

CHANGES IN THE BILE-ACIDS COMPOSITION OF BILE JUICE AFTER FAT INTAKE

SCHIMBĂRI ÎN COMPOZIȚIA ACIZILOR BILIARI AI SUCULUI BILIAR DUPĂ INGESTIA DE GRĂSIME

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A high fat intake increases the flow of bile or changes the composition of bile acids in refluxed duodenal contents, and then plays an important role in the developmental precancerous lesion of BE and leading to esophageal adenocarcinoma EAC. Wistar rats were divided into three groups based on their diet: a control group (fed with standard diet, containing 4.20% soybean oil), a second group (fed with a low cow-fat diet, containing 4.20% cow fat) and the third group (fed with a high cow-fat diet, containing 16.8% cow fat). The TCA value detected in the animals fed the high cow-fat diet (median concentration, 13.8 ± 2.42 mmol/L) was significant increased comparative with those detected of animals fed the standard diet (8.15 ± 1.22 mmol/L). The TDCA value in the high cow-fat group (2.64 ± 0.97 mmol/L) was significantly increased comparative with those detected of animals fed the standard diet (1.66 ± 0.50 mmol/L).

Keywords: fat intake, bile acids

Introduction

A high level of dietary-fat intake increases the flow of bile into the intestine, and bile or bile acids promote gastroesophageal reflux disease (GERD) and gastric carcinogenesis (El-Serag et al., 2005). It is widely accepted that bile acids, which are important constituents of duodenal fluid, are associated with the development of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC), (Wild et al., 2003). The dietary fat (especially, the consumption of red fat meat) promotes the excretion of bile acids, which can be converted into carcinogens. Miwa et al. (1996) reported that rats with duodenal-contents reflux that were fed high-fat food showed an increased incidence of gastric adenocarcinoma.

The aim of this study was to observe the quantitative modifications of bile acids after high fat intake because they play an important role in the development of a precancerous lesion of BE and leading to esophageal adenocarcinoma EAC.

Materials and Methods

All procedures complied with the ethical guidelines for animal experimentation were used in our laboratory. Twenty four Wistar rats weighing about 120 g from Biobaza Cantacuzino were divided into three groups based on their diet as follows: a control group (animals fed with standard diet, containing 4.20% soybean oil), a second group (animals fed with a low cow-fat diet, containing 4.20% cow fat) and the third group (animals fed with a high cow-fat diet, containing 16.8% cow fat). Their constituents are summarized in table 1.

Table 1.

Comparison of the components of each diet:
standard diet, low cow-fat diet and high cow-fat diet

Diet	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrate (%)	Energy (Caleries/100 g)
Standard	26.8	4.2	5.5	6.5	50.3	355.4
Low cow fat	26.8	4.2	5.5	6.5	50.3	355.4
High cow fat	23.2	16.8	4.5	5.5	45.2	440.3

After six months and 24 h last feeding, the bodyweights of the rats were measured. The animals underwent an upper-median incision under inhalation anesthesia with diethylether. To examine the effect of various types of dietary intake on the metabolism of bile acids, the bile juice was collected directly from the common bile duct using a needle connected to a syringe. It aspirates bile juice to a volume of approximately 2.0 mL. The collected bile juice was stored at -80°C until the further analysis. Total bile acid was assayed enzymatically using commercially available reagent kits Roti-Star Gold. The bile acids were evaluated using high-performance liquid chromatography with analyser type LC-2060 Plus, Jasco sign. The bile acids included: cholic acid, deoxycholic acid, lithocholic acid, chenodeoxycholic acid, ursodesoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, glycochenodeoxycholic acid, glyoursodesoxycholic acid, taurocholic acid (TCA), taurodeoxycholic acid (TDCA), tauroolithocholic acid, taurochenodeoxycholic acid, and taoursodesoxycholic acid. After the animals were sacrificed, we collected and measured the pH of the gastric contents of the animals from the three groups using a pH meter.

Results for the bile acids in bile juice, bodyweights of the animals, and the pH of the gastric contents were expressed as mean \pm SD. Statistical evaluation of these parameters was carried out using Student's t-test.

Results and Discussion

The average bodyweight of the animals fed the high cow-fat diet (420.5 ± 36.3 g) was higher than that of the animals fed the standard diet (320.4 ± 24.6 g) and low cow-fat diet (336.3 ± 18.0 g). The TCA value (table 2) detected in the animals fed the high cow-fat diet (median concentration, 13.8 ± 2.42 mmol/L) was significant increased comparative with those detected of animals fed the standard diet (8.15 ± 1.22 mmol/L). The TDCA value in the high cow-fat group (2.64 ± 0.97 mmol/L) was significantly increased comparative with those detected of animals fed the standard diet (1.66 ± 0.50 mmol/L). On the contrary, there were no significant differences in the concentrations of TCA and TDCA among the animals fed standard diet and the low cow-fat diet.

Table 2

Bile acid composition and total bile acid (mmol/L)
of bile juice aspirated from the common bile duct of the animals

File of bile acids	Standard diet	Low cow fat diet	High cow fat diet
Free bile acids			
Cholic acid	0.52 ± 0.26	0.56 ± 0.30	0.60 ± 0.32
Deoxycholic acid	n.d.	n.d.	n.d.
Lithocholic acid	n.d.	n.d.	n.d.
Chenodeoxycholic acid	n.d.	n.d.	n.d.
Ursodesoxycholic acid	n.d.	n.d.	n.d.
Glycine conjugates			
Glycocholic acid	3.35 ± 1.32	3.40 ± 1.35	3.80 ± 1.78
Glycodeoxycholic acid	0.46 ± 0.30	0.48 ± 0.46	0.52 ± 0.32
Glycolithocholic acid	n.d.	n.d.	n.d.
Glycochenodeoxycholic acid	n.d.	n.d.	n.d.
Glycoursodesoxycholic acid	0.10 ± 0.00	n.d.	n.d.
Taurine conjugates			
Taurocholic acid	8.15 ± 1.22	8.63 ± 1.46	13.8 ± 2.42
Taurodeoxycholic acid	1.66 ± 0.50	1.82 ± 0.59	2.64 ± 0.97
Tauroolithocholic acid	n.d.	n.d.	n.d.
Taurochenodeoxycholic acid	0.96 ± 0.09	0.99 ± 0.09	0.93 ± 0.08
Tauroursodesoxycholic acid	0.18 ± 0.10	0.20 ± 0.11	0.20 ± 0.12
Total bile acid	15.38 ± 1.70	16.08 ± 1.60	22.49 ± 2.10

n.d., not determined: less than the limit of sensitivity
(the value of the detection limit is under 0.1 mmol/L);

On opening the stomach for collecting and measuring the pH of its contents we found the duodenogastric reflux. The fats presence in the duoden and the increase of the bile quantity interfere in the decrease of motility in the distal area of the stomach and in the mechanism of changes of pyloric local neurotransmitters. Two main peripheral neurons mediate active contraction and relaxation of the pilor sphincter and the lower esophageal sphincter: acetylcholine being the excitatory neurotransmitter and nitric oxide the main inhibitory neurotransmitter. There was a certain kind of positive correlation between nitric oxide in rats' pylorus and bile acid to the stomach, for nitric oxide could loosen the pyloric sphincter and increase the bile acid to the stomach. The reflux of duodnal contents into the stomach also includes the pancreatic and intestinal enzymes. In these circumstances the duodenogastric reflux contents (pepsine, gastric acid and bile) favorise later the gastroesophageal reflux and the production of esophagitis.

The pH of refluxed duodenal contents into stomach cavity was measured in the three animal groups. The pH of this mixture contents of the high cow-fat group (median 6.2 ± 0.51 ; range 5.2–6.8) was lower than that of the control group (7.10 ± 0.41 ; range 6.4–7.4).

A significant increase in the concentration of taurine-conjugated bile acids, especially TCA and TDCA, was detected in the high cow-fat group compared with animals fed the standard diet. Nehra et al. reported that only TCA among the primary bile acids significantly increased in the BE group. TDCA was found in significantly higher concentrations in the esophageal aspiration of patients with erosive esophagitis and strictures (Nehra et al., 1999). Moreover, they reported that mixed reflux (gastric acid and bile) is more harmful than acid gastric reflux alone with possible toxic synergism between the taurine conjugates and acid, and that the pH range 4–7 wherein most bile acids exist in a two-phase state, ionized and unionized. In the present study, the pH of the gastric contents of the high cow-fat group was around these values.

Because of their hydrophobic and cytotoxic properties, bile acids in humans are amidated with glycine or taurine to form bile salts before their excretion into bile. The conjugation of bile acids are governed by the pKa and pH conditions into duoden. The conjugation of bile acids with glycine or taurine significantly decreases their pKa compared with that of unconjugated bile acids in the pH range 7.4-8 found within the intestinal tract.

In the pH range 4–7 most bile acids are unionized, and this implies that most bile acids can enter the gastroesophageal epithelia (Stamp et al., 2002). However, in humans with intact gastric acid production, whose gastric and esophageal pH is mostly low, only taurine-conjugated bile acids are unionized and can enter the epithelia, and play a crucial role in the development of epithelial diseases such as BE and EAC. Bile acids are known to promote the growth of gastrointestinal cancer, but the entire mechanism still remains unclear. Bile acids are known to be extremely toxic at high doses, probably through damage to cell or mitochondrial membranes or interference with cellular function. This toxicity may play a role in stimulating proliferation and hence may contribute to cancer. Bile acids are known

to stimulate cell-signaling effects involving c-myc (Tselepis et al., 2003) and cyclooxygenase (COX)-2 (Shirvani et al., 2000) which contributes to bile acid-driven carcinogenicity.

In our study, the bodyweight of the animals fed the high cow-fat diet was slightly higher than low cow fat group and control group after five weeks. El-Serag et al. reported that high saturated fat, cholesterol, or fat in the diet is associated with GERD symptoms only in participants with a body mass index (BMI) of more than 25 kg/m² (El-Serag et al., 2005). These results imply that a high-fat diet is probably linked to increases in weight and BMI.

Conclusions

1. The dietary components are responsible for the development of esophageal and gastric lesions.

2. High fat intake induces a significant increase in taurine-conjugated bile acids in bile juice, which are unionized and can enter the gastroesophageal epithelia.

3. An increase in the amount of taurine-conjugated bile acids induced by high cow-fat intake must be a main causative factor of GERD and EAC.

References

1. El-Serag H.B, Satia JA, Rabeneck L. 2005 - Dietary intake and the risk of gastro-oesophageal reflux disease. *Gut.*, **54**: 11–17.

2. Miwa K, Kinami S, Miyazaki I, Hattori T. 1996 - Positive association between dietary fat intake and risk of gastric carcinoma in rats. *Carcinogenesis*, **17**: 1885–9.

3. Nehra D, Howell P., Williams CP. 1999 - Toxic bile acids in gastro-oesophageal reflux disease: influence of gastric acidity. *Gut.*, **44**: 598–602.

4. Shirvani VN, Ouatu-Lascar R, Kaur BS. 2000 - Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: Ex vivo induction by bile salts and acid exposure. *Gastroenterology*, **118**: 487–96.

5. Stamp DH. 2002 - Three hypotheses linking bile to carcinogenesis in the gastrointestinal tract: certain bile salts have properties that may be used to complement chemotherapy. *Med Hypotheses*, **59**: 398–405.

6. Tselepis C, Morris CD, Wakelin D et al. 2003 - Upregulation of the oncogene c-myc in Barrett's adenocarcinoma. *Gut*; **52**: 174–80.

7. Wild CP, Hardie LJ. 2003 - Reflux, Barrett's oesophagus and adenocarcinoma: burning questions. *Nat Rev Cancer*; **3**: 676–84.