Alteration of Bile Acid Metabolism by a High-Fat Diet Is Associated with Plasma Transaminase Activities and Glucose Intolerance in Rats

Reika Yoshitsugu, Keidai Kikuchi, Hitoshi Iwaya, Nobuyuki Fujii, Shota Hori, Dong Geun Lee and Satoshi Ishizuka

Laboratory of Nutritional Biochemistry, Research Group of Bioscience and Chemistry, Division of Fundamental Agriscience Research, Research Faculty of Agriculture, Hokkaido University, Sapporo 060–8589, Japan

Summary Ingestion of a high-fat (HF) diet is known to enhance bile acid (BA) secretion, but precise information about the BA molecular species is lacking, especially information on the conjugated BAs in enterohepatic circulation. As cholesterol is the precursor of BAs, we analyzed alterations of the entire BA metabolic pathway in response to a HF diet without the addition of cholesterol and BA in the diet. Additionally, we evaluated the relationships between BA metabolism and some disorders, such as plasma transaminase activities and glucose intolerance induced by the HF diet. Acclimated WKHA/HkmSlc male rats (3 wk old) were divided into two groups fed a control or the HF diet for 22 wk. Fasting blood glucose was measured during the experimental period, and an intraperitoneal glucose tolerance test was performed at week 21. As a result, ingestion of the HF diet selectively increased the concentration of taurocholic acid in the bile and small intestinal contents as well as deoxycholic acid in the large intestinal contents and feces. These results indicated a selective increase of 12α-hydroxylated BA concentrations in response to the HF diet. Moreover, fecal 12α-hydroxylated BA concentration was positively correlated with cumulative energy intake, visceral adipose tissue weight, and glucose intolerance. The present study suggests that fecal 12α-hydroxylated BA is a non-invasive marker that can detect the early phase of glucose intolerance.

Key Words bile acids, high-fat diet, energy intake, liver damage, glucose tolerance

The expansion of noncommunicable diseases (NCDs) has increased, and overweight and unhealthy diets are factors associated with the incidence of disease (1). Consumption of an energy-dense diet is considered to be related to NCDs. Actually, obesity (2) and various disorders (3), such as insulin resistance and dyslipidemia, related to energy metabolism are observed in animal models fed a high-fat diet. Many researchers have attempted to reveal the underlying mechanisms in the development of these disorders, but these efforts are still underway.

Ingestion of a high-fat diet promotes bile acid (BA) secretion as shown by fecal excretion of secondary BAs in animal and human studies (4, 5). In these studies, BAs were primarily analyzed by gas chromatography (GC) and GC/mass spectrometry (MS). In this method, deconjugation and derivatization steps of the extracted BAs are necessary before analysis with GC/MS, which makes it difficult to distinguish unconjugated and conjugated BAs in the BA profile. Those preparatory steps are not required in current methods with liquid chromatography (LC)/MS (6, 7), and the whole BA profile can be analyzed at the same time. The development of this instrumental analysis enables us to accurately evaluate BA metabolism, including conjugated BAs.

During the development of instrumental analysis, information on BA metabolism has gradually been revealed. For example, BAs undergo deconjugation and/or 7α-dehydroxylation by some intestinal bacteria (8). Aging increases the ratio of cholic acid (CA) to chenodeoxycholic acid (CDCA) in rodents (9). The CA/CDCA ratio in primary BAs in humans has not been systematically investigated. However, there are some reports showing the BA composition in bile, serum, or feces, which enabled us to calculate the CA/CDCA ratio of 0.3–0.5 in infants until 72 h after birth (10), of ~0.5 in children ages 12–59 mo (11), and of >1.1 in adults 24–41 y of age (12). Taurocholic acid (TCA) is the taurine-conjugated form, and deoxycholic acid (DCA) is the 7α-dehydroxylated form of CA (13). Both TCA and DCA possess an α-hydroxyl group in the steroid ring. In rodents, there is an additional BA synthetic pathway from CDCA to α- and β-muricholic acids in the liver (14). Considering the diversity of primary BA synthesis, it is appropriate to use the term “proportion of 12α-hydroxylated BAs (12αBAs)” as a more general expression rather than the CA/CDCA ratio.

BA metabolism is closely related to several disorders, such as carcinogenesis in the liver and intestine (15),

*To whom correspondence should be addressed. E-mail: zuka@chem.agr.hokudai.ac.jp

J Nutr Sci Vitaminol, 65, 45–51, 2019
type 2 diabetes (16) and cardiovascular diseases (17). However, fundamental information on BA metabolism on a high-fat diet is still unclear despite the attention that has been paid to the relationship between BAs and the development of NCDs. In this study, we assessed BA metabolism in rats fed a high-fat diet and explored the relationship between BA metabolism and several disorders observed with high-fat diet consumption.

MATERIALS AND METHODS

Animals. The study was approved by the Institutional Animal Care and Use Committee of National Corporation Hokkaido University (approved number: 14-0026), and all animals were maintained in accordance with the Hokkaido University Manual for Implementing Animal Experimentation. Wistar King A Hokkaido male rats (3-wk-old NBRP Rat No: 0154) (WKAH/Hkmslc, Japan SLC, Inc., Shizuoka, Japan), an inbred strain of Wistar rats, were housed in an air-conditioned room at 22 ± 2°C with 55 ± 5% humidity, and the light period was from 8:00 to 20:00. The rats were housed individually in wire-bottomed cages and allowed ad libitum access to diet and water. The rats were acclimatized with a control diet (C) based on the AIN-93G formulation (Table 1) (18) for 2 wk. The acclimatized rats (n = 20) were then divided into two groups and were fed either a C (n = 10) or a high-fat diet (HF) (n = 10) (Table 1) for another 22 wk. Tail vein blood was collected every 2 wk from week 1 to week 15 and at week 21 during the test period after food deprivation for 16 h to measure fasting plasma glucose concentration. Additionally, feces were collected every 2 wk from week 2 to week 22 to measure fecal BA compositions. At week 22, bile juice was collected through a polyvinyl catheter (SV-35; id 0.5 mm, od 0.9 mm; Natsume Seisakusyo, Tokyo, Japan) inserted into the common bile-pancreatic duct under anesthesia with sodium pentobarbital (Somnopentyl, 50 mg/kg body weight, Kyoritsu Seiyaku Corporation, Tokyo, Japan). Immediately after the bile collection for 10 min, aortic blood was collected from the aorta abdominis of the rats. An anticoagulant agent (heparin, 50 U/mL blood, Nakarai Tesque, Inc., Kyoto, Japan) and a protease inhibitor (aprotinin, 500 kIU/mL blood, Wako Pure Chemical Industries, Ltd., Osaka, Japan) were added to the collected blood, and the plasma was separated. The rats were killed by exsanguination thereafter. The liver, visceral adipose tissues, and intestinal contents (jejunum, ileum, cecum, and colon) were collected and weighed. Feces were collected through the last day of the experimental period.

BA analysis. The levels of each BA in the bile, intestinal contents, and feces were extracted and measured as previously reported (6, 7). The individual BA concentrations were measured with nordeoxycholic acid (23-nor-5β-cholanic acid-3α,12α-diol) as the internal standard. The BAs measured in this experiment were as follows: CA (5β-cholanic acid-3α,7α,12α-triol), Urscholic acid (5β-cholanic acid-3α,7β,12α-triol, UCA), α-muricholic acid (5β-cholanic acid-3α,6β,7α-triol, αMCA), β-muricholic acid (5β-cholanic acid-3α,6β,7β-triol, βMCA), α-muricholic acid (5β-cholanic acid-3α,6α,7β-triol, αMCA), CDCA (5β-cholanic acid-3α,7α-diol), DCA (5β-cholanic acid-3α,12α-diol), hyocholic acid (5β-cholanic acid-3α,6α,7α,7-triol, HCA), hyodeoxycholic acid (5β-cholanic acid-3α,6α-diol, HDCA), ursodeoxycholic acid (5β-cholanic acid-3α,7α-diol, UDCA), lithocholic acid (5β-cholanic acid-3α-ol, LCA), hyocholic acid (5β-cholanic acid-3α,6α,7α-triol, HCA), TCA (5β-cholanic acid-3α,7α,12α-triol-N-2-sulphoethyl-amine), tauro-α-muricholic acid (5β-cholanic acid-3α,6β,7α-triol-N-2-sulphoethyl-amine), taumuricholic acid (5β-cholanic acid-3α,6α,7β-triol-N-2-sulphoethyl-amine, TMA), tauro-β-muricholic acid (5β-cholanic acid-3α,6β,7β-triol-N-2-sulphoethyl-amine, TBMCA), tauro-α-muricholic acid (5β-cholanic acid-3α,6α,7β-triol-N-2-sulphoethyl-amine, TMA), taurocholanic acid (5α-cholanic acid-3α,6α-diol-N-2-sulphoethyl-amine, TDCA), taurodeoxycholic acid (5α-cholanic acid-3α,12α-diol-N-2-sulphoethyl-amine, TDCA), taurohyodeoxycholic acid (5β-cholanic acid-3α,6α-diol-N-2-sulphoethyl-amine, TDCA), glycocholic acid (5β-cholanic acid-3α,7α-diol-N-(carboxymethyl)-amide, GCA), glycocholanic acid (5β-cholanic acid-3α,7α-diol-N-(carboxymethyl)-amide, GCDA), glycodeoxycholic acid (5β-cholanic acid-3α,12α-diol-N-(carboxymethyl)-amide, GDCA), glycodeoxycholic acid (5β-cholanic acid-3α,12α-diol-N-(carboxymethyl)-amide, GDCA), glycodeoxycholic acid (5β-cholanic acid-3α,12α-diol-N-(carboxymethyl)-amide, GDCA), glycodeoxycholic acid (5β-cholanic acid-3α,12α-diol-N-(carboxymethyl)-amide, GDCA), glycocholic acid (5α-cholanic acid-3α,6α-diol-N-(carboxymethyl)-amide, GLCA), 7-oxodeoxycholic acid (5β-cholanic acid-3α,12α-diol-7-one, 7oDCA), 7-oxo-lithocholic acid (5β-

<table>
<thead>
<tr>
<th>Table 1. Diet composition.</th>
<th>C</th>
<th>HF</th>
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<tbody>
<tr>
<td>Dextrin</td>
<td>529.5</td>
<td>299.5</td>
</tr>
<tr>
<td>Casein</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
<td>Soybean oil</td>
<td>70.0</td>
<td>70.0</td>
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<tr>
<td>Lard</td>
<td>230.0</td>
<td></td>
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<tr>
<td>Cellulose</td>
<td>50.0</td>
<td>50.0</td>
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<tr>
<td>Mineral mixture</td>
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<td>35.0</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.0</td>
<td>3.0</td>
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1 TK-16 (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan).
2 NZMP Acid Casein (Fonterra Co-Operative Group Limited, Auckland, New Zealand).
3 Nippon Beets Sugar Manufacturing Co., Ltd., Tokyo, Japan.
4 J-Oil Mills, Inc., Tokyo, Japan.
5 Crystalline cellulose (Celoz PH-102, Asahi Kasei Chemicals Corp., Tokyo, Japan).
6 AIN-93G mineral mixture (18).
7 AIN-93 vitamin mixture (18).
8 Wako Pure Chemical Industries, Ltd., Osaka, Japan.
cholic acid-3α-ol-7-one, 7oLCA), and 12-oxo-lithocholic acid (5β-cholanic acid-3α-ol-12-one, 12oLCA). All BAs, with the exception of UCA, were purchased from Steraloids, Inc. (Newport, RI, USA); UCA was obtained from Toronto Research Chemicals (Toronto, Ontario, Canada). The 12αBAs in this study are as follows: CA, UCA, DCA, TCA, TDCA, GCA, GDCA, 7oDCA and 12oLCA. Among these BAs, 12oLCA possesses an oxo group at position 12 on the steroid ring and is not a genuine 12αBA, but we included 12oLCA in the 12αBA family in the present study because this molecule can originate from one of the 12αBAs, DCA (19).

Blood aminotransferase activities, glucose, and insulin. Blood plasma was stored at −80˚C until it was used in the analyses. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using a Transaminase CII-test Wako (Wako). Plasma glucose and insulin concentrations were analyzed using a Glucose CII-test kit (Wako) and a Rat Insulin ELISA kit (AKRIN-010T) (Shibayagi Co. Ltd., Shibukawa, Japan), respectively.

Intraperitoneal glucose tolerance test (IPGTT). We performed an IPGTT (20) at week 21 during the experimental period. The rats were fasted for 16 h, and then, the blood was collected from the tail vein. A glucose solution was injected intraperitoneally (1 g/kg), and blood samples were collected from the tail vein at the indicated time points after glucose injection. The blood samples were collected into tubes containing heparin (final concentration 50 IU/mL; Ajinomoto Co., Inc., Tokyo, Japan) and aprotinin (final concentration 500 Kallikrein inhibitor units/mL; Wako). The plasma was separated by centrifugation at 2,500 × g for 10 min at 4˚C and stored at −80˚C until analysis. The relative value of the homeostasis model assessment of insulin resistance (HOMA-IR) (21) was calculated by using the data in week 21.

Statistics. The significance of differences between the control and HF groups was determined using Student’s t-test. For correlation analysis, linear regression was analyzed by using the least squares method. A p value <0.05 was considered to be significant. JMP version 12.2.0 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analyses.

RESULTS

The HF diet reduced diet consumption, probably due to its high energy density (Table 2). In contrast, total energy intake in the test period was significantly higher
in the HF-fed rats than in the control rats. Consistent with the energy intake, body weight in the HF-fed rats was greater than that in the C-fed rats, along with an increased weight in visceral adipose tissues. The relative liver weight was lower in the HF-fed rats, but the actual values of the liver weight were almost identical in both groups (13.39 ± 0.42 in C, 13.41 ± 0.43 in HF).

We determined changes in BA excretion in the feces of the rats fed the C or HF diet (Fig. 1). HF diet consumption enhanced total and secondary BA excretions from week 10, but an increase in the concentration of 12αBAs could be detected much earlier in week 4. These levels were almost stable during the last half of the experimental period. In contrast, there was no clear alteration in the primary BA excretion. Next, we analyzed BA composition in bile juice, intestinal contents, and feces at the end of the experimental period (Fig. 2). TCA was the major component of BAs in the bile and small intestine (jejunal and ileal contents), especially in the HF-fed rats. The concentration was approximately 10–20 mM in the C group and 25–40 mM in the HF group at these sites. The concentration of BAs was much lower in the large intestine (cecal and colonic contents). The most abundant BA in the large intestine and feces of the HF-fed rats was DCA, whereas the concentration of MCAs (sum of βMCA and ωMCA) appeared greater than that of DCA in the control rats. An increase in the 12αLCA concentration was also observed in the colon and feces. The concentration of ωMCA was nearly the same as that of DCA, especially in the large intestinal contents and feces of the rats fed the HF diet.

Significantly higher values in the HF group were detected for both AST and ALT from week 5 (Fig. 3).
time point was almost the same (in weeks 11 and 12) when the difference in 12αBAs was observed. The value was continuously increased throughout the experimental period in the rats fed the HF diet. There was a significant increase in the highest glucose concentration in the IPGTT performed at week 21 (Fig. 4). Likewise, an increase in insulin secretion was observed at 30 min in the HF-fed rats in response to the glucose injection. Similar changes were found in the glucose AUC as well as the ΔAUC (Table 3). There was no difference in the HOMA-IR, although a decrease was observed in the fasting glucose of the HF-fed rats. We analyzed the relationships among energy intake, glucose tolerance, and fecal 12αBA concentration (Fig. 5). The total energy intake was significantly correlated with glucose tolerance. Interestingly, there was a significant correlation between total energy intake and fecal 12αBA concentration. Moreover, there was a positive correlation between fecal 12αBA concentration and glucose ΔAUC in the IPGTT.

**DISCUSSION**

Pioneering studies performed by Reddy and colleagues (4, 5, 12) demonstrated an increase in BA secretion following HF diet consumption in experimental animals and in humans considering the relationship between BA metabolism and gastrointestinal carcinogenesis. The researchers observed an increase in bile CA concentration as well as fecal DCA concentration in the rats fed a high-fat and high-sucrose diet, suggesting an increase in BA secretion by a high-fat diet. However, as a consequence of the technical limitations at that time, there was no information on conjugated BAs in the profiles. The bile CA in the rat study (4) should be mainly TCA, which was confirmed in the present study. Additionally, we observed a selective increase in bile TCA concentration by the HF diet. TCA possesses high water solubility, and the critical micellar concentration is approximately 8–12 mM (22). The present study suggests that the bile TCA concentration in the control is sufficient to form emulsions and contributes to lipid digestion as well as absorption. The TCA concentration in the HF-fed rats might increase in response to the addition of high levels of dietary lipids.

Secreted BAs in the duodenum are reabsorbed by an apical sodium-dependent BA transporter (ASBT) at the ileal epithelial cells (23). The present study demonstrated a higher proportion of TCA than MCAs in the small intestinal contents and in the portal blood of the HF-fed rats despite comparable proportions of 12αBAs and non-12αBAs in the large intestine and feces. TCA might be highly available in the small intestine because

![Fig. 4. Changes in glucose and insulin concentrations in the blood plasma taken from the tail vein of the rats fed the C and HF diets during IPGTT at week 21.](image)

After collection of tail vein blood in fasting conditions (16 h food deprivation) at time 0, a glucose solution was intraperitoneally injected (1 g/kg rats), and tail vein blood was collected at the indicated time points to measure glucose and insulin concentrations. Values are shown as the mean with the SE (n=10). Open circles, C; filled circles, HF. Asterisks indicate significant differences compared to C (p<0.05).

![Fig. 5. Correlations among total energy intake, fecal 12αBA concentration, and glucose tolerance of the rats fed the C and HF diets.](image)

Total energy intake was calculated from diet consumption throughout the experimental period and the energy density of each diet. Glucose ΔAUC was calculated from the data shown in Fig. 4. The fecal 12αBA concentration was calculated from the data in week 22 as shown in Fig. 2. Individual data are shown as circles (open circles, n=10 for C; filled circles, n=10 for HF).

<table>
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<tr>
<th>Parameter</th>
<th>C</th>
<th>HF</th>
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<tr>
<td>Fasting glucose (mg/dL)</td>
<td>92.3±3.6</td>
<td>80.0±2.4*</td>
</tr>
<tr>
<td>Glucose AUC (mg/dL·h)</td>
<td>242.9±7.3</td>
<td>276.8±6.2*</td>
</tr>
<tr>
<td>Glucose ΔAUC (mg/dL·h)</td>
<td>58.3±8.7</td>
<td>124.3±8.6*</td>
</tr>
<tr>
<td>Fasting insulin (nm)</td>
<td>0.69±0.14</td>
<td>0.80±0.12</td>
</tr>
<tr>
<td>Insulin AUC (nm·h)</td>
<td>1.67±0.14</td>
<td>2.32±0.24</td>
</tr>
<tr>
<td>Insulin ΔAUC (nm·h)</td>
<td>0.60±0.11</td>
<td>0.72±0.07</td>
</tr>
<tr>
<td>Relative HOMA-IR</td>
<td>1.00±0.14</td>
<td>1.38±0.12</td>
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*Significant different from the values in C (Student’s t-test, p<0.05, n=10).
the absorption rate of TCA by ASBT is relatively high compared to CA (13, 24), which results in increased TCA concentration in organs related to enterohepatic circulation. These results suggest that TCA is preferentially used to support lipid absorption even in rodents that synthesize substantial amounts of MCAs. There are two pathways for BA synthesis: the classical (neutral) pathway and alternative (acidic) pathway (13, 14); Cyp8b1 synthesizes 12αBAs in the classical pathway, and Cyp27a1 synthesizes non-12αBAs in the alternative pathway. Therefore, the present study suggests that an HF diet activates the classical pathway rather than the alternative pathway in liver BA synthesis.

In this study, we observed an increase in plasma AST and ALT activities at week 5 when the fecal 12αBA concentration was already elevated in the HF-fed rats. The increased TCA is used as the precursor of DCA in the large intestine. Various 12αBAs, such as CA and DCA, are involved in the increase of these transaminase activities (25). Especially, DCA provokes DNA damage and cell death via induction of reactive oxygen and nitrogen species (26). The changes in the transaminase activities of the present study were consistent with these observations. The increase in DCA, the 7α-dehydroxylated product of CA in the 12αBAs, absorbed in the distal digestive tract is a possible factor to increase plasma transaminases via cell death of hepatocytes. Additionally, there was an interesting correlation between the fecal 12αBAs and glucose intolerance. Interestingly, the influence of the HF diet was on the glucose-related parameters but not on the insulin-related parameters (Table 3). Haesler and colleagues reported a significant correlation between serum 12αBAs and insulin tolerance in a human study (16). It is also possible that an increase in 12αBAs enhances glucose intolerance because abrogation of Cyp8b1 ameliorates glucose intolerance (27). Cyp7a1 deficiency also improves glucose tolerance in a high-fat fed condition via increased respiratory exchange ratio (28). These results suggest abrogation of 12αBA synthetic pathway enhances fecal excretion of triacylglycerols and/or promoted energy expenditure, but in the Cyp8b1 deficient study (27), no difference in the energy expenditure is observed. The precise mechanism remains to be elucidated in view of classical pathway in glucose tolerance. Nevertheless, 12αBAs can be a suitable marker for glucose intolerance. This study suggests that fecal 12αBAs measured in a non-invasive manner are not only a marker for an early phase of glucose intolerance but also a marker that reflects energy consumption status.

Presumably, the repeated food deprivation in the present study would induce lipolysis in adipose tissues. In response to noradrenaline that represents fasting condition, less lipolytic rate is reported in retroperitoneal adipose tissue regardless of repeated fasting.

We performed repeated food deprivation for 16 h during the experiment and measured fasting glucose in the present study, but no difference was found until week 15. Later, we observed a significant difference in fasting glucose at week 21, but the value in the HF-fed rats was lower than that in the control rats. There was no difference in the HOMA-IR, but significantly higher values in the AUC of the plasma glucose levels were found in the HF-fed rats in the IPGTT. Food deprivation is necessary for the glucose tolerance test to obtain a steep alteration of blood glucose levels during IPGTT. However, a small effect of dietary intervention on glucose tolerance might disappear following frequent food deprivation. One example in this study might be some of the insulin-related parameters in the IPGTT. Notably, the fasting condition of a treatment with a weak effect, especially on glucose regulation, should be carefully determined in terms of duration and frequency in the experiment to obtain accurate results.

Dietary cholesterol is the precursor of BAs (13), and a high-cholesterol diet may hide the intact BA metabolism in response to an increase in energy consumption. In this study, we selected a HF diet without the addition of dietary cholesterol or BAs to elucidate sterol metabolism on an HF diet. The present study demonstrated that BA metabolism reflects energy consumption status as well as glucose intolerance in the body. In conclusion, this study suggests that 12αBA is a reliable marker to evaluate the onset of glucose tolerance rather than fasting glucose, fasting insulin, and HOMA-IR.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number 24658114.

REFERENCES

Bile Acids in High-Fat Diet-Induced Glucose Intolerance


