the lungs is low suggest the potential clinical value of PET for tumor staging. Another clinical application of FDG-PET may be early detection of locally recurrent tumors. After surgery and radiotherapy, recurrent lesions are usually difficult to detect in the early phase. Physical examination and Roentgen techniques are hampered by scar tissue and distortion of the normal tissue planes, disadvantages that PET does not have. Future PET investigations should also be aimed at the application of other radiopharmaceuticals such as labeled amino acids, DNA substrates and chemotherapeutic drugs. Evaluation of radiotherapeutic and chemotherapeutic results for soft-tissue sarcoma also seem attractive fields for future PET studies.

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Dynamic Cholescintigraphy: Induction and Description of Gallbladder Emptying

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The main purposes of this study were to investigate the best parameter for describing gallbladder emptying and whether gallbladder bile emptying should be induced with a bolus injection or continuous infusion of cholecystokinin-octapeptide (CCK-8). **Methods:** Gallbladder emptying was measured by dynamic cholescintigraphy. Twelve healthy subjects and six patients with gallstones were examined twice with CCK-8 infusion cholescintigraphy, 0.3 ng CCK-8 kg per min for 60 min under identical circumstances. Another six healthy subjects randomly received bolus injection (0.04 μg/kg) and infusion of CCK-8 (0.3 ng/kg per min for 60 min), respectively, during cholescintigraphy on two separate occasions. The choice of bolus dose was based on recommendations from the CCK-8 manufacturer. The infusion dose was chosen to produce plasma CCK concentrations similar to postprandial plasma CCK levels. **Results:** A parameter of gallbladder emptying, mean ejection fraction (EF), was defined as 100% minus the area under the time-activity curve normalized to 100% and divided by the time interval from maximum to minimum counts per minute. This parameter proved superior to the well known parameters, EF_{max} and EF_{30}, in regard to reproducibility in healthy subjects. The slope of the regression line for the mean EF was 0.998 and the intercept value approximately 0% ($p = 0.0001$). The mean coefficient of variation was 4%. Apart from a higher mean coefficient of variation, similar reproducibility results were seen in the six patients. The measurements of EF_{30} in healthy subjects scattered more widely around the mean compared to the mean EF and EF_{max}, which indicates poorer ability to separate normal from abnormal gallbladder emptying. Intravenous bolus injection of CCK-8 resulted in incomplete gallbladder emptying with a mean EF value of 16% (s.d. 9%; range 7%–32%) compared to 49% (s.d. 7%; range 37%–57%) following CCK-8 infusion ($p = 0.004$). Abdominal discomfort was observed in all subjects after administration of the bolus injection, whereas no complaints were reported during infusion. **Conclusion:** Mean EF is the best parameter for describing gallbladder emptying. Moreover, slow infusion of a physiological dose of CCK-8 is preferable to induce gallbladder emptying because it results in more complete emptying and has no side effects.

Key Words: dynamic cholescintigraphy; gallbladder emptying; cholecystokinin-octapeptide

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Cholescintigraphy has been used to assess gallbladder bile emptying in healthy subjects and patients with gallstones since the method was introduced by Englert and Chiu in 1966 (*1*). In several studies, intravenous administration of cholecystokinin (CCK) and cholecystokinin-octapeptide (CCK-8) has been used to elicit gallbladder emptying. Various dose rates and methods of infusion have been utilized with administered doses ranging from 0.6 to 39 U/kg of CCK and from 10 to 43 ng/kg of CCK-8, infused rapidly as a bolus or slowly as a continuous infusion over 30–60 min (*2–9*).

There is no unambiguous parameter to describe gallbladder emptying. Most frequently, ejection fraction (EF) (*3,5–7,9*) and the half-emptying time (*2,4*) are used to express the degree of gallbladder emptying but with varying definitions. The best parameter for describing gallbladder emptying would be a parameter with a high degree of reproducibility combined with limited variation in healthy subjects, thereby making it possible to separate ill from healthy subjects. A few studies do reveal information about reproducibility of the cholescintigraphic method. In a recently published study, Xynos et al. (*10*) reported a correlation coefficient of 0.84 for repeated EF measurements in 28 healthy subjects. Two earlier studies using the half-emptying time and EF, respectively, to evaluate gallbladder emptying, reported mean interstudy variations of 12% (20 healthy subjects) (*6*) and 15% (7 patients with gallstones) (*4*) but without specifying the range intervals and, in the latter case, the degree of gallbladder emptying. In patients with impaired gallbladder emptying, the half-emptying time is not usable as the emptying parameter because it will exceed the duration of the cholescintigraphic emptying period.

Furthermore, the cholescintigraphic technique may vary with recordings performed sequentially (*8–10*) or continuously (*2–3,5,6*). With dynamic cholescintigraphy, it is possible to create continuous time-activity curves during gallbladder filling and emptying and to use the entire time-activity curve in describing gallbladder emptying.

In this study we used dynamic cholescintigraphy to determine the best single parameter to describe gallbladder emptying, to assess the intraobserver, interobserver and interstudy variations of the method and to determine whether bolus injection or continuous infusion of CCK-8 is preferable.

METHODS

Subjects

We studied 18 healthy volunteers (4 men, 14 women) and 6 patients with cholesterol stones of the gallbladder (1 man, 5 women) aged 21–56 yr (mean age 30 yr) and 29–59 yr (mean 44 yr), respectively. None of the healthy subjects had gastrointestinal symptoms or gallstones, as determined by ultrasonography. Informed consent was obtained from each participant after the protocol had been approved by the local ethical committee.

Dynamic Cholescintigraphy

After fasting overnight, each subject was positioned supine beneath a gamma camera equipped with a 140-keV low-energy, all-purpose, parallel-hole collimator. The gamma camera was connected to a computer, which enabled simultaneous data acquisition in a 64 × 64 matrix. Counts rates were recorded continuously with one frame per minute with the photopeak window set at 140 keV (±15%). The detector was placed anteriorly over the right hypochondrium to enable visualization of the liver, gallbladder, bile ducts and the upper small intestine. Subject and camera positioning was kept constant during the entire study.

Dynamic acquisition was started at time 0 min with simultaneous administration of a bolus injection of 100 MBq ^{99m}Tc-EHIDA (diethylphenylcarbamoylmethyliminoacetate) prepared immediately before injection. Continuous recordings were obtained for 60 min. During this time, gallbladder filling was observed. At time 60 min, an infusion of CCK-8 0.3 ng/kg per min for 60 min, or a bolus of CCK-8, 0.04 μg/kg injected intravenously over 1 min, were administered to stimulate gallbladder emptying. An infusion pump was used for the 60-min CCK-8 infusion. During the following 60 min, dynamic acquisition with one frame per min was continued during gallbladder emptying. Recording and CCK-8 infusion ended at 120 min (Fig. 1).

After data collection, images were replayed from the computer and the region of interest corresponding to the gallbladder was defined. Time-activity curves for the gallbladder region corrected for background and radioactivity decay were generated. To correct for background, the frame containing the maximal liver area was identified. The maximal gallbladder area (from frame 61–71) was duplicated into this frame. A small area comprising 16 pixels was drawn just adjacent to the duplicated gallbladder area with an identical proportion covering the liver. Attention was paid to avoid interference with the bile ducts. A time-activity curve for the small area was constructed. This curve was divided by 16 and multiplied by the number of pixels comprising the maximal gallbladder area. The final curve representing time-background activity was subtracted from the gallbladder time-activity curve.

We calculated the following parameters to describe gallbladder emptying:

1. Time for maximum counts per minute (CPM) during the time interval 60–120 min, $t_{max.\,CPM}$ (min).
2. Mean ejection fraction during the emptying period, mEF, defined as 100% minus the area under the time-activity curve from maximum to minimum CPM, normalized to 100% (i.e., maximum CPM = 100%), divided by the timedifference from $t_{min.CPM}$ (i.e., time for minimum CPM during the time interval 60–120 min) to $t_{max.\,CPM}$:

$$mEF\ (\%) = 100\% - \frac{\text{AUC (from maximum CPM to minimum CPM in the period 60–120 min)}}{t_{min.\,CPM} - t_{max.CPM}}. \quad \text{Eq. 1}$$

3. The maximal gallbladder ejection fraction, $EF_{max.}$:

$$EF_{max.}\ (\%) = \frac{\text{max. CPM (in the period 60–120 min)} - \text{min. CPM (in the period } t_{max.\,CPM} - \text{120 min)}}{\text{max. CPM (in the period 60–120 min)}} \times 100. \quad \text{Eq. 2}$$

4. Gallbladder ejection fraction from 60 to 120 min, EF_{60-120}:

$$EF_{60-120}\ (\%) = \frac{\text{CPM (at time 60 min)} - \text{CPM (at time 120 min)}}{\text{CPM (at time 60 min)}} \times 100. \quad \text{Eq. 3}$$

5. Gallbladder ejection fraction from $t_{max.}$ and the following 30 min, EF_{30}:

$$EF_{30} = \frac{\text{max. CPM (in the period 60–120 min)} - \text{CPM (after 30 min)}}{\text{max. CPM (in the period 60–120 min)}} \times 100. \quad \text{Eq. 4}$$

Study Design

In both subjects and patients, cholescintigraphy was performed twice, 2 wk apart.

In six subjects, gallbladder emptying in one cholescintigraphic study was initiated by an intravenous bolus of CCK-8 administered at time 60 to 61 min, whereas an infusion of CCK-8 from time 60 to 120 min was used in the other study. Both methods were administered randomly.

In another 12 subjects and in the 6 patients, repeat cholescintigraphy was performed under identical circumstances. In both studies, gallbladder emptying was induced by an infusion of CCK-8.

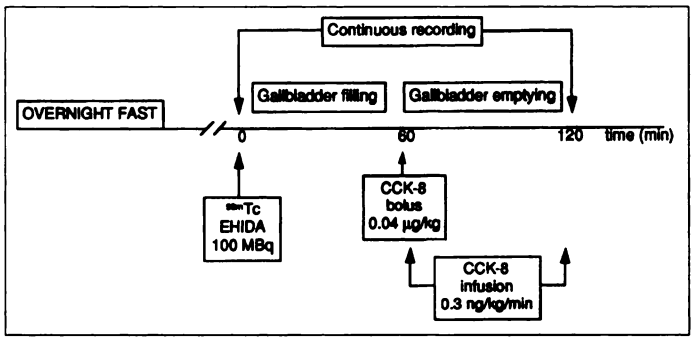

FIGURE 1. Protocol for dynamic cholescintigraphy with gallbladder emptying induced by either bolus injection or continuous infusion of CCK-8.

The selection of a bolus dose of CCK-8 was based on manufacturer recommendations to achieve satisfactory gallbladder emptying. A 0.3 ng/kg per min infusion dose of CCK-8 was chosen because it is known to induce CCK concentrations similar to postprandial CCK levels (*11,12*). The subjects were not allowed to move during the entire imaging procedure, which would make an examination time of more than 120 min intolerable. Therefore, the gallbladder emptying period was set at 60 min.

To determine interobserver variation, the mean EF was calculated independently by three separate observers in six randomly selected recordings. In another series of six recordings, the mean EF was calculated from the same recording on two separate occasions by a single observer to detect intraobserver variation.

Laboratory Analysis

In six subjects receiving bolus and infusion administration of CCK-8, plasma concentrations were measured during CCK-8 infusion.

Blood was sampled from an arm vein at 10, 30, 45, 62, 65, 70, 75, 80, 90, 100, 110 and 120 min into ice-chilled tubes containing 5 µmole ethylenediaminetetra-acetate (EDTA) per ml of blood. Samples were centrifuged (1200 × g, 10 min) at 4°C within 1 hr, and the plasma stored at −20°C. The CCK concentrations were measured using antiserum (Ab) G-160. This antiserum binds bioactive CCK peptides containing the tyrosine-*O*-sulfated sequence 25–30 of CCK-33. The relative affinities of Ab G-160 to sulfated CCK-8, CCK-33, CCK-39, sulfated and non-sulfated gastrin-17 are 1.0, 0.40, 0.40, 0.015 and <0.001, respectively. The method is described in detail by Cantor (*13*). The detection limit of the assay was 0.3 pmole/liter of CCK-8. Basal plasma CCK concentration was calculated as mean of the three values at 10, 30 and 45 min.

Dosimetry

For cholescintigraphy, a standard dose of 100 MBq ^{99m}Tc-EHIDA was administered with an effective dose equivalent of 0.024 mSv/MBq (*14*). This resulted in an effective dose equivalent of 2.4 mSv.

Statistical Analysis

Linear regression and the coefficient of variation were used to express reproducibility. The slope (coefficient of regression) and intercept values of the regression line were calculated. Probability values of <0.05 were considered significant using analysis of variance for linear regression. The coefficient of variation was defined as the standard deviation divided by mean. The Mann-Whitney test was used for between group comparisons and probability values of $p < 0.05$ were considered significant.

RESULTS

Gallbladder Emptying Parameters

In 12 healthy subjects examined twice with CCK-8 infusion cholescintigraphy, a high degree of reproducibility was observed using the mean EF with a regression coefficient of 0.998 and an intercept value of 0.3% ($p = 0.0001$) (Fig. 2). The corresponding mean values for mean EF were 48.8% and 49.0%. Although, excellent reproducibility was achieved with EF_{30}, this parameter was inferior to the mean EF. When gallbladder emptying was expressed as $EF_{max.}$ or EF_{60-120}, considerably lower but significant coefficients of regression were achieved, 0.684 and 0.622, respectively ($p = 0.0001$). The intercept values were 26.6% for $EF_{max.}$ and 31.8% for EF_{60-120}; these values are proportional to the EF values between 75.1% and 79.8%. No significant differences were observed between $EF_{max.}$ and EF_{60-120} in these 12 subjects.

The first CCK-8 infusion cholescintigram resulted in a mean $t_{max.CPM}$ of 64 min (s.d. 5 min; range 60–75 min). A mean value of 67 min (s.d. 6 min; range 61–83 min) for the second infusion cholescintigram did not differ significantly ($p = 0.15$). Although the mean $t_{max.CPM}$ values did not differ significantly between the two studies, no correlation of paired values was found (regression coefficient = 0.265, $p = 0.4989$). The coincidence between $t_{max.CPM}$ and the time for the start of the CCK-8 infusion (60 min) was observed in only two subjects. All results are listed in Table 1. Eighteen healthy subjects underwent cholescintigraphy with CCK-8 infusion (Table 2). Compared to the mean EF and $EF_{max.}$, significant intrasubject variation was observed in EF_{30}.

Mean EF measurements in the six patients who underwent

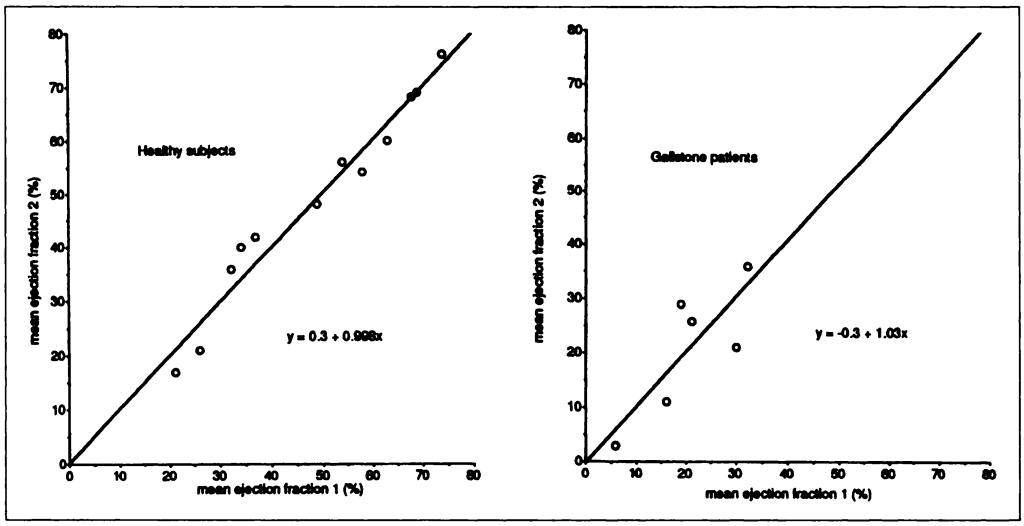

FIGURE 2. Paired data for mean EF in 12 subjects and 6 patients examined with CCK-8 infusion cholescintigraphy twice under identical circumstances, 2 wk apart. The regression lines and formulas are indicated.

TABLE 1
Results of Repeat Gallbladder Emptying Induced by Continuous Infusion of CCK-8 Measured by Dynamic Cholescintigraphy in 12 Healthy Subjects

Variable	Cholescintigraphy 1 mean (s.d.; range)	Cholescintigraphy 2 mean (s.d.; range)	Slope value of regression line	Intercept value of regression line	p value for linear regression
$t_{max.CPM}$	64 min (5 min; 60–75 min)	67 min (6 min; 61–83 min)*	0.265	0.27%	0.4989
Mean EF	48.8% (18.3%; 21%–74%)	49.0% (18.7%; 17–76%)*	0.998	0.3%	0.0001
$EF_{max.}$	77.8% (21.0%; 46%–99%)	79.8% (15.9%; 50–98%)*	0.684	26.6%	0.0001
EF_{60-120}	75.1% (24.0%; 40%–99%)	78.5% (17.0%; 49–98%)*	0.622	31.8%	0.0002
EF_{30}	61.3% (23.7%; 20%–99%)	61.2% (23.3%; 21–95%)*	0.886	8.0%	0.0002

*No significant difference compared to cholescintigraphy 1.

repeat CCK-8 infusion cholescintigraphy showed a high degree of reproducibility, with a coefficient of correlation of 1.03 and an intercept value of −0.3% (p = 0.0001) (Fig. 2). The mean value for both examinations was 21% (range 6%–32% and 3%–36%, respectively).

Interstudy Variations

When time-activity curves from regions of interest over the gallbladder were performed by the same investigator, repeat CCK-8 infusion cholescintigraphy in 12 healthy subjects resulted in a mean interstudy variation of mean EF of 4% (range 0%–7%). A higher interstudy variation of 25% (range 8%–47%) was seen in the six patients examined twice with CCK-8 infusion cholescintigraphy. For the patient with an interstudy variation of 47%, repeat mean EFs of 6% and 3% were measured. Mean interobserver and intraobserver variations were 2% (range 0%–5%) and 1% (range 0%–3%), respectively.

Comparison of Bolus Injection CCK-8 Infusion

After bolus injection of CCK-8, all six subjects experienced transient mild-to-moderate abdominal cramping and nausea. One subject complained of sweating and headache. Neither subjects nor patients experienced adverse reactions during CCK-8 infusion.

Significantly lower mean EFs and $EF_{max.}$ were observed with bolus injection compared to CCK-8 infusion (p = 0.004). The mean value of the mean EF after bolus injection was 15.8% (s.d. 8.7%; range 7%–32%) compared to 48.7% (s.d. 7.4%; range 37%–57%) after infusion. Likewise, $EF_{max.}$ increased from 23.5% (s.d. 10.5%; range 9%–42%) after bolus injection of CCK-8 to 85.3% (s.d. 6.5%; range 77%–95%) after infusion. Gallbladder emptying curves achieved with CCK-8 infusion were more continuous and complete, whereas the curves obtained after bolus administration showed incomplete and rapid emptying followed by a plateau (Fig. 3).

TABLE 2
Results of Gallbladder Emptying Induced by Continuous Infusion of CCK-8 Measured by Dynamic Cholescintigraphy in 18 Healthy Subjects

	Mean EF	$EF_{max.}$	EF_{30}
Mean	48.8%	80.3%	57.8%
s.d.	15.4%	17.7%	22.7%
Range	21%–74%	46%–99%	20%–99%

Plasma CCK Concentrations

After start of CCK-8 infusion, plasma CCK concentrations increased gradually in six healthy subjects from 0.8 ± 0.1 pmole/liter (mean ± s.e.m.) to a peak of 6.3 ± 0.4 pmole/liter 30 min later, whereafter the CCK concentration stabilized at a level of approximately 5.6 pmole/liter (Fig. 4).

DISCUSSION

We found that the best parameter for describing gallbladder emptying is the mean EF. The main purpose for introducing this parameter was to create one that uses the entire gallbladder emptying curve and not just one or two points. Mean EF was thought as an analog to the well known physiological parameter mean transit time used for measuring blood flow in different organs (*15*). Mean EF was characterized by a high degree of reproducibility expressed by linear regression and coefficients of variation in healthy subjects. Thus, the coefficient of regression was 0.998, the regression intercept value approximately 0 and the interstudy variation 4%. The consistent results obtained in the subjects were reproducible in the patients, some of whom had severely impaired gallbladder emptying. The interstudy variation was higher in patients than subjects. In one patient, the mean EF on two separate occasions was 6% and 3%, respectively. Both examinations showed extremely impaired gallbladder emptying, but nevertheless, the interstudy variation was 47%. Consequently, this indicates that the lower the mean EF the higher the interstudy variation. Forgacs et al. (*4*) achieved an interstudy variation of 15% in seven patients with gallstones compared to 25% in our six patients. They do not, however, report any data on the degree of gallbladder emptying in their patients.

Compared to the mean EF and $EF_{max.}$, EF_{30} showed considerable scatter around the mean, which indicated poor ability to separate normal from abnormal gallbladder emptying. This implies that EF_{30} is not a useful parameter, despite an acceptable reproducibility. The $EF_{max.}$ proved to have limited variation between healthy subjects but was inferior in regard to reproducibility. In contrast, the mean EF seemed to fullfill both criteria.

According to our results, defining gallbladder EF is extremely important. Some studies calculate EF from pre-CCK and post-CCK gallbladder CPM (*5–7*). In these cases, the risk of underestimating EF exists because the time for $t_{max.CPM}$ during gallbladder emptying is usually not the same time at which the CCK infusion was started. Thus, we found mean $t_{max.CPM}$ values of 64–67 min, which indicate that the maximum CPM were achieved 4–7 min after CCK-8 infusion was

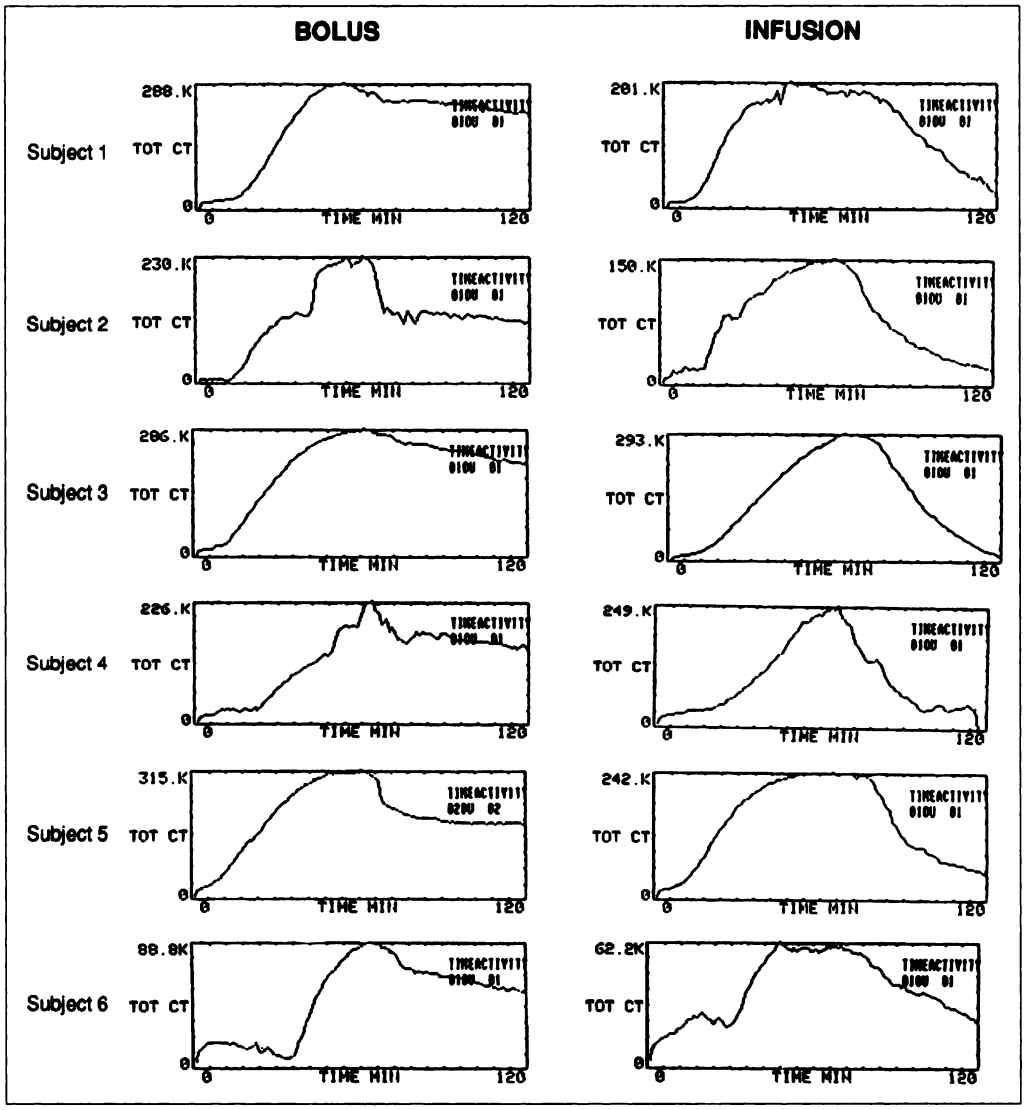

FIGURE 3. Gallbladder filling and emptying curves from six subjects who received a bolus injection of CCK-8 over 1 min (left) and an infusion of CCK-8 for 60 min (right) on two separate occasions.

started. Liddle et al. (*11*) demonstrated that no significant gallbladder emptying occurred until the plasma CCK concentration exceeded at least 2 pmole/liter. In six of our subjects, we found that the plasma CCK level rose above this limit approximately 5 min after the start of CCK-8 infusion. Consequently, it seems the delay before a decrease in activity reflects the time it takes to reach the threshold CCK level required to induce gallbladder emptying. Although, we did not observe significant differences between $EF_{max.}$ (i.e., EF calculated from max.CPM to min.CPM) and EF_{60-120} (i.e., EF calculated from pre-CCK-8 CPM to post-CCK-8 CPM), slightly better reproducibility was seen with $EF_{max.}$. In view of reduced reproducibility and possible underestimation, EF calculated from pre-CCK-8 CPM to post-CCK-8 CPM cannot be used to describe gallbladder emptying.

In this study, we did not investigate the half-emptying time parameter because it is inapplicable. Based on the degree of gallbladder emptying after a meal or CCK infusion, our patients seem to fall into two groups: "contractors," those with normal gallbladder emptying and "noncontractors," those with impaired gallbladder emptying (*16–18*). The reason for this difference in not clear, but a decreased number of CCK receptors in the gallbladder wall or decreased sensitivity of these receptors to CCK in the noncontractors have been suggested (*19*). Thus, in a subgroup of patients with impaired gallbladder emptying, the half-emptying time will exceed the duration of the study. Since this parameter would not allow comparison of gallbladder emptying between subjects and patients, it should not be used.

After ingestion of a mixed liquid/solid meal, plasma CCK concentrations slowly increase to a peak of 7–8 pmole/liter at 90 min postprandially, after which the CCK concentration declines but remains elevated for up to 3 hr (*12*). Following a mixed liquid meal, plasma CCK levels promptly increase within 10 min to a peak of approximately 5 pmole/liter and remain constant at this level for 2 hr (*11*). In our subjects, plasma CCK concentration gradually reached a peak of 6.3 pmole/liter 30 min after the start of the CCK-8 infusion and plateaued at a slightly decreased plasma CCK level. Thus, our time-plasma CCK curve showed a pattern similar to that seen postprandially, which means that our infusion dose of CCK-8 produced a physiological stimulus for gallbladder emptying.

When a bolus injection of CCK-8, 0.02 μg/kg is injected intravenously over 1–3 min, serum CCK peaks rapidly at supraphysiological levels and then promptly returns to baseline with a serum half-life time of approximately 2.5 min. If the same dose of CCK-8 is infused over a 30-min period, a substantially lower peak plasma CCK concentration is reached, but an increased level of plasma CCK is observed for a longer

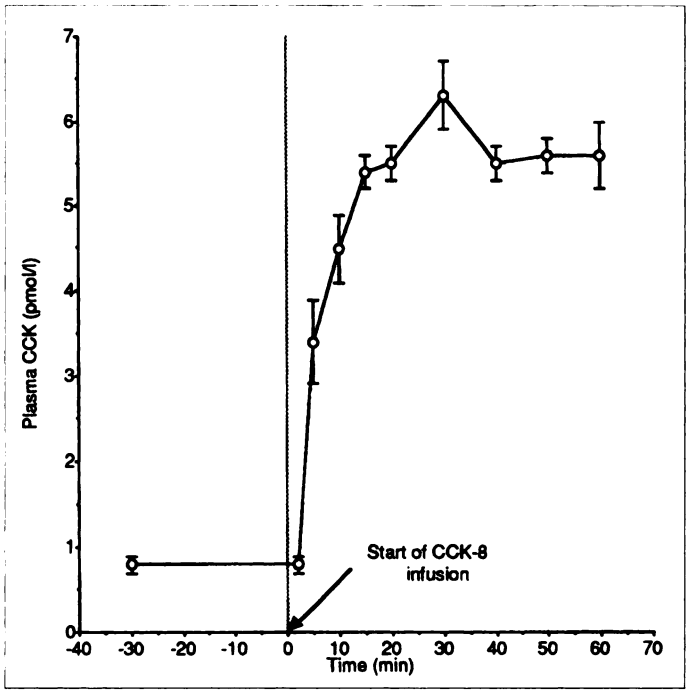

FIGURE 4. Plasma CCK concentrations during CCK-8 infusion for 60 min in six healthy subjects. Values are mean ± s.e.m.

period following infusion rather than bolus injection. More complete gallbladder emptying is seen with a slow infusion compared to a bolus injection resembling the emptying pattern after a meal (*11,20–21*). It therefore seems that both the degree and duration of the rise in plasma CCK are important for inducing gallbladder emptying (*20*). Nevertheless, the package insert accompanying the kit recommends a CCK-8 dose of 0.02 μg/kg infused over 30 or 60 sec to induce gallbladder emptying. Another recommendation is to increase the CCK-8 dose to 0.04 μg/kg if satisfactory gallbladder emptying does not occur. Krishnamurthy et al. (*22*) demonstrated that a larger dose of CCK-8 does not necessarily elicit a greater emptying response all the time; in some subjects, it produces a lesser degree of emptying (*22*). The relative ineffectiveness of the large increase in plasma CCK after bolus injection in inducing more efficient gallbladder emptying may be related to spasms of the cystic duct induced by supraphysiological plasma CCK concentrations (*23*). Although it has been known for some time that a 0.02–0.04 μg/kg bolus injection of CCK-8 in cholescintigraphy (*24–25*) results in nonphysiological effects, this stimulus for gallbladder emptying has been used and recommended in recent articles (*7,26,27*). Consequently, we wanted to emphasize this fact by comparing the nonphysiological effect of a bolus dose (0.04 μg/kg) versus a physiological infusion of CCK-8 (~0.02 μg/kg over 60 min). In our study, subjects complained of abdominal discomfort after bolus injection of CCK-8 and incomplete gallbladder emptying was observed. Slow infusion resulted in more complete gallbladder emptying without any discomfort. The presence of side effects following bolus injection cannot be explained by differences in the total bolus and infusion doses since similar side effects and emptying curves have been demonstrated following 0.02 μg/kg bolus injections of CCK-8 (*21,22*). Therefore, a 0.04 μg/kg dose of CCK-8 injected as a bolus is nonphysiological because it results in incomplete gallbladder emptying and produces side effects (*20,21*).

CONCLUSION

Mean EF measured by dynamic CCK-8 infusion cholescintigraphy is superior to $EF_{max.}$ and EF_{30} for describing gallbladder emptying, primarily because of a high degree of reproducibility. Compared to slow infusion, a bolus dose of CCK-8 of 0.04μg/kg is unphysiological and should not be used during cholescintigraphy.

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