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学術論文

Effects of Tumor-Bearing on Serum and Liver Lipid Levels in Sato Lung Carcinoma (SLC)-Implanted Rats

佐藤肺癌移植ラットにおける血清および肝臓脂質レベルに対する担癌の影響

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The effects of tumor-bearing on serum and liver lipid levels were investigated in Sato lung carcinoma (SLC)-implanted rats by comparing to normal rats fed a diet *ad libitum* (control) and pair-fed groups. Tumor-bearing rats were implanted with 5×10^6 SLC cells and maintained for 21 days. Serum total cholesterol, and especially high-density lipoprotein (HDL)-cholesterol and esterified cholesterol concentrations, were significantly decreased following tumor implantation as compared to the control group. Serum HDL-cholesterol and esterified cholesterol concentrations in the pair-fed group were also significantly decreased as compared to the control group. These results suggest that in SLC-implanted rats, serum cholesterol concentrations undergo change, resulting in abnormal lipid metabolism, and that these serum cholesterol concentration-reductive actions might be due, at least in part, to the decrease in the food intake accompanied by the tumor-bearing state in addition to the tumor-bearing effect itself.

Keywords: *cancer, serum and liver lipids, tumor-bearing*
癌，血清および肝臓脂質，担癌

INTRODUCTION

The tumor cell has a very distinctive metabolism. Several previous studies have considered the metabolic changes that take place in the host after tumor implantation. Metabolic abnormalities arising from malnutrition and malignancy are seen in animals or patients with cancer cachexia. Various cancers affect the lipid metabolism.[1-7] Rats subcutaneously implanted with the ascites hepatoma cell line AH109A show hyperlipidemia with a notable decrease in the high-density lipoprotein (HDL) fraction and an enormous increase in the very-low-density lipoprotein (VLDL) plus low-density lipoprotein (LDL) fractions[5-7] during growth of the solid tumor.[5] AH109A is a hepatoma and originates from the liver. The liver carries out major lipid metabolism, including cholesterol, fatty acid, and triglyceride biosyntheses, as well as VLDL production. It is therefore considered that hepatoma cells may affect lipid metabolism in the host body and that the cancer state affects lipid metabolism.

Sato lung carcinoma (SLC) is a transplantable solid pulmonary tumor induced in a male Donryu strain rat with 4-nitroquinoline 1-oxide. SLC cells injected intravenously have been found to produce metastatic foci only in the lung, with all injected rats dying of pulmonary metastases.[8,9]

In the present study, to investigate the effects of the SLC implantation on lipid metabolism, changes in lipid levels in serum and liver were examined in SLC-implanted rats. The SLC is a lung cancer and originates from the lung; therefore, the effects of the implanted tumor cells on lipid metabolism of SLC may be small as compared to the hepatoma,[5-7] which originates from the liver and is active in various types of lipid metabolism. In addition, in the tumor-bearing state, decreases in food intake and body weight are seen as a result of a cancer-induced cachexia. Therefore, a pair-feeding study was conducted to investigate the effects of food restriction on tumor-bearing in SLC-implanted rats.

MATERIALS AND METHODS

Animals and diets. This animal experiment was conducted with the approval of the Iwate Prefectural University Research Ethics Committee.

Male Donryu rats (four weeks of age, Japan SLC, Inc., Shizuoka, Japan) were individually housed in stainless steel cages with wire bottoms in an air-conditioned room with a temperature of 22±2℃, a relative humidity of 55±5%, and an 8:00 a.m. to 8:00 p.m. light cycle and were kept on a stock pellet diet (MF; Oriental Yeast Co., Tokyo, Japan), followed by a basal diet containing 20% casein.[10] The composition of the basal diet was as follows: 20% casein (Oriental Yeast Co.), 13.2% α-cornstarch (Oriental Yeast Co.), 39.75% cornstarch (Oriental Yeast Co.), 10% sucrose (Nissin Sugar Manufacturing Co., Tokyo, Japan), 5% cellulose powder (Oriental Yeast Co.), 7% soybean oil (Oriental Yeast Co.), 3.5% mineral mixture (AIN 93G composition)[10] (Oriental Yeast Co.), 1% vitamin mixture (AIN 93 composition)[10] (Oriental Yeast Co.), 0.25% choline bitartrate (Wako Pure Chemical Industries, Osaka, Japan), and 0.3% L-cystine (Wako Pure Chemical Industries). After preliminary feeding, the rats

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Abbreviations: HDL, high-density lipoprotein; LCAT, lecithin-cholesterol acyl transferase; LDL, low-density lipoprotein; PBS(-), phosphate-buffered saline; TBARS, thiobarbituric acid-reactive substance; VLDL, very-low-density lipoprotein.

Table 1. Initial body weight, food intake, body weight gain, and weights of liver and epididymal adipose tissue in normal rats fed *ad libitum* (Control), tumor-bearing rats fed *ad libitum* (Tumor-bearing), and normal rats pair-fed with tumor-bearing rats (Pair-fed).

Measurement	Control	Tumor-bearing	Pair-fed
Initial body weight (g)	157.0 ± 4.1	157.0 ± 2.4	166.0 ± 3.2
Food intake (g/21d)	464.5 ± 14.3	451.8 ± 12.2	446.6 ± 2.1
Body weight gain (g/21d)	185.7 ± 6.7	178.2 ± 5.5	167.8 ± 1.0
Liver weight (g/whole body)	17.1 ± 0.7	16.0 ± 0.4	16.3 ± 0.6
Epididymal adipose tissue weight (g/whole body)	5.89 ± 0.46	5.37 ± 0.28	5.22 ± 0.19

Values represent the means ± standard errors for seven rats. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Bonferroni test.

were divided into three groups (n=7) of similar body weights, with the first group receiving a subcutaneous implantation of 5×10^6 SLC cells (provided by the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan) suspended in phosphate-buffered saline (PBS(-)) (0.5 ml/rat) in the back to produce a solid tumor (tumor-bearing group), with the second group receiving a sham injection of PBS(-) alone (0.5 ml/rat), this group being designated normal rats (control group). Normal rats of the last group were given the same amount of diet which group of the tumor-bearing rats ate on the day before, and this group designates pair-fed group. The SLC cells were prepared by means of a cell culture system. The SLC cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum. The rats of each group were maintained for a further 21 days on the basal diet. The solid tumor diameter was measured to estimate the tumor volume every day. Water and diet were offered at 7:00 p.m. every day and remained available at all times. Animals were deprived of their diet at 9:00 a.m. on the 21st day, but allowed free access to water until sacrifice, which was performed 4 hours later. Blood was collected and left to clot at room temperature to obtain serum. The liver, epididymal adipose tissue, and solid tumor were quickly removed, washed with cold 0.9% NaCl, blotted on filter paper, and weighed. The serum and liver were stored at −30°C until lipid concentration analyses were performed. Aliquots of the liver were also preserved in methanol and stored at 4°C until analyses of the lipid contents were performed.

Lipid analyses. The lipoprotein separation of serum was as follows, HDL was separated from VLDL plus LDL by precipitation method using sodium phosphotungstic acid and MgCl,[11] and VLDL was separated from LDL plus HDL by ultracentrifugation.[12]

Serum total, HDL-, and (HDL+LDL)-cholesterol, free cholesterol, triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations were determined by an enzymatic method using a Cholesterol E-test, Free cholesterol E-Test, Triglyceride E-test, Phospholipid C-test, and NEFA C-test (Wako Pure Chemical Industries), respectively. The difference between the total cholesterol concentration and HDL-cholesterol concentration was regarded as the (VLDL+LDL)-cholesterol concentration. The difference between the (HDL+LDL)-cholesterol concentration and HDL-cholesterol concentration was regarded as the LDL-cholesterol concentration. The difference between the (VLDL+LDL)-cholesterol concentration and LDL-cholesterol concentration was regarded as the VLDL-cholesterol concentration. The ratio of the (VLDL+LDL)-cholesterol concentration to HDL-cholesterol concentration is designated as the atherogenic index. The difference between the total cholesterol concentration and free cholesterol concentration was regarded as the esterified cholesterol concentration, and the ratio of the esterified cholesterol concentration to total cholesterol concentration is designated as the cholesterol ratio.

Total lipids were extracted according to the procedure of Folch *et al.*[13] from the liver. After portions of the chloroform phase had been dried under nitrogen, the contents of cholesterol,[14] triglyceride,[15] and phospholipid[16] were determined.

The serum and liver thiobarbituric acid-reactive substance (TBARS) values were measured according to the method described by Yagi[17] and Mihara *et al.*,[18] respectively.

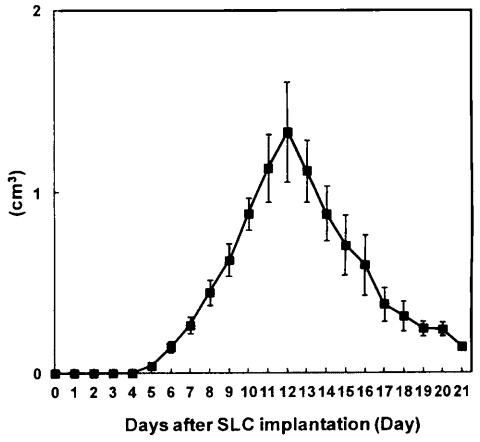

Fig. 1. Estimated solid tumor volume in tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors.

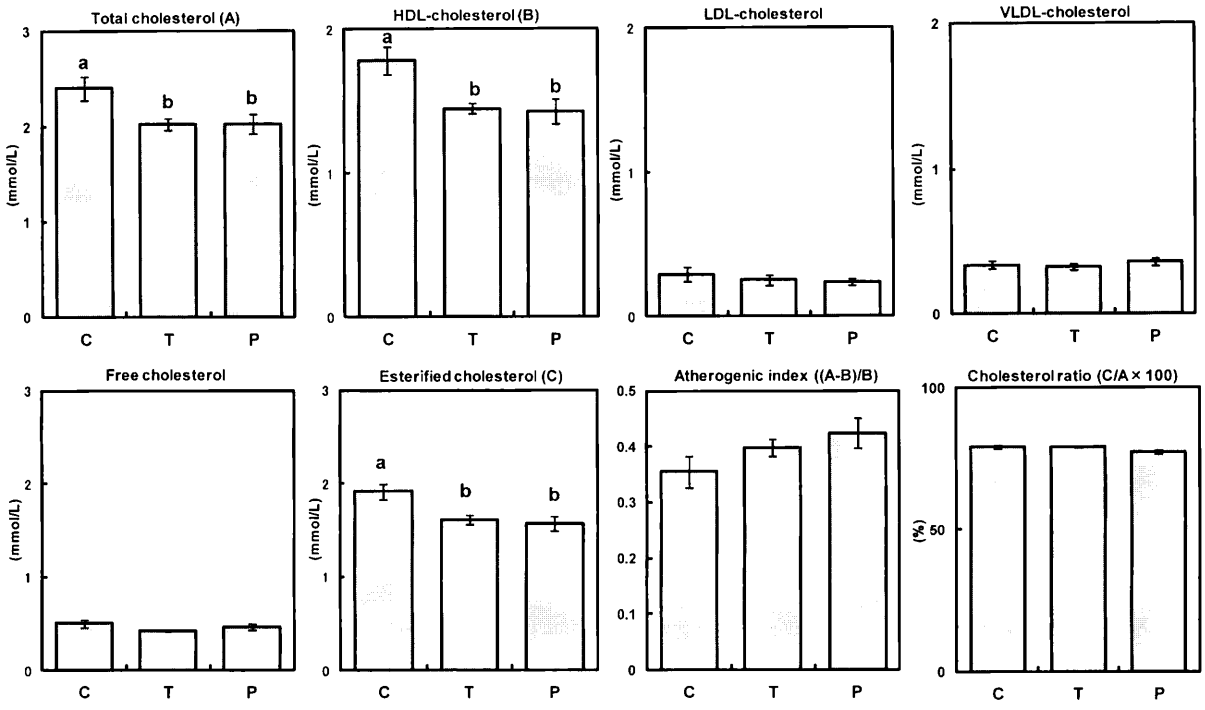

Fig. 2. Serum cholesterol concentration, atherogenic index, and cholesterol ratio in normal and tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Bonferroni test. C, normal rats fed *ad libitum* group (control); T, tumor-bearing rats fed *ad libitum* group; P, normal rats pair-fed with tumor-bearing rats.

Statistical analysis. Results were expressed as mean ± standard error. Statistical analysis was carried out by one-way analysis of variance followed by Bonferroni test using the SPSS Statistics, version 20 (IBM Japan, Ltd., Tokyo, Japan). A significance level of $p < 0.05$ was used for all the comparisons.

RESULTS

Table 1 shows the initial body weight, food intake, and body weight gain for the 21-day duration of the experimental period, and weights of the liver and epididymal adipose tissue at the end of the experimental period. There were no significant differences between the control and tumor-bearing groups with regard to the food intake, body weight gain, and liver and epididymal adipose tissue weights. In the pair-feeding study, the food intake, body weight gain, and weights of the liver and epididymal adipose tissue in the pair-fed group were not changed as compared to the control group.

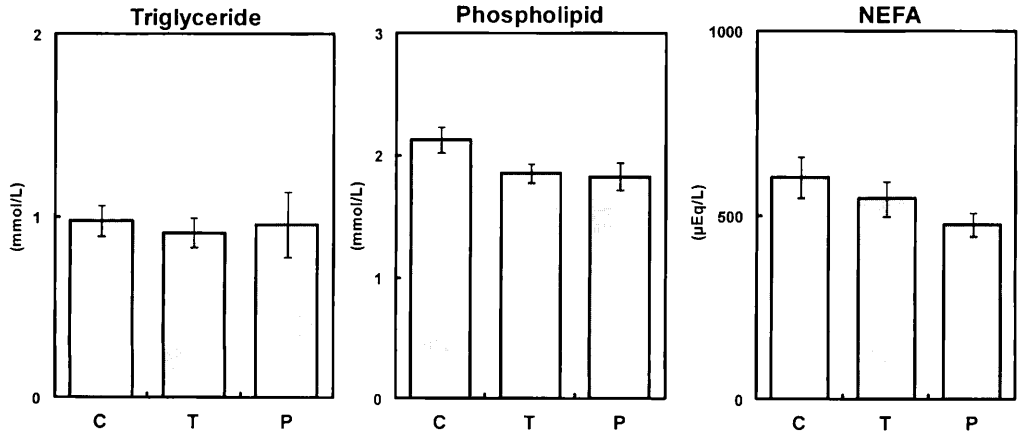

Fig. 3. Serum triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations in normal and tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors. C, normal rats fed *ad libitum* group (control); T, tumor-bearing rats fed *ad libitum* group; P, normal rats pair-fed with tumor-bearing rats.

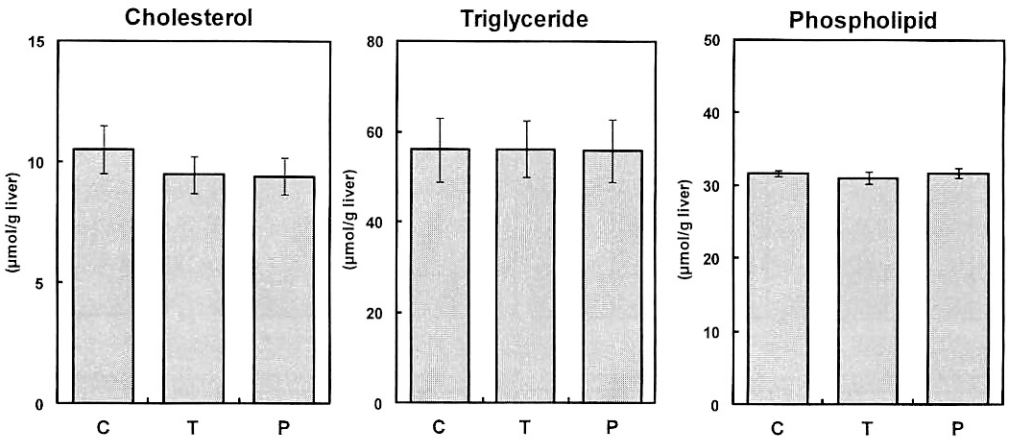

Fig. 4. Liver lipid contents in normal and tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors. C, normal rats fed *ad libitum* group (control); T, tumor-bearing rats fed *ad libitum* group; P, normal rats pair-fed with tumor-bearing rats.

The absolute and relative weights of the solid tumor at the end of the experimental period were 0.89±0.12g and 0.27±0.24% of body weight, respectively.

Fig. 1 shows the estimated solid tumor volume in tumor-bearing rats after SLC implantation. The solid tumor could be observed 3–5 days after SLC implantation, and continued to grow with time. On approximately the 12th day after SLC implantation, however, solid tumor stopped growing and then began to reduce in size until the 21st day after SLC implantation.

Serum cholesterol concentrations are shown in Fig. 2. The tumor-bearing rats significantly decreased the total cholesterol concentration as compared to the control group. In lipoprotein cholesterol, HDL-cholesterol and esterified cholesterol concentrations in the tumor-bearing group were significantly lower than those in the control group, while LDL-, VLDL- and free cholesterol concentrations were not changed in response to tumor-bearing. The atherogenic index and cholesterol ratio in the tumor-bearing group were not significantly changed as compared to the control group. In the pair-feeding study, the total cholesterol concentration in the pair-fed group was significantly lower than that in the control group. In lipoprotein cholesterol, HDL-cholesterol and esterified cholesterol concentrations in the pair-fed group were significantly lower than those in the control group, while LDL-, VLDL- and free cholesterol concentrations were not changed between the control and pair-fed groups. These cholesterol concentrations in the pair-fed group were not changed as compared to the tumor-bearing group. The atherogenic index and cholesterol ratio in the pair-fed group were not significantly changed as compared to the control group.

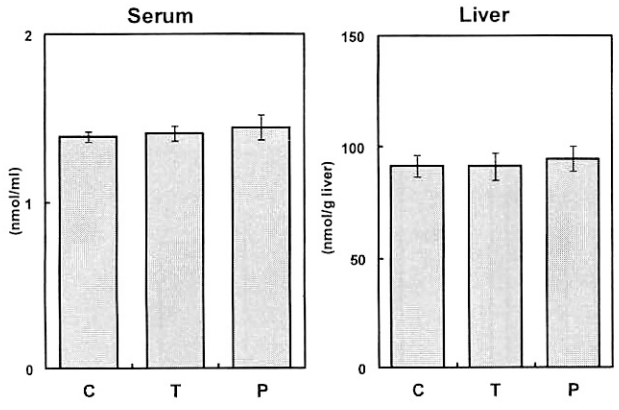

Fig. 5. Serum and liver thiobarbituric acid-reactive substance (TBARS) values in normal and tumor-bearing rats. Values represent the means for seven rats. Vertical bars indicate standard errors. C, normal rats fed *ad libitum* group (control); T, tumor-bearing rats fed *ad libitum* group; P, normal rats pair-fed with tumor-bearing rats.

Serum triglyceride, phospholipid, and NEFA concentrations are shown in Fig. 3. There were no significant differences between the control and tumor-bearing groups with regard to the serum triglyceride, phospholipid and, NEFA concentrations. In the pair-feeding study, the serum triglyceride, phospholipid, and NEFA concentrations were not significantly different between the control and pair-fed groups.

Liver lipid contents are shown in Fig. 4. The hepatic cholesterol, triglyceride, and phospholipid contents were not significantly different between the control and tumor-bearing groups. In the pair-feeding study, there were also no significant differences between the control and pair-fed groups with regard to the liver cholesterol, triglyceride, and phospholipid contents.

Serum and liver TBARS values are shown in Fig. 5. There were no significant differences between the control and tumor-bearing groups with regard to the serum and liver TBARS values. In the pair-feeding study, both serum and liver TBARS values were not significantly changed between the control and pair-fed groups.

DISCUSSION

The present study examined the effects of tumor-bearing on serum

lipid concentrations and liver lipid contents in SLC-implanted rats, and SLC implantation to rats significantly decreased the total cholesterol concentration, and especially esterified cholesterol and HDL-cholesterol concentrations, as compared to the control group. The free cholesterol esterification is catalyzed by lecithin-cholesterol acyl transferase (LCAT). LCAT catalyzes the transfer of a fatty acid residue from the 2 position of lecithin to cholesterol to form esterified cholesterol and is considered to be responsible for much of the esterified cholesterol in plasma lipoproteins. It is likely that, in the SLC-implanted rats, the esterification of free cholesterol might be reduced by the suppressed LCAT activity, and hence, the serum esterified cholesterol concentration is decreased. LCAT is also an important enzyme in reverse cholesterol transport, playing a central role in the transport of excess cholesterol from peripheral tissues to HDL. It has been reported that a significant decrease in plasma HDL-cholesterol concentration and a suppression of plasma LCAT activity are seen simultaneously in hemodialysis patients.[19] It is considered that if the suppression of serum LCAT activity is seen, it might be the cause of the significant decrease in serum HDL-cholesterol concentration seen in SLC-implanted rats.

It is well known that some cancers affect lipid metabolism. For example, serum total cholesterol and (VLDL+LDL)-cholesterol concentrations increased in ascites hepatoma cell line AH109A-bearing rats, while the serum HDL-cholesterol concentration decreased.[5-7] In the present study, the SLC implantation did not change the most of parameters related to lipid metabolism except for the above-mentioned measurements. In the tumor-bearing state, the disorder of lipid metabolism occurred during growth of the solid tumor.[5] In a previous study of tumor-bearing rats, an increase in plasma triglyceride concentration and a suppression of activities of tissue lipoprotein lipase, the hydrolytic enzyme in serum triglyceride, were seen with increased tumor burden, and tumor removal completely reversed these changes.[4] These studies provide evidence that the tumor-induced changes in lipid concentrations in blood are stimulated by the presence of tumor. In the present study, the solid tumor was observed within a short time after SLC implantation, though the solid tumor stopped growing and solid tumor weight was only less than 1% of the body weight on the 21st day after SLC implantation. Therefore, most of the parameters related to lipid metabolism examined in the present study might not be changed by SLC implantation. The reason why implanted tumor cells stopped growing is unclear. The tumor cell has a very distinctive metabolism with the potential for infinite growth. The SLC cell originates from Donryu rats; it is therefore difficult to cause a rejection in the present study because SLC cells were implanted into the Donryu rats.

The present study investigated the effects of diet restriction of tumor-bearing rats in addition to the effects of tumor implantation. The SLC implantation significantly decreased the total cholesterol, and especially HDL-cholesterol and esterified cholesterol concentrations, as compared to the control group. At the same time, these serum cholesterol concentrations were reduced by the diet restriction. Therefore, these serum cholesterol concentration-reductive actions might be due, at least in part, to the suppression of food intake accompanied by the tumor-bearing state in addition to the tumor-bearing effect itself.

In conclusion, SLC implantation to rats changes some serum cholesterol concentrations. The mechanisms responsible for the observed changes in these serum cholesterol concentrations that occur as a result of the SLC implantation were not determined in the present study. Questions regarding the mechanisms of these changes in lipid levels in response to SLC implantation remain to be answered in a further study.

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和文要旨　佐藤肺癌（SLC）移植ラットにおける血清および肝臓脂質レベルに対する担癌の影響を，食餌を自由摂取させた正常ラット（対照群）およびペアフィーディング正常ラットとの比較で検討した。担癌ラットにはSLC細胞を5×10^6移植し21日間飼育した。血清総コレステロール濃度ならびにその中で高密度リポタンパク質（HDL）コレステロールおよびエステル結合型コレステロール濃度が癌の移植後対照群と比較して有意に低下した。ペアフィーディング群のHDLコレステロールおよびエステル結合型コレステロール濃度も対照群と比較して有意に低下した。SLC移植ラットにおいては血清コレステロール濃度が変動し脂質代謝異常が生じたが，これら血清コレステロール濃度の低下には少なくとも一部として担癌そのものの影響に加えて担癌による飼料摂取量の低下が要因となっていることが示唆された。