Modified Lipoprotein Lipase Activities, Rates of Lipogenesis, and Lipolysis as Factors Leading to Lipid Depletion in C57BL Mice Bearing the Preputial Gland Tumor, ESR-586

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ABSTRACT

The biochemical basis for the observed depletion of adipose tissue in C57BL mice bearing a transplantable nonmetastasizing preputial gland tumor, ESR-586, has been investigated. The results have shown that there are a number of significant changes in both deposition and mobilization of lipid as the tumor grows. The first change, before the tumor reached 2 g, was a decline in the activity of adipose tissue lipoprotein lipase to levels normally found in starved animals. This was accompanied by a slight increase in lipoprotein lipase activity in heart and appearance of substantial activity in large tumors. Together, these would result in impaired uptake of exogenous fatty acids by adipose tissue, and dietary lipid would be directed away from storage. This was followed by a marked decline in endogenous lipid synthesis in adipose tissue which commenced when the tumor weighed between 2 and 3 g, as measured in vivo by the incorporation of radioactivity into lipid from tritiated water. The basal rate of lipolysis was enhanced 2-fold in epididymal fat pads from mice bearing tumors that weighed between 2 and 4 g, although there was no difference in the epinephrine-stimulated activity.

INTRODUCTION

The depletion of lipid from the fat stores has been frequently observed in animals bearing either spontaneous or transplanted tumors (1–4, 7, 14, 18, 19) and is a common observation in human cancer patients as well (20). The metabolic basis for this depletion of lipid has not been fully elucidated. Several possible mechanisms could be involved. These include modifications of the rate of lipid deposition or of the rate of lipid mobilization, which taken singly or together could result in the observed depletion of the adipose lipid. Furthermore, because there is more than one source of adipose lipid, the rate of lipid deposition can be affected by several factors. Exogenous triacylglycerols of dietary origin or those synthesized in adipose cells can enter the adipose cells. The endogenous lipid is synthesized primarily from glucose, and here many potential sites of regulation exist, not the least being the availability of glucose itself.

All these possibilities have been suggested previously, and there is some evidence in support of each of them. Rats bearing the Walker 256 carcinoma have increased plasma free fatty acid levels, and their epididymal adipose tissue has been shown to have basal lipolytic rates 2 to 3 times higher than those of tissue from normal rats (18), which suggests increased mobilization of lipid. Earlier reports have shown that the incorporation of labeled glucose (3, 16) and labeled acetate (24) into carcass lipids of tumor-bearing animals is depressed when compared with normal animals. Trew and Begg (27) have also shown impaired incorporation of labeled acetate into fatty acids in a mince prepared from adipose tissue of tumor-bearing rats. All of these studies, however, have each addressed only a single parameter, and in none has the relative importance of all the possible mechanisms leading to lipid depletion been determined. Furthermore, none of these studies has related the appearance of these changes with the stage in tumor growth.

In the present paper, 3 major mechanisms have been investigated in C57BL mice bearing a transplantable, nonmetastasizing preputial gland tumor, ESR-586 (9, 11, 22). Results show that while both enhanced mobilization and impaired storage contributed to the depletion of carcass lipid in the mice with large tumors, the earliest modification detected was the decline in adipose tissue lipoprotein lipase activity. This could be responsible for a redistribution of dietary lipid away from storage in the adipose tissue to the other host tissues and, as the tumor grows, to the tumor itself.

MATERIALS AND METHODS

Chemicals. Tritiated water (5 Ci/ml), glycerol tri[9,10-3H]-oleate (500 mCi/mmol), and [U-14C]glucose (284 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Buckinghamshire, United Kingdom. Hyamine hydroxide (1 M in methanol) was obtained from Koch-Light Laboratories, Ltd., Colnbrook, Buckinghamshire, United Kingdom. Butyl-PBD3 was obtained from Ciba-Geigy, (N.Z.), Ltd.

Epinephrine (1-ml ampul for injection; 1 mg/ml) was from McGaw Ethicals, Ltd., Auckland, New Zealand. Heparin (5-ml ampul for injection; 500 units/ml) was from Weddel Pharmaceuticals, Ltd., London, United Kingdom.

Bovine serum albumin (Fraction V), triolein, enzymes, and coenzymes were obtained from Sigma Chemical Company, St. Louis, Mo. All other reagents used were of analytical grade.

Animals. Male and female C57BL mice, supplied by the University of Otago Animal Breeding Station, were used in this study. The mice were 4 to 6 months old when used. Mice bearing the transplantable preputial gland tumor, ESR-586, were inoculated with tumor, as described by Grigor (10). The growth rate of the tumor was not linear with time. There is a lag period of up to 3 weeks, after which there is rapid growth, and...
tumor weights of 8 to 9 g are reached by 6 weeks after inoculation (10). The present studies were carried out with both male and female mice bearing tumors that had been transplanted at least 2 weeks previously. All the measurements have been related to tumor weight. In all experiments, tumor-bearing mice were compared with age-matched normal mice of the same sex.

The mice had free access to a standard pelleted diet (A. E. Reeves, Ltd., Dunedin, New Zealand) unless otherwise stated. Meal-fed mice were given access to pellets from 9:00 a.m. to 12 noon daily. At least 1 week was allowed for the mice to adjust to the regimen before being inoculated and used for experiments. Food consumption by normal mice over the 3-hr period [mean food intake of 30 mice, 3.44 ± 0.65 (S.D.) g/mouse] was equal to consumption ad libitum (mean food intake of 8 mice, 3.3 g/mouse). Thirty mice bearing small tumors (less than 3 g) consumed 3.67 ± 0.73 g/mouse over the 3-hr period.

Total Lipid Content of Carcass. Female mice bearing tumors of different weights were killed and liver and tumor removed. The remaining carcass was homogenized in a Waring Blender for 60 sec in 150 ml of methanol:chloriform:water (20:10:6, by volume). The homogenate was filtered, and the lipid was extracted using the method of Bligh and Dyer (4). The chloroform layer was evaporated to dryness, and the total weight of lipid was determined gravimetrically.

**Lipolysis in Vitro.** Intact epididymidal fat pads were incubated in plastic vials with 2 ml Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 2.5% bovine serum albumin, in the absence and presence of epinephrine (10 μg/ml), to test for stimulatory activity in lipolysis. The reaction was linear for at least 3 hr, dependent on enzyme concentration and was characteristic of lipoprotein lipase, namely, dependent on preincubation of emulsion with serum and inhibited by 1 M NaCl (23). The difference in activity with and without NaCl was taken to correspond to the lipoprotein lipase activity.

**Lipogenesis in Vivo.** The rates of lipogenesis in vivo were determined by measuring the incorporation of 3H from 3H2O into the lipids of the tissues under study. Meal-fed mice were used. At the end of the feeding period (12 noon), the mice were given i.p. injections of 3H2O (2 μCi in 0.5 ml 0.9% NaCl solution). After 2 hr, the mice were decapitated, and blood samples were collected. The specific activity of the body water was determined by counting a suitably diluted aliquot of the plasma. The liver, parametrial or epididymidal fat pads, and, where appropriate, the tumor were quickly removed and weighed, and the lipid was extracted using the method of Bligh and Dyer (4).

The lipid samples were washed thoroughly, dried, and redissolved in chloroform. Appropriate aliquots of these samples were transferred to scintillation vials and evaporated to dryness, and 5 ml scintillation fluid (9 g butyl-PBD and 500 ml Triton X-100 per 1000 ml toluene) were added for counting, using a Packard Tri-Carb liquid scintillation spectrometer.

The rate of lipid synthesis in the tissues was calculated from the quotient:

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\frac{\text{[^3]H in total lipid (cpm)}}{\text{Specific radioactivity of plasma}}
\]

and converted into mol of newly synthesized fatty acid by dividing by 13.3 (15, 28). The results were expressed as μmol fatty acid synthesized per hr per g tissue or per total tissue.

The time course of lipid synthesis determined in liver and adipose tissue of normal mice was linear for at least 2 hr after the administration of the 3H2O.

In some cases, the lipid samples were analyzed by thin-layer chromatography on Silica Gel G plates developed with hexane: diethyl ether:glacial acetic acid (50:50:1, by volume), and the percentage of incorporation of label into the various lipid fractions was determined (25).

**RESULTS**

Total Lipid Content of Carcass, Liver, and Tumor. Both male and female mice bearing the transplantable preputial gland tumor became noticeably devoid of fat tissue. During growth of the tumor, there was a progressive depletion of total carcass lipid to amounts less than 0.25 g compared with a mean of 2.7 g for 6 normal mice (Chart 1). There appeared to be a sharp decline in lipid content in the early stages of tumor growth (tumors < 0.5 g). This may be attributed to the lag period in tumor growth (10); therefore, by the time the tumors reach between 0.5 and 1.0 g they have already been growing for 2 weeks.

It has been reported previously that there was no significant change in the total lipid content of the liver (5) and that the lipid...
content of the tumor, expressed either as mg lipid per g, wet weight, or as the total lipid content, increased during tumor growth (10). However, the greatest lipid content recorded was 237 mg for a very large tumor, and this was considerably lower than the 2 g lost from the carcass of mice with tumors of comparable weights.

Lipoprotein Lipase. An important potential point of control is that of the uptake and subsequent esterification of exogenous fatty acids from triacylglycerols of the circulating lipoproteins (chylomicrons from the gut and very-low-density lipoproteins from the liver). This process depends primarily on the presence of active lipoprotein lipase in the endothelial cells of the blood capillaries within the adipose tissue.

Chart 2A shows the activities of lipoprotein lipase in epididymal fat pads from normal fed and 48-hr-starved mice and from mice bearing tumors of increasing weight. It has been assumed that any changes in activity of lipoprotein lipase in this tissue would reflect the changes in all of the body fat depots.

Fasting for 48 hr resulted in a substantial decrease in activity from 35 to less than 10 μmol/hr/g, wet weight. In tumor-bearing mice, the activity fell to starved levels before the tumor had reached 2 g, even though the mice were feeding normally. This was the earliest change in lipid metabolism that was observed.

On the other hand, the lipoprotein lipase activity of the heart increased slightly (Chart 2B). Substantial activity also appeared in the tumor itself (Chart 2C). In tumors weighing less than 4 g, the activity was about 10 μmol/hr/g; but in tumors weighing between 6 and 8 g, the activity ranged between 25 to 48 μmol/hr/g. The latter represented a considerable total activity of lipoprotein lipase because of the relatively large size of the tissue. It is apparent that the tumor has the capacity to compete effectively for dietary triacylglycerols or those synthesized by the liver.

Lipogenesis in Vivo. The rates of lipogenesis in adipose tissue, in liver, and in tumor were determined in vivo by measuring the incorporation of label from 3H2O into lipid. Tritiated water has the advantage over other labeled precursors for measuring lipogenesis, because the specific activity of the body water remains constant for the duration of the experiment; therefore, the actual rates of synthesis, regardless of the sources of carbon, can be determined.

Of the label recovered in the lipid of the parametrial fat pads of normal mice, 64 ± 8% (5 mice) was present as triacylglycerol and 30 ± 7% as diacylglycerol. A similar distribution was observed in the tumor-bearing mice [79 ± 4 and 21 ± 3% (3 mice), respectively]. This is consistent with the role of adipose tissue as the major site of triacylglycerol storage.

The rates of lipogenesis in the parametrial fat pads were determined (Chart 3A). The mean rate for the normal parametrial fat pads was 8.6 μmol/hr/g, wet weight. The rates of lipogenesis in the adipose tissue declined as the tumor weight increased (Chart 3A). The decline commenced when the weight of the tumor was between 2 and 3 g. Beyond 4 g, the rates were less than 0.5 μmol/hr/g, wet weight, and some of the fat pads had disappeared totally. The total amount of lipid synthesized in the fat pads showed a similar pattern of decline (Chart 3a).

The lipogenic rate in the parametrial fat pads of mice fasted for 48 hr was also determined, and it was found to be negligible.

In liver, the rates of lipogenesis per g, wet weight, of tissue gradually declined with tumor growth (linear correlation coefficient, \( r = 0.63 \); Chart 3B). However, the total amount of lipid synthesized was not significantly altered in tumor-bearing mice (Chart 3B). This could be accounted for by the fact that the liver weight increases as the tumor grows (5). These results parallel the previously reported observations that the lipid content per g wet weight liver declined with tumor growth and
that the total lipid content was constant throughout (5). There was no significant alteration in the types of lipid synthesized by livers of normal and tumor-bearing mice. In both cases, approximately 15% of the label was incorporated into di- and triacylglycerols and 80% appeared in the phospholipids.

The rates of lipogenesis in the tumors decreased as the tumor grew (Chart 3C; linear correlation coefficient, r = 0.80), whereas the total amount of lipid synthesized per hr in the tumor increased, reached a maximum when the tumor weighed between 3 and 4 g, and then declined (Chart 3c). The increase occurred during the period of most rapid tumor growth (10). With the onset of necrosis in large tumors, lipid synthesis would be expected to decline as observed.

The other experiments reported in this paper used mice that were feeding ad libitum, whereas these experiments were carried out on 3-hr meal-fed mice. This approach was initially used to eliminate any variability in lipogenic rates between mice that could occur simply on the basis of different period of time since last feeding. However, this approach has also revealed a very interesting finding, namely, that mice bearing tumors greater than 4 g do not carry out lipogenesis even in the face of the normal caloric load of a meal. In some preliminary experiments, the food intake of normal mice was restricted to 75% of that normally consumed. There was no significant difference in the total amount of lipid synthesized by epididymal fat pads from food-restricted mice [1.29 ± 0.67 (9 mice) µmol/hr] compared to that from normally fed mice [1.15 ± 0.58 (6 mice) µmol/hr]. Refeeding normal mice that had been starved for 40 hr caused a 10-fold stimulation in the total rate of lipogenesis. In other words, both food-restricted and starved mice responded to a meal with active lipogenesis. In contrast, a normal response to a meal was impaired in the tumor-bearing mice.

Lipolysis. To determine whether any change in lipolysis had occurred the rates of glycerol release were measured in isolated epididymal fat pads from mice bearing tumors at the stage of growth when lipid depletion was occurring, namely, between 2 and 4 g, and compared with age-matched normal mice (Table 1).

For the normal mice, the basal rate of glycerol release was stimulated about 3-fold by epinephrine. For mice with tumors between 2 and 4 g, the basal rate had increased to twice normal, but the epinephrine-stimulated values were not different from those for normal mice. The rate of lipolysis in fat pads from 24-hr-fasted mice was similar to that from mice bearing 2- to 4-g tumors (results not shown).

The average weight of a single fat pad from these tumor-bearing mice was 134 ± 57 mg (11 observations) compared to 159 ± 50 mg for 14 normal mice. The difference in weight was not significant; therefore, the observed increase in basal lipolysis was most likely to be a real effect, not simply an apparent one due to smaller adipocytes, for example.

DISCUSSION

The depletion of adipose tissue lipid stores in C57BL mice bearing the preputial gland tumor is not the result of a single event but rather the result of concerted changes in both deposition and mobilization. The first and perhaps critical change that occurs during the growth of the tumor is a dramatic decline in lipoprotein lipase activity of the adipose tissue. This occurs when the tumor is less than 5% body weight (less than 2 g). It is followed by changes in lipogenic and lipolytic rates which both occur when the tumor is between 2 and 4 g. Liver changes [depletion of glycogen (5) and the modification of several enzyme activities (12)] are also noted from this time. From growth curves for this tumor in these mice, these events may be separated by several days (10). The only other change detected to date at the very early stages of tumor growth is a mitogenic stimulus to the liver. This has been investigated by measuring the uptake of [3H]thymidine into liver DNA and by

![Table 1](cancerres.aacrjournals.org)
measuring the activity of thymidine kinase in these livers (21).

The decrease in lipoprotein lipase activity implies that the ability of the adipose tissue to take up exogenous fatty acids from the circulating triacylglycerol is also decreased. A number of studies using different tissues under a variety of physiological conditions have shown that a correlation exists between the activity of the enzyme and the ability to take up lipid (23). In our tumor-bearing mice, the changes in lipoprotein lipase activity occur in a tissue-specific manner, and the loss of adipose tissue activity is accompanied by increases in the activity in both the heart and the tumor itself. These findings suggest that the plasma triacylglycerols are directed away from storage in the adipose tissue and possibly toward uptake by the heart and the tumor. This kind of redistribution of lipoprotein lipase activity has been noted in starved rats where adipose tissue activity falls and the heart activity rises (6, 23) and in lactating rats where the adipose tissue activity falls while that of the mammary gland rises (13).

It is still a matter of speculation as to how the presence of the tumor brings about these changes in lipoprotein lipase activity and rates of lipogenesis and lipolysis. An early explanation for lipid depletion in tumor-bearing animals was that a situation analogous to starvation resulted from increased fuel demands by the tumor. The accumulated evidence, however, suggests that the depletion of carcass lipid in tumor-bearing animals cannot be explained solely on nutritional grounds. For example, the depletion of lipid in tumor-bearing rats also occurred when they were force-fed on high-fat, high-carbohydrate, or high-protein diets (26). In experiments with rats bearing Walker carcinoma 256, it was shown that the decreased incorporation of labeled acetate into fatty acids of minced preparations of adipose tissue persisted even after force-feeding the tumor-bearing rats (27). Furthermore, the inability of adipose tissue from our tumor-bearing mice to carry out lipogenesis in response to a meal (this report) provides another important difference.

It is possible that the tumor secretes a humoral factor which either has a direct effect on the adipose tissue of the host or can perturb the normal endocrine system of the host in such a way as to cause the observed metabolic responses. As this manuscript was being prepared for submission, it was reported that the serum from AKR mice with lymphomas contains an active lipolytic factor (17). We have reported previously the presence of a mitogen in the serum of mice with the preputial tumor which stimulated the incorporation of [3H]thymidine into liver DNA when injected into control mice (21).

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REFERENCES

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