Gallbladder bile supersaturated with cholesterol in gallstone patients preferentially develops from shortage of bile acids

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Running title: Gallbladder bile acids are deficient in gallstone disease

Abbreviations: BA, bile acid; GSD, cholesterol gallstone disease; CSI, cholesterol saturation index; GB, gallbladder; GS, gallstone; GSF, gallstone free; PLs, phospholipids;

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Abstract
Gallstone formation requires that bile is supersaturated with cholesterol, estimated by a cholesterol saturation index (CSI) calculated from gallbladder total lipids and the mol % bile acids, cholesterol and phospholipids. Whereas CSI indicates gallstone risk, we hypothesized that additional comparisons of gallbladder lipid mol % data are inappropriate to identify why CSI is increased in gallstone disease. We anticipated gallbladder lipid mmol/L levels should instead identify that and therefore retrieved gallbladder mmol/L data for bile acids, cholesterol and phospholipids from a study on 145 gallstone and 87 gallstone-free patients and compared them with the corresponding mol % data. Bile acid and phospholipid mmol/L levels were 33 and 31% lower in gallstone patients, while cholesterol was unaltered. CSI was higher in gallstone patients and correlated inversely with gallbladder levels of bile acids and phospholipids but not with cholesterol. Literature search confirmed in 13 studies from 11 countries, that gallbladder bile acid levels, and to a certain extent phospholipids, are strongly reduced in gallstone patients while cholesterol levels are not elevated.

Our findings show that shortage of bile acids is a major reason why gallbladder bile is supersaturated with cholesterol in gallstone patients. These results are sustainable since they are also valid from a global perspective.

Key words: Bile acid metabolism, Bile physical chemistry/Gallstone formation
Cholesterol metabolism, Gallstone formation, Obesity
Introduction

Cholesterol gallstone disease (GSD) is a common multifactorial gastrointestinal condition. A prerequisite for gallstone (GS) development is that gallbladder (GB) bile is supersaturated with cholesterol (1-3). A common way to monitor this is from a cholesterol saturation index (CSI), calculated from the mol% values of the 3 major lipids in GB bile; bile acids (BAs), cholesterol and phospholipids (PLs) together with the total lipids (4). CSI is an established indicator of GSD risk. However, a high CSI value does not tell if increased cholesterol or reduced BAs and/or reduced PLs actual levels in GB bile is causing it, a crucial question for identification of the major most common cause for supersaturated bile. Nevertheless, a frequent view is that increased CSI in GB bile is primarily due to hepatic hypersecretion of cholesterol into bile (5-10). In studies on lipid levels in GB bile authors often compare mol% data of GB lipids from patients with and without gallstones. From such comparisons authors frequently conclude that the mol% cholesterol is increased in GB bile from patients with GSD, a finding often considered to support that hypersecretion of cholesterol is the primary and major cause for why GB bile from patients with GSD is supersaturated with cholesterol (2, 9, 11-14). We hypothesized that such comparisons of relative mol% data of GB lipids (12, 13, 15, 16) do not provide information as to whether cholesterol supersaturation results from excess hepatic secretion of biliary cholesterol, decreased biliary secretion of bile acids or phospholipids, or a combination of both. We reasoned that straightforward mmol/L levels of GB bile lipids should instead provide more relevant direct information to answer this question.

To investigate this, we aimed to bring forward the mmol/L levels of GB lipids in GS and gallstone free (GSF) patients to compare the results with those obtained using the corresponding mol% values. This was feasible from the option of getting access to data from a previously published report comprising 145 patients with GSD and 87 GSF patients, one of the largest patient materials published where pure GB bile, collected at surgery, was analyzed (12). In that article the relative mol% for cholesterol, PLs and BAs in GB bile were reported. We here expand that report with previously unpublished individual data on the mmol/L levels of GB lipids, also allowing for the evaluation of the correlations between GB bile CSI and the mmol/L levels of GB BAs, cholesterol and PLs respectively. Finally, we
compared our results with those from 13 published studies where GB lipids were reported for GS and GSF patients from 11 countries, putting our results into a global perspective.

We show that in GSD globally, the mmol/L levels of BAs in GB bile are strongly reduced while cholesterol is not increased. We conclude that the major cause for increased CSI in GSD is reduced BAs in GB bile.
Materials and methods

Collection of samples: GB bile were consecutively collected from gallbladders of patients subjected to elective cholecystectomy between 1981 and 1998, as described in detail including basal clinical data (12). The study was approved by The ethical committee at Karolinska Institutet and informed consent was obtained from all patients. There were 145 patients with cholesterol gallstones (111 women, 34 men) and 87 GSF (73 women, 14 males). The GSF patients were cholecystectomized due to polyps, adenomyomatosis or because of recurrent symptoms suggesting GB dysfunction. BMI of GSD patients was significantly higher (P< 0.05) than for controls (24.8±0.3 versus 23.5±0.4 kg/m²). GB bile samples were analyzed for cholesterol, total BAs, PLs and total lipids. Individual BAs were analyzed with gas-chromatography after alkaline hydrolysis as described.(12) Cholesterol saturation of GB bile was calculated as described by Carey (4). GB lipids and mol% data of GB BAs, PLs and cholesterol were determined as described (12).

Data were also retrieved from 13 reference’s tabular data and graphs (17). All sorts used in the original publications were kept. In two reports (18) and (15) BAs, cholesterol and PLs in GBs were only available in mol% together with total lipid levels in mg/dL. In those studies, we calculated the corresponding levels in mmol/L assuming the molecular weights of BAs, cholesterol and PLs being 491, 387 and 775 respectively. All data presented are means and SD. In one study referred to (19) (Fig. 2, Panel D1) only mean data was available for GB mol% data. Differences between groups were determined by unpaired student t-test using Graphpad Prism 7 version 7.03.
Results

We first compared GB lipids in GS and GSF patients shown in mol% (Fig. 1, Panel A1) versus that in mmol/L (Fig. 1, Panel A2). The mol% BAs were slightly but significantly (P=0.002) lower in GB bile from GS patients (68.7%±0.50) compared to GSF patients (71.2%±0.63), while the mol% cholesterol was higher (7.8% vs 5.5%, P< 0.0001) in GS patients while PLs were unaltered. When the corresponding mmol/L data was examined, BAs and PLs were 33% and 31% lower respectively (both P<0.0001) in GB bile from GS patients, while GB cholesterol was unaltered in GS patients (P= 0.265). CSI% was 52% higher (P<0.0001) in GS patients (113±3.85) compared to GSF patients (74.6±2.55) (Mean±SEM). Since supersaturated bile is a prerequisite for the precipitation of cholesterol gallstones (GSs) and precedes GS formation (10), we next investigated the correlation between CSI of GB bile and GB mmol/L levels of cholesterol in the individual patients (Fig. 1, Panel A3). There was no correlation (P=0.542). In contrast, a correlation was found between the CSI of GB bile and the GB mmol/L level of BAs (P<0.0001) (Fig. 1, Panel A4). A strong correlation (P<0.0001) was also present between GB CSI values and PL mmol/L levels (Fig. 1, Panel A5).

Comparisons of present results with those from 13 published reports

We next compared our findings with those from two studies on GB lipids in subjects with and without GSD where individual data on GB lipids were reported. First, in a Swedish study by Ahlberg et al. (18) the individual mol% of GB BAs, cholesterol and PLs were reported together with total GB lipids and CSI (Fig. 1, Panels B). There were 10 GSF controls (8 women and 2 men) and 12 GS patients (9 women and 3 men). Since mmol/L data were not available, we calculated them since total GB lipids were given. When data were expressed in mol%, GB BAs relative values were on borderline significance (P= 0.056) for a slight reduction in GS patients (64.5% vs 69.4% in GSF) (Fig. 1, Panel B1). PLs were unchanged while GB cholesterol relative values were increased in GSD (10.2% vs 6.8%) (P = 0.005). When data were shown in mmol/L, BA levels were 38% lower in GB bile from GS subjects (P= 0.027) while cholesterol levels were unaltered (P = 0.968) (Fig. 1, Panel B2). PL levels were 27% lower in GS patients but nonsignificant. The calculated CSI% was 52% higher (P= 0.004) in
GS patients (138±11.11) compared to GSF (91.1±8.25) (Mean±SEM). We next evaluated the correlations between GB CSI and mmol/L levels of GB cholesterol and found, in line with the above results, no correlation (P= 0.823) (Fig. 1, Panel B3). However, also in this study there were strong correlations between CSI and GB mmol/L levels of BAs (P= 0.0007) (Fig. 1, Panel B4) but not between CSI and GB mmol/L of PLs (P=0.111, Fig. 1, Panel B5).

Second, in a study from New Zealand by Pattinson (20), individual GB BAs, cholesterol, and PLs in both mol% and mmol/L levels and CSI values were reported for 31 cholecystectomized GS patients and 10 GSF controls (Fig. 1, Panels C). GB lipids in mol% showed no differences for any of the lipids (Fig. 1, Panel C1). When expressed in mmol/L BAs were 19% lower in GS patients, on borderline significance (P= 0.070), while cholesterol and PLs were 16 (P= 0.202) and 23% lower (P= 0.102) respectively in GB bile from GS patients (Fig. 1, Panel C2). In this study CSI was not significantly different between GS (1.4±0.06) and GSF (1.2±0.10) (Mean±SEM) subjects presumably due to the fact that 8 of the 10 control subjects had supersaturated bile. Importantly, there was no correlation between CSI and mmol/L levels of cholesterol (P= 0.910) (Fig. 1, Panel C3). However, there was a clear correlation between CSI and the mmol/L levels of GB BAs (P= 0.002) and between CSI and mmol/L of PLs (P= 0.001) (Fig. 1, Panels C4 and C5).

We next analyzed five studies where mean mol% and mmol/L levels of GB lipids were available. First, in a study by Miquel et al.(15) on 52 Chilean GS patients and 40 GSF subjects, the mol% data were reported along with total GB lipids. When those data were presented in mol%, (Fig. 2, Panel A1) GB cholesterol values were 18% higher (P=0.013) in patients with GSD (7.2 mol% vs 6.1 mol%) while BAs and PLs were unaltered as compared to GSF subjects. When we calculated the corresponding mmol/L levels from these data, GB BAs and PLs in subjects with GSD were then both significantly (P<0.0001) reduced by 29 and 28% respectively (Fig. 2, Panel A2). Interestingly, GB mmol/L of cholesterol was now 15% lower (P=0.012) in GB bile from GS patients compared to GSF patients (Fig. 2, Panel A2), results similar to the three above studies. CSI% in the GS patients (130±5) was 19% higher (P=0.011) than in GSF subjects (109±7) (Mean±SEM).
Second was a study by Ho et al. (21) from Taiwan (Fig. 2, Panels B). The composition of GB bile from 10 controls and 4 patients with mixed stones was reported. When shown in mol% (Fig. 2, Panel B1) GB BAs were lower (P=0.014) in GS patients than in GSF individuals. PLs were unaltered but the mol% cholesterol was doubled (P<0.0001). When results were shown in mmol/L (Fig. 2, Panel B2), BAs were 53% lower (P=0.015) in GB bile from GS patients and PLs were 45% lower in GB bile from GS patients but not significant. Cholesterol was unaltered. CSI% was 91% higher (P=0.0005) in GS patients (201±23) than in GSF subjects (105.1±23) (Mean±SD).

Third, in a Swedish study by Cahlin et al.(22) (Fig. 2, Panels C1 and C2) GB bile mol% data were evaluated. There were no differences for any of the three major GB lipids but a 35% increase in cholesterol in GB bile on borderline significance (P=0.061). However, when the mmol/L levels were examined GB BAs were 32% lower (P=0.005) in the GS group. PLs (lecithin) were 30% lower (P=0.043) while cholesterol was now 24% lower in the GS group, although not significant. There were no CSI data in this study.

Fourth, we retrieved a study by Sadaruddin et al.(19) (Fig. 2, Panels D1 and D2) on 23 Pakistan patients with mixed stones, and 6 controls. Only GB lipid mean values without variations were reported for the mol% data. Nevertheless, the mean mmol/L data for GB lipids were in line with the above reports. Thus, GB mmol/L of BAs were reduced by 50% (P=0.036) in the GS group and PLs by 29% (P=0.0007) while mmol/L levels of GB cholesterol were unaltered. There were no CSI data in this study.

Fifth, was an American report by Chuang et al.(23) (Fig. 2, Panels E1 and E2) where GB levels in mol% and mmol/L and CSI were reported from a study on 42 GSF subjects (14 men/28 women) and 11 GS patients (2 men/9 women). The mol% for BAs were 17% lower in the GS patients (P = 0.009) and cholesterol was 133% higher (P=0.004) while PLs did not differ between GS and GSF subjects. However, when presented in mmol/L, GB BAs in GS patients were 42% lower (P=0.012) while cholesterol and PLs were unaltered. The CSI of GB bile was increased by 82% in GS patients (3±3 vs 1.7±0.56) (Mean±SD) (P=0.002).
We finally analyzed six studies where only mmol/L concentrations of GB lipids were reported. The first, a German report by Schentke et al.(24) (Fig. 2F) on 18 GS and 10 GSF patients. The mmol/L levels of GB BAs were 36% lower (P<0.010) in GS patients while GB bile cholesterol did not differ between the two groups. PLs were 34% lower in the GS group but nonsignificant (P=0.057). There was no CSI data. The second was a Danish study by Dam et al.(25) (Fig. 2G) on 26 GS and 27 GSF subjects. GB bile from GS patients had 47% lower mmol/L BA levels (P<0.0001), 36% lower cholesterol levels (P=0.003) and 32% lower PL levels (P=0.001) than bile from GSF subjects. There were no CSI data. The third study by Halpern et al.(26) (Fig. 2H) was from USA on 6 GS patients and 3 GSF controls. Despite being a small study, GS patient’s GB mmol/L levels of BAs were 48% lower (P=0.018), cholesterol and PLs were unaltered although the latter showed a nonsignificant 41% decrease as compared to GSF patients. The CSI of GB bile was 41% higher but nonsignificant (P=0.074) in the GS patients (1.5±0.14 vs 1.1±0.02) (Mean±SEM). The fourth was a Japanese study by Hirota et al.(27) (Fig. 2I) on 16 GS and 9 GSF controls. GB bile from GS patients had 33% lower mmol/L BA levels (P=0.007) while cholesterol and PLs were not altered. The CSI of GB bile was higher (P=0.0007) in the GS patients (1.1±0.26 vs 0.7±0.14) (Mean±SD). The fifth was an Australian study by Whiting et al.(28) (Fig. 2J) where 18 GS patients and 14 GSF controls were studied. GB BAs, cholesterol and PLs were all highly significantly reduced in the GS patients by 39, 34 and 43% respectively. There was no CSI data. The sixth was an English study by Jazrawi, et al.(17) (Fig. 2K) where 45 GS and 19 GSF patients were reported. As compared to GSF patients, GB bile from GS patients showed 46% reduced mmol/5mL levels of BAs (P=0.0003) while the levels of cholesterol and PLs were unaltered. CSI of GB bile from GS patients was 75% higher in GS patients (1.7±0.1 vs 0.97±0.06) (Mean±SEM) (P<0.0001).

Finally, for a robust overview of the results from the 13 studies referred to and our current data in Fig. 1A, we presented them all in Table 1.
Discussion

Much effort has been made to understand the prerequisites for cholesterol GS formation in the GB. Important landmarks are that GB bile must be supersaturated with cholesterol for gallstone precipitation (3), and that this precedes gallstone formation (3, 29-31). However, a high CSI value does not answer the question whether high cholesterol and/or reduced BAs and/or PLs in the GB is causing it. We brought forward the mmol/L levels of GB bile lipids for a straightforward comparison to the respective mol% levels. To the best of our knowledge, such a comparison on a substantial number of patients has not been previously reported. We retrieved unpublished mmol/L data from a previous study (12), and compared our results with those from 13 published studies on GB lipids in GS and GSF patients. We made four sets of important conclusions, all of global validity:

1) GB BAs are strongly reduced in GSD. The mmol/L levels of GB BAs and PLs were reduced by 33 and 31% respectively in GS patients as compared to GSF subjects (Fig. 1, Panel A2, Table 1). In line with these findings, 19 to 53% reduced GB mmol/L BA levels presented in patients with GSD in 12 of the 13 referred studies from 11 different countries including Australia (28), Chile (15), China (21), Denmark (25), England (17), Germany (24), Japan (27), Pakistan (19), Sweden (12, 22), and USA (23, 26). GB mmol/L PLs were significantly reduced in 5 of the 13 referred studies (Table 1), while in the remaining 8 studies PLs were unaltered in GS patients.

2) GB bile cholesterol levels are not elevated in GSD. GB cholesterol mmol/L levels in GS patients in the present study were not increased when compared to GSF patients (Table 1). In line with this, GB mmol/L cholesterol levels were not increased in any of the 13 studies referred to; in three studies GB bile cholesterol was instead significantly reduced in GS patients as compared to GSF patients. These results, from measuring GB bile lipid mmol/L levels, are difficult to fit with the thinking that increased secretion of cholesterol into bile is the primary and major cause for why CSI is increased in gallbladder bile in GSD. This is because if cholesterol is secreted into bile at increased rates in GSD this should presumably serve to increase the mmol/L levels of cholesterol in GB bile, which was not observed in any of the 14 studies.
3) GB lipids mmol/L levels disclose a different picture than those from mol% data. When displayed in mol%, of the seven studies where mol% data and variations were available, mol% cholesterol was significantly increased in five (Table 1) and on borderline significance for an increase in one study (Table 1, Cahlin). However, when shown in mmol/L these six studies did not reveal any increases in GB bile cholesterol in GS patients, in one study GB cholesterol was instead reduced (Table 1, Miquel et al., P=0.012). Further, in these six studies, mmol/L levels of BAs were significantly reduced by 29 to 53% (Table 1). Apparently, and in line with our hypothesis, comparing relative mol% data of biliary lipids (12, 13, 15, 16) results in severe misconceptions when to identify the major reason/s for why GB bile is supersaturated with cholesterol in GSD.

4) Supersaturated GB bile in patients with GSD is chiefly due to BA deficiency that precedes GS formation. The increased CSI of GB bile from GS patients was predominantly due to reduced BAs in GB bile (13 of 14 studies, Table 1) and to a certain extent to reduced PLs (6 of 14 studies), the latter in line with that the secretion of BAs modulates the secretion of PLs (32, 33). GB cholesterol levels had apparently a limited role for the increased CSI observed in the GS patients. Since it is well established that GB bile must be supersaturated with cholesterol and that this presents prior to GS formation (3, 29-31) our results imply that reduced mmol/L levels of GB BAs and PLs also present prior to GS formation. Overall, our findings and those from the 13 reports referred to consistently show that the major reason for why CSI is increased in GS patients is that the actual mmol/L levels of GB BAs are reduced 19 to 53%. This is also in line with reports showing 30-50% reduced total BA pool sizes in GSD (29, 34-38). The lack of a major role of GB cholesterol mmol/L levels for elevated CSI in patients with GSD was further supported by the lack of correlation between CSI and mmol/L levels of GB bile cholesterol in the present study (Fig. 1, Panel A3) as also seen in the reports by Ahlberg et al. (18) (Fig. 1, Panel B3) and by Pattinson et al (20) (Fig. 1, Panel C3). Instead, there were strong negative correlations between CSI and BAs and PLs separately (Fig. 1, Panel A4,5), confirmed in the reports by Ahlberg et al. (18) and by Pattinson et al. (20). Indeed, all these reports (12, 15, 17-29, 34-37) show that GB BAs are deficient in GSD, evident when the actual mmol/L levels are considered, which does not align well with the view that the initial and major step in the generation of supersaturated bile is
increased GB cholesterol due to elevated hepatic secretion of cholesterol fueled by hepatic overproduction of cholesterol (6, 8-10). The latter view is frequently linked to parallel comparisons of mol% data of GB bile lipids (12, 13, 15, 18). Surely, cholesterol secretion is increased in certain groups of patients with GSD. This is supported by reports on cholesterol secretion in GSD showing divergent results, from no differences in biliary lipid secretion (39) or reduced BA secretion with no changes in cholesterol secretion (40-42) to increased cholesterol secretion together with reduced secretion of BAs in GSD (43). When the latter report is cited for demonstrating increased cholesterol secretion in GSD, it seems as it has been overlooked that this frequently cited study (104 citations) also contains data showing strong reductions in the secretion rates for BAs in GSD. 8 GS women were compared to 14 GSF. Cholesterol secretion was 43% higher in GS women (0.77 vs 0.54 mg/h/kg). At the same time, BA secretion was reduced by 51% in GS patients 10.2 vs 20.9 mg/h/kg. Thus, reports with cholesterol secretion data reveal results in line with our present findings of a major deficiency of BAs in GSD.

Why then are BAs deficient in GB bile in patients with GSD? This is an important question to answer in future studies. We can only speculate on this based on published reports. BA deficiency can develop from reduced BA production or from a higher intestinal loss of BAs. Both ways will themselves lead to a lower return of BAs from gut to liver at steady state. In the search for an anticipated reduced production of BAs in GSD it was unexpectedly found that BA synthesis in 41 patients with GSD was 40% higher than in 72 GSF controls (44). Similar results have been published by Sauter et al.(45, 46); from investigation of 106 GSF (46) and 51 GS patients (45); BA synthesis was 31% higher in GS patients. Further, in a study on 165 Chilean women (47) BA synthesis was 37% higher in women with GSD as compared to GSF women. These 4 studies on 435 subjects from Sweden, Germany and Chile consistently show that BA synthesis is significantly induced by 31 to 40% in patients with GSD. Thus, reduced BA synthesis is not likely to explain why BAs are deficient in GB bile from GS patients. The remaining possibility then seems to be that the major primary event should be an enhanced fecal loss of BAs as has indeed been reported for GSD (48). This should lower the total BA pool (29, 34-38) leading to a compensatory induced BA synthesis consuming liver cholesterol which will induce
cholesterol synthesis, a finding reported for patients with GSD (47, 49). A particular situation when fecal loss of BAs is increased is bile acid diarrhea – a condition reported to be strongly linked to increased risk for GSD (50). Thus, considering above mentioned findings, GSD may be a disease of intestinal origin to a larger extent than previously anticipated. Possible causes for elevated fecal losses of BAs may be genetic and/or due to environmental factors. Interestingly recent genetic studies have identified that loss of function mutations in the SLC10A2 gene, encoding for the apical sodium dependent bile acid transporter (ASBT), demonstrates that reduced BA transport by ASBT is linked to GSD (51, 52). Regarding environmental factors, one overlooked such is the level of intake of food mass (2, 53). Apparently, while reduced food intake reduces both fecal loss of BAs (2, 53) and BA production (54) increased food intake increases the loss of fecal BAs (2, 53) as well as BA production (54). Since overeating is a basal prerequisite for increased BMI, a very strong risk factor for GSD (55), it is of particular interest to note that BMI has been identified as a causal factor for GSD in a Mendelian randomization study (52). Evidently, the reason(s) why BMI associates so strongly to GSD certainly warrants further study.

Strengths and weaknesses of the study. Since bile was operatively obtained at cholecystectomy in the current study, as well as in the 13 studies referred to, this eliminated uncertainties whether gallstones were present or not. Another strength of our study is the unusual large number of patients (n=232). The GB bile mmol/L levels of cholesterol in GB bile from GSD patients were neither elevated in our study nor in any of the 13 studies referred to. It may be speculated that this could in part be due to a hampered ability to concentrate bile in gallbladders from GS patients. Such a possibility does however not explain why there were no correlations between CSI and GB bile cholesterol mmol/L levels in contrast to the strong correlations seen between CSI and BA mmol/L levels in the three studies where individual data were available (Fig. 1, Panel A4, B4 and C4).
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| AUTHOR                  | No  | % change of mol  | % values in GS versus GSF subjects | % change of mmol/L levels in GS versus GSF subjects | % increase in GS versus GSF subjects |
|------------------------|-----|------------------|-----------------------------------|---------------------------------------------------|-------------------------------------|
| GUSTAFSSON (PRESENT STUDY) | 232 | 43% <0.0001      | -4% 0.002                         | -7% 0.265 <0.0001                                   | 52% <0.0001                         |
| AHLBERG                 | 22  | 50% 0.005        | -7% 0.056                         | 1% 0.968 <0.0001                                   | 52% 0.004                           |
| PATTINSON               | 41  | 6% 0.506         | 1% 0.833                         | -16% 0.202 <0.0001                                  | 16% 0.150                           |
| MIQUEL                  | 92  | 18% 0.013        | -2% 0.147                         | -15% 0.012 <0.0001                                  | 19% 0.011                           |
| HO                      | 14  | 98% <0.0001      | -8% 0.014                         | 0% >0.999 <0.0001                                  | 91% 0.0005                          |
| CAHILIN                 | 27  | 35% 0.061        | -6% 0.172                         | -24% 0.241 <0.0001                                  | no data                             |
| SADARUDDIN              | 29  | 135% mean        | -26% mean                         | 12% 0.514 0.003                                    | no data                             |
| CHUANG                  | 53  | 133% 0.004       | -17% 0.009                         | 13% 0.540 0.012                                    | 82% 0.002                           |
| SCANTNELKE              | 28  | -                | -                                 | -15% 0.501 0.003                                   | no data                             |
| DAM                     | 53  | -                | -                                 | -36% 0.003 0.000                                   | no data                             |
| HALPERN                 | 9   | -                | -                                 | -26% 0.365 0.018                                   | 41% 0.074                           |
| HIROTA                  | 25  | -                | -                                 | 3% 0.840 -33%                                      | 50% 0.0007                          |
| WHITING                 | 32  | -                | -                                 | -34% 0.003 -39%                                    | no data                             |
| JAZRAWI                 | 64  | -                | -                                 | 27% 0.450 0.003                                    | 75% <0.0001                         |

Table 1. Gallbladder lipids in mol% bring focus on cholesterol in GSD while cholesterol mmol/L levels in gallbladder bile are never increased in patients with GSD. Overview of differences in lipids in gallbladder bile from patients with GSD compared to the respective data from GSF patients in the present study (Gustafsson), and in 13 other reports indicated by first author. Left section, % changes of mol % values for cholesterol (Chol), BAs and PLs in gallbladder bile from patients with GSD in relation to those for GSF patients. The rightmost section likewise shows the % changes of the mmol/L levels of Chol, BAs and PLs between GS and GSF patients. Red boxes, significant increases; blue boxes, significant reductions. P values are from unpaired student t-test. In five studies, there were no CSI data. The percentage increases of CSI in GS patients versus GSF are indicated in the 9 studies where CSI data were available.
Figure 1. GB bile lipids in the present study, Gustafsson (12); and in 2 studies referred to, Ahlberg (18), and Pattinson (20) where individual data were available. (A1,B1,C1), presented as mol%: (A2,B2,C2), shown as mmol/L; (A3,B3,C3), correlations between CSI and total GB bile cholesterol; (A4,B4,C4), correlations between CSI and total GB bile BAs; (A5,B5,C5), correlations between CSI and total PLs. Means and SD are shown with P values (* <0.05, ** <0.01; *** <0.001; **** <0.0001) from unpaired student t-test between GS and GSF patients. \( r^2 \) = Pearson's correlation coefficient. Patient numbers are found in Table and Results.
Figure 2. GB bile lipids in 11 studies. (Panels A-E), GB bile lipids from 5 studies on GS and GSF patients where mol% and mmol/L levels were reported. Results are presented row-wise and indicated by first author name and reference. (Panels F-K), GB bile lipids from 6 studies on GS and GSF patients where only mmol/L levels were reported. Studies are indicated by first author name and reference. See Table 1 for number of patients. The P values (*<0.05, **<0.01; ***<0.001; ****<0.0001) from unpaired student t-test between GS and GSF subjects are indicated.