Low risk	Participants lost to follow-up were accounted for in the analyses.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	Low risk	Funding source: no funding received.

Moran 1997

Methods	Randomised comparison of UDCA versus fibre.
Participants	• Mean age: 39 years (UDCA), 38 years (fibre). • Females: 83% (UDCA), 83% (fibre). • Mean weight: 89.7 kg (UDCA), 85.8 kg (placebo). • Mean BMI: 34 kg/m^2 (UDCA), 35 kg/m^2 (fibre).
Interventions	• Intervention: 750 mg UDCA/d plus fibre placebo for 8 weeks. • Control: 15 g fibre as psyllium plantago plus UDCA placebo for 8 weeks.
Outcomes	Formation of gallstones, crystal determination, weight loss, compliance.
Duration of follow up	8 weeks.
Collateral interventions	Both groups followed a low calorie diet (calculated as - 500 kcal their total energy requirements. This diet comprised 15% protein, 60% carbohydrate, 25% fat, 15% fibre [20g]).
Notes	• Most information was extracted from the 1997 publication. • Additional information received from the primary investigators. • Country: Mexico.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the intervention or placebo (by an external monitor).
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	Low risk	All participants completed the trial and were reported on.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	Low risk	Source of funding: partially funded by National Council on Science and Technology in Mexico (CONACyT).

Shiffman 1995

Methods	Randomised comparison of UDCA versus placebo.
Participants	• Mean age: 40.3 years (UDCA), 39.8 years (placebo). • Females: 66% (UDCA), 66% (placebo). • Mean weight: 127.8 kg (UDCA), 128.7 kg (placebo). • Mean BMI: 44.1 kg/m^2 (UDCA), 44.5 kg/m^2 (placebo).
Interventions	• Intervention: 300/600/1200 mg UDCA/day for 16 weeks. • Control: identical placebo for 16 weeks.
Outcomes	Formation of gallstones (or crystals or microstones), gallbladder sludge, bile analysis, weight loss and compliance.
Duration of follow up	16 weeks.
Collateral interventions	All groups followed a very low calorie diet (520 kcal, 50 g protein, 79 g carbohydrate, 1g to 3 g fat, plus supplemental vitamins and minerals supplying 100% to 150% US recommended daily allowance were provided and consumption of non-caloric fluids (water, diet drinks) was unlimited.
Notes	• The three intervention groups with different doses of UDCA were combined to form one group and were compared to the placebo group. • Additional information received from the primary investigators. • Country: USA.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	High risk	Participants lost to follow-up from each intervention group were not clearly described or accounted for in the analyses.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	High risk	The sponsoring pharmaceutical company carried out the data collection/analysis.

Sugerman 1995

Methods	Randomised comparison of UCDAversus placebo.
Participants	• Mean age: 36.4 years (UDCA), 37.4 years (placebo). • Females: 80% (UDCA), 79% (placebo). • Mean weight: 137 kg (UDCA), 144 kg (placebo). • Mean BMI: 49.0 kg/m^2 (UDCA), 50.7 kg/m^2 (placebo).
Interventions	• Intervention: 300/600/1200 mg UDCA/day for 24 weeks. • Control: identical placebo for 24 weeks.
Outcomes	Formation of gallstones, efficacy of 3 doses of UDCA, weight loss, compliance.
Duration of follow up	6 months (12 months in a subset of participants but this is not included in the review).
Collateral interventions	None, however all participants received Roux-en-Y gastric bypass 4 days before intervention initiation.
Notes	• The three intervention groups with different doses of UDCA were combined to form one group and were compared to the placebo group. • Additional information received from the primary investigators. • Country: USA.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	Low risk	Participants lost to follow-up were accounted for in the analyses.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	High risk	Funding source: pharmaceutical company.

Williams 1993

Methods	Randomised comparison of UCDA versus placebo.
Participants	• Mean age: not given. • Females: not given. • Mean weight: not given. • Mean BMI: not given.
Interventions	• Intervention: 10 mg/kg UDCA/day for up to 18 months. • Control: identical placebo for up to 18 months.
Outcomes	Formation of gallstones, weight loss, compliance.
Duration of follow up	18 months.
Collateral interventions	None, however all participants received vertical banded gastroplasty six weeks before intervention initiation.
Notes	• No additional information received from the primary investigators. • Country: Canada.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	High risk	Although all the numbers and reasons for drop-outs in all intervention groups were described, a high non-compliance was observed.
Selective reporting (reporting bias)	High risk	Clinically relevant outcomes not clearly defined and reported.
Other bias	High risk	Funding source: partially sponsored by a pharmaceutical company.

Worobetz 1993

Methods	Randomised comparison of UCDA versus placebo.
Participants	• Mean age: 33.5 years (UDCA), 33.9 years (placebo). • Females: 69% (UDCA), 69% (placebo). • Mean weight: 146.8 kg (UDCA), 142.5 kg (placebo). • Mean BMI: not given.
Interventions	• Intervention: 1000 mg UDCA/day for 12 weeks. • Control: identical placebo for 12 weeks.
Outcomes	Formation of gallstones (or cholesterol crystals), bile composition, blood parameters (complete blood count, electrolytes, fasting cholesterol and triglycerides, fasting glucose, creatinine, albumin, biliruin, prothrombin time, aspartate aminotransferase, alkaline phosphatase), weight loss, compliance.
Duration of follow up	12 weeks.
Collateral interventions	None, however all participants received vertical band gastroplasty 4 days before intervention initiation. By the 4th postoperative day most participants tolerated a pureed diet (832 kcal, 26.0% protein, 52.0% carbohydrate, 22.0% fat) consumed as 9 meals per day for 4 weeks. They then advanced to a soft diet (995 kcal, 24.5% protein, 44.0% carbohydrate, 31.5% fat) consumed as 3 meals per day.
Notes	• No additional information received from the primary investigators. • Country: Canada.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	Low risk	Participants lost to follow-up were described for each group and accounted for in the analyses.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	Low risk	Funding source: partial funding provided by the Medical Research Council of Canada.

Wudel 2002

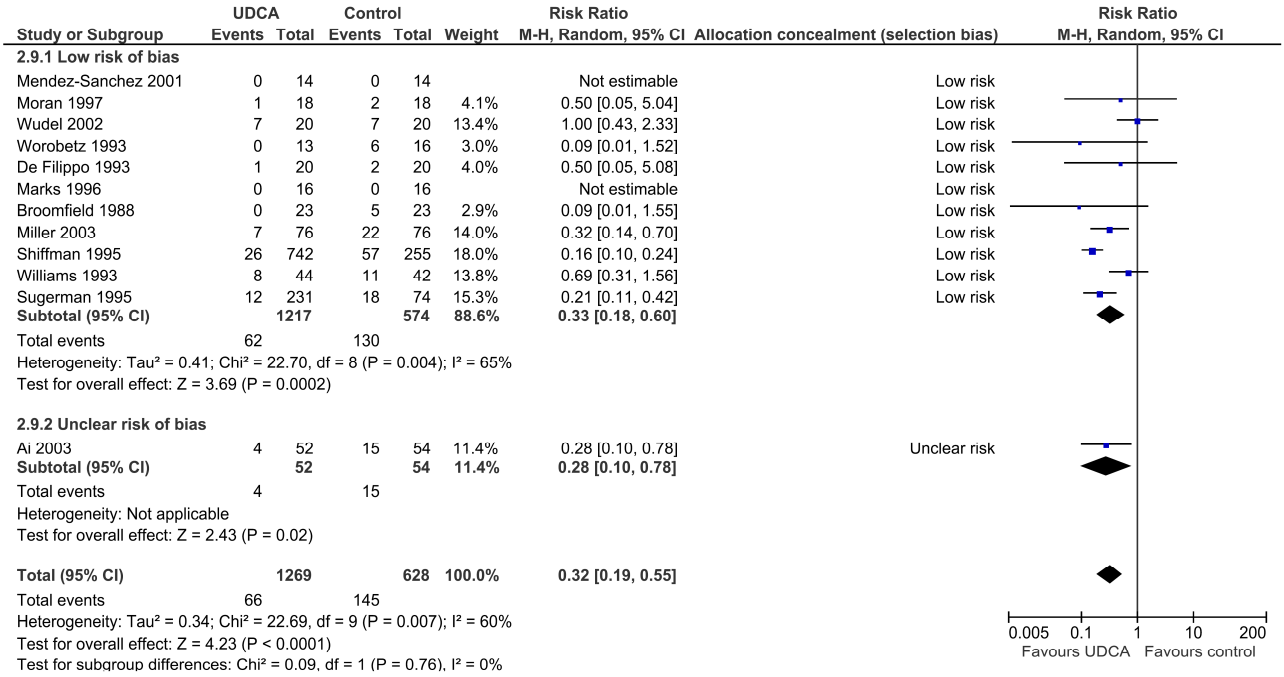
Methods	Randomised comparison of UCDA versus placebo.
Participants	• Mean age: 38 years (UCDA and placebo). • Females: 85% (UCDA and placebo). • Mean weight: 159 kg (UCDA and placebo). • Mean BMI: not given.
Interventions	• Intervention: 600 mg UDCA/day for 24 weeks. • Control: identical placebo for 24 weeks.
Outcomes	Formation of gallstones, gallbladder emptying, cholesterol saturation index, weight loss, compliance.
Duration of follow up	12 months.
Collateral interventions	None, however all participants received Roux-en-Y gastric bypass before intervention initiation.
Notes	• The trial includes a third intervention group (600 mg ibuprofen/day) that was excluded from the main meta-analyses. • No additional information received from the primary investigators. • Country: USA.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Table of random numbers.
Allocation concealment (selection bias)	Low risk	Administration of blinded containers with the active intervention or placebo.
Blinding of participants and personnel (performance bias)	Low risk	Placebo controlled.
Blinding of outcome assessment (detection bias)	Low risk	Blinded outcome assessment.
Incomplete outcome data (attrition bias)	High risk	Participants lost to follow-up were not clearly accounted for in the analyses, and a high non-compliance was observed.
Selective reporting (reporting bias)	Low risk	Clinically relevant outcomes defined and reported.
Other bias	High risk	Funding source: partial funding provided by a Clinical Nutrition Research Unit Award and partially funded by a pharmaceutical company.

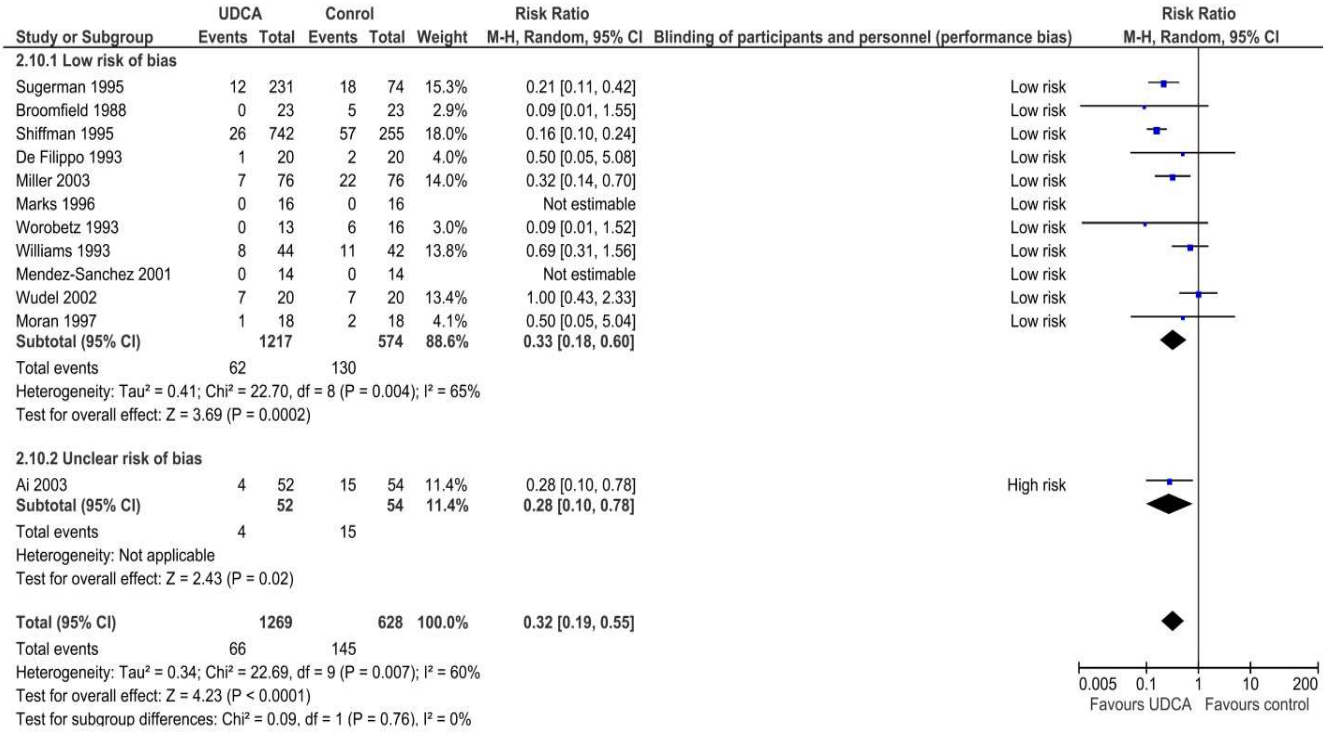
Appendix 5. Meta-analyses assessing risk of bias

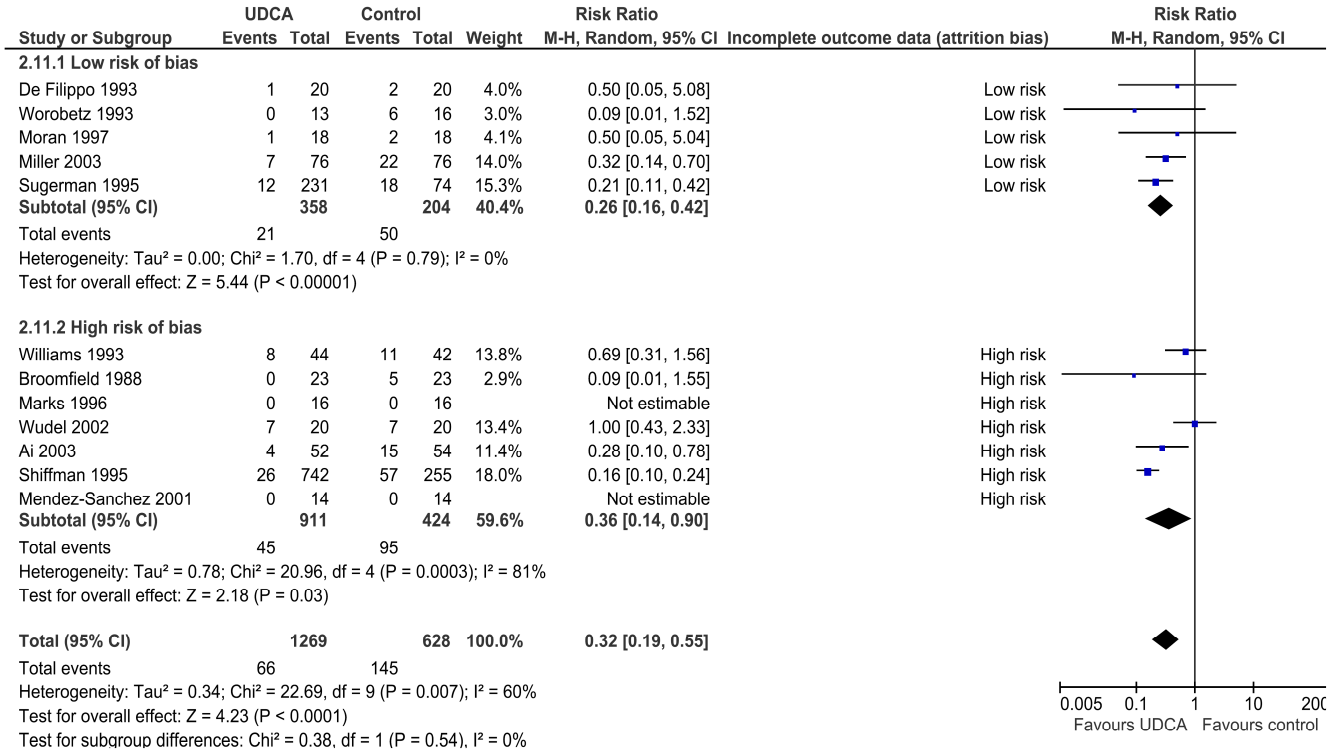
Meta-analyses assessing different components of bias with regard to gallstone formation in patients receiving UDCA versus control interventions based on:

Allocation concealment stratification

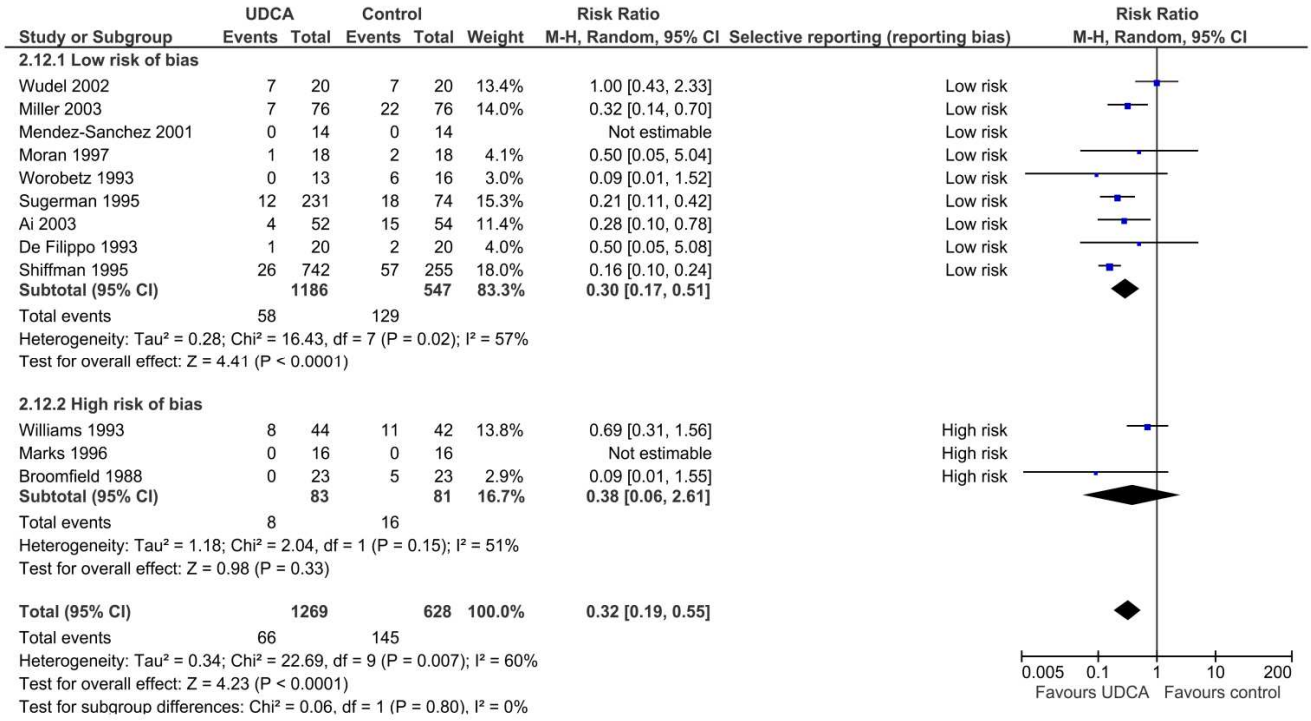


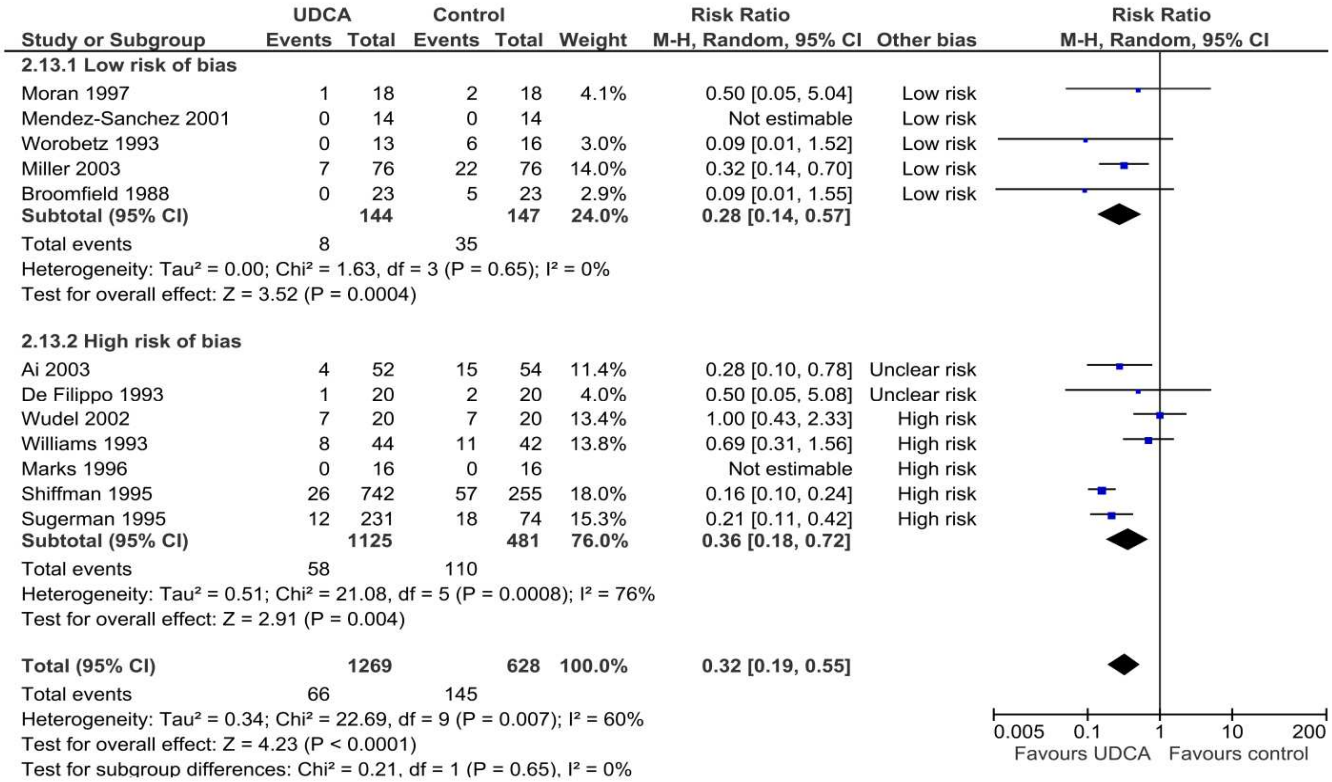
Blinding methods stratification (upper) and attrition bias stratification (lower)





Outcomes reporting stratification (upper) and other bias (lower)

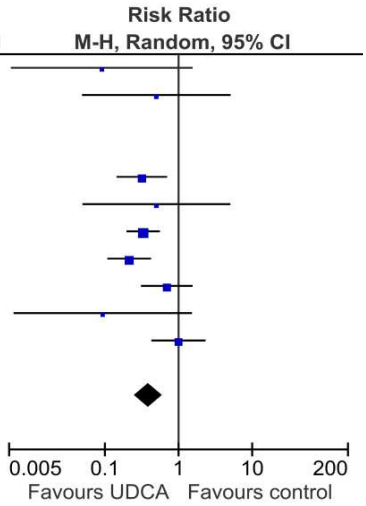




Good outcome (upper) and poor outcome analysis (lower)

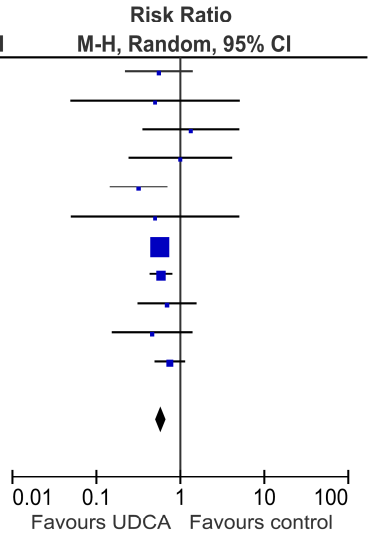
Study or Subgroup	UDCA Events	UDCA Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Broomfield 1988	0	23	5	23	2.2%	0.09 [0.01, 1.55]
De Filippo 1993	1	20	2	20	3.2%	0.50 [0.05, 5.08]
Marks 1996	0	16	0	16		Not estimable
Mendez-Sanchez 2001	0	14	0	14		Not estimable
Miller 2003	7	76	22	76	16.2%	0.32 [0.14, 0.70]
Moran 1997	1	18	2	18	3.2%	0.50 [0.05, 5.04]
Shiffman 1995	26	742	27	255	23.4%	0.33 [0.20, 0.56]
Sugerman 1995	12	231	18	74	18.8%	0.21 [0.11, 0.42]
Williams 1993	8	44	11	42	15.8%	0.69 [0.31, 1.56]
Worobetz 1993	0	13	6	16	2.2%	0.09 [0.01, 1.52]
Wudel 2002	7	20	7	20	15.0%	1.00 [0.43, 2.33]
Total (95% CI)		**1217**		**574**	**100.0%**	**0.39 [0.25, 0.60]**
Total events	62		100			

Heterogeneity: Tau² = 0.14; Chi² = 12.61, df = 8 (P = 0.13); I² = 37%
Test for overall effect: Z = 4.27 (P < 0.0001)



Study or Subgroup	UDCA Events	UDCA Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Broomfield 1988	5	23	9	23	2.3%	0.56 [0.22, 1.41]
De Filippo 1993	1	20	2	20	0.4%	0.50 [0.05, 5.08]
Marks 1996	4	16	3	16	1.1%	1.33 [0.35, 5.03]
Mendez-Sanchez 2001	3	14	3	14	1.0%	1.00 [0.24, 4.13]
Miller 2003	7	76	22	76	3.2%	0.32 [0.14, 0.70]
Moran 1997	1	18	2	18	0.4%	0.50 [0.05, 5.04]
Shiffman 1995	182	742	110	255	55.3%	0.57 [0.47, 0.69]
Sugerman 1995	66	231	36	74	20.5%	0.59 [0.43, 0.80]
Williams 1993	8	44	11	42	3.0%	0.69 [0.31, 1.56]
Worobetz 1993	3	13	8	16	1.6%	0.46 [0.15, 1.40]
Wudel 2002	12	20	16	20	11.2%	0.75 [0.49, 1.14]
Total (95% CI)		**1217**		**574**	**100.0%**	**0.59 [0.51, 0.68]**
Total events	292		222			

Heterogeneity: Tau² = 0.00; Chi² = 6.24, df = 10 (P = 0.80); I² = 0%
Test for overall effect: Z = 7.37 (P < 0.00001)



The Cochrane Hepato-Biliary Group
Title Registration Form

Please complete this form to outline your proposal for a Cochrane systematic review. E-mail the completed form to dnikolov@ctu.rh.dk, or send to Dimitrinka Nikolova, Managing Editor, Cochrane Hepato-Biliary Group, Rigshospitalet, Dept. 33.44, Blegdamsvej 9, DK-2100 Copenhagen Ø Denmark, Tel. +45 3545 7169. Fax +45 3545 7101. http://ctu.rh.dk/chbg.

Proposed title (see Handbook section 4.2.1)

Non-pharmacological and pharmacological interventions for the primary prevention of gallbladder stones

Contact person (see Handbook section 4.2.3)

Name: Caroline Stokes

Review proposal and inclusion criteria: (see Handbook chapter 5)

Motivation for the review:	There are two main motivators for this review: 1. To ascertain whether existing evidence supports the use of non-pharmacological and pharmacological interventions (as opposed to surgical) in the prevention of gallbladder stones. 2. To contribute to the development of the European clinical practice guidelines for gallbladder stones through the Cochrane review publication.
Review objective:	The objective of this review is to evaluate the efficacy of non-pharmacological and pharmacological interventions in the primary prevention of gallbladder stones in adults.
Types of study: (section 5.5)	In our analysis we plan to include all randomized controlled trials (RCTs) irrespective of blinding, language, sample size, or publications status. Controlled clinical trials (CCTs) where quasi-randomization methods have been used, such as day of the week, date of birth, medical record number may also be considered.
Participants / population: (section 5.2)	This review will include both hospital and community based male and female adults (>18 years) from all ethnicities, who have a BMI > 25 kg/m^2 and who do not have gallbladder stones as confirmed by ultrasonography. However studies using self-report measures will also be included when relating specifically to cholecystectomy as they are deemed reliable. The time frame when searching the literature will therefore include studies from 1970 to present, coinciding with the introduction of ultrasonography.

Intervention: (section 5.3)	Trials will be considered where at least one arm of the study has been allocated to receive a pharmacological intervention (irrespective of the time, dose, or pharmacological class of the administered drug) or a non-pharmacological intervention for prophylaxis against gallstone formation following a standard (within trial) protocol. This may include the following interventions (single or multiple per trial): • pharmacological (e.g. ursodeoxycholic acid, non-steroidal anti inflammatory drugs) • non-pharmacological (e.g. calorie restriction, dietary fibre or lipid modification, physical activity interventions). Settings: both community and hospital based interventions will be considered. For the above interventions, special consideration will be given to what is delivered (e.g. drug preparation), as well as to the intensity, frequency and route of administration. Pharmacological regimens for gallstone prevention are usually taken once or twice per day – this discrepancy is deemed unlikely to cause substantial differences in treatment effect thus will not be separated. Duration: the duration period will vary between studies, however minimum standards will be set for determining inclusion criteria (e.g. interventions lasting ≥ 3 months). Therefore, in terms of dietary-related interventions, single meal studies will be excluded as these are deemed to have a minimal impact on prevalence of gallbladder stones. Between-study variation may also exist for the dosage or quantity of an intervention, particularly with regards pharmacological interventions. Where varying pharmacological doses exist, these may be grouped together if significances are observed in all treatment levels, alternatively doses may be grouped based on predetermined clusters such as low, medium or high. For example, ursodeoxycholic acid is often prescribed as mg/kg body weight or in standard doses ranging from 300, 500, 600,1000 or 1200 mg/day and can therefore be grouped into the following: low dose (≤ 600 mg/day) and medium dose > 600 mg/day). Our understanding is that high doses of ursodeoxycholic acid have not been investigated in RCTs. Acceptable comparator groups will include: (1) inactive control intervention such as placebo, no treatment (2) active control intervention such as a variant of the same intervention, other pharmacological or non-pharmacological treatments.

Outcomes and adverse effects: (section 5.4)	**Types of outcomes** **Main outcomes:** • Formation of gallbladder stones • Cholecystectomy treatment • Adverse events (e.g. mortality, morbidity) • Quality of life • Attrition rates/compliance • Effect of interventions on weight loss (which will inform part of our review looking at gallstone prevention during weight loss) **Primary outcomes:** (1) Formation of gallbladder stones (asymptomatic or symptomatic) as assessed by ultrasonography. Results reporting gallstones cases per group will be included (e.g. percentage of gallstones cases per group; Odds Ratio values; 95% Confidence Intervals, P values). Studies that do not report on gallstone formation as an outcome will not be included. (2) Treatment with cholecystectomy (which is also a proxy for symptomatic gallstones), as measured by ultrasonography or self report measures. Results on patients who underwent cholecystectomy which are reported as per the first primary outcome will be included. (3) Occurrence (number and type) of adverse events during or within a reasonable post intervention time frame and will include for example, patient and clinician reporting of side effects, poor quality of life scores, morbidity, and mortality. An adverse event based on the ICH *definitions and standards for expedited reporting* (ICH 1995) is defined as any untoward medical occurrence that does not have a causal relationship with the particular treatment and can include any unfavourable and unintended sign (e.g. abnormal laboratory findings), symptoms or disease which is temporarily associated with the use of the respective medicinal product under investigation, regardless of whether it is related to this medicinal product. The ICH describe a severe adverse event as that which results in death; is life threatening; requires hospitalisation or prolongation of existing hospitalisation; results in persistent or significant disability/incapacity or any medical event which may jeopardise the patient or require an intervention to prevent it. Evidence of adverse events will not only be sought from RCTs but also from open studies and case reports. **Secondary outcomes:** 2. The effect of an intervention on bile lithogenicity (changes in physiological parameters of bile composition indicative of an increased risk of gallstones, e.g. bile nucleation time, biliary cholesterol saturation, cholesterol crystals, bile acid synthesis/pool – assessed through the analysis of patient bile). 3. The effect of the intervention on weight loss (e.g. weight, BMI and percent weight loss from baseline weight). 4. Attrition rates and/or compliance with study intervention (to determine potential bias in the data analysed from remaining subjects [e.g. was an Intention To Treat analysis used], as well as the feasibility of an intervention in clinical practice). 5. Quality of life (e.g. subjective measures with validated questionnaires such as Short Form 36, Ware Med Care 1995 33:AS264-AS279).

	Timing of outcome measurements Outcome measures will be assessed post intervention. For studies with differing time frames (e.g. for follow up) the following intervals will be used: short term (≤ 3 months), medium term (between 4-9 months) and long term (> 10 months).
Subgroup analyses: (section 9.6)	If enough studies are found to justify subgroup analyses, the following subgroups could be investigated further for effect modification: 1. Intervention type a. pharmacological therapies (different types and dosage) b. non-pharmacological therapies (in combination and/or alone) c. pharmacological versus non-pharmacological therapy 2. Intervention type based on patients receiving bariatric surgery versus those not receiving bariatric surgery 3. Intervention type based on patients actively trying to lose weight versus those not trying to lose weight 4. Outcome of symptomatic gallstones and/or cholecystectomy versus asymptomatic gallstones 5. Quality of bias control 6. Treatment duration/duration of follow up 7. Gender - as gallstones are more prevalent amongst females (Portincasa et al, Lancet 2006 368:230-239) 8. Different geographical locations and/or ethnicities due to the fact that both ethnic and geographical disparities exist regarding the prevalence of gallstones (Lammert & Miquel Liver Int 2008 28:935-947) A discussion of cost and cost-effectiveness of the interventions will be included in our review. **Sensitivity Analysis** If appropriate, a sensitivity analysis will be performed to explore the influence of the following factors on effect size: • Publication status (excluding unpublished studies) • Study quality (excluding studies of low quality e.g. that do not provide the drop out rate) • Blinding (removal of unclear or inadequate) • Allocation concealment (removal of unclear or inadequate allocation procedure) • Duration (impact of short and long duration) • Language of publication
Other information:	From our experience in clinical practice, consumers who are at a high risk of gallstones are often reluctant to undergo prophylactic cholecystectomy but frequently request for alternative (particularly non-pharmacological) interventions instead. We hope this review will help to inform clinical practice by providing a systematic evaluation of the evidence base relating to non-surgical (pharmacological and non-pharmacological) interventions for the primary prevention of gallbladder stones.

Authors' responsibilities

By completing this form, you accept responsibility for preparing, maintaining and updating the review in accordance with Cochrane Collaboration policy. The Cochrane Review Group (CRG) will provide as much support as possible to assist with the preparation of the review.

A draft protocol must be submitted to the CRG within six months. If drafts are not submitted before the agreed deadlines, or if we are unable to contact you for an extended period, the CRG has the right to de-register the title or transfer the title to alternative authors. The CRG has the right to de-register or transfer the title if it does not meet the standards of the CRG and/or The Cochrane Collaboration.

You accept responsibility for maintaining the review in light of new evidence, comments and criticisms, and other developments, and updating the review at least once every two years, or, if requested, transferring responsibility for maintaining the review to others as agreed with the CRG.

Publication in the *Cochrane Database of Systematic Reviews*

The support of the CRG in preparing your review is conditional upon your agreement to publish the protocol, finished review and subsequent updates the *Cochrane Database of Systematic Reviews*. By completing this form you undertake to publish this review in the *Cochrane Database of Systematic Reviews* before publishing elsewhere (concurrent publication in other journals may be allowed in certain circumstances with prior permission from the CRG).

I understand the commitment required to undertake a Cochrane review, and agree to publish first in the *Cochrane Database of Systematic Reviews*.

Signed on behalf of the authors:

Form completed by: Caroline Stokes	**Date:** 23.12.10

Do the authors have any potential conflict of interest?

Yes ☐ No ☒

If yes, please give details. Authors should declare and describe any present or past affiliations or other involvement in any organisation or entity with an interest in the outcome of the review that might lead to a real or perceived conflict of interest. This includes acting as an investigator of a study that might be included in this review. Authors should declare potential conflicts even if they are confident that their judgement is not influenced (see Handbook section 2.6 and www.cochrane.org/docs/commercialsponsorship.htm).

Review context

Is the review subject to any specific funding?	No
Is there a deadline for completing the review?	No
Has the review already been completed or published elsewhere?	No

Proposed deadlines

Date you plan to submit a draft protocol: (within 4 months)	30 April 2011 (dependent on start date)
Date you plan to submit a draft review: (within 12 months)	31 December 2011 (dependent on start date)

Review authors (see Handbook section 4.2.2.)

Each person named as an author must make a substantial contribution to the conception and design, or analysis and interpretation of the data in the review. Please attach a brief cv for each author.

Contact person / Author 1 (see Handbook section 4.2.3)

Is the contact person an author of the review?	Yes ☒ No ☐		
Prefix (e.g. Ms, Dr):	Ms	Given name (名字 míngzi):	Caroline
Middle initial(s):	S	Family name (姓 xìng):	Stokes
Suffix (e.g. MD, PhD):	RD	Web address:	
Preferred full name for review byline:	Stokes CS		
Do you already have a user account and password for the Archie database?	Yes ☐ No ☒		
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Telephone number:	+49 6841 16 23299	Fax number:	+49 6841 16 23570
Mobile/cell number:			
Privacy:	As the contact person, your address and email will be published with the completed protocol or review. Your details will be stored on our central database, known as 'Archie', and may be accessed by members of The Cochrane Collaboration. Details of our privacy policy are available at www.cc-ims.net/Archie/archie-privacy-policy. Within Archie, would you like to: Hide your address and phone numbers: ☐ Hide your email address: ☐		
Country of origin:	United Kingdom	Gender:	Female ☒ Male ☐
What expertise do you bring to the review?	(e.g. clinical, review methods, statistics) Knowledge of the subject area; experience in scientific writing and publishing original and review articles; data collection and synthesis		
Have you prepared a systematic review before?	Yes ☐ No ☒		
If yes, have you prepared a Cochrane review? (please state most recent title)	Yes ☐ No ☒		
Are you already a member of another Cochrane Review Group? Which one(s)?	Yes ☐ No ☒		
At what level are you able to speak and write English?	First language		

Author 2

You must have at least two authors to register a title. Copy this table for additional authors.

Prefix (e.g. Ms, Dr):	Prof	Given name (名字 míngzi):	Frank
Middle initial(s):		Family name (姓 xìng):	Lammert
Suffix (e.g. MD, PhD):	Dr med	Web address:	
Preferred full name for review byline:	Lammert F		
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Email address(es):	1) frank.lammert@uks.eu 2)		
Job Title/Position:	Director/Head		
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Organisation:	Saarland University Hospital		
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State/Province:	Saarland	Country:	Germany
Telephone number:	+49 6841 16 23201	Fax number:	+49 6841 16 23267
Mobile/cell number:			
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Country of origin:	Germany	Gender:	Female ☐ Male ☒

What expertise do you bring to the review?

(e.g. clinical, review methods, statistics)

In-depth knowledge of the subject area and experience in developing clinical practice guidelines, experience of systematic reviews

Select publications from 2005 onwards:

Reviews

1. **Lammert F**, Sauerbruch T. Molecular mechanisms of disease: the genetic epidemiology of gallbladder stones. **Nat Clin Pract Gastroenterol Hepatol** 2005; 2 (9): 423-433 [IF 4,4]
2. Grünhage F, **Lammert F**. Pathogenesis of gallstones: a genetic perspective**. Best Pract Res Clin Gastroenterol** 2006; 20 (6): 997-1015 [IF 2,1]
3. Wittenburg H, **Lammert F**. Genetic predisposition to gallbladder stones. **Semin Liver Dis** 2007; 27 (1): 109-121 [IF 3,8]
4. **Lammert F**, Miquel JF. Gallstone disease: from genes to evidence-based therapy. **J Hepatol** 2008; 48 (S1): S124-S135 [IF 7,8]
5. Höblinger A, **Lammert F**. Genetics of biliary tract diseases: new insights into gallstone disease and biliary tract cancers. **Curr Opin Gastroenterol** 2008; 24 (3): 363-371 [IF 3,0]
6. Krawczyk M, Müllenbach R, Weber S, Zimmer V, **Lammert F**. Genome-wide association studies and genetic risk assessment of liver diseases **Nat Rev Gastroenterol Hepatol** 2010; 7: 669-681 [IF 4,4]

Original Research

1 Katsika D, Grjibovski A, Einarsson C, **Lammert F**, Lichtenstein P, Marschall HU. Genetic and environmental influences on symptomatic gallstone disease: a Swedish study of 43141 twin pairs. **Hepatology** 2005; 41 (5): 1138-1143 [IF 11,4]

2. Grünhage F*, Acalovschi M*, Tirziu S, Walier M, Wienker TF, Ciocan A, Mosteanu O, Sauerbruch T, **Lammert F**. Increased gallstone risk in humans conferred by common variant of the hepatic ABC transporter for cholesterol. **Hepatology** 2007; 46 (3): 793-801 [IF 11,4]

3. Buch S, Schafmayer C, Völzke H, Becker C, Franke A, von Eller-Eberstein H, Kluck C, Bäßmann I, Brosch M, **Lammert F**, Miquel JF, Nervi F, Wittig M, Rosskopf D, Timm B, Höll C, Seeger M, ElSharawy A, Lu T, Egberts J, Fändrich F, Fölsch UR, Krawczak M, Schreiber S, Nürnberg P, Tepel J, Hampe J. A genome-wide association scan identifies the hepatic cholesterol transporter ABCG8 as a susceptibility factor for human gallstone disease. **Nat Genet** 2007; 39 (8): 995-999 [IF 25,6]

4. Schafmayer C, Völzke H, Buch S, Egberts J, Spille A, von Eberstein H, Franke A, Seeger M, Hinz S, ElSharawy A, Rosskopf D, Brosch M, Krawczak M, Fölsch UR, Schafmayer A, **Lammert F**, Schreiber S, Fändrich F, Hampe J, Tepel J. Investigation of the *Lith6* candidate genes *ABOBEC1* and *PPARG* in human symptomatic gallstone disease. **Liver Int** 2007; 27 (7): 910-919 [IF 2,6]

5. Zúñiga S, Molina H, Azocar L, Amigo L, Arrese M, Nervi F, **Lammert F**, Miquel JF. Ezetimibe prevents cholesterol gallstone formation in mice. **Liver Int** 2008; 28 (7): 935-947 [IF 2,6]

6. Kovacs P, Kress R, Rocha J, Kurtz U, Miquel JF, Nervi F, Méndez-Sánchez N, Uribe M, Bock HH, Schirin-Sokhan R, Stumvoll M, Mössner J, **Lammert F**, Wittenburg H. Variation of the gene encoding the nuclear bile salt receptor FXR and gallstone susceptibility in mice and humans. **J Hepatol** 2008; 48: 116-124 [IF 7,8]

7. Acalovschi M, Tirziu S, Chiorean E, Krawczyk M, Grünhage F, **Lammert F**. Common variants of *ABCB4* and *ABCB11* and plasma lipid levels: a study in sib pairs with gallstones and controls. **Lipids** 2009; 44 (6): 521-526 [IF 1,4]

8. Katsika D, Magnusson P, Krawczyk M, Grünhage F, Lichtenstein P, Einarsson C, **Lammert F**, Marschall HU. Gallstone disease in Swedish twins is associated to *ABCG8* D19H risk genotype. J Int Med 2010; epub [IF 5,4]

Guidelines

1. **Lammert F**, Neubrand MW, Bittner R, Feussner H, Greiner L, Hagenmüller F, Kiehne KH, Ludwig K, Neuhaus H, Paumgartner G, Riemann JF, Sauerbruch T. S3-guidelines for diagnosis and treatment of gallstones. German society for digestive and metabolic diseases and german society for surgery of the alimentary tract. **Z Gastroenterol** 2007; 45 (9): 971-1001 [IF 0,8]

2. Beuers U, Boberg KM, Chapman RW, Chazouillères O, Invernizzi P, Jones DE, **Lammert F**, Parès A, Trauner M. EASL Clinical Practice Guidelines: Management of cholestatic liver diseases. **J Hepatol** 2009; 51 (2): 237-267 [IF 7,0]

Have you prepared a systematic review before?	Yes ☒ No ☐
If yes, have you prepared a Cochrane review? (please state most recent title)	Yes ☐ No ☒
Are you already a member of another Cochrane Review Group? Which one(s)?	Yes ☒ No ☐
Cochrane Hepato-Biliary Review Group	
At what level are you able to speak and write English?	Equivalent to a native speaker

Roles and responsibilities

Please advise who has agreed to undertake each of the following tasks:

Draft the protocol	CS and FL
Develop and run the search strategy	CS and FL with input from the TSC
Obtain copies of studies	CS
Select which studies to include (2 people)	CS and FL
Extract data from studies (2 people)	CS and FL
Enter data into RevMan	CS
Carry out the analysis	CS
Interpret the analysis	CS and FL
Draft the final review	CS and FL
Update the review	CS and FL

Team resources

Have you read the *Cochrane Handbook for Systematic Reviews of Interventions*? (see www.cochrane.org/resources/handbook)	Yes ☒ No ☐
Do you require training?	Yes ☒ No ☐
If yes, on which topics? For author 1: Preparing a systematic review protocol; Formulating the methods section of the protocol; Understanding meta-analyses	
Have you attended a Cochrane review training workshop?	Yes ☐ No ☒
If no, do you plan to? (see www2.cochrane.org/news/workshops.htm)	Yes ☒ No ☐
Which workshop did you/will you attend? RA1 - *Beginning a systematic review protocol* (Feb 23 2011, UK) RA2 - *Methods section of the protocol* (Feb 24 2011, UK) The workshops in Germany are in the German language, therefore being a UK national, the UK Cochrane Centre has agreed for author 1 to attend these workshops at the protocol stage.	
Which computer operating system do you use?	Windows
Have you downloaded and installed RevMan, the Cochrane review software? (see www.cc-ims.net/RevMan)	Yes ☒ No ☐
Have you seen the Cochrane Hepato-Biliary Review Group website (see http://ctu.rh.dk/chbg)?	Yes ☒ No ☐
Do you have access to these electronic databases: *The Cochrane Library*	Yes ☒ No ☐
MEDLINE	Yes ☒ No ☐
EMBASE	Yes ☒ No ☐
Do you have access to a medical library?	Yes ☒ No ☐
If yes, can you order journal articles not held in the library?	Yes ☒ No ☐

Do you have access to advice from a medical librarian?	Yes ☒ No ☐
Do you have access to reference management software (e.g. Endnote)? If yes, which software, and what version? Endnote X4	Yes ☒ No ☐
Do you have access to a statistician? If yes, who? We have access to statistical/biometric consultancy from the Director PD Dr S Gräber of the Institute for Medical Biometry, Epidemiology and Medical Computer Science at Saarland University Hospital. www.uniklinikum-saarland.de/einrichtungen/fachrichtungen-theoretische-und-klinische-medizin/imbei/wissenschaftliche-kooperation/	Yes ☒ No ☐
Do you have contact with consumer groups relevant to this review? If yes, which one(s)? Patients with identified genetic and environmental risk factors for gallbladder stones; patients diagnosed with gallbladder stones	Yes ☒ No ☐
Have you identified appropriate time and resources to complete the review? The authors plan on seeking guidance from a meta analysis expert for this review.	Yes ☒ No ☐
Would you like to be assigned a mentor (an experienced author who has volunteered to help new authors)?	Yes ☒ No ☐

Acknowledgements

I am extremely grateful to my supervisor, Prof. Dr. Frank Lammert for providing me with the opportunity to dabble in the wonderful world of ‘gallstones’ and for his unwavering trust, and precious guidance throughout. It has been a real privilege. A special thank you to Dr. Lisa Lotte Gluud for her incredible support and inspiration - I am tremendously grateful. I would also like to warmly thank Sarah Klingenberg, the Cochrane Trial Search Co-ordinator, for carrying out the searches of the electronic databases so quickly and efficiently; to Christian Gluud and Dimitrinka Nikolova from the Cochrane Hepato-Biliary Group for their support and guidance. I would also like to express my gratitude to Nadine Godel for so diligently reviewing the titles from the search outcome with me; to Markus Casper for being my ally in the data extraction; to Silvia Zuniga and Marcin Krawczyk for their help in translating the Spanish and Italian trials and to Alexander Olbricht for coming to the rescue when my computer was crashing! I am also very grateful to my family – to my partner Dietrich for his patience, understanding and endless support and to my parents Lili and Trevor, my sister Debbie and my grandmother Mary for their endless support and encouragement throughout.

Lastly but certainly not least, I would like to thank all the authors of the studies and the pharmaceutical companies who kindly responded to my questions and requests for more information. I really appreciate you taking the time to reply to my burdensome emails!

Related Scientific Publications

Krawczyk M*, **Stokes CS***, Lammert F. Bile duct stones: new approaches to genetics and treatment. *Current Opinion in Gastroenterology* 2013;29:329-35 **Both authors contributed equally*

Krawczyk M, Miquel JF, **Stokes CS**, Hampe J, Balraj M, Lammert F. Genetics of biliary lithiasis from an ethnic perspective. *Clinics and Research in Hepatology and Gastroenterology* 2013;37:119-25

Stokes CS, Lammert F. Non-pharmacological and pharmacological interventions for primary prevention of gallbladder stones in adults (Protocol). *Cochrane Database of Systematic Reviews* 2012; Issue 6. Art No.: CD009918. DOI: 1002/14651858.CD009918

Stokes CS, Lammert F. Transporters in Cholelithiasis. *Biological Chemistry* 2012;393:3-10

Stokes CS, Krawczyk M, Lammert F. Gallstones: environment, lifestyle and genes. *Digestive Diseases* 2011;29:191-201

Curriculum Vitae

Education

2008-10 **PG DIP** (Postgrad. Degree) Clinical Nutrition/Dietetics, King's College London, UK
2001-03 **MMedSci** (Master of Medical Sciences) Human Nutrition, Sheffield University, UK
1996-99 **BSc (Hons)** (Bachelor of Science) Psychology, Southampton Solent University, UK
1992-96 **International Baccalaureate**, American Community Schools, Athens, Greece

Employment

Since 07/10 **Clinical Scientist,** Saarland University Medical Center, Homburg Germany

- **Doctoral studies**/PhD topic: Meta-analysis (Cochrane Review) of pharmacological and non-pharmacological interventions for primary prevention of gallbladder stones
- **Supervisor: Prof. Dr. med. Frank Lammert**, Department of Internal Medicine II
- Research on the role of vitamin D in chronic liver diseases
- Internal Medicine Teaching Coordinator for Saarland University Medical School

01/09-12/09 **Nutrition Consultant,** Pfizer SMA Nutrition, UK
01/08-06/08 **Nutrition Scientist,** British Nutrition Foundation, London, UK
01/06-12/07 **Research Scientist,** Medical Research Council, Human Nutrition Research, Cambridge, UK
12/05-12/06 **Director,** Fareshare South Yorkshire, Barnsley, UK
02/04-12/06 **Research Nutritionist,** Doncaster and South Humber National Health Service, Sheffield, UK

Awards

2012 Saarland University Excellence Programme for scientists striving to be Professors
2010 Studienstiftung Saar – PhD Stipend
2010 Van Den Berghs-Prize for most distinguished graduate on clinical nutrition course
2007 'Rising Stars' Programme in public communication, Cambridge University
2005 National Health Service (NHS) Journal Award
2005 Certificate of Commendation from NHS Trust Chief Executive