Basic nutritional investigation

Lysophospholipid profile in serum and liver by high-fat diet and tumor induction in obesity-resistant BALB/c mice

Hyang Yeon Kim M.S., Minhee Kim M.S., Hye Min Park Ph.D., Jiyoung Kim Ph.D., Eun Ji Kim Ph.D., Choong Hwan Lee Ph.D.,* Jung Han Yoon Park Ph.D.,**

*Department of Bioscience and Biotechnology, Konkuk University, Seoul, Korea
**Department of Food Science and Nutrition, Hallym University, Chuncheon, Korea

Abstract

Objective: Our previous study revealed that chronic consumption of a high-fat diet (HFD) stimulates colon cancer progression in obesity-resistant BALB/c mice. The aim of the present study was to investigate the significant alteration of metabolites caused by tumor progression and an HFD in the serum and liver in the same mouse model.

Methods: Male BALB/c mice were fed either a control diet or a HFD for 20.5 wk. The syngeneic CT26 colon carcinoma cells were injected into the right rear flank of mice after 16 wk of feeding. Metabolites in serum and liver samples were analyzed by ultra-performance liquid chromatography-quadrupole-time-of-flight-mass spectrometry-based metabolomics.

Results: HFD feeding and tumor injection induced changes in the choline-containing phospholipids, namely, phosphatidylcholines and lysophosphatidylcholines (lysoPCs), and lysophosphatidylethanolamines in the serum and liver. The majority of these metabolite changes were due to HFD feeding (11 in sera and 5 in livers) rather than tumors (3 in sera and 1 in livers).

Conclusion: The HFD- and tumor-related metabolite alterations of phospholipids, especially lysoPCs, in the liver and serum of obesity-resistant mice, suggesting that the lysoPCs are potential biomarkers for the chronic consumption of HFD in nonobese individuals.

Introduction

Colorectal cancer (CRC) is one of the most common cancers in the United States [1] and its incidence appears to be rising worldwide. The prognosis is directly related to the stage of the cancer at diagnosis; however, at the time of diagnosis, metastasis is already present in many patients [2]. Therefore, there is an urgent need for new prevention measures that will reduce the progression and metastasis of colon cancer. For effective prevention of colon cancer, controlling the risk factors for cancer is important. Intake of a high-fat diet (HFD) often leads to weight gain and obesity in rodents [3] and humans [4], and is associated with a higher risk for CRC [5]. Results from epidemiologic studies indicate that overweightness and obesity are positively associated with several cancers, including colon cancer [6–8]. Although some individuals appear to be genetically predisposed to staying lean no matter what or how much they eat, others become obese. However, it is hard to determine whether the intake of an HFD indirectly affects CRC by inducing obesity or if it is an independent risk factor for this disease.

Previously, we conducted an animal study to determine whether the chronic consumption of an HFD without substantial weight gain stimulated the promotion and progression of colon cancer [9], BALB/c mice do not become obese on an HFD [10,11]. Consumption of an HFD led to a small increase in epididymal fat pad mass and the number of fat cells in tumor tissues without any discernible weight gain in BALB/c mice injected with CT26.
colon cancer cells that were derived from a spontaneously arising tumor. In those HFD-fed mice, tumor growth, as well as tumor angiogenesis and lung metastasis, was increased [9].

Recently, to better understand the biochemical mechanisms underlying obesity and the related dysfunction, metabolomic profiles of body fluids have been investigated using nuclear magnetic resonance spectroscopy or ultra-performance liquid chromatography-quadrupole-time-of-flight-mass spectrometry (UPLC-Q-TOF-MS). It has been shown that the levels of lyso-phosphatidylcholine (lysoPC) 14:0 and 18:0 were higher, but those of lysoPC 18:1 were lower in the plasma of obese men who had a high-fat intake with a lower ratio of polyunsaturated fatty acids to saturated fatty acids compared with the levels in the plasma of lean men [12]. It has been reported that metabolites such as phosphatidylcholines (PCs), lysoPCs, fatty acids, and branched amino acids were altered in the liver and plasma of obese C57BL6 mice fed an HFD [13]. HFD may induce changes in metabolites, which may subsequently influence tumor promotion and progression in obesity-resistant BALB/c mice. In the present study, using the same BALB/c mouse model, we attempted to identify the altered metabolites in the sera and livers when tumor growth and metastasis were stimulated by chronic consumption of an HFD.

Materials and methods

Animals, dietary treatment, and CT26 cell injection

Three-wk-old, male BALB/c mice were purchased from Orient Bio Inc. (Gapyung, Korea). Mice were maintained in a pathogen-free animal facility at Hallym University to acclimatize them to the laboratory conditions for 1 wk. Animals were then randomly divided into two groups and fed purified diets (Research Diets Inc., New Brunswick, NJ, USA); the HFD contained 60 kcal% fat (D12450B) and the control diet (CD) contained 10 kcal% fat (D12492). Sixteen wk after the initiation of feeding, the two dietary groups were further subdivided into four groups: sham-injected CD (SC; n = 15), sham-injected HFD (SH; n = 29), tumor-injected CD (TC; n = 15), and tumor-injected HFD (TH; n = 29). Mouse colon carcinoma CT26 cells (5 × 106 cells) were suspended in 0.1 mL of Matrigel (BD Biosciences, San Jose, CA, USA) and subcutaneously injected into the right rear flanks of the TC and TH mice. The SH received a subcutaneous injection of 0.1 mL of Matrigel. The mice were fed continuously on the same diets. Thirty-one d after tumor cell injection, the mice were anesthetized via an intraperitoneal injection of 2.5% avertin, and blood was collected from the orbital plexus.

Histochemical analyses

Paraffin-embedded liver and adipose tissues were sectioned, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). For trichrome staining, a Trichrome Stain (Masson) kit (Sigma Chemical Co., St. Louis, MO, USA) was used.

Data processing, multivariate analysis, and other statistical analyses

UPLC-Q-TOF-MS analysis

A Waters Micromass Q-TOF Premier with UPLC Acquity system containing an Acquity UPLC BEH C18 column (100 × 2.1 mm with a 1.7 μm particle size; Waters, Milford, MA, USA) was performed. The mobile phase was acetonitrile and water. The initial gradient of the mobile phase was maintained at 5% acetonitrile for 1 min, after which the concentration of acetonitrile was gradually increased to 100% for 9 min. The acetonitrile was held at 100% for 1 min and gradually decreased to 0% for 2 min. Five mL of the sample was injected and the flow rate was maintained at 0.3 mL/min. Electrospray ionization (ESI)-Q-TOF-MS was performed in the negative- and positive-ion mode within a range of m/z 50 to 1000. The source temperature was set at 120 °C, the nebulizer gas flow at 600 L/h, and the cone gas at 50 L/h. The capillary and sample cone voltages were set at 3000 and 40 V, respectively. Leucine enkephalin was used as the lock mass at a concentration of 0.2 mg/L and a flow rate of 0.3 μL/min. Argon was employed as the collision gas at a flow rate of 0.3 μL/min and the collision energy was set at 50 eV.

To prepare serum extracts, 150 μL of serum was mixed with 450 μL ice-cold methanol containing internal standards (0.5 mg/L of iodocaine and D-camphorsulfonic acid) and extracted with a mixer mill for 1 min (frequency, 30). The samples were centrifuged at 3220g for 5 min, and the supernatants were collected in Eppendorf tubes. Before UPLC-Q-TOF-MS analysis, the supernatants (160 μL) were added to 80 μL water.

Liver extracts were prepared according to a previously described method [14]. Briefly, liver samples (50 mg) were homogenized in 1.5 mL of ice-cold methanol/water (1:1) with a mixer mill for 2 min (frequency 30) and centrifuged at 3220g for 5 min. After centrifugation, the supernatants were collected in Eppendorf tubes and dried. Before the UPLC-Q-TOF-MS analysis, liver extract samples were resuspended in 500 mL of methanol/water (1:1) and filtered through a 0.45-μm polytetrafluoroethylene membrane.

Effect of a high-fat diet and tumor injection on body weights, organ weights, and food intakes

Animals, dietary treatment, and CT26 cell injection

Effect of a high-fat diet and tumor injection on body weights, organ weights, and food intakes

Table 1

<table>
<thead>
<tr>
<th>Description</th>
<th>SC (n = 15)</th>
<th>SH (n = 29)</th>
<th>TC (n = 15)</th>
<th>TH (n = 29)</th>
<th>Diet effect (P-value)</th>
<th>Tumor effect (P-value)</th>
<th>Interaction (P-value)</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>30.3 ± 0.4a</td>
<td>33.8 ± 1.2b</td>
<td>31.5 ± 0.3ab</td>
<td>33.1 ± 0.8ab</td>
<td>0.002</td>
<td>0.647</td>
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<td>Body weight-tumor weight (g)</td>
<td>30.3 ± 0.4b</td>
<td>33.8 ± 1.2a</td>
<td>30.4 ± 0.3b</td>
<td>31.1 ± 0.6a</td>
<td>0.008</td>
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<td>Tumor (g)</td>
<td>1.26 ± 0.18b</td>
<td>1.87 ± 0.11ab</td>
<td>1.93 ± 0.05a</td>
<td>1.95 ± 0.05a</td>
<td>0.017</td>
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<td>0.038</td>
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<td>Liver (g)</td>
<td>1.74 ± 0.05b</td>
<td>1.87 ± 0.11ab</td>
<td>1.93 ± 0.05a</td>
<td>1.95 ± 0.05a</td>
<td>0.017</td>
<td>0.032</td>
<td>0.038</td>
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<td>Spleen (mg)</td>
<td>151 ± 10.3a</td>
<td>170 ± 7.8a</td>
<td>224 ± 10.9b</td>
<td>307 ± 14.3a</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td>Lung (mg)</td>
<td>223 ± 11.7a</td>
<td>220 ± 5.2a</td>
<td>216 ± 2.8a</td>
<td>219 ± 3.8a</td>
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<td>Epididymal fat (mg)</td>
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<td>ND</td>
<td>242 ± 25b</td>
<td>446 ± 43.8a</td>
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CD, control diet; HFD, high-fat diet; SC, sham-injected; CD, SH, sham-injected, HFD; TC, tumor-injected, CD; TH, tumor-injected, HFD; ND, not determined

The data were evaluated using Student’s t test (for two groups) or Duncan’s multiple range test (for four groups). Means with the different letters, e.g., a, b, or c are statistically different. Differences were considered significant for P < 0.05.
Results

Changes in body and organ weights in mice due to HFD feeding and/or tumor injection

At the time of sacrifice, the mean body weight was higher in the SH group than in the SC group. However, there was no difference in mean body weight and body weight (body weight−tumor weight) between the TC and TH groups. The ad libitum food intake in the SC, SH, TC, and TH groups was 4.17 ± 0.38, 3.48 ± 0.23, 4.36 ± 0.46, and 3.46 ± 0.28 g/d, respectively. The food intake (g/d) was lower, whereas the energy intake (kJ/d) was significantly higher in the HFD groups than in the CD groups. Consistent with previous results [9], tumor weights were significantly higher in mice fed the HFD. Liver weights were increased in tumor-bearing mice, but the difference between the SH and TH groups was not statistically significant. The weights of the spleens were significantly increased in tumor-bearing mice compared with the SH mice and were further increased in the TH group compared with the TC group. Lung weights did not vary among the four groups. The epididymal fat pad weights were increased in the TH mice compared with the TC mice (Table 1).

Histological changes in the liver and adipose tissue due to HFD feeding and tumor injection

H&E staining of liver sections revealed that the number of lipid vacuoles increased in the SH and TH groups. In fact, few lipid vacuoles were detected in the liver in the SC and TC groups. Consistent with previous results [9], tumor weights were significantly higher in mice fed the HFD. Liver weights were increased in tumor-bearing mice, but the difference between the SH and TH groups was not statistically significant. The weights of the spleens were significantly increased in tumor-bearing mice compared with the SH mice and were further increased in the TH group compared with the TC group. Lung weights did not vary among the four groups. The epididymal fat pad weights were increased in the TH mice compared with the TC mice (Table 1).
the blue color (collagen contents) was also increased in the SH and TH groups compared with that in the SC and TC groups. The size of the adipocytes in epididymal fat tissue was significantly increased in the TH group relative to the TC group (Fig. 1).

**Changes in metabolite profiles in mouse sera and livers due to HFD and/or tumor injection**

In negative mode, 386 (in sera) and 614 (in livers) variables were used to compare the group differences resulting from the consumption of the HFD and tumor progression via PCA and PLS-DA (Figs. 2 and 3). In the serum samples, PCA and PLS-DA score plots from the first two dimensions of vector t[1] explained 22.9% and 12.7% of the variation and vector t[2] explained 10.6% and 11.2% of the variation among the four groups, respectively. Each group was clearly separated into four clusters in the PLS-DA score plot by diet and tumor effects. According to the diet and tumor effects, two groups were located in the opposite direction of the scores (data not shown). Arbitrary thresholds of variable importance for the projection (VIP) > 1.0 and P < 0.05 were chosen to select the variables, and 99 variables were determined.

In the liver samples, PCA and PLS-DA score plots from the first two dimensions of vector t[1] explained 17.4% and 15.6% of the variation and vector t[2] explained 14.1% and 12.8% of the variation among the four groups. In the PLS-DA score plot, the TC group discriminated from other groups. However, each group was located in the opposite direction of the scores in the comparison with the metabolite difference between the groups by diet or tumor effects (data not shown). Among the variables, 147 were selected by VIP value (>1.5) and P-value (<0.05).

**The discriminative metabolites in mouse sera and livers due to HFD and/or tumor injection**

Sixteen serum and 8 liver peaks were annotated as significant variables with major influences on the group differences (Tables 2 and 3). The most discriminative metabolites between HFD-fed and CD-fed mice or tumor-injected and sham-injected mice in the serum included intermediates of lipid metabolism,
levels of metabolites in the serum and liver after chronic con- and lung metastasis of CT26 CRC cells in obesity-resistant BALB/c of an HFD increased tumor angiogenesis, solid tumor growth, Discussion

<table>
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<tr>
<th>Group</th>
<th>tR (min)</th>
<th>Tentative compound</th>
<th>Measured MS (m/z)</th>
<th>Molecular formula</th>
<th>Error (mDa)</th>
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<td>4.56</td>
<td>2-Octenoic acid (12)</td>
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<td>SM d18:0/16:1 (16)</td>
<td>703.5732</td>
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<td>1.6</td>
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FA, formic acid; lysoPC, lysophosphatidylcholine; lysoPE, lysophosphatidylethanolamine; PE-NMe, monomethylphosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; UPLC-Q-TOF-MS, ultra-performance liquid chromatography-quadrupole-time-of-flight-mass spectrometry

Components with a P-value ≤ 0.05. Moderate evidence against H0

- Group affected by chronic consumption of a high-fat diet (A), tumor (B), and tumor and high-fat diet (C).
- tR, retention time.

such as lysoPCs, PCs, lysophosphatidylethanolamines (lysoPE), monomethylphosphatidylethanolamine (PE-NMe), and sphingomyelin (SM).

In the serum samples, the levels of five lysoPCs with C14:0, C16:1, C20:3, C16:0, and C18:1 were significantly decreased in the SH and TH groups, whereas the levels of four lysoPCs with C22:6, C20:4, C18:2, and C18:0, lysoPE 20:0, and PE-NMe 18:1/18:1 were increased (Fig. 4A). The levels of 2-octenoic acid, PC 20:0/18:4, and PC 18:2/18:0 were decreased in the TC and TH groups (Fig. 4B). LysoPE 22:6 and SM d18:0/16:1 were major metabolites influenced by tumors and a HFD (Fig. 4C).

In the liver samples, the levels of five lysoPCs with C22:6, C20:4, C18:2, C16:0, and C18:0 (Fig. 5A) were significantly increased in the SH and TH groups. The level of lysoPE 18:1, and lysoPE 16:0 was lower in the SC group (Fig. 5B and C).

Discussion

Our previous study demonstrated that chronic consumption of an HFD increased tumor angiogenesis, solid tumor growth, and lung metastasis of CT26 CRC cells in obesity-resistant BALB/c mice [9]. In the present study, we investigated the changes in the levels of metabolites in the serum and liver after chronic consumption of a HFD and/or tumor injection in the same model. HFD has been well known to be associated with obesity [16,17], diabetes [18], and inflammation [19]. Additionally, HFD-induced obesity was reported to affect lipid metabolism, including reduction of the levels of phospholipids and triglycerides in the liver [16] and increase in total cholesterol and triglyceride concentrations in the plasma [20]. We noted an accumulation of lipids in the liver and adipose tissues of the SH and TH groups (Fig. 1) even though HFD feeding slightly increased the body weight of the SH mice. The results from the trichrome staining of the liver tissues revealed that collagen contents (blue color) were increased in the SH and TH groups indicating that HFD feeding induced fibrotic changes without changes in liver weight (Table 1). Previously, we demonstrated, using the same mouse tumor models, that HFD feeding increased lipid vacuoles in the tumor tissues, as well as in infiltration of macrophages into tumor and adipose tissues [9]. These results indicate that HFD feeding can induce changes in tissue and body composition, as well as inflammatory changes, without substantial alteration in tissue and body weight.

In mice fed an HFD, the number of adipose cells was increased in both liver and adipose tissues, whereas the size of adipocytes was increased only in the adipose tissues but not in the liver (Fig. 1). A previous study demonstrated that an HFD increases in the size of lipid vacuoles in the liver of obesity-prone C57BL/6N mice [21]. Current results cannot explain these differential

Table 2
Tentative identification of significantly altered metabolites in serum of high-fat diet-fed and tumor-bearing mice by UPLC-Q-TOF-MS analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>tR (min)</th>
<th>Tentative compound</th>
<th>Measured MS (m/z)</th>
<th>Molecular formula</th>
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FA, formic acid; lysoPC, lysophosphatidylcholine; lysoPE, lysophosphatidylethanolamine; UPLC-Q-TOF-MS, ultra-performance liquid chromatography-quadrupole-time-of-flight-mass spectrometry

Components with a P-value ≤ 0.05. Moderate evidence against H0

- Group affected by chronic consumption of a high-fat diet (A), tumor (B), and tumor and high-fat diet.
- tR, retention time.
Fig. 4. Box-and-whisker plots of metabolite level changes in mouse sera. (A) Chronic consumption of a HFD, (B) Tumor, and (C) Tumor and HFD. SC: sham-injected, CD (n = 15); SH: sham-injected, HFD (n = 29); TC: tumor-injected, CD (n = 15); TH: tumor-injected, HFD (n = 29). Variables were selected by VIP value (>1.0) and P-value (<0.05). CD, control diet; HFD, high-fat diet; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; UPLC-Q-TOF-MS, ultra-performance liquid chromatography-quadrupole-time-of-flight-mass spectrometry; VIP, variable importance for the projection.
changes in adipose cell size between the two tissues of these obesity-resistant BALB/c mice. One possible explanation is the finding that the weights of the liver did not change due to HFD feeding, whereas those of adipose tissues increased (Table 1). Because in these obesity-resistant BALB/c mice, energy intakes were only slightly increased by the HFD feeding and their body weight increases were minimal (Table 1), the majority of small extra energy was probably stored in the adipose tissues but not in the liver. Nevertheless, the extra energy increases were probably enough to induce increases in adipose cell number in the liver.

In addition to lipid accumulation, there were changes in metabolites, primarily lipid or lipid-related, such as lysoPCs, lysoPEs, PCs, SM, and fatty acids, in the liver and serum in HFD-fed mice. LysoPCs accounted for approximately 60% of the metabolites whose levels were altered. Several studies suggested that lysoPCs generated from phospholipase A2-catalyzed hydrolysis of PC were involved in chronic inflammatory diseases,

Fig. 5. Box-and-whisker plots of metabolite level changes in mouse livers. (A) Chronic consumption of a HFD, (B) Tumor, and (C) Tumor and HFD. SC: sham-injected, CD (n = 15); SH: sham-injected, HFD (n = 29); TC: tumor-injected, CD (n = 15); TH: tumor-injected, HFD (n = 29). Variables were selected by VIP value (>1.0) and P-value (<0.05). CD, control diet; HFD, high-fat diet; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; UPLC-Q-TOF-MS, ultra-performance liquid chromatography-quadrupole-time-of-flight-mass spectrometry; VIP, variable importance for the projection.
including atherosclerosis [22], rheumatoid arthritis [23], and ischemia [24], as well as tumor progression [25,26]. It has been reported that the chemotactic ability of lysoPCs that act as chemoattractants in T lymphocytes was determined by the chain length and number of double bonds in the fatty acids of lysoPC [27]. Our results demonstrate that the chronic intake of an HFD had a stronger effect on lysoPCs than tumor injection, and variations in the levels of lysoPCs differed depending on the fatty acid composition in the serum and liver samples. Our previous results revealed that HFD feeding led to increases in the levels of various inflammatory markers, including cyclooxygenase-2 and inducible nitric oxide synthase in tumor tissues, and various cytokines/chemokines in the sera [9]. These previous results, together with the present ones, suggest that increased lysoPCs by HFD act as proinflammatory lipid mediators, thereby regulating the secretion of proinflammatory cytokines/chemokines and the expression of inflammatory enzymes. Although we noted that changes in the levels of lysoPCs and PCs were closely associated with HFD and tumor progression, future studies are needed to examine whether lysoPCs indeed regulate inflammation, as well as tumor growth and progression. It is also necessary to determine whether different lysoPCs containing various fatty acids differentially regulate these processes, because acyl distribution and composition of fatty acids within each PC are continually modified by the actions of multiple phospholipases and lyso-phosphatidylcholine acyltransferases in the PC biosynthesis pathway.

We found that the levels of four lysoPCs with C22:6, C20:4, C18:2, and C18:0 were simultaneously increased in the serum and liver of HFD-fed mice (Figs. 4A and 5A). Contrary to our results, previous findings [28] showed that the levels of most lysoPCs were reduced by HFD in the plasma and liver of HFD-induced obese mice. It has also been reported that HFD caused a decrease in the levels of most lysoPCs and lysoPEs in the liver and serum in obese C57BL6 mice, whereas the levels of PCs were increased [13]. The difference between the present results and those of other studies [13,28] suggests that metabolic responses to HFD feeding differ between obese-prone and obese-resistant animals.

Interestingly, the levels of lysoPC 16:0 decreased in the serum of HFD-fed mice, but increased in the liver (Figs. 4A and 5A). LysoPC 16:0 was the more predominant form of the lysoPCs in all analyzed samples. One study demonstrated that lysoPC 16:0 can induce growth-factor gene expression at nontoxic concentrations in cultured endothelial cells [29]. It was also demonstrated that lysoPC 16:0 exhibits very potent chemotactic properties in vitro [27] and induces inflammation via the production of cytokines, including interleukin (IL)-5, IL-6, and prostaglandin E2 in ICR mice [30]. We also reported that HFD increases the serum levels of epidermal growth factor, insulin-like growth factor-I, insulin, and leptin, as well as IL-1ra, monocyte chemotactant protein-1, interferon-γ, and stromal cell-derived factor-1 in tumor-bearing, BALB/c mice [9]. And, previous results [13] showed that the level of lysoPC 16:0 was suppressed in both serum and liver in HFD-induced obese mice. In human plasma with ovarian cancer, the level of lysoPC 16:0 depended on cancer staging [31]. Compared with various reports on change tendency of lysoPC 16:0 in plasma and serum, information concerning lysoPC 16:0 in liver is sparse, particularly in the tumor progression. So, the opposite alteration of lysoPC 16:0 level in serum and liver is not fully explained due to lack of references. Therefore, further biochemical and molecular studies on the relationship between lysoPC 16:0 and liver, as well as between lysoPC 16:0 and serum according to tumor progression is needed to evaluate and confirm the direct effects and metabolic pathway in depth.

Nevertheless, these results suggest that changes in lysoPC 16:0 may have contributed to the HFD stimulation of inflammation and lysoPC 16:0 potential as biomarkers for the chronic consumption of HFD in tumor-bearing, obesity-resistant mice.

In the serum and liver of tumor-bearing mice, the levels of 2-ocatenoic acid, PCs, and lysoPE, were significantly reduced compared with those in the SH mice (Figs. 4B and 5B). It has been reported that in breast and ovarian cancer cell lines, phosphocholine (PCho) levels were increased in the PC-cycle compared with normal cell lines [32]. PCho is converted to PC by cytidyllytransferase, which mediates the rate-limiting step of the pathway. There may have been changes in the activity of this enzyme in the liver of tumor-bearing mice, which in turn led to a decrease in PC levels in the liver and serum. PCs are reported to induce apoptosis of cells, including colon cancer cells, vascular endothelial cells, macrophages, and adipocytes [33]. Additionally, it has been reported that the fat tissue in the phosphatidylcholine-injected rabbits showed necrosis [34]. Future studies are needed to examine the direct effect of PC on CRC cell proliferation and apoptosis.

The present results revealed that lysoPCs and PCs were the major metabolites altered in the liver and serum of HFD-fed and tumor-bearing mice and HFD played a greater role than tumors in metabolite changes. Together, these results imply that the stimulation of tumor growth and progression in HFD-fed, obesity-resistant mice may be mediated via alterations in lysoPCs. Our results clearly demonstrate that HFD induces changes in metabolite profiles, especially lysoPCs, without inducing significant changes in body or tissue weight.

**Conclusion**

In this study, HFD has a greater effect on metabolite changes in the serum and liver of obesity-resistant BALB/c mice. The lysoPCs possess potential as biomarkers for the chronic consumption of HFD in nonobese individuals.

**References**


