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Metabolic and Morphologic Characteristics of Adipose Tissue Associated with the Growth of Malignant Tumors

Osamu Ishiko,[1] Sadako Nishimura, Tomoyo Yasui, Toshiyuki Sumi, Kouzo Hirai, Ken-ichi Honda and Sachio Ogita

Department of Obstetrics and Gynecology, Osaka City University Medical School, 1 Asahimachi, Abeno-ku, Osaka 545-8585

Changes in total body fat and the metabolic and morphologic characteristics of adipose tissue were sequentially investigated in individual rabbits implanted with VX2 tumors to elucidate the pathology of the fat reduction in animals with malignant tumors as compared with that of diet-restricted rabbits. Lipogenesis in normal, VX2-implanted, and diet-restricted rabbit groups on day 40 after the start of the experiments was 19.1±2.9, 13.3±3.5, and 41.7±6.0×10^5 cpm/g/h, respectively, and glycerol liberation by their adipose tissue was 199±21, 528±94, and 301±45 nmol/g/h, respectively. In addition, apoptotic cells were noted in the adipose tissue of VX2-implanted rabbits on days 20–30 after implantation, but not in diet-restricted rabbits. The results showed clear differences between the total body fat reduction profiles of VX2-implanted rabbits and diet-restricted rabbits, suggesting a characteristic lipid metabolism with enhanced lipolysis and diminished lipogenesis in VX2-implanted rabbits. The results strongly suggest that adipocyte apoptosis might be involved in these phenomena.

Key words: Adipose tissue — Apoptosis — Cancer cachexia — Lipid metabolism — Weight loss

The pathology of the body weight loss of patients with terminal cancer in the final stage of their disease has been studied from the standpoints of protein, carbohydrate, and lipid metabolism, and their lipid metabolism has been found to be characterized by hyperlipidemia and disappearance of depot fat.[1, 2] Although many metabolic approaches have already been attempted, there has been no study on the time course of changes in the total body fat of individual animals in parallel with the progression of the cancer. The major reason for this was the lack of a noninvasive method that would allow repeated sampling of adipose tissue to measure total body fat in experimental animals. We previously reported the establishment of a noninvasive method for determining total body fat in rabbits.[3] This method was used in the present study to investigate the course of changes in body weight and total body fat level in VX2-tumor-bearing rabbits as an experimental model for studying differences in phenomena and metabolism related to the body weight reduction associated with the growth of malignant tumors, in comparison with the weight loss in diet-restricted rabbits.

MATERIALS AND METHODS

Experimental animals Male Japanese White rabbits weighing about 3 kg were used. VX2-tumor-bearing rabbits were prepared by implanting 1×10^5 VX2 tumor cells into the right thigh. Rabbits given one-third of the diet normally given to rabbits were used as diet-restricted animals. Throughout the experimental period, the animals had access to water and food *ad libitum*, except for the restriction of food intake in the diet-restricted animals. The amount of food the animals actually consumed was measured daily.

Measurement of total body fat The animals were anesthetized with intravenous pentobarbital (25 mg/kg), and their total body electrical conductivity (TOBEC) values were measured by using the TOBEC Small Animal Body Composition Analysis System (Model 3152, EM-SCAN Inc., Springfield, IL, 203 mm in inner diameter and 617 mm in length). The measurements were made by observing changes in impedance in a measurement chamber with a 10-MHz magnetic field. The total body fat (g) of a rabbit was calculated by using the formula: Wt (g) − (1.536 × TOBEC value + 475.1).[3]

Measurement of lipogenesis and lipolysis in subcutaneous adipose tissue Under pentobarbital anesthesia, adipose tissue was removed from the back of each rabbit. Adipocytes were isolated from about 50 mg of adipose tissue and incubated in 2 ml of Krebs-Ringer bicarbonate buffer, pH 7.4 (containing 4 m*M* glucose, 0.5 m*M* palmitate, 2.5 m*M* $CaCl_2$, and 4% (w/v) bovine serum albumin) for 1 h at 37°C, equilibrated with 95%O_2-5%CO_2, with the addition of 20 μCi of D-[U-^{14}C]glucose. The buffer was removed, and 5 ml of 2:1 (vol/vol) chloroform:methanol (freshly distilled solvents) was added to adipocytes to extract the lipid. The solvent was evaporated with an N_2

[1] To whom requests for reprints should be addressed.
E-mail: ishikoo@msic.med.osaka-cu.ac.jp

gas flow, and scintillation fluid (Emulsifier Safe, Packard Instrument, B.V., Groningen, the Netherlands) was added to the residue to measure incorporation of D-[U-^{14}C]glucose into the adipose tissue as [^{14}C]triglyceride.[4, 5] To measure glycerol release, the glycerol concentration in the buffer after incubation for 1 h without addition of D-[U-^{14}C]glucose was determined by the method of Wieland *et al*.[6]

Observation of apoptotic cells in adipose tissue from the back of the rabbit An adipose tissue specimen was collected from the back of each rabbit, before, and on days 10, 20, 30, and 40 after implantation of VX2-tumor, and before and on days 10, 20, and 30 after the start of diet-restriction experiments, and fixed with 10% neutral formalin to prepare paraffin sections of the tissue. Apoptotic cells were counted microscopically by the TdT-mediated dUTP-biotin nick-end labeling (TUNEL) method.[7] The apoptotic index (AI) was defined as the ratio of TUNEL-positive cells in a 200-times magnified field, and the AI values of the experimental animal groups were compared.

Data analysis Student's *t* test was used to analyze the data; differences were considered statistically significant when the *P* value was <0.05. All data are expressed as means±SD.

RESULTS

Changes in food intake, body weight, and total body fat Food intake, body weight, and total body fat values measured in individual animals before the start of the experiments were compared with those on days 10, 20, 30, 40, 50, and 60, and expressed as ratios to the values before the start. As shown in Table I, food intake in the tumor-bearing group significantly diminished to 0.68±0.25 (P<0.01) on day 40 and further diminished to 0.22±0.23 on day 60. Body weight in the tumor-bearing group decreased to 0.94±0.02 on day 20, 0.90±0.04 on day 30, 0.89±0.06 on day 40, 0.81±0.19 on day 50, and 0.72±0.09 on day 60, as opposed to decreases in the diet-restricted group to 0.89±0.07 on day 20, 0.85±0.06 on day 30, and 0.77±0.02 on day 40 (Fig. 1A). Total body fat in the tumor-bearing group was 0.82±0.09 on day 20, 0.79±0.11 on day 30, 0.76±0.18 on day 40, 0.70±0.09 on day 50 and 0.58±0.21 on day 60, while in the diet-restricted group the values were 0.78±0.11 on day 20, 0.52±0.18 on day 30, and 0.39±0.25 on day 40 (Fig. 1B).

Table I. Food Intake of Control, VX2-tumor-bearing, and Diet-restricted Rabbits

Day	Control (n=15)	Tumor-bearing (n=12)	Diet-restricted (n=10)
10	1.01±0.05	0.98±0.04	0.33
20	1.08±0.11	0.99±0.19	0.33
30	1.12±0.18	0.96±0.16	0.33
40	1.00±0.31	0.68±0.25[a)]	0.33
50	1.18±0.29	0.54±0.21[b)]	—
60	1.13±0.37	0.22±0.23[b)]	—

Data are shown as ratios to the value on day 0, and are expressed as means±SD.
a) P<0.01, vs the control group.
b) P<0.001, vs the control group.

Lipogenesis and lipolysis in adipose tissue Adipose tissue was removed from the back of the rabbits in the tumor-bearing group on day 60 and the diet-restricted group on day 30, when both groups had similar levels of total body fat, with normal rabbits before the experiments serving as a control. Incorporation rates of D-[U-^{14}C]glucose into triglycerides in the adipose tissue in the control,

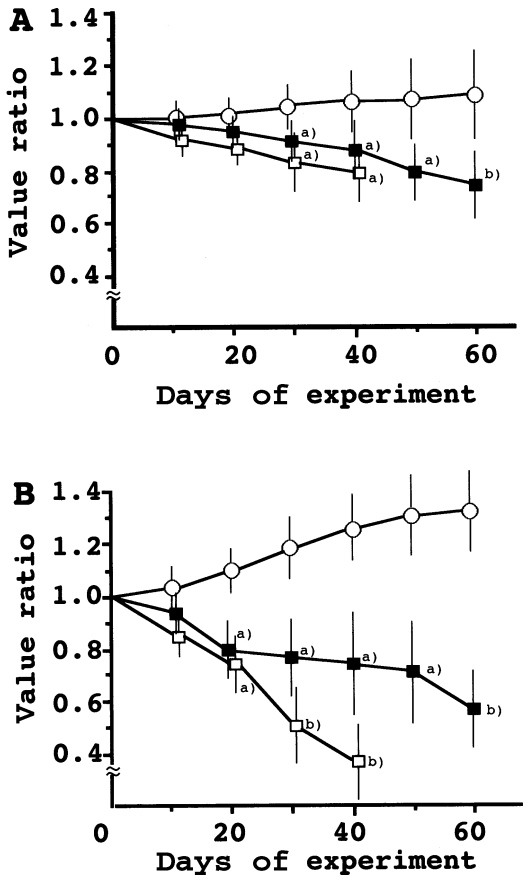

Fig. 1. Changes in body weight (A) and total body fat (B) in normal, tumor-bearing, and diet-restricted rabbits. Data are shown as ratios to the values on day 0, and are expressed as means±SD. The open circles, closed squares, and open squares show the values of the control (n=15), tumor-bearing (n=12), and diet-restricted rabbits (n=10), respectively. Differences from the values on day 0 are indicated as a) P<0.05 and b) P<0.01.

Table II. Incorporation of D-[U-^{14}C]Glucose into Glyceride in the Adipose Tissue and Glycerol Liberated from the Adipose Tissue in Control, Tumor-bearing, and Diet-restricted Rabbits

	D-[U-^{14}C]Glucose uptake (10^5 cpm/g/h)	Glycerol (nmol/g/h)
Control (*n*=7)	19.1±2.9	199±21
Tumor-bearing (*n*=7)	13.3±3.5[a)]	528±94[c)]
Diet-restricted (*n*=7)	41.7±6.0[b)]	301±45[d)]

Differences are indicated as: *a*) $P<0.001$ vs diet-restricted rabbits, *b*) $P<0.001$ vs control rabbits, *c*) $P<0.001$ vs control and diet-restricted rabbits, *d*) $P<0.05$ vs control rabbits.
Values are expressed as means±SD.

Table III. Apoptotic Index in Control, Tumor-bearing, and Diet-restricted Rabbits

	Day				
	0	10	20	30	40
Control (*n*=5)	0	0	0	0	0
Tumor-bearing (*n*=5)	0	1.9±0.8	28.7±9.5	16.5±7.8	9.2±3.3
Diet-restricted (*n*=5)	0	0	0	0	0

Values are expressed as means±SD.

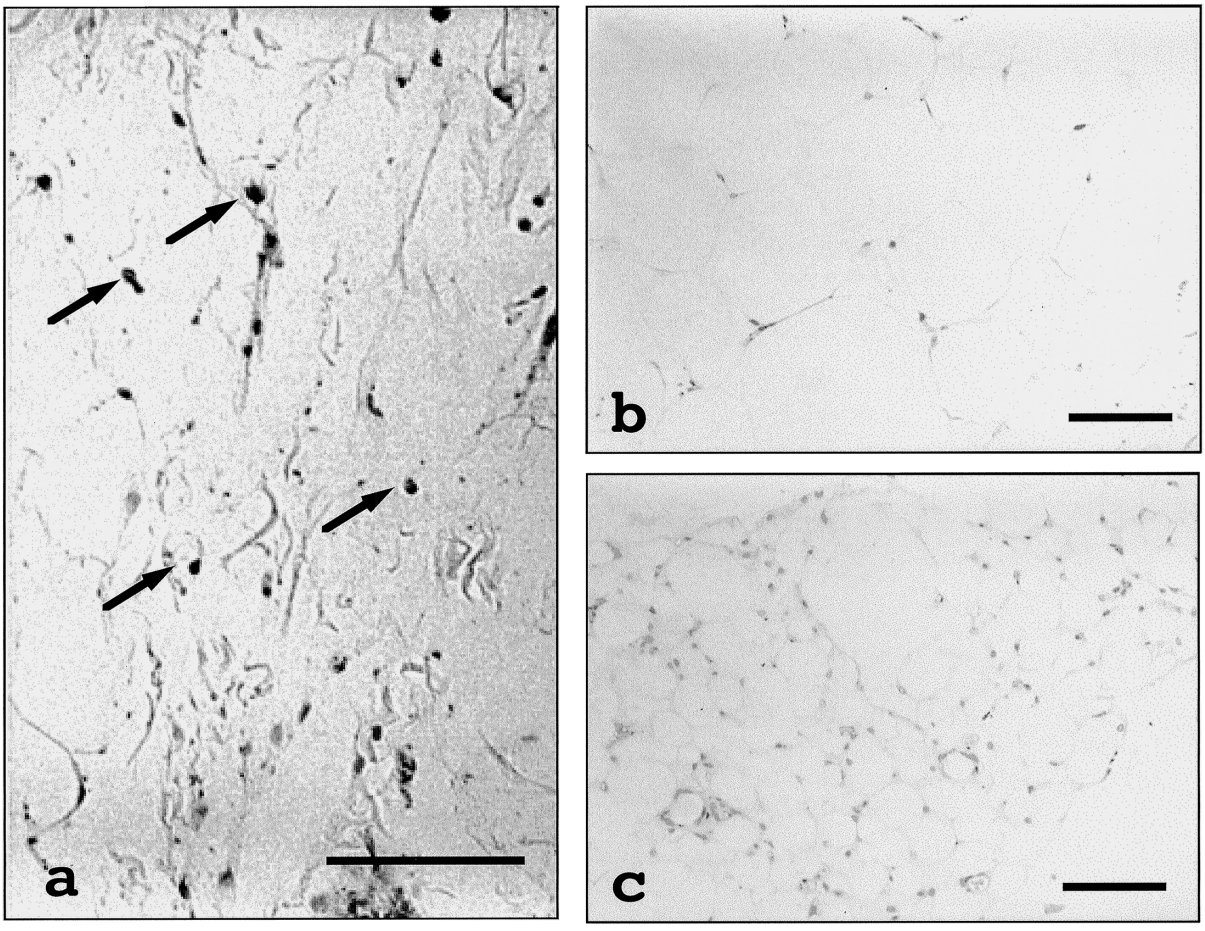

Fig. 2. Adipocyte apoptosis in VX2-bearing rabbits. TUNEL signals are demonstrated in the nuclei of adipocytes in VX2-bearing rabbit on day 20 after implantation with an AI of 31.2% (arrows in a). No apoptotic adipocytes were detected in the adipose tissue of the control (b) and diet-restricted rabbits on day 30 after experiment (c). The thick lines show the scale bars (50 μm).

tumor-bearing, and diet-restricted groups were 19.1±2.9×10^5, 13.3±3.5×10^5, and 41.7±6.0×10^5 cpm/g/h, respectively. Lipogenesis in the tumor-bearing group was decreased by 30.4% in comparison with the control group, but was increased by 118.3% in the diet-restricted group. Glycerol liberation rates from the adipose tissue in the control,

tumor-bearing, and diet-restricted groups were 199±2.9, 528±94, and 301±45 nmol/g/h, respectively. These results indicated that lipolysis increased by 165.3% in the tumor-bearing group as compared with the control group, and by 51.3% in the diet-restricted group (Table II).

Apoptotic cells and AI in adipose tissue No apoptotic cells were detected by the TUNEL method in the adipose tissue of the control and diet-restricted groups. By contrast, the AI values in the tumor-bearing group on days 10, 20, 30, and 40 after implantation were 1.9±0.8, 28.7±9.5, 16.5±7.8, and 9.2±3.3, respectively (Table III, Fig. 2).

DISCUSSION

The pathology of cancer cachexia is so complex that it may be unreasonable simply to attribute the reduction of body weight to anorexia or digestive disorders due to cancer. This view is supported by the results of the present study, showing that body weight and total body fat started to diminish earlier in the VX2-tumor-bearing rabbits than the decrease in food intake. This finding suggests that humoral factors are probably involved in the reduction of body weight and total body fat. Nakahara and Fukuoka[8] proposed toxohormone secreted by cancerous tissue as a causative agent. Kawakami and Cerami[9] reported that cachectin/tumor necrosis factor (TNF) produced by macrophages inhibits lipoprotein lipase and thereby decreases depot fat. Hirai *et al*.[10] reported lipid-mobilizing factor as a causative agent. In our previous studies,[11–13] anemia-inducing substance (AIS), which induces anemia and immunodeficiency and inhibits the growth of skeletal muscle cells *in vivo*, was isolated from the plasma of a patient with advanced cancer. There are also reports of studies that show possible involvement of interleukin (IL)-1, IL-6,[14, 15] and LIF.[16]

Our previous studies using VX2-tumor-bearing rabbits showed that anemia, immunodeficiency, and abrupt reduction of body weight were first recognized around day 30 to 40 after implantation, a condition quite similar to cachexia of cancer patients.[17] The causative cytokines of cancer cachexia were not elucidated, though we showed that AIS has lipolytic activity in VX2-tumor-bearing rabbits.[18] Although some studies have used patients with advanced cancer or a cachexia model in which reduction of body weight was already apparent,[19, 20] no studies in which total body fat levels were followed over time from the initial phase of tumor bearing have been reported. The present study using the rabbit model made it possible to measure changes of lipid metabolism periodically, while studies using rats or mice can observe only a few points.[21, 22] This was mainly because there was no noninvasive method available to accurately measure fat levels in small animals. We have successfully established a method of measuring total body fat in rabbits by the TOBEC method. This method allowed us to establish for the first time in this study that a remarkable reduction in total body fat occurs in the very early stage of tumor progression, whereas the most profound reduction of total body fat had been reported to occur after the cancer had become advanced in previous studies. In addition, experiments on D-[U-^{14}C]-glucose incorporation and glycerol liberation conducted under identical conditions showed clear differences in lipid metabolism between the tumor bearing and diet-restricted groups: the former exhibited lipid metabolism characterized by enhanced lipolysis and diminished lipogenesis, when compared with the latter group. It is not yet clear why and how apoptosis was induced in the adipose tissue cells of the tumor-bearing rabbits and what it means. However, the coincidence in the timing of the reduction of total body fat and the appearance of apoptotic cells strongly suggests involvement of apoptosis in the changes of lipid metabolism.

The above results suggest that growth of malignant tumors may alter lipid metabolic pathways, although the physiological role of this is still unknown. However, the lipolysis occurring in the initial phase of tumor bearing may in part assist the growth of the cancer. A new approach to systemic control of nutrition for tumor-bearing patients could be developed based on this hypothesis.

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