papers and notes on methodology

A new method for the measurement of bile acid turnover and pool size by a double label, single intubation technique

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Summary A new method is described for measuring the cholic acid turnover and pool size by a single duodenal intubation technique. The method is based on determination in a single bile sample of the ratio of the specific activities of [^{14}C]cholic acid and [^{3}H]cholic acid administered intravenously with an interval of 24 hr. With this ratio the fractional turnover rate (k) of cholic acid can easily be calculated as well as the half-life and pool size. Studies in ten normal subjects indicate that the cholic acid half life and pool size, determined by this single intubation technique, correlate very well ($r \geq 0.98$) with the results obtained by Lindstedt's method. Unlike the other methods using a single intubation, this method allows a good estimate of the bile acid turnover as well as the bile acid pool size.—**Vantrappen, G., P. Rutgeerts, and Y. Ghoos.** A new method for the measurement of bile acid turnover and pool size by a double label, single intubation technique. *J. Lipid Res.* 1981. **22:** 528–531.

Supplementary key words [^{14}C]cholic acid · [^{3}H]cholic acid

Important changes in bile acid pool size and turnover may occur in patients with diseases of gallbladder (1, 2), liver (3), and intestinal tract (4–6). The variability of the bile acid pool size in relation to the recycling rate was demonstrated recently (7) in normal subjects. The measurement of bile acid turnover and pool size by the isotope dilution method described by Lindstedt (8) is a standard procedure in bile acid research. At least four bile samples have to be collected by duodenal intubation over a period of 5–7 days, following the administration of a labeled bile acid. To overcome these sampling problems several authors (9, 10) have used a method for measuring the bile acid pool size by a single sampling 3 to 12 hr after the injection of the labeled bile acid. A single early point on the specific activity decay curve is determined and the specific activity at that time is assumed to represent the specific activity at time zero. This method gives a crude estimate of the pool size and is able to distinguish patients with small pools due to gallstones (11) or unoperated Crohn's disease (12, 13) from healthy control subjects. The turnover of bile acids, however, cannot be measured with this technique. The aim of the present study was to validate a new method for estimating both bile acid turnover and pool size by a single intubation technique.

MATERIALS AND METHODS

The method is based on the determination of the ratio of specific activities of [^{14}C]cholic acid and [^{3}H]cholic acid, administered intravenously with a 24-hr interval.

Subjects

Ten subjects, four females and six males, without gastrointestinal, hepatic, or metabolic disease were studied. The mean age of the subjects was 45 years (19–66). All subjects were on an 80-g fat diet 3 days before and during the study and took their meals at regular intervals. The subjects gave their informed consent for the investigation and the study was approved by the ethical committee of the University Hospital of Leuven.

Radioactive material

[2,4-^{3}H]Cholic acid was purchased from New England Nuclear (Boston, MA) and [carboxyl-^{14}C]cholic acid was obtained from the Radiochemical Center (Amersham, England). The products were checked for chemical purity by thin-layer chromatography in isooctane–ethylacetate–acetic acid 5:5:1. The radiopurity was determined by radioscanning (Berthold, model LB 2723, Germany) and was found to be greater than 99.5%. The radioactivity measurements were carried out with a liquid scintillation counter (Packard, model 2450 Downers Grove, IL). Quenching was corrected by external standardization.

Study design

On day 1 (t_1), [24-^{14}C]cholic acid was administered intravenously before breakfast and the patient took three meals a day containing 80 g fat. On day 2 (t_2), exactly 24 hr after the first injection, [2,4-^{3}H]cholic acid was administered intravenously before breakfast while the patient continued his diet.

On day 3 (t_3), after an overnight fast, a tube was passed down to the second part of the duodenum.

Exactly 48 hr after the administration of [^{14}C]cholic acid and 24 hr after the administration of [^{3}H]cholic acid, a vigorous gallbladder contraction was induced by intravenous injection of cerulein (Montedison) 0.0064 μg/kg over a 2-min period. Bile was collected over 40 min, mixed, and a sample of 5 ml was obtained, while the remainder was returned to the duodenum.

In order to compare this double label, single intubation technique with the method of Lindstedt, bile samples were obtained in the same manner in the fasting state on 3 subsequent days.

Principle of the method

The principle of the method is based on the assumption that, in normal subjects, a tracer dose of a radioactive bile acid mixes homogenously with the endogenous bile acid pool and that the decrease of the specific activity of the labeled bile acid in bile follows first order kinetics. The decay of the specific activity of labeled cholic acid can be expressed: $SA_t = SA_0e^{-kt}$, where SA_0 and SA_t are the specific activities at time 0 and time t, respectively; k is the fractional turnover rate (days^{-1}). The half-life, $T_{1/2}$, is calculated by the formula $T_{1/2} = 0.693/k$.

Assuming that the decay constants of [^{14}C]cholic acid and [^{3}H]cholic acid are equal, a constant 24-hr interval exists at each point of the specific activity decay curve for the specific activities $SA_{t\,^{14}C}$ and $SA_{t\,^{3}H}$. For the sake of clarity, the specific activity related to ^{14}C is expressed in capital letters (SA), the specific activity related to ^{3}H in lower case letters (sa).

The calculation of the pool size and turnover of cholic acid using two labeled bile acids, [^{14}C]cholic acid and [^{3}H]cholic acid, can be described as follows:

$$SA_t = SA_0e^{-kt} \qquad 1)$$

$$sa_t = sa_0e^{-kt} \qquad 2)$$

where:

$$SA_0 = \frac{\text{dpm}^{14}\text{C}}{\text{miscible cholic acid pool}}$$

$$sa_0 = \frac{\text{dpm}^{3}\text{H}}{\text{miscible cholic acid pool}}$$

The ratio between Equation 1 and Equation 2 at the time of sampling (t_3) is:

$$\frac{SA_{t_3}}{SA_{t_3}} = \frac{SA_0e^{-k2}}{sa_0e^{-k1}} = \frac{\text{dpm}^{14}\text{C}}{\text{dpm}^{3}\text{H}}e^{-k} \qquad 3)$$

By replacing

$$\frac{SA_{t_3}}{sa_{t_3}} = R_{t_3} \quad \text{and} \quad \frac{SA_0}{sa_0} = R_0,$$

Equation 3 can be written:

$$R_{t_3} = R_0e^{-k} \qquad 4)$$

As R_0 is known (dpm^{14}C/dpm^{3}H) and R_{t_3} can be determined, the decay-constant can easily be calculated.

$$\ln \frac{R_0}{R_{t_3}} = k \qquad 5)$$

and

$$T_{1/2} = \frac{0.693}{k}$$

When k is known, the miscible cholic acid pool size can be calculated from the general formula:

$$SA_t = SA_0e^{-kt}$$

and

$$sa_t = sa_0e^{-kt}$$

Methods of analysis

Bile acids were separated on an X.A.D.-2 column and were washed from the column with methanol. The methanol solution was dried and the bile acids were redissolved in 1 ml of methanol. Three-tenths ml was spotted on a Silicagel G-plate (Merck, Darmstadt, Germany) and the bile acids were separated by two-dimensional chromatography. The first solvent was isooctane–diisopropylether–acetic acid–n-butanol–isopropanol–water 10:5:5:3:6:1. The plates were dried in air overnight; the following day, the bile acids were run in the second solvent: propionic acid–isoamylacetate–propanol–water 15:20:10:5. The radioactivity was localized by scanning (Berthold LB 2723). The spots, containing cholic acid conjugates, were extracted with methanol.

The ^{14}C and ^{3}H radioactivity of each spot was separated by oxidation (oxydizer, Packard, IL) and the bile acid content was determined enzymatically by the α-hydroxysteroiddehydrogenase method. The specific activities of [^{14}C] and [^{3}H]cholic acid were calculated and the R_t value was determined.

Statistics

Results were expressed as mean ± SD. Regression analysis was carried out between the data obtained by the single intubation method and Lindstedt's method.

RESULTS

The data on the kinetics and the pool size of cholate obtained by the double-label single intubation technique are compared with those obtained by the Lindstedt method in **Table 1.**

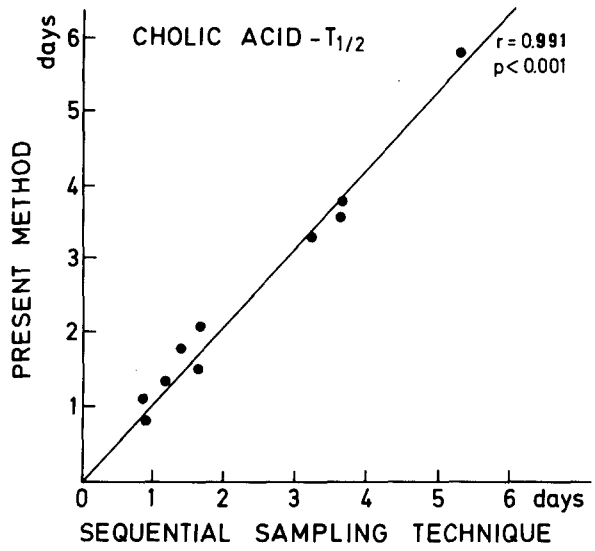

Fig. 1. Correlation between the cholic acid half life measured with the single intubation method and Lindstedt's technique in ten normal subjects.

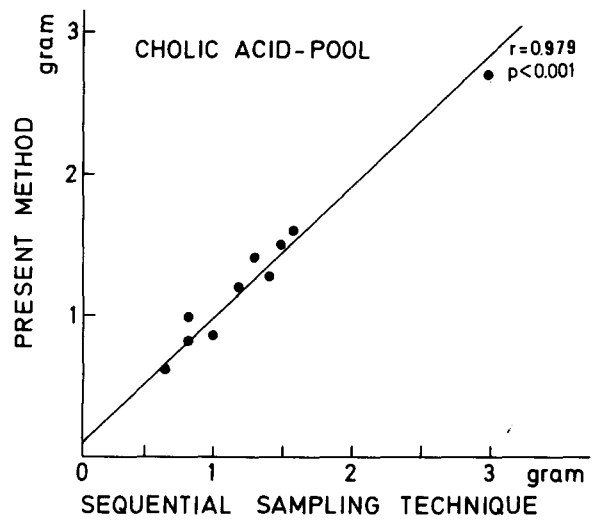

Fig. 2. Correlation between the cholic acid pool size measured with the single intubation method and Lindstedt's method in ten normal subjects.

The construction of the specific activity time plot on semi-logarithmic axes in the multiple-sampling technique was done with the aid of eight specific activity values (four for each isotope). The correlation coefficients of the cholic acid decay curves were excellent (r ranging from 0.955 to 0.999). The correlation ($r = 0.991$) between the half-lives measured with the single intubation and the Lindstedt technique and the correlation ($r = 0.979$) between the cholate pool size measured with the single intubation and the Lindstedt method (**Figs. 1 and 2**) were very significant (both $P < 0.001$). The half-life of labeled cholate in these normal subjects averaged 2.4 ± 1.5 days, and the cholate pool size was 1.336 ± 0.687, using Lindstedt's technique; the corresponding values using the single intubation technique were 2.5 ± 1.6 days and 1.344 ± 0.598, respectively.

The reproducibility of the method was excellent. There was a good correlation of the ratio determined at day 3 and the three subsequent days: $r = 0.96$, $P < 0.001$. In addition, in two normal volunteers, the value of the turnover and the pool size of cholic acid were determined with 1-month interval. The data are summarized in **Table 2.**

DISCUSSION

The present method allows accurate measurement of bile acid pool size and turnover by a single

TABLE 1. Cholate, turnover and pool size in normal subjects

Subjects	Ratio Method		Sequential Sampling Method		
	$T_{1/2}$	Pool Size	$T_{1/2}$	Correlation	Pool Size
	days	*g*	*days*	*r*	*g*
C.G.	5.8	1.516	5.4	−0.984	1.466
S.L.	1.3	0.840	1.2	−0.999	0.803
N.R.	0.8	0.859	0.9	−0.992	1.010
G.A.	1.8	1.431	1.4	−0.990	1.254
D.M.	3.6	1.179	3.7	−0.966	1.209
F.M.	2.1	0.618	2.2	−0.986	0.631
D.W.	1.1	1.023	0.9	−0.991	0.808
D.F.	1.5	2.714	1.7	−0.981	3.049
C.L.	3.8	1.642	3.7	−0.958	1.555
S.X.	3.3	1.615	3.3	−0.955	1.577
Mean ± S.D.	2.5 ± 1.6	1.344 ± 0.598	2.4 ± 1.5		1.336 ± 0.687

TABLE 2. Reproducibility of cholic acid turnover and pool size in two healthy volunteers (The studies were done with one month interval)

Subject		$T_{1/2}$	Pool
		days	*g*
R.V.	At start	5.6	1.395
	After 1 month	5.2	1.069
D.T.	At start	3.7	1.818
	After 1 month	4.2	1.945

intubation. The method is based on the calculation of the ratio of specific activities of [^{14}C]cholic acid and [^{3}H]cholic acid administered intravenously with a 24-hr interval period. The method has been validated by comparison with the results obtained with Lindstedt's method. The results for half-life and pool size obtained with both methods correlated perfectly. The single intubation method previously developed for measuring the bile acid pool size is inaccurate and does not allow determination of bile acid turnover. The present method allows a good estimate of the bile acid pool size as well as the bile acid turnover. Complete mixing of the labeled bile acids with the endogenous bile acid pool is guaranteed because sampling is done 24 hr after the second and 48 hr after the first administration of the labeled bile acid. Multiple tube positioning and duodenal sampling are avoided. The method can also be used for simultaneous kinetic studies of cholic acid and chenodeoxycholic acid when deoxycholic acid and chenodeoxycholic acid are separated appropriately (14). In conclusion, the above described method is a simple method for the measurement of bile acid kinetics.

The authors acknowledge the technical assistance of Mrs. S. Rutten and Mr. L. Verhaegen. This research was sponsored by grants from the "Fonds voor Wetenschappelijk Geneeskundig Onderzoek van België", Brussels, Belgium.

Manuscript received 17 June 1980 and in revised form 4 November 1980.

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