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# Postprandial Gallbladder Motor Function: Refilling and Turnover of Bile in Health and in Cholelithiasis

RIADH P. JAZRAWI,* PAOLO PAZZI,*,† M. LETIZIA PETRONI,* NAPOLEONE PRANDINI,† CATHERINE PAUL,* JANE A. ADAM,* SERGIO GULLINI,† and TIM C. NORTHFIELD*

*Departments of Medicine and Radiology, St. George's Hospital Medical School, London, England; and †Ospedale S. Anna, Ferrara, Italy

***Background & Aims:*** **Impaired gallbladder emptying is implicated in gallstone disease. Ultrasonography and scintigraphy have shown conflicting results because the former is influenced by postprandial refilling, whereas the latter is not influenced by refilling. The aim of this study was to measure postprandial refilling and turnover of bile by combining the two techniques.** ***Methods:*** **Simultaneous scintigraphy and ultrasonography were used in 14 patients with gallstones and 11 healthy controls. Measurements were performed while the patients were fasting and at 10-minute intervals after a standard meal for 90 minutes, and the measurements were used to calculate postprandial refilling, turnover of bile (in milliliters), and turnover index.** ***Results:*** **Ultrasonography and scintigraphy provided different gallbladder emptying patterns. Compared with controls, patients with gallstones had impaired emptying by both scintigraphy ($P < 0.0001$) and ultrasonography ($P < 0.01$). Postprandial refilling and turnover were both reduced between 60 and 90 minutes ($P < 0.05$), and the turnover index was markedly reduced (1.8 vs. 3.5; $P < 0.001$).** ***Conclusions:*** **Simultaneous scintigraphy and ultrasonography provide a new model of gallbladder motor function showing that refilling begins immediately postprandially. In healthy controls, the gallbladder postprandially handles up to six times its basal volume within a period of 90 minutes, but this turnover of bile is markedly reduced in cholelithiasis causing a reduced washout effect of the gallbladder contents, including cholesterol crystals.**

The concept that impaired gallbladder motor function may be important in the pathogenesis of gallstones is more than a century old.[1–3] The recent cholesterol nucleation theory[4] provides a rationale that the stasis of gallbladder bile plays an important role in the development of gallstones by providing the time necessary for the precipitation of cholesterol crystals.

Impaired gallbladder emptying has been implicated in gallstone formation in patients during pregnancy,[5,6] in patients administered female sex hormones,[6,7] and in patients after receiving parenteral nutrition therapy.[8,9] The converse is also true because patients with gallstones have abnormal gallbladder emptying.[10–19] Although these latter studies cannot distinguish whether abnormal gallbladder emptying is caused by the presence of gallstones or vice versa, there are several reports of humans and of animals that propose that abnormal gallbladder emptying precedes the development of gallstones.[17,18,20–25] These reports have been based on the fact that, in animals,[21,23–25] gallbladder muscle contractility and gallbladder emptying are impaired before and during gallstone formation and that, in humans, the defect in gallbladder motor function persists after complete gallstone dissolution by bile acids[17] or by extracorporeal shock wave lithotripsy.[18]

Previous studies of gallbladder motor function in patients with gallstones have yielded conflicting results. Increased,[10,19,26] normal,[27] and impaired gallbladder emptying have all been described previously.[12–18,28] These conflicting results cannot be explained only on the basis of the different techniques used to assess gallbladder emptying (i.e., cholecystography, ultrasonography, and cholescintigraphy) because three studies using ultrasonography[19,27,13] have reported increased, normal, or impaired gallbladder emptying in patients with gallstones, respectively. With regard to scintigraphic studies, although the majority of studies have reported impairment in gallbladder emptying,[13–16] some have reported normal gallbladder function in at least a subgroup of patients,[14,16] and at least one study has reported increased emptying in patients with gallstones.[29]

We believe that there are two main problems responsible for these contrasting results: a conceptual problem and a methodological problem. The conceptual problem relates to the way in which the gallbladder handles bile in response to a meal or other stimuli. Traditionally, it

***Abbreviations used in this paper:*** **GB $Count_n$, postprandial gallbladder count at different 10-minute intervals; GB $Volume_n$, postprandial gallbladder volume at different 10-minute intervals; $GE_{GC}$, gallbladder emptying by gamma camera; $GE_{US}$, gallbladder emptying by ultrasonography.**



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has been assumed that the gallbladder empties in a steady, progressive fashion postprandially and refills between meals. This assumption was originally challenged by studies using dual isotope scintigraphy[30] that showed that both emptying and refilling occurred simultaneously during the postprandial period. This observation was later confirmed by measuring simultaneous postprandial emptying and refilling, using a combination of duodenal perfusion and scintigraphy.[31] The observation of intermittent postprandial emptying and refilling has also been reported in the prairie dog using scintigraphy[25] and in humans using minute-by-minute ultrasonography.[26] However, none of the previous studies of gallbladder motor function in gallstone disease has been designed to enable assessment of both emptying and refilling because neither ultrasonography nor scintigraphy is capable of assessing both events simultaneously when used alone.

The methodological problem relates to the incorrect assumption that both ultrasonography and scintigraphy measure similar gallbladder functions (e.g., emptying). In fact, ultrasonography measures changes in gallbladder volume that are dependent on refilling, whereas scintigraphy measures the reduction in total gallbladder isotope counts that is independent of refilling. This methodological difference has been highlighted recently in a letter to the editor of *Gut* by Krishnamurthy[32] who used only scintigraphy in assessing gallbladder motor function, disputing the results of other investigators[26] who used ultrasonographic techniques.

Postprandial gallbladder refilling must be considered when evaluating gallbladder motor function, especially in the context of gallstone pathogenesis. Rapid alternations in emptying and refilling and the consequent increase in the turnover of bile within the gallbladder are likely to provide a washout effect that is more important in eliminating cholesterol crystals than gallbladder emptying alone.

We reasoned that, by using ultrasonography and scintigraphy simultaneously to measure gallbladder emptying, the difference in their measurements would provide an index of postprandial gallbladder refilling; therefore, this latter measurement would allow a calculation of the turnover of bile within the gallbladder. Our aims were to measure the turnover of bile within the gallbladder in healthy patients and to test the hypothesis that there is a slower turnover rate of bile within the gallbladder in patients with gallstone disease compared with normal patients, indicating a reduced washout effect and, hence, stasis within the gallbladder.

## Materials and Methods

### Patients

Fourteen patients with gallstone disease and 11 healthy volunteers were studied. In both groups, the patients were women of similar age and body mass index. The studies were performed in the first 10 days of the menstrual cycle in premenopausal women. The patients with gallstones were studied after complete dissolution of their stones, and at the time of the studies, 6 patients had had gallstone recurrence, whereas 8 patients were still gallstone free. The recurrent stones in all the patients were small and occupied <10% of the gallbladder volume. None of the patients with gallstones was taking bile acids or any other medication. The study was approved by the District Hospital Ethical Committee, and all patients gave written informed consent to participate in the study.

### Experimental Procedure

After an overnight fast, each patient was administered an intravenous bolus injection of 1 mCi (37 MBq) $^{99m}$Tc iminodiacetic acid. Measurements were obtained between 90 and 120 minutes after the injection, which was the time necessary for more than 95% of the $\gamma$-labeled isotope to clear the liver.[33] To assess the reproducibility of the two techniques, two baseline ultrasonography and gamma camera scans were performed 5 minutes apart. The patients were then asked to ingest, within 10 minutes, a semisolid meal (370 kcal; 55% fat, 25% carbohydrates, and 20% proteins) consisting of two slices of toasted bread, 15 g of butter, 10 g of a commercially available amino acid preparation (Casilan 90; Crooks, Nottingham, England), and 200 mL of whole milk. Time 0 was defined as the time immediately after meal ingestion. Postprandially, gallbladder ultrasonography followed immediately by a gamma camera scan were performed with the patient lying in the same position and were repeated at 10-minute intervals for a period of 90 minutes.

Ultrasound was performed using a real time machine with a linear 3.5 MHz transducer (EUB; Hitachi, Uchi Kanda, Japan). Longitudinal and transverse scans of the gallbladder were obtained, always after deep inspiration. Abdominal markers were used to standardize as much as possible the position from which the gallbladder views were obtained. The largest diameters of the gallbladder (axial length, width, and height) were recorded during ultrasonography by using a distance caliper and were photographed using an attached Polaroid camera (Mitsubishi, Tokyo, Japan). Gallbladder volume was calculated using the ellipsoid method.[34] The average scan time was 70 seconds.

A gamma camera with a high-resolution collimator interfaced with a microcomputer system was used (Sigma 410; Technicare, Cleveland, OH). Anterior and posterior scans were performed, and the geometric mean of the number of counts was used to abolish the effect of differing distances of the gallbladder from the gamma camera crystal, as has been validated previously.[33] The gamma camera scans were performed for 60 seconds, and the data were recorded as 64 × 64 digital images and stored on 8-in floppy disks for later analysis. The analysis of scintigraphic images was performed by selecting the following areas of interest: gallbladder, liver (gallbladder subtracted), common bile duct, intestine, urinary bladder, and whole abdomen. All counts were corrected for background and radioactive decay. The values for the absorbed dose of the whole body lie within World Health Organization category 1. This is of the same order of magnitude as the level of background radiation exposure for the general population for 12 months.

### Measurements and Calculations

The following measurements were performed: fasting gallbladder volume (in milliliters) measured by ultrasonography immediately before meal administration (considered to be 100%) and fasting gallbladder count (in counts per minute) measured by gamma camera scan immediately before meal administration (considered to be 100%). Both of the above measurements were performed in duplicate to assess reproducibility.

Gallbladder emptying by ultrasonography ($GE_{US}$) was determined according to the following formula:

$$GE_{US} = \frac{\text{Fasting GB Volume} - \text{GB Volume}_n}{\text{Fasting GB Volume}} \times 100,$$

where *GB Volume$_n$* is the postprandial gallbladder volume at the different 10-minute intervals.

The results were expressed in percentage terms with the fasting gallbladder volume represented as 100%. The results were also expressed in terms of volume ejected (in milliliters) by the following equation:

$$\text{Fasting GB Volume (mL)} \times \%GE_{US}.$$

Gallbladder emptying by gamma camera ($GE_{GC}$) was determined according to the following formula:

$$GE_{GC} = \frac{\text{Fasting GB Count} - \text{GB Count}_n}{\text{Fasting GB Count}} \times 100,$$

where *GB Count$_n$* is the postprandial gallbladder count at the different 10-minute intervals. The results were also expressed in percentage terms with the fasting gallbladder count represented as 100%, and the results were also expressed in terms of volume ejected (in milliliters) by the following equation:

$$\text{Fasting GB Volume (mL)} \times \%\ GE_{GC}$$

The postprandial gallbladder refilling (in milliliters) was calculated at each 10-minute interval from the difference between the simultaneously measured $GE_{GC}$ and $GE_{US}$ because the former measurement is not influenced by gallbladder refilling, whereas the latter measurement is influenced by gallbladder refilling. The results were expressed in milliliters by transformation of the percentage values for $GE_{GC}$ and $GE_{US}$, using the fasting gallbladder volume. The sequential 10-minute values were added to calculate the cumulative postprandial refilling. This was used as an index of the total amount of hepatic bile entering the gallbladder during the 90-minute study period.

Postprandial cumulative gallbladder turnover (in milliliters) was defined as the volume of bile handled by the gallbladder in the postprandial period. It was calculated at each time interval from the sum of cumulative gallbladder refilling and $GE_{GC}$ at that time interval. The results were expressed in absolute terms (in milliliters).

Gallbladder turnover index at 90 minutes was derived by dividing the cumulative turnover at 90 minutes by the fasting gallbladder volume. This should indicate the relative performance efficiency of the gallbladder at the end of the study period. The latter three parameters of gallbladder motor function are novel and have not been quantified previously.

### Statistical Analysis

The results for the different parameters measured were expressed as the mean ± SE. The comparison between the simultaneous measurements using ultrasonography and gamma camera scan and between the different groups was performed by Student's *t* test and repeated-measure ANOVA, where appropriate, with $P$ values of $<0.05$ considered significant. Correlation coefficients were calculated using the method of least squares. Coefficient of variation (CV) was calculated for reproducibility studies.

## Results

### In Vivo Reproducibility

There was a close agreement between duplicate measurements for fasting gallbladder volume by ultrasonography (mean ± SE, 19.6 ± 1.8 mL and 20.3 ± 2.3 mL, respectively; CV, 8.6%) and for fasting gallbladder count by gamma camera scan (mean ± SE, 39,203 ± 3785 cpm and 38,993 ± 3892 cpm, respectively; CV, 4.1%). The same was true for the total abdominal count (mean ± SE, 99,157 ± 8466 and 99,111 ± 8679 cpm, respectively; CV, 1.9%).

### Fasting Gallbladder Volume

Fasting gallbladder volume tended to be higher in the patients with gallstones by comparison with the controls, but the difference was not significant (mean ± SE, 23.8 ± 3.8 mL vs. 15.8 ± 1.4 mL; $P = 0.09$) (Table 1). Taking both groups together, fasting gallbladder volume correlated with body mass index ($r = 0.58$; $P < 0.005$). A similar correlation was found between the two parameters in the patients with gallstones ($r = 0.66$; $P < 0.05$) but not in the healthy controls alone.

### Gallbladder Emptying Patterns in Healthy Controls

Figure 1 shows the patterns for percent reduction in postprandial gallbladder isotope activity (cpm%) as measured by scintigraphy and in volume (mL%) as measured simultaneously by ultrasonography in the same group of healthy controls. In each case, the first data point represents the fasting value (100%). As expected, the isotope activity over the gallbladder area decreased in a biexponential fashion, with an initial fast phase followed by a late slow phase. There was a very different pattern for gallbladder volume measured at the same

**Table 1.** Gallbladder Emptying Parameters

| | Gallbladder empyting | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Scintigraphy | | | | | | Ultrasonography | | | | | |
| | Ejection fraction | | | Emptying volume | | | Ejection fraction | | | Emptying volume | | |
| Time (*min*) | Con[a] (%) | GS[b] (%) | *P* value[c] | Con[a] (*mL*) | GS[b] (*mL*) | *P* value[c] | Con[a] (%) | GS[b] (%) | *P* value[c] | Con[a] (*mL*) | GS[b] (*mL*) | *P* value[c] |
| 0 | 20.2 | 11.7 | NS | 3.4 | 2.3 | NS | 5.3 | 4.8 | NS | 1.9 | 2.0 | NS |
| 10 | 39.6 | 25.1 | <0.01 | 6.2 | 5.0 | NS | 23.9 | 14.8 | NS | 4.9 | 4.0 | NS |
| 20 | 55.9 | 33.1 | <0.0005 | 8.7 | 6.8 | NS | 42.6 | 26.9 | <0.01 | 7.8 | 6.9 | NS |
| 30 | 66.2 | 38.4 | <0.0001 | 10.4 | 6.8 | NS | 55.2 | 37.6 | <0.005 | 9.8 | 9.3 | NS |
| 40 | 71.4 | 44.4 | <0.0005 | 11.2 | 9.0 | NS | 60.2 | 41.6 | <0.001 | 10.6 | 10.4 | NS |
| 50 | 76.8 | 50.1 | <0.0005 | 12.0 | 10.4 | NS | 53.9 | 42.5 | <0.01 | 9.5 | 10.6 | NS |
| 60 | 80.1 | 52.8 | <0.0001 | 12.6 | 11.3 | NS | 49.4 | 39.6 | <0.05 | 8.8 | 10.0 | NS |
| 70 | 84.6 | 55.1 | <0.0001 | 13.2 | 11.9 | NS | 44.7 | 35.2 | <0.05 | 8.1 | 9.1 | NS |
| 80 | 86.7 | 57.6 | <0.0001 | 13.6 | 12.6 | NS | 40.1 | 32.0 | <0.05 | 7.4 | 8.5 | NS |
| 90 | 89.0 | 59.8 | <0.0001 | 13.9 | 12.9 | NS | 34.0 | 29.5 | NS | 6.4 | 8.0 | NS |

NOTE. Values are expressed as the mean values.
Con, controls; GS, patients with gallstones.
[a]n = 11.
[b]n = 14.
[c]Differences between the two groups by ANOVA.

time intervals. The volume decreased progressively for up to 40 minutes and then began to increase, indicating net refilling. At all time intervals, the percent reduction in isotope activity was greater than the corresponding percent reduction in volume, and this was significant between 50 and 90 minutes ($P < 0.001$). The difference between the two measurements at each time interval represents postprandial gallbladder refilling.

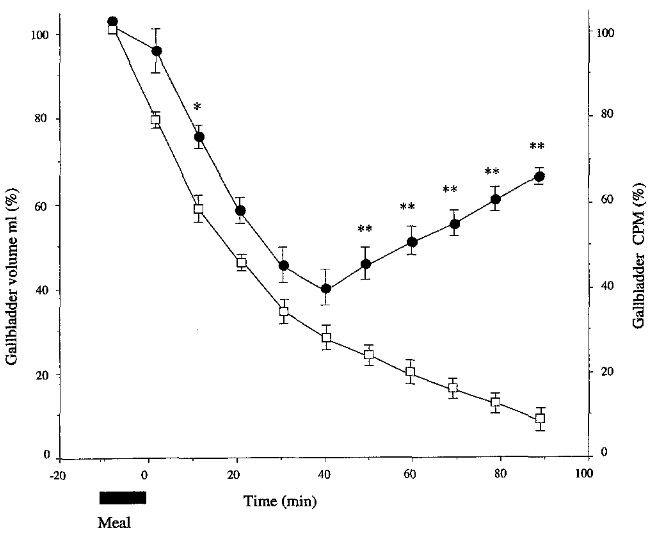


**Figure 1.** Gallbladder emptying patterns in healthy controls. Mean and SE bars represent percentages of fasting gallbladder volume or number of counts per minute (CPM). Differences between the two respective measurements at each time interval represent postprandial refilling. ●, Ultrasonographic measurements; □, scintigraphic measurements. *$P < 0.05$; **$P < 0.0001$.

## Gallbladder Emptying Patterns in Gallstone Disease

Figure 2 shows a comparison between the patients with gallstones and healthy controls with regard to the patterns for postprandial gallbladder isotope activity and volume. In the patients with gallstones, the postprandial gallbladder isotope activity (Figure 2*A*) also decreased in a progressive biexponential manner, whereas postprandial gallbladder volume (Figure 2*B*) decreased for up to 40 minutes and then began to increase, similar to the healthy controls. However, the postprandial reduction in activity was significantly smaller in the patients with gallstones than in the healthy controls at all time intervals between 10 and 90 minutes (Figure 2*A*), and the postprandial reduction in gallbladder volume was smaller in the patients with gallstones between 20 and 80 minutes. When the same results were expressed in terms of emptying volumes, the differences between the two groups disappeared for both the scintigraphic and ultrasonographic measurements (Table 1). This occurred because the values for the fasting gallbladder volume were greater in the patients with gallstones.

## Postprandial Gallbladder Refilling

There was a slow increase in cumulative postprandial refilling (up to 8–11 mL) in healthy controls between 0 and 40 minutes (Figure 3). However, the increase began to accelerate after 40 minutes, reaching values of 41 ± 4.1 mL at 90 minutes. By contrast, there was minimal postprandial refilling in the patients with gallstones (up to 5 mL by 50 minutes), reaching values of 22 ± 5.3 mL at 90 minutes. The values for the patients with gallstones were less than for controls at all time intervals, with the difference between the two groups being significant at 60–90 minutes.

### Gallbladder Turnover of Bile

There was a steady increase in cumulative gallbladder bile turnover in the healthy controls (Figure 4*A*). Once again, the values for the patients with gallstones were less than for the healthy controls at all time intervals, with the difference between the two groups being significant at 50–90 minutes. At 90 minutes, the cumulative turnover in the healthy controls was (54.9 ± 5.1 mL compared with 35.2 ± 5.9 mL in the patients with gallstones; $P < 0.05$).

The mean turnover index in the healthy controls at 90 minutes was about twice that in the patients with gallstones (3.5 ± 0.3 vs. 1.8 ± 0.3; $P < 0.001$; Figure 4*B*). Under our experimental procedure (measurements performed at 10-minute intervals for 90 minutes), the majority of healthy subjects (8 of 11) achieved a turnover index of >3 (three times the fasting gallbladder volume) compared with only 2 of 14 patients with gallstones. This greater difference between the two groups was because the determination of the turnover index takes into account the fasting gallbladder volume, which is greater in the patients with gallstones. There was a significant inverse relationship between the mean fasting gallbladder volume and the cumulative turnover at 90 minutes ($r = -0.60$; $P < 0.002$; Figure 5).

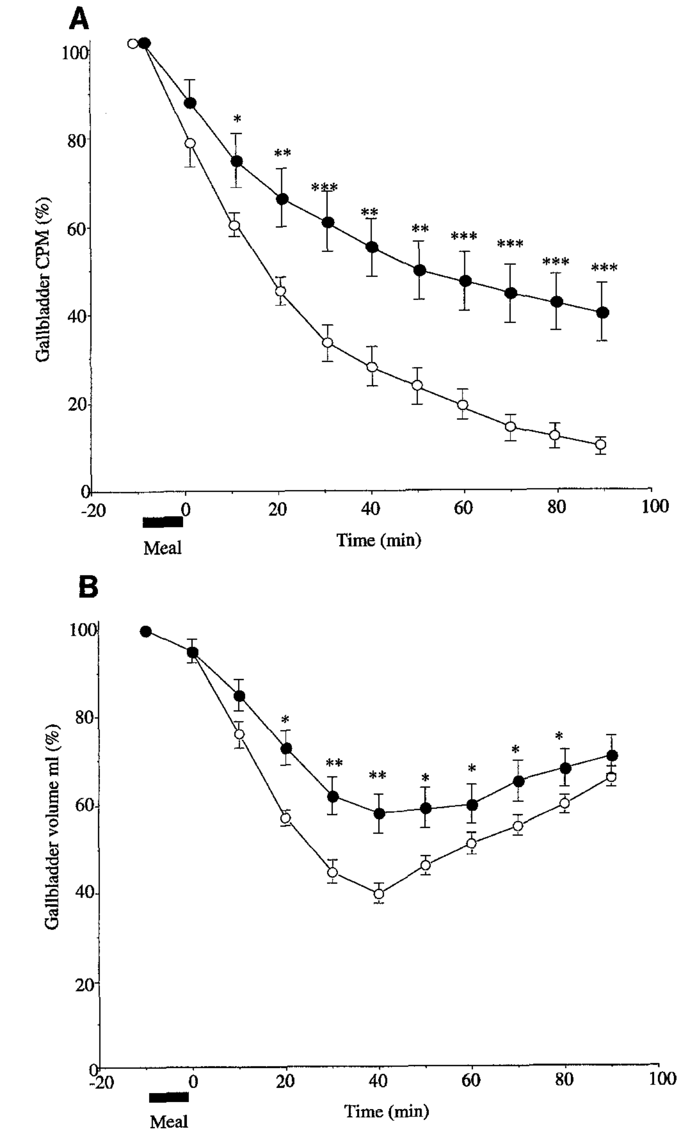


**Figure 2.** Comparison of gallbladder emptying patterns between healthy controls (○) and patients with gallstones (●). (*A*) Scintigraphic measurements and (*B*) ultrasonographic measurements. Results are expressed as means with SE bars and represent the percent change in (*A*) gallbladder counts per minute (CPM) ($^{*}P < 0.01$; $^{**}P < 0.0005$; $^{***}P < 0.0001$) or (*B*) gallbladder volume ($^{*}P < 0.05$; $^{**}P < 0.005$) in the postprandial period.

## Discussion

Gallbladder motor functions were assessed in a group of patients with gallstones and a group of healthy controls matched for sex, age, and body mass index. These factors have been reported to affect gallbladder emptying[35–38] and may explain, in part, some of the contrasting results from previous reports. Howard et al.[26] compared gallbladder emptying in young, male, nonobese, healthy volunteers and old, obese women with gallstones. They report that postprandial gallbladder bile outputs tended to be higher in the patients with gall-

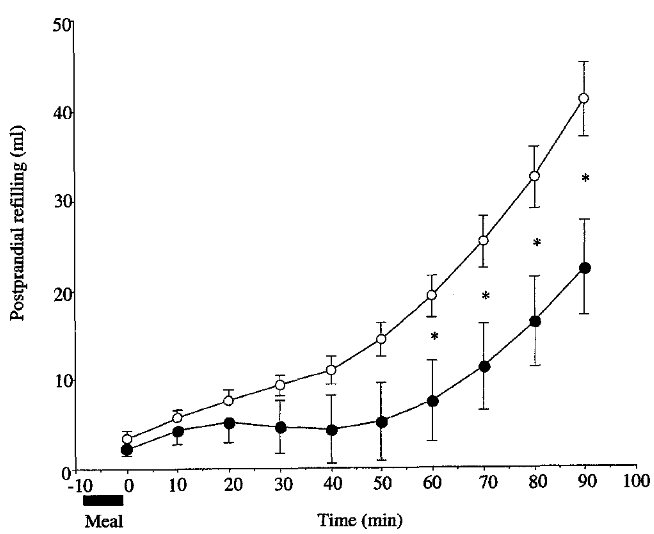


**Figure 3.** A comparison of cumulative postprandial gallbladder refilling between healthy controls (○) and patients with gallstones (●). *Asterisks* indicate significant differences between the two groups at the respective time intervals (ANOVA; $^{*}P < 0.05$).

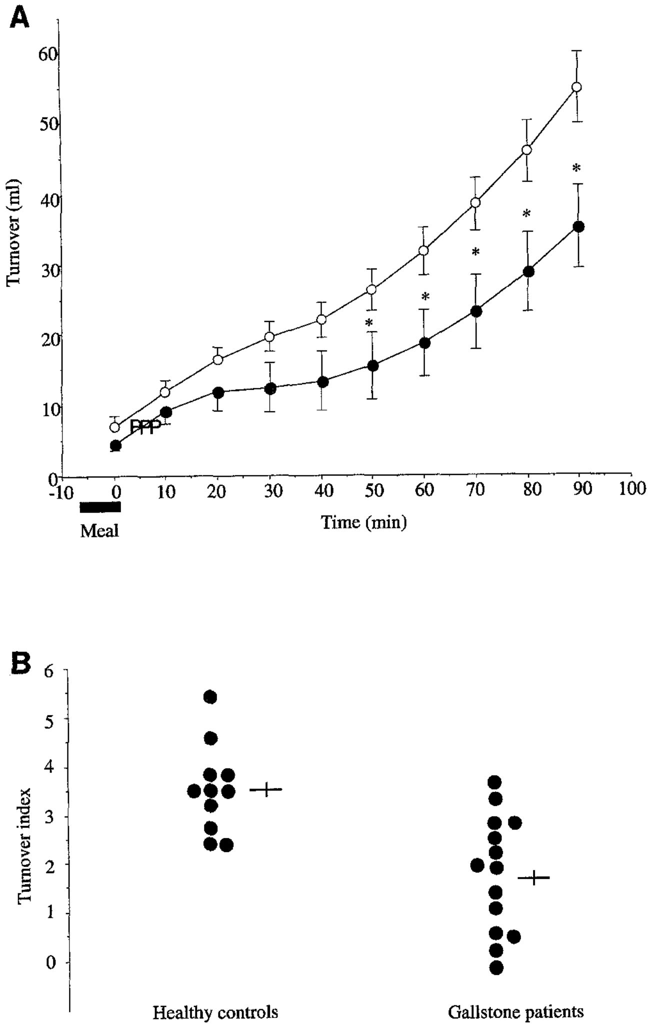


**Figure 4.** (*A*) Comparison of cumulative postprandial gallbladder turnover of bile between healthy controls (○) and patients with gallstones (●). *Asterisks* indicate significant differences between the two groups at the respective time intervals (ANOVA; $^{*}P < 0.05$). (*B*) Comparison of the turnover index between healthy controls and patients with gallstones ($P < 0.001$).

stones than in healthy volunteers. Other studies[17,18,28] have reported impaired gallbladder emptying. The differences cannot be explained simply by the presence of a larger fasting gallbladder volume in patients with gallstones because this was the case in all of the latter studies. Gallbladder emptying patterns can also be affected by the dose and type of gallbladder stimuli,[35,39–41] the route of administration,[41,42] and the meal composition.[43–45] In the present study, we chose a semisolid meal with a small caloric value of 370 kcal because we were attempting to assess early gallbladder refilling in the postprandial period.

The patients studied were all women. To overcome fluctuations in gallbladder motor functions due to the effect of hormones, studies were performed in the first 10 days of the menstrual cycle in premenopausal women. Therefore, it is likely that the observations made could be generalized to men. Indeed, early validation studies included 2 men who showed motility patterns similar to those observed in the present study.

The fasting gallbladder volume tended to be larger in the patients with gallstones compared with healthy controls, but the difference was not statistically significant. However, it is not known whether the greater gallbladder volume in patients with gallstones is actually a result of the gallbladder motor defects in these patients. Indeed, a recent study[19] has shown that, in a subgroup of patients with gallstones with normal gallbladder contraction, the fasting gallbladder volume was still significantly greater than in a matching group of healthy controls. Fluctuations in fasting gallbladder volume could occur because of the interdigestive motor cycle.[46] Theoretically, this could affect results in the present study by affecting the fasting gallbladder volume in the two groups to different extents. However, it is most unlikely because all studies were performed after a similar period of fasting. Hence, the differences between the population with gallstones and the healthy controls are true and have been shown previously by several other studies.[17,18,26]

In the healthy subjects, the measurement of postprandial gallbladder emptying by ultrasonography and scintigraphy showed different patterns. With scintigraphy, there was a continuous decrease in activity over the gallbladder area for the entire postprandial period. As reported previously.[34,47] With the simultaneous ultrasonography measurements, the reduction in postprandial gallbladder volume followed the fast (scintigraphic) emp-

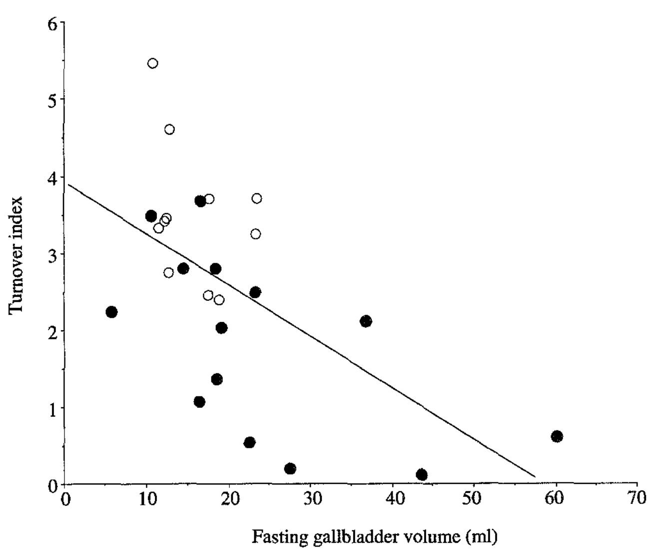


**Figure 5.** There was a significant inverse correlation between postprandial gallbladder turnover and fasting gallbladder volume ($r = -0.6$; $P < 0.001$). The data *points* represent the cumulative turnover at 90 minutes in both groups. ○, Controls; ●, patients with gallstones.

tying phase in the early part of the postprandial period but then diverged, showing the effect of postprandial refilling (Figure 1). However, the fact that the percent reduction in gallbladder volume was less than the corresponding percent reduction in gallbladder isotope count at all time periods suggests that gallbladder refilling began immediately after the meal and continued throughout the entire postprandial study period.

The difference between the two simultaneous measurements became significant during the latter part of the study period (60–90 minutes), suggesting increased refilling during that latter part. Indeed, when assessed directly, gallbladder refilling (Figure 4) increased slowly during the first 40 minutes of the postprandial period (corresponding to the fast emptying phase), but afterwards, there was a faster increase. This pattern is not surprising because both emptying and refilling take place in a rapid, alternating fashion through a single channel (cystic duct). Therefore, it is logical to assume that there is a limit to the overall traffic of bile (input plus output) through this channel. Hence, increased output (emptying) during the fast emptying phase leads to a reduction in the input (refilling) and vice versa.

The human liver secretes about 1000 mL of bile per day (40–50 mL/h). Thus, our finding in the healthy controls of gallbladder refilling of 41 mL at 90 minutes confirms the observation of Lanzini et al.[31] that the majority of hepatic bile enters the gallbladder before reaching the duodenum even in the postprandial period. The large discrepancy between cumulative refilling and residual gallbladder volume suggests that the hepatic bile that enters the gallbladder in the postprandial period leaves quickly, confirming the washout effect that Howard et al. postulated in a previous study.[26]

The cumulative turnover of bile in the gallbladder (postprandial emptying plus refilling) indicates the volume of bile handled by the gallbladder in the postprandial period. This was more than five times the fasting gallbladder volumes in health, suggesting that the gallbladder is a dynamic organ with complex motor functions and not a mere storage sac for bile that empties postprandially. Lanzini et al.[31] have shown that rapid alternations in gallbladder filling and emptying occur not only postprandially but also during fasting. They suggest that these rapid alternations contribute to the mixing of the gallbladder contents. The results of this study confirm that these rapid alternations are indeed associated with a high turnover of bile within the gallbladder (i.e., a washout effect) postprandially. Therefore, they are likely to have a similar effect in the fasting state.

The patients with gallstones had patterns of gallbladder emptying by ultrasonography and scintigraphy that were qualitatively similar to those patterns observed in healthy controls, suggesting that refilling in patients with gallstones also began in the immediate postprandial period. However, quantitatively and as shown in previous studies,[11–15] gallbladder emptying by scintigraphy was found to be impaired in the patients with gallstones only when the results were expressed in terms of percent emptying but not when the results were expressed in terms of absolute volumes ejected. Similarly, the ultrasonographic measurements of percent gallbladder emptying showed a difference between the two groups, albeit less clear cut. This is because ultrasonography measures net changes in volumes caused by both emptying and refilling that occur within the same 10-minute interval. Thus, the lack of difference between the two groups is because of the fact that, in controls, there was not only greater emptying but also greater refilling, so that the net volume change within the 10 minutes was small. Furthermore, when the ultrasonography results were expressed in terms of absolute ejection volumes (in milliliters), not only was there no difference between the two groups but there was also a trend for larger ejection volumes in the patients with gallstones. This discrepancy is shown clearly in Table 1 in which the same results are expressed both as the percent emptying and as the ejection volumes, and the discrepancy is caused by the larger fasting gallbladder volumes in the patients with gallstones, a finding reported by several ultrasonography-based studies.[17–19,26,28] It also explains why some investigators have reported unimpaired[27] or even increased[19] gallbladder emptying when the results are expressed in terms of ejection volumes, whereas other investigators have reported impaired emptying when the results were expressed in percentage terms.

There was a very marked difference between patients with gallstones and healthy controls in the volume of bile turned over in the gallbladder. By 90 minutes, the gallbladders in healthy controls handled more than 1.5 times the volume of bile handled in the patients with gallstones despite the fact that the patients with gallstones had larger fasting volumes. When the results were expressed in terms of the turnover index, the difference between the two groups was even greater. The turnover index may provide a way of assessing the performance efficiency of the gallbladder, and its impairment may suggest gallbladder failure by analogy with cardiac failure, in which the myocardial performance is impaired and the heart is enlarged. The very sluggish gallbladder turnover in patients with gallstones reflects stasis of gallbladder contents and contributes to the effect of cholesterol nucleation promoting factors[48] in enhancing cholesterol crystal precipitation and gallstone formation. On

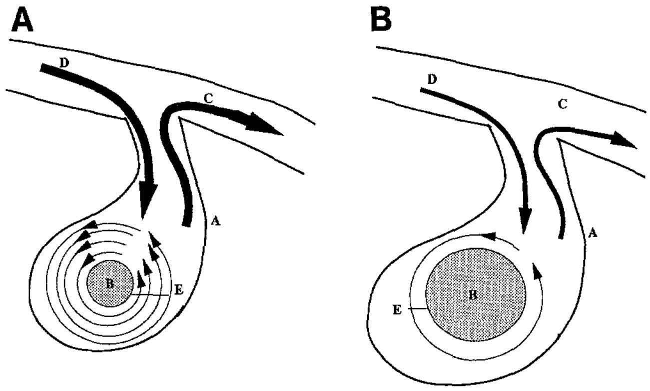


**Figure 6.** Proposed model for gallbladder motor function in (*A*) controls and (*B*) patients with gallstones. Both fasting volumes (A) and residual volumes (B) are shown to be larger for the patients with gallstones. The *arrows* in and out of the gallbladder represent postprandial gallbladder emptying (C) and refilling (D) that are both reduced in patients with gallstones. The difference between the fasting and residual gallbladders represents bile turnover (E), which is markedly impaired in the patients with gallstones.

the other hand, the turnover index represents the efficiency of the gallbladder in eliminating its contents, including cholesterol crystals. Indeed, the strong inverse correlation between gallbladder turnover and fasting gallbladder volume (Figure 5) suggests that the reduced turnover of bile occurs in the larger and flabbier gallbladders of the patients with gallstones where stasis is more likely.

Our proposed model for the postprandial gallbladder motor function is shown in Figure 6. According to our results, the patients with gallstones have greater fasting and residual gallbladder volumes and impairment in both emptying and refilling, resulting in a marked reduction in the turnover of bile in the gallbladder.

Postprandial refilling and turnover are new parameters of gallbladder motor function. Their quantification was made possible by using ultrasonography and scintigraphy simultaneously. Together with gallbladder emptying, they may be of value in studying the pathogenesis of gallstones. The concept of using ultrasonography and scintigraphy simultaneously to assess gallbladder motor function is not new. Qvist et al.[49] used ultrasonography and scintigraphy simultaneously to assess gallbladder storage and emptying in the fasting state. However, because Qvist et al. used a continuous infusion of $^{99m}$Tc–iminodiacetic acid and not an intravenous bolus as in the present study, they were assessing the similarity between the two techniques. The fact that Qvist et al. found a concordance of only 30% during filling and 46% during emptying may be because of several reasons. First, it confirms that ultrasonography and scintigraphy measure different parameters. Second, Qvist et al. used only posterior gamma camera scans instead of anterior and posterior scans. This could lead to errors in isotope counting caused by differing distances of the gallbladder from the gamma camera crystal.[33] Third, as Qvist et al. propose in their article, fluid transport across the gallbladder epithelium could theoretically affect volume measurements by ultrasonography without affecting isotope counts by scintigraphy. However, this is hardly likely to be the case both in the present study or in the study of Qvist et al.[49] The liver secretes about 1000 mL of bile per 24 hours,[50,51] i.e., 60 mL in 90 minutes. If we consider the healthy controls in the present study, the bile turnover in 90 minutes was 55 mL, and the residual gallbladder volume was 10 mL (total volume, 65 mL). This volume could account for the sum of the fasting gallbladder volume (15 mL) and the majority of the 60 mL secreted by the liver in the 90 minutes. Even assuming that all hepatic bile entered into the gallbladder, at most, only 5–10 mL of fluid in 90 minutes (or 0.05–0.1 mL/min) could be transported across the epithelial layer. These values are negligible in the context of the present study and cannot account for the differences between the ultrasonographic and scintigraphic measurements.

There are two possible explanations in regard to the overall mechanism for the motility defects in patients with gallstones. The first possiblility is that the differences in gallbladder tone reflect the inherent compliance of the smooth muscle and fibroelastic tissue within its wall. According to pressure and/or volume measurements, LaMorte et al.[20] reports that the compliance of the gallbladder should remain constant over 2–3 hours despite large fluctuations in volume in healthy controls. This property of tone adjustment, which would normally allow refilling after gallbladder contraction, may be impaired in patients with gallstones. Animal studies have shown that even before gallstone formation, an increase in the amount of cholesterol crystals in gallbladder bile affects gallbladder wall motility and impairs gallbladder emptying. The second possibility is impaired cystic duct function. Because the cystic duct is the only channel through which emptying and refilling occur, mechanical or functional impairment wcould account for the defects in both of the parameters that we observed in patients with gallstones. Indeed, Doty et al.[23] have noted that cystic duct resistance was increased in the prairie dog that was fed lithogenic bile, and they concluded that gallbladder stasis is probably secondary to relative outflow obstruction.

The present study confirms first that the concept of gallbladder emptying during meals and refilling between meals is not correct because refilling begins immediately after a meal and continues during the postprandial period. Second, this study confirms that the present methodology for assessment of gallbladder motor functions (ultrasonography or scintigraphy alone) is not capable of evaluating both emptying and refilling in a quantitative manner; hence, there is a need for improved methodology. Third, the present study provides evidence that postprandial refilling and turnover of bile are important factors that need to be assessed when addressing gallbladder motor function and its role in the pathogenesis of cholesterol gallstone disease.

We conclude that postprandial gallbladder handling of bile is complex and cannot be measured reliably by using ultrasonography or scintigraphy alone. When used in combination, these two techniques can be used to quantify refilling and turnover of bile in the gallbladder and also to calculate a turnover index that represents the overall gallbladder performance efficiency. In healthy controls, this was up to about six times the fasting gallbladder volume within a period of 90 minutes. However, it was markedly reduced in patients with gallstones, suggesting a reduction in the ability of the gallbladder to eliminate its contents including cholesterol crystals. We postulate that impaired turnover of bile coupled with enhanced cholesterol nucleation leads to gallstone formation in these patients.

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**Address requests for reprints to: Riadh P. Jazrawi, M.D., Division of Biochemical Medicine, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, England. Fax: (44) 81-767-4696.**

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