The Synthesis and Activity of Lipoprotein Lipase in the Subcutaneous Adipose Tissue of Patients with Musculoskeletal Sarcomas

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Abstract. *The purpose of this study was to explore the triacylglycerol (TG) deposition and lipoprotein lipase (LPL) activity in the adipose tissue of patients with muculoskeletal sarcoma. Subcutaneous adipose tissue was obtained from the thighs of 19 patients with musculoskeletal sarcomas (sarcoma group) and 20 patients with osteoarthritis of the hip joint (control group) at surgery. The adipose tissue was homogenized and aliquots of the homogenate were used to measure the TG content and to prepare an acetone/ether powder to measure the LPL activity. The TG content was higher, but not significantly, in the sarcoma group than in the control group. The LPL activity of the sarcoma group was significantly higher than that of the control group. The TG content of the sarcoma group correlated positively with the LPL activity. [^{35}S]Methionine incorporation investigation showed that the rate of LPL synthesis was significantly higher in the sarcoma group than in the control group. These results indicated that LPL was up-regulated at the transcriptional/translational level, thus resulting in an increased TG deposition in the adipose tissue of patients with muculoskeletal sarcoma.*

Cancer cachexia is a complex metabolic disorder, involving the loss of body fat, the loss of skeletal muscle mass, an elevation of the resting energy expenditure and anorexia, and it is often associated with a poor prognosis and decreased survival time (1-3). About half of all cancer patients show cachexia (4). Cachexia occurs more commonly in patients with lung and upper gastrointestinal cancer and less commonly in patients with breast and lower gastrointestinal cancer (5).

The loss of body fat involves two distinct lipolytic systems. One system is an increased lipolysis of triacylglycerol (TG) in adipocytes catalyzed by hormone-sensitive lipase. An increased mobilization of body fat has been observed early in the growth of tumors, thus resulting in decreased body fat in tumor-bearing mice (6, 7). The other system is a decreased uptake of fatty acids from circulating TG into adipocytes catalyzed by lipoprotein lipase (LPL). LPL is synthesized in adipocytes and it is transported from adipocytes to the luminal surface of capillaries in adipose tissues where it hydrolyzes TG in chylomicrons and very low density lipoproteins into fatty acids and monoacylglycerols. These fatty acids are re-esterified and then are accumulated in the TG pool of adipose tissues. There have been some reports on adipose tissue LPL in cancer patients (8) and tumor-bearing rodents (9-12). In tumor-bearing mice, the LPL activity in the epididymal fat pad decreased significantly with the increasing tumor burden (12). Our previous study showed that the LPL activity of the adipose tissue of patients with carcinomas was lower than that of the adipose tissue of control subjects (8).

To date there has been no report addressing the relationship between body fat and the LPL activity in patients with musculoskeletal sarcomas. In the present study, the TG content and LPL activity were measured in subcutaneous adipose tissues of patients with musculoskeletal sarcomas. In addition, the correlations between the LPL activity and the adipose tissue TG content and body mass index (BMI) were also examined.

Materials and Methods

Materials. A chicken antiserum to bovine LPL was generously donated by Dr. Thomas Olivecrona (Umea University, Sweden). Glycerol tri[9,10(n)-^{3}H]oleate, and L-[^{35}S]methionine were

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obtained from GE Healthcare Bio-Science Co. (Tokyo, Japan). An ELISA kit for LPL mass was purchased from Daiichi Pure Chemicals (Tokyo, Japan). The Wako triglyceride E-test was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Subjects and adipose tissue. Nineteen patients with musculoskeletal sarcomas (7 males and 12 females) constituted the sarcoma group (Table I). The mean age of the patients was 46.1 years (range, 15-79 years). Their mean height and body weight were 160.1±1.8 cm and 61.3±1.9 kg, respectively. Twenty patients with osteoarthritis of the hip joint (4 males and 16 females) were the control group. Their mean age was 63.2 years (range, 49-74 years). Their mean height and body weight were 158.0±1.9 cm and 58.8±1.7 kg, respectively.

Both the sarcoma group and the control group were sampled in the late morning after fasting overnight. Subcutaneous adipose tissue was obtained from the thigh at surgery and then transported to the laboratory within 10 min after the excision in Dulbecco's modified Eagle's medium (DMEM) containing 5 μg/ml of insulin, 100 U/ml of penicillin, 0.1 mg/ml streptomycin, 0.25 μg/ml of amphotericin B and 10% fetal bovine serum (FBS). Neither metastases nor tumor infiltration was present in the adipose tissue.

The protocol was approved by the Ethics Committee of the Ehime University School of Medicine and informed consent was obtained from all the participants before their inclusion in the study.

Measurements of DNA and TG contents and LPL activity. Each sample of 500 mg of adipose tissue was homogenized with a Teflon-glass homogenizer in 1.5 ml of 50 mM NH_4Cl/NH_4OH buffer (pH 8.2) containing 2% bovine serum albumin and 20 μg/ml of heparin at 10˚C and sonicated briefly at 0˚C. Aliquots of the homogenates were used for the DNA and TG measurements. The DNA was measured fluorometrically by the method of Hinegardner (13) and the TG was measured using a Wako triglyceride E-test. Another aliquot of the homogenate was used to prepare an acetone/ether powder as described previously (14).

The LPL was extracted by adding the powder to 1 ml of ice-cold 50 mM NH_4Cl/NH_4OH buffer (pH 8.2) containing 20 μg/ml of heparin as described previously (14). A stock substrate emulsion containing 5 mCi of tri[9,10(n)-^{3}H]oleoylglycerol, 1.13 mmol of trioleoylglycerol, 60 mg of phosphatidylcholine and 9 ml of glycerol was prepared. A total of 1 volume of the stock substrate emulsion, 19 volumes of 3% bovine serum albumin in 0.2 M Tris/HCl buffer (pH 8.2) and 5 volumes of heat-inactivated (56˚C, 10 min) serum from starved rats was mixed and incubated for 15-30 min at 37˚C. For the assay, 100 μl of this activated substrate mixture was added to 100 μl of diluted extract and incubated for 60 min at 37˚C. One milliunit (mU) of lipolytic activity was defined as that releasing 1 nmol of fatty acid/min at 37˚C.

Synthesis of LPL. Each sample of 500 mg of adipose tissue was incubated for 2 h at 37˚C with 200 μCi of [^{35}S]methionine in methionine-deficient DMEM containing 5 μg/ml of insulin, 100 U/ml of penicillin, 0.1 mg/ml of streptomycin, 0.25 μg/ml of amphotericin B, and 10% FBS that had been dialyzed against methionine-deficient DMEM. The tissue specimens were washed once with ice-cold Dulbecco's phosphate-buffered saline, homogenized in lysis buffer (pH 7.5) containing 0.2 M Tris, 3% Triton® X-100, 1% N-lauroylsarcosine, 0.15 M NaCl and 1 mM phenylmethylsulfonyl fluoride at 10˚C, sonicated briefly at 0˚C and centrifuged for 20 min at 12000×g at 4˚C. The ^{35}S-labeled LPL was then immunoprecipitated from an aliquot of the infranatant with a chicken antiserum to bovine LPL and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (14). The gels were stained with Coomassie blue, destained, and dried on a sheet of cellophane under vacuum. Autoradiographs were obtained by exposing Kodak X-Omat film to the dried gels at –80˚C for 14 days. The band corresponding to LPL was cut out from the gel and dissolved in 1 ml of H_2O_2 at 65˚C overnight. The radioactivity was determined in a liquid scintillation counter.

Statistical analysis. Differences between two independent groups were analyzed by Student's *t*-test. Pearson *r* was used to calculate any correlations between the LPL activity and the adipose tissue TG and BMI. A *p*-value of <0.05 was considered to indicate a significant difference. All data were expressed as the mean±SE.

Results

Clinical data of sarcoma patients and adipose tissue TG content. The clinical data of the patients with musculoskeletal sarcoma are shown in Table I. The diagnosis was established histopathologically using specimens obtained by either a needle biopsy or open biopsy. The histopathological types of the tumors are shown in Table I. Eight patients were overweight (BMI: 25< to <30 kg/m^2) and the remaining 11 patients were normal-weight (BMI: 18.5< to <25 kg/m^2). Four patients developed hypertriglyceridemia and 2 patients developed both hypertriglyceridemia and hypercholesterolemia. This is consistent with the findings of others that the elevation of plasma TG was often observed in cancer patients (15-18) and tumor-bearing rodents (9-11). The BMI of the hypertriglyceridemic patients was higher than that of the normolipemic patients (BMI (n=6), 26.0±0.5 kg/m^2 and BMI (n=13), 23.3±0.5 kg/m^2, respectively; $p<0.01$).

In the control group, 7 subjects were overweight (BMI 26.6±0.4 kg/m^2) and the remaining 13 subjects were normal-weight (BMI 22.0±0.7 kg/m^2) (data not shown). The plasma TG levels of all the control subjects were normal (51-146 mg/dl), while the plasma total cholesterol (TC) levels of 2 patients were 220 and 223 mg/dl.

The TG content in the adipose tissue homogenates of the sarcoma patients is shown in Table I. The mean TG content of the adipose tissue of the sarcoma group was 1.7 times higher, but not significantly, than that of the control group (control group, 988±110 mg/μg DNA (n=20); sarcoma group, 1638±369 mg/μg DNA (n=19)).

Activity and synthesis of LPL. The LPL activity in the aqueous extract of the acetone/ether powder of the adipose tissues is shown in Table II. When the LPL activity of all the patients in the sarcoma group was compared with that of all the patients in the control group, the mean LPL activity was significantly higher in the sarcoma group than in the control group. The patients were divided into two subgroups, namely

Table I. *Clinical data of 19 patients with musculoskeletal sarcoma.*

Pt. No.	Age (years)	Gender	BMI (kg/m^2)	AT-TG (mg/μg DNA)	Plasma TG (mg/dl)	Plasma TC (mg/dl)	Histopathological type
1	15	M	21.5	0.49	68	211	Osteosarcoma
2	16	M	24.0	4.30	165	191	Osteosarcoma
3	31	M	23.4	1.32	71	153	Osteosarcoma
4	37	F	24.4	0.72	96	149	Osteosarcoma
5	48	M	24.1	0.82	201	236	Liposarcoma
6	59	F	25.2	1.38	72	173	Liposarcoma
7	62	F	26.2	1.78	87	219	Liposarcoma
8	69	F	26.1	2.99	265	188	Liposarcoma
9	62	F	25.7	3.72	89	203	MFH
10	72	F	25.4	0.81	134	196	MFH
11	76	F	24.5	0.67	168	184	MFH
12	79	M	26.2	5.02	219	213	MFH
13	30	F	20.6	0.51	130	216	Synovial sarcoma
14	57	F	28.0	4.52	174	234	Synovial sarcoma
15	39	F	23.2	0.64	84	194	Leiomyosarcoma
16	52	M	22.2	0.41	106	207	Leiomyosarcoma
17	20	M	25.1	0.34	124	139	Chondrosarcoma
18	64	F	24.7	0.42	63	207	Chondrosarcoma
19	19	F	19.1	0.26	52	144	Ewing's sarcoma

BMI, Body mass index; AT-TG, adipose tissue triacylglycerol; TC, total cholesterol; MFH, malignant fibrous histiocytoma.

Table II. *LPL activity in the adipose tissues of the control and sarcoma groups.*

	LPL activity (mU/mg DNA)[a]		
	All patients	Normal-weight subgroup	Overweight subgroup
Control group	15.0±1.6 (20)	12.3±1.7 (13)	20.0±2.5* (7)
Sarcoma group	25.9±2.9** (19)	21.4±2.6** (11)	32.1±5.2 (8)

[a]Mean±S.E; the numbers in parentheses show the number of patients. *$p<0.05$ (*vs.* the normal-weight subgroup in the control group); **$p<0.01$ (*vs.* the corresponding group of the control group).

Table III. *Correlation coefficient between BMI and adipose tissue TG and LPL activity.*

	Control group (n=20)		Sarcoma group (n=19)	
	AT-TG (μg/mg DNA)	LPL activity (mU/mg DNA)	AT-TG (μg/mg DNA)	LPL activity (mU/mg DNA)
BMI (kg/m^2)	0.725**	0.734**	0.603**	0.514*
AT-TG (μg/mg DNA)	-	0.918**	-	0.887**

AT-TG, Adipose tissue triacylglycerol; *$p<0.05$, **$p<0.01$.

a normal-weight subgroup and an overweight subgroup. In the normal-weight subgroup, the mean LPL activity of the sarcoma group was 1.7 times higher than that of the control group. In the overweight subgroup, the mean LPL activity of the sarcoma group was higher, but not significantly, than that of the control group.

Next, the sarcoma group was divided histopathologically into the 7 types of tumors and the LPL activity of each type was compared with that of the control group (Figure 1A). The mean LPL activities of the 4 patients with osteosarcoma and the 4 patients with liposarcoma were 1.8 and 2.0 times higher ($p<0.01$), respectively, than that of the control group. Out of the 4 patients with malignant fibrous histiocytoma, the mean LPL activity of two of the patients was very high, but that of the other 2 patients was within the normal range. The LPL activity of the 2 patients with synovial sarcoma tended to be higher than that of the control group. The LPL activities of the 2 patients with leiomyosarcoma, the 2 patients with chondrosarcoma and the patient with Ewing's sarcoma were within the normal range.

The synthesis of LPL in the adipose tissues of the patients with osteosarcoma was compared with that in the control group. The ^{35}S-labeled LPL of the osteosarcoma group moved on SDS-PAGE with the same mobility (Mr=57 kDa) as that of the control group (Figure 1B, upper panel), thus indicating that the adipose tissue specimens of the osteosarcoma group synthesized a normal-sized LPL. The amount of ^{35}S incorporated into LPL of the osteosarcoma group was 1.6 times higher than that of the control group (Figure 1B, lower panel).

Correlation of adipose tissue TG, BMI and LPL activity. Table III shows the correlation coefficients between adipose tissue TG, BMI and LPL activity. In both the control and sarcoma groups, the adipose tissue TG content correlated positively with the BMI and the LPL activity showed a positive correlation with the adipose tissue TG content and BMI.

Discussion

Cancer patients and tumor-bearing animals frequently show a striking depletion of body lipid (4, 6, 7, 19, 20). Our previous study showed that in patients with carcinoma, the TG content of omental adipose tissue was 31% of that of control subjects, and the TG content of subcutaneous adipose tissue was also lower, but not significantly, than that of control subjects (8). In the present study, in the patients with musculoskeletal sarcoma, the TG content of subcutaneous adipose tissue was higher, but not significantly, than that of control subjects, suggesting that the lipid metabolism in the adipose tissue of the patients with musculoskeletal sarcoma

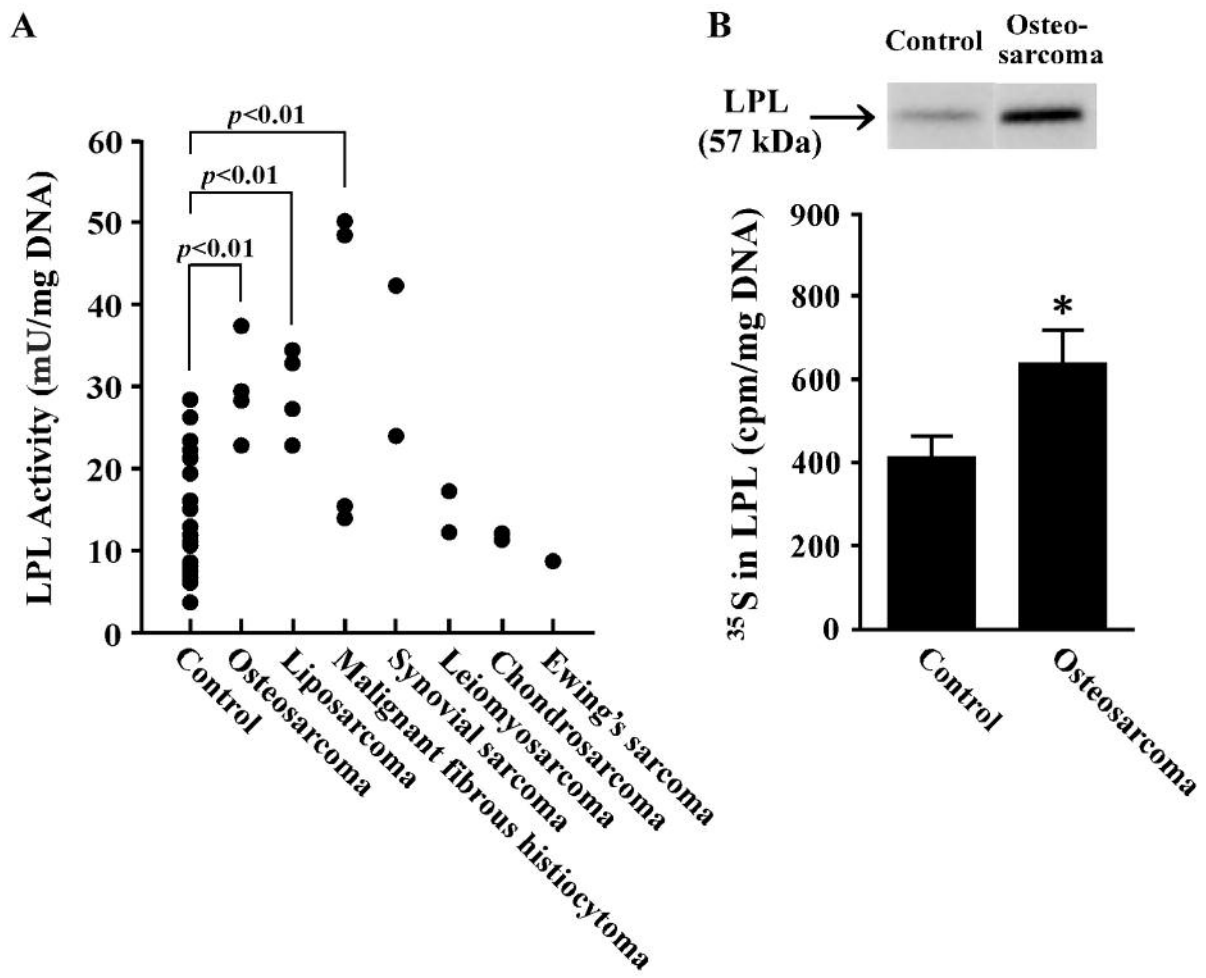

Figure 1. *Activity and synthesis of LPL in adipose tissue. (A) LPL activity in adipose tissue. The adipose tissue was processed into acetone/ether powders, and the LPL activity in an aqueous extract of the powder was measured. The data show the LPL activity of each type of musculoskeletal sarcoma. (B) LPL synthesis in adipose tissue from control subjects and patients with osteosarcoma. The adipose tissues of the control subjects and the patients with osteosarcoma were incubated for 2 h with [^{35}S]methionine, and ^{35}S-labeled LPL was immunoprecipitated with a chicken antiserum to bovine LPL and resolved on SDS-PAGE. The upper panel shows autoradiographs of ^{35}S-labeled LPL. The values given are the mean±SE for four patients. *p<0.01 (compared with the value of the control group).*

may be different from that in the adipose tissue of the patients with carcinoma. Recently, Mansky *et al.* (21) have also reported an increased body fat content in pediatric sarcoma patients. However, the mechanisms responsible for the increased fat content in these patients remain unclear.

Human adipose tissue derives most of the lipids for storage from circulating TG, therefore, LPL is a key regulator of TG accumulation. In the present study, the LPL activity of the control group correlated positively with adipose tissue TG content and BMI, which was consistent with earlier work showing that LPL regulates the TG pool of adipose tissue (22). The down-regulation of LPL activity is thought, in part, to contribute to cancer cachexia (9). The elevated adipose tissue TG levels found in the sarcoma patients raised the question of whether musculoskeletal sarcoma affects the adipose tissue LPL activity. The LPL activity of the sarcoma group was significantly higher than that of the control group and additionally the LPL activity of the sarcoma group correlated positively with the adipose tissue TG. These results indicated that the LPL activity was up-regulated in the adipose tissue of the patients with musculoskeletal sarcoma, resulting, at least in part, in an increase in TG deposition in the adipose tissue, in contrast to down-regulation of LPL and decreased TG deposition in the adipose tissue of patients with carcinoma (8).

The adipose tissue of obese subjects has been shown to contain a higher level of LPL activity than does that of lean individuals (23-25), which was confirmed by the present

finding that in the control group, the LPL activity of the overweight subgroup was significantly higher than that of the normal-weight subgroup. Since the BMIs of the osteosarcoma group were normal, while their LPL activity was significantly higher than that of the control group, the synthesis of LPL was examined in the adipose tissues of the osteosarcoma group and the control group. The incorporation of [^{35}S]methionine into LPL showed that the rate of LPL synthesis in the osteosarcoma group was significantly higher than that of the control group. However, there was no difference in the amount of ^{35}S incorporated into the total protein in the adipose tissues between the osteosarcoma group and control group (data not shown), indicating that the protein synthesis in the adipose tissues of the osteosarcoma group was normal. Thus the higher LPL activity in the adipose tissue of the osteosarcoma group might be due to increased LPL synthesis.

Human LPL is a glycoprotein with two *N*-linked oligosaccharide chains (26), one complex type and one high-mannose type chain (27), and the expression of LPL activity involves posttranslational modifications, such as glycosylation (28-31), as well as LPL gene transcription, mRNA processing and translation. The use of endoglycosidase H, which cleaves the high mannose-type oligosaccharide chain, but not the complex type chain of glycoproteins (32), showed that there was no difference in the processing of the oligosaccharide chains of LPL between the osteosarcoma group and the control group (data not shown). The present findings indicated that the LPL activity of the adipose tissues of the patients with osteosarcoma was up-regulated at the transcriptional/translational level.

In conclusion, LPL activity is significantly increased in the adipose tissue of musculoskeletal sarcoma patients and adipose tissue TG levels tend to be elevated, which is in contrast to carcinoma patients.

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