The contribution of newly synthesized cholesterol to bile salt synthesis in rats quantified by mass isotoeppomer distribution analysis

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Abstract

A new stable isotope procedure has been developed and validated in rats, applying [1-13C]acetate infusion to quantify the production of bile salts from de novo synthesized cholesterol making use of the mass isotoeppomer distribution analysis (MIDA) principle. Ions (m/z) 458-461, 370-373 and 285-288 were monitored by GC/MS (EI-mode) for the methyl trimethylsilyl ether derivatives of cholate, chenodeoxycholate and β-muricholate, respectively. Rats with intact exteriorized enterohepatic circulation and rats with chronic bile diversion were infused with [1-13C]acetate for up to 14 h. After 10 h of infusion the enterohepatic circulation of the intact group was interrupted to deplete the existing bile salt pool (acute bile diversion). The fractions of biliary cholesterol and individual bile salts derived from newly synthesized cholesterol were determined by MIDA at t=14 h. In rats with acute bile diversion, these fractions were 20, 25, 27 and 23% for biliary cholesterol, cholate, chenodeoxycholate and β-muricholate, respectively. After bile diversion for 8 days to induce hepatic cholesterol and bile salt synthesis, these fractions increased significantly to 32, 47, 41 and 47%, respectively. Calculated enrichments of the acetyl-CoA precursor pools were similar for all bile salts and biliary cholesterol within the two rat groups. However, chronic enterohepatic interruption decreased the acetyl-CoA pool size almost two-fold. We conclude that MIDA is a validated new stable isotope technique for studying the synthetic pathway from acetyl-CoA to bile salts. This technique provides an important new tool for studying bile salt metabolism in humans using stable isotopes. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: MIDA; Bile salt; Bile salt synthesis; Cholesterogenesis; Stable isotope; Mass spectrometry

1. Introduction

Bile salts are of key importance for the generation of bile flow, for biliary secretion of cholesterol and phospholipids and for efficient absorption of dietary fats. In addition, bile salts are crucial for the maintenance of cholesterol homeostasis: conversion of
cholesterol to bile salts comprises a major route for removal of cholesterol from the body.

Little is known about the origin and regulation of the cholesterol pool(s) used for bile salt synthesis. Preformed and newly synthesized cholesterol do not necessarily form a homogenous pool in the liver for secretion into bile or plasma or for use in bile salt synthesis. In fact, the observation that enzymes controlling the rate-limiting steps of cholesterol and bile salt synthesis, i.e. HMG CoA-reductase, cholesterol 7α-hydroxylase and sterol 27-hydroxylase, are physically separated in the liver [1], indicates that these processes may not be functionally linked to each other. Nevertheless, close linkage in activity appears to exist for these enzymes under most experimental conditions [2,3].

Isotopic studies have been performed in rats and hamsters, using mainly 3H2O and 14C-labeling techniques, to quantify the relative amount of biliary bile salts and biliary cholesterol derived from newly synthesized cholesterol [4-9]. Comparison of the different studies reveals a great variability in outcome and has led to different conclusions about the predominant origin of bile salts and biliary cholesterol under in vivo conditions. A problem of most studies was that they were performed using animal models in which the enterohepatic circulation of bile salts was chronically interrupted. It is well known that interruption of the enterohepatic circulation leads to an upregulation of cholesterol and bile salt synthesis [3,9,10]. In fact, Schreibner et al. have shown by 3H2O-incorporation techniques that such an interruption causes a significant increase in the proportion of bile salts derived from newly synthesized cholesterol [9]. Finally, to our knowledge, no stable isotope technique exists to determine metabolic origin of bile salts which can be applied both in animals as well as in human subjects.

Recently, Hellerstein et al. [11] developed a stable isotope method, termed mass isotopomer distribution analysis (MIDA), to study synthesis rates of biological polymers in vivo. An additional major advantage of MIDA is that it allows determination of the actual precursor pool enrichment of these polymers [11]. This method has already been used for studying synthesis of cholesterol [12,13], glucose [14], fatty acids [15] and albumin [16]. Quantification of bile salt synthesis from newly synthesized cholesterol is theoretically also possible with an adaptation of this technique. The use of non-radioactive labeling materials would allow its use in human studies on bile salt metabolism.

Our aim was to develop and validate a MIDA procedure for individual primary bile salts that can be applied in humans. Furthermore we wanted to quantify the contribution of newly synthesized cholesterol to bile salt synthesis under physiological conditions. Finally we wanted to compare the acetyl-CoA precursor pools from which cholesterol and bile salt are synthesized, which cannot be determined by existing techniques. This would allow us to gain insight into the interactions between the metabolic pathways of cholesterol and bile salt synthesis. For our validation studies, we used a rat model that allows measurements under physiological conditions, i.e. without upregulation of bile salt synthesis, and compared the results with those in rats with chronic interruption of the enterohepatic circulation.

2. Materials and methods

2.1. Animals

Male Wistar rats (290–410 g) were maintained in a light- (lights on 06.00–18.00 h) and temperature- (21°C) controlled room and fed normal laboratory chow (RMH-B, Hope Farms BV, Woerden, the Netherlands) ad libitum. Animals were equipped with permanent catheters in bile duct and duodenum. The two catheters were connected to each other during the operation session to maintain an intact enterohepatic circulation. During the same operating session, animals were provided with a heart catheter via the left jugular vein. The catheter was used for continuous infusion of [1-13C]acetate [10]. In a number of rats, the enterohepatic circulation was interrupted after day 1 by connecting the bile duct catheter to a fraction collector. Rats were used for experiments 8 days after surgery, i.e. when preoperative body weights and normal food intake were re-established. Experimental procedures were approved by the local Ethics Committee for Animal Experiments.
2.2. **Experimental procedures**

Rats were divided into two groups. Rats with an initial intact enterohepatic circulation (control group) were infused with $^{[1-13]C}$acetate at a rate of about 1.5 mmol/h/kg b.wt. from 10.00 to 24.00 h. A small bile sample (150 µl) was taken at the start of the infusion for baseline calculations. The enterohepatic circulation in these rats was interrupted during the last 4 h of the infusion period to deplete the bile salt pool. Bile was collected continuously in 0.5 h samples after the interruption. The second group consisted of rats with a chronically interrupted enterohepatic circulation. These rats were infused with $^{[1-13]C}$acetate at a lower rate of about 0.6 mmol/h/kg b.wt. from 12.00 to 24.00 h. Bile was collected continuously in 1 h samples. The difference in acetate infusion between the two groups was necessary, since $^{13}$C enrichments turned out to be too low in the bile salts of the control group when infused at a rate of 0.6 mmol/h/kg b.wt. Bile production was determined gravimetrically. During the experiments, food was removed.

2.3. **Analytical methods**

2.3.1. **Biliary lipids and bile salts**

Cholesterol was extracted from bile samples using a Bligh and Dyer extraction [17] and derivatized to the TMS derivative using $N,O$-bis-(trimethylsilyl)trifluoracetamide with 1% trimethylchlorosilane at room temperature [12]. Samples were dried under nitrogen and dissolved in hexane. The bile salts were hydrolyzed enzymatically with cholyglycine hydrolase and extracted with Seppak C-18 cartridges (Waters Millipore, Milford, MA, USA), as described by Setchell et al. [18], followed by elution of the free bile acids with methanol/water (75:25 v/v). Bile acids were subsequently methylated with methanol/acetylchloride, 20:1 (v/v) at 60°C for 30 min. Silylation was accomplished by adding a solution containing $N,O$-bis-(trimethylsilyl)trifluoracetamide, pyridine and trimethylchlorosilane, 5:4:1 (v/v/v) and derivatizing for 60 min at room temperature. Samples were evaporated to dryness and redissolved in hexane.

2.3.2. **Gas chromatography-mass spectrometry**

Samples were analyzed by gas chromatography-mass spectrometry. Cholesterol TMS derivatives were separated with a HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA), using a 25 m ×0.25 mm (0.1 µm film thickness) OV1 capillary column (CP-Sil 5 CB, Chrompack, Middelburg, the Netherlands) directly inserted into the ion source of a magnet sector mass spectrometer, model 70-250S (Micromass, Manchester, UK). The oven temperature was programmed from 120 to 260°C at 20 °/min, to 280°C at 2.5 °/min, and then to 300°C at 20 °/min. A splitless injection was applied. The mass spectrometer was used in the EI-mode at 70 EV. The mass fragments $m/z$ 368, 369, 370 and 371 were monitored by selected ion recording as described before [19]. The area ratios $m/z$ 369/368, 370/368, and 371/368 were calculated and introduced into the MIDA calculation software.

The bile salt derivatives were separated with a HP 5890 gas chromatograph, using a 50 m ×0.25 mm (0.2 µm film thickness) capillary OV1701 column (CP-Sil 19 CB, Chrompack, Middelburg, the Netherlands) directly inserted into the ion source of a Finnigan SSQ 7000 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA). The oven temperature was programmed from 80°C (1 min) to 280°C at 30 °/min. A splitless injection was applied. The mass spectrometer was used in the EI mode at 70 EV. Isotope abundance measurements were carried out by selected ion recording for cholate, chenodeoxycholate and β-muricholate simultaneously. Various mass fragments of the three bile salts were tested to study their suitability for MIDA analysis, as defined by reproducibility and comparison to theoretical values for isotopomer distributions. The mass fragments $m/z$ 458, 368 and 253 were tested for the trihydroxylated cholate, where the 458 fragment contains a single TMSOH group, which is lost in the $m/z$ 368 fragment. Loss of the side chain leads to the 253 fragment. The mass fragment $m/z$ 370, which has lost all TMSOH groups, was tested for the dihydroxy bile salt chenodeoxycholate. The fragments $m/z$ 195 and 285 tested for the trihydroxylated β-muricholate are formed by the A ring and C-6 of the basic bile salt nucleus with one and two TMSOH groups, respectively. Dwell times at the various mass ions were adjusted to the chromatographic peak width ensuring minimally 20 data points on each chromatographic peak. Furthermore, conditions
were adjusted to obtain sufficient ion intensity. The area ratios were calculated and introduced into the MIDA calculation software.

2.4. Calculations

The theoretical background of the MIDA technique is described in detail elsewhere [11,12]. Briefly, MIDA allows to determine the enrichment of the pool of acetyl-CoA precursor units that has entered newly synthesized cholesterol or bile salt molecules during the course of the [1-13C]acetate infusion. This is achieved by analysis of the isotopomer pattern of the molecules of interest and comparison with a theoretical table generated using binomial expansion and known isotope frequencies of the atomic isotopes. During the [1-13C]acetate infusion, the pool of acetate will reach a stable enrichment and newly synthesized cholesterol molecules will contain a certain amount of labeled acetate units and thereby yield different isotopomers (M0, M1, M2...). From the isotopomer pattern that is thus formed, the acetyl-CoA pool enrichment (P) can be calculated. From the value of P, the maximal enrichment of each isotopomer, i.e. the theoretical situation in which all molecules are newly synthesized, can be calculated. Comparison of this maximal enrichment with the measured enrichment of a certain isotopomer during the experiment reveals the fraction of newly synthesized molecules (f). For cholesterol, f represents the fraction of newly synthesized free cholesterol. In the case of bile salts, fbs represents the relative amount of bile salts made from cholesterol molecules synthesized during the period of the [1-13C]acetate infusion. Absolute bile salt synthesis rates were calculated by multiplying bile flow with bile salt concentrations at the end of the experiment (24.00 h). Total synthesis of newly synthesized bile salts was calculated by multiplying total bile salt synthesis rates with fbs.

2.5. Statistical analysis

All values are expressed as mean ± S.D. Differences of f values and total synthesis values between experimental groups were analyzed using the non-parametric Mann-Whitney test. Differences of P values and f values within experimental groups were analyzed using the non-parametric Friedman test for related samples with Wilcoxon as post hoc test, with correction for sample size of four.

3. Results

3.1. Mass spectra

The EI mass spectra of cholate, chenodeoxycholate and β-muricholate are shown in Fig. 1. The mass fragments m/z 253, 368 and 458 for cholate, m/z 370 for chenodeoxycholate and m/z 195 and 285 for β-muricholate were initially selected as potential candidates for selected ion monitoring analysis. For cholate, m/z 253 appeared unsuitable due to un-

Table 1

| Isotope ratios of mass fragments for MIDA analysis for cholesterol, cholate, chenodeoxycholate and β-muricholate obtained from basal, unenriched rat bile samples |
|-----------------|-----------------|-----------------|-----------------|
|                  | Cholesterol     | Cholate         | Chololate       |
|                  | m/z 368, 369, 370, 371 | m/z 458, 459, 460, 461 | m/z 370, 371, 372, 373 |
| M1/M0            | 30.63 ± 0.12 (0.40) | 31.19           | 37.40 ± 0.18 (0.49) |
| M2/M0            | 5.08 ± 0.07 (1.32)  | 4.69            | 10.09 ± 0.04 (0.45) |
| M3/M0            | 0.74 ± 0.02 (3.11)  | 0.45            | 1.98 ± 0.02 (2.02)  |
|                  |                  |                 |                  |
| Chenodeoxycholate|                  | β-Muricholate   |                  |
| m/z 370, 371, 372, 373 |                | m/z 285, 286, 287, 288 |
| M1/M0            | 29.75 ± 0.04 (0.14) | 28.90           | 26.03 ± 0.11 (0.41) |
| M2/M0            | 4.80 ± 0.03 (0.72)  | 4.43            | 10.30 ± 0.04 (0.36) |
| M3/M0            | 0.44 ± 0.02 (2.64)  | 0.48            | 1.78 ± 0.03 (1.26)  |

Data are expressed as mean percentage ± S.D., (covariance percentage) and theoretical values.
Fig. 1. Electron impact mass spectra of the methyl-TMS-derivatives of cholate (A), chenodeoxycholate (B) and β-muricholate (C). Arrows indicate fragments used for MIDA analysis.
known interference at \( m/z \) 256. Large deviations from theoretical values were observed for all isotope ratios based on \( m/z \) 368. The mass ions \( m/z \) 458 (cholate), 370 (chenodeoxycholate) and 285 (\( \beta \)-muricholate) appeared to give precise and accurate isotope abundance values when sufficient ion intensities were obtained (Table 1). The data for the isotope abundance measurements for cholesterol are included for comparison. No effect of biological interferences was observed when measurements were compared between artificial bile salt mixtures or rat bile samples. The concentration of the final analysis sample, sample injection volume and mass spectrometric conditions were adapted to ensure sufficient ion intensities in all cases.

3.2. Effect of acute enterohepatic interruption on bile production

Fig. 2 shows bile flow, cholesterol and total bile salt output in control rats immediately after interruption of the enterohepatic circulation, representing the time span from the 10th to the 14th hour of the \([1-13C]\) acetate infusion. Bile flow decreased after interruption from 0.54 ± 0.15 ml/100 g/h to 0.24 ± 0.05 ml/100g/h after four h. Bile salt and cholesterol output decreased during this time course by 85 and 55%, respectively. These data are compatible with previous studies from our laboratory [3,10]. Arrows indicate samples used for the MIDA analysis.

3.3. Fractional synthesis values

The \( f \) of biliary cholesterol and \( f_{bs} \) are shown in Fig. 3 for control rats after acute pool depletion (see arrows Fig. 2) as well as for the chronically bile-diverted animals. The \( f_{bs} \) increased significantly after long-term bile diversion compared to short-term bile diversion. Although a significant difference existed between the \( f_{bs} \) values and \( f \) values of biliary cholesterol in all rats with acutely and chronically depleted bile salt pools, post hoc testing with corrected \( \alpha \) of each subgroup revealed no significant difference.

3.4. Absolute bile salt synthesis

Chronic bile diversion led to a significant upregu-
lation of absolute bile salt synthesis compared to acute bile diversion (Table 2). More cholate than chenodeoxycholate or \(\beta\)-muricholate was produced. The absolute amount of the three main bile salts produced from newly synthesized cholesterol increased significantly by chronic bile diversion: approximately 0.29 and 2.54 \(\mu\)mol/100 g/h of newly synthesized cholesterol was converted to the three bile salts in control and bile-diverted rats, respectively.

### 3.5. Acetyl-CoA pool enrichments

Precursor pool enrichments \((P)\) of the individual bile salts and biliary cholesterol were compared (Table 3). Enrichments reached stable values after 3 h of \([1-13C]\)acetate infusion in cholesterol and all bile salts and did not change during the course of the infusion period (data not shown). There were no significant differences between the different bile salts within each experimental group nor existed a significant difference between the \(P\) values of the bile salts and biliary cholesterol. Since \([1-13C]\)acetate infusion rates for both rat groups were different no direct comparison between the \(P\) values of those groups was possible. However, they could be compared by dividing \(P\) values by \([1-13C]\)acetate infusion rates. These ratios are also shown in Table 3. Average \(P\) per mmol/kg/h infused \([1-13C]\)acetate was significantly higher \((P < 0.05)\) in rats with chronic bile diversion compared to acutely interrupted rats.

### 4. Discussion

No stable isotope technique is available for studying the metabolic pathway from acetyl-CoA to bile salts which can be applied in human studies. Bile salts can be synthesized from several sources: newly formed or preformed, hepatic or lipoprotein-derived cholesterol. Since an important function of bile salt synthesis is the removal of excess cholesterol from the body into the feces, it is important to gain insight

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**Table 2**

Total synthesis and synthesis from newly synthesized cholesterol of cholate (C), chenodeoxycholate (CDC) and \(\beta\)-muricholate (MC) in rats with acute (control, \(n = 4\)) and chronic (\(n = 4\)) interruption of the enterohepatic circulation measured at midnight

<table>
<thead>
<tr>
<th></th>
<th>Total synthesis ((\mu)mol/100 g/h)</th>
<th>Total synthesis from newly synthesized cholesterol ((\mu)mol/100 g/h)</th>
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</thead>
<tbody>
<tr>
<td><strong>Acute interruption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.59 ± 0.11</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>CDC</td>
<td>0.41 ± 0.22</td>
<td>0.12 ± 0.10</td>
</tr>
<tr>
<td>MC</td>
<td>0.11 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>(\Sigma)</td>
<td>1.12 ± 0.08</td>
<td>0.29 ± 0.10</td>
</tr>
<tr>
<td><strong>Chronic interruption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.82 ± 0.21(^a)</td>
<td>1.80 ± 0.32(^a)</td>
</tr>
<tr>
<td>CDC</td>
<td>1.54 ± 0.12(^a)</td>
<td>0.63 ± 0.24(^a)</td>
</tr>
<tr>
<td>MC</td>
<td>0.23 ± 0.04(^a)</td>
<td>0.11 ± 0.07(^b)</td>
</tr>
<tr>
<td>(\Sigma)</td>
<td>5.59 ± 0.08(^a)</td>
<td>2.54 ± 0.48(^a)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of four rats in each group.

\(^a\)Significantly different \((P < 0.05)\) from rats after acute interruption of the enterohepatic circulation.
into factors that determine the contribution from different sources.

Various methods have been used to study the metabolic origin of biliary cholesterol and bile salts [4-9] in experimental animals, using mainly $^3$H$_2$O incorporation. These studies have yielded wide variations in outcome for the relative amount of bile salts derived from newly synthesized cholesterol. The disadvantage of using radiolabeled tracers and the difficulty of establishing with certainty the amount of $^3$H atoms transferred from H$_2$O into each cholesterol molecule [20,21], present certain limitations to the use of these methods and interpretation of results obtained. Additionally, no information is gathered on the actual acetyl-CoA pool(s) from which bile salts and cholesterol are being synthesized. Finally, most of these studies have made use of rats with a chronically interrupted enterohepatic circulation. It is well known that chronic bile diversion leads to strong upregulation of both cholesterol and bile salt synthesis [3,9,10].

In this study, we tried to adapt the MIDA technique for studying bile salt metabolism and to validate the technique in rats. MIDA has been used to study de novo lipogenesis, cholesterogenesis, gluconeogenesis and protein synthesis in humans and experimental animals [11,12,14-16]. This technique has various advantages over existing, mostly radiolabeled, techniques. Stable isotopes are used, which makes it safe for use in human subjects. Furthermore, short-term effects can be detected and finally, enrichments of the actual precursor pools from which the polymers are synthesized can be calculated. Since this technique depends on accurate GC-MS measurements of the isotope ratios of the selected mass fragments, we compared isotope ratios measured for various mass fragments of the bile salt derivatives. The isotope ratio values of the fragments that were finally chosen closely resembled theoretical values and were highly reproducible both in reference standards and in bile samples.

In the present study, we used rats with acute or long-term interruption of the enterohepatic circulation. We have shown previously that 4 h of bile diversion is sufficient to deplete the bile salt pool in rats [3,10], after which time point, all of the secreted bile salts are newly synthesized by definition. Furthermore, increases in hepatic cholesterogenesis and bile salt synthesis do not occur during this short-term bile diversion [9,22]. From the results here we conclude that, under physiological conditions as well as during upregulation of cholesterol and bile salt synthesis through long-term bile diversion, most of the bile salts are made from preformed cholesterol rather than from newly synthesized cholesterol. Using the new MIDA technique, we were able to obtain values comparable with values found by Scheibner et al. [9] (12-19%) in rats after 6 h of bile diversion.

Conflicting results have been reported concerning the hepatic cholesterol pool(s) used for biliary cholesterol secretion and bile salt synthesis [4,7-9,23]. In a number of studies [5,8,9] different proportions for the relative contribution of de novo cholesterol used for cholate, chenodeoxycholate and $\beta$-muricholate synthesis have been found, suggesting that different cholesterol pools are used for the formation of the different bile salts. Our study shows a trend where the $f_{bs}$ of individual bile salts are higher than $f$ for biliary cholesterol, both in acute bile diversion and in the chronically bile-diverted animals. The $f_{bs}$ of indi-

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### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Acute bile diversion</th>
<th>Chronic bile diversion</th>
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<tr>
<td></td>
<td>$P$</td>
<td>$P/[1-^{13}C]$acetate infusion rate</td>
</tr>
<tr>
<td>Chol</td>
<td>0.09 ± 0.03</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>C</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>CDC</td>
<td>0.09 ± 0.02</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>MC</td>
<td>0.10 ± 0.02</td>
<td>0.07 ± 0.01</td>
</tr>
</tbody>
</table>

*Corrected* $P$ values were calculated by dividing $P$ values by mmol/kg/h of infused [1-^{13}C]acetate. Values are expressed as mean ± S.D. of four rats in each group. There was no significant difference between the individual bile salts nor between bile salts and cholesterol within each rat group.

*Significantly $(P<0.05)$ different from acutely interrupted rats.
individual bile salts within each group were similar, providing evidence against the existence of different cholesterol subpools for formation of individual bile salts.

An advantage of the MIDA technique is that it allows determination of the $^{13}$C enrichments of the acetyl-CoA pools from which biliary cholesterol and bile salts are synthesized. Our results show similar precursor pool enrichments ($P$) for biliary cholesterol and the individual bile salts. In an earlier study in rats [19], we have shown similar $P$ values for plasma cholesterol and biliary cholesterol. These results indicate that plasma cholesterol, biliary cholesterol and cholesterol used for bile salt synthesis originate from the same, or at least from an isotopically well-mixed, acetyl-CoA pool. Furthermore, the higher $P$ values corrected for infusion rates in rats with chronic interruption of the enterohepatic circulation compared to acutely interrupted animals could be related to the greatly elevated sterol synthesis rates in these animals, which may decrease the acetyl-CoA pool size.

Thus, in the present study, we have developed and validated a MIDA technique that can be applied to quantitate metabolic pathways leading to bile salt formation in rats. Applying this technique, we have found that newly synthesized cholesterol is preferentially used for bile salt synthesis rather than for direct secretion as free cholesterol into bile, but the data do not support the existence of separate precursor pools for formation of individual bile salts. The MIDA technique is based on use of stable isotopes and therefore preferable over techniques using radiolabeled compounds for use in humans. In humans, however, it is obviously not possible to deplete the bile salt pool as we did in the current study. This problem can be circumvented by measuring the bile salt pool size and bile salt synthesis rate using an additional pool dilution approach [24]. We are therefore currently adapting the MIDA bile salt technique, in combination with the isotope dilution technique to evaluate the origin of cholesterol used for bile salt synthesis in human subjects.

Acknowledgements

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