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Heterogeneous response of adipose tissue to cancer cachexia

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Abstract

Cancer cachexia causes disruption of lipid metabolism. Since it has been well established that the various adipose tissue depots demonstrate different responses to stimuli, we assessed the effect of cachexia on some biochemical and morphological parameters of adipocytes obtained from the mesenteric (MES), retroperitoneal (RPAT), and epididymal (EAT) adipose tissues of rats bearing Walker 256 carcinosarcoma, compared with controls. Relative weight and total fat content of tissues did not differ between tumor-bearing rats and controls, but fatty acid composition was modified by cachexia. Adipocyte dimensions were increased in MES and RPAT from tumor-bearing rats, but not in EAT, in relation to control. Ultrastructural alterations were observed in the adipocytes of tumor-bearing rat RPAT (membrane projections) and EAT (nuclear bodies).

Key words
- Cancer cachexia
- Adipose tissue heterogeneity
- Adipocyte heterogeneity

Introduction

Cancer cachexia is a common paraneoplastic syndrome inducing intense catabolism, which is associated with a poor prognosis and decreased survival time (1). Fatty acids arising from lipolysis in the peripheral adipose tissue constitute the major component of weight loss, followed by amino acids from protein breakdown in skeletal muscle (2). Lipid metabolism is markedly affected, and besides the reduction of adipose tissue mass, increased plasma non-esterified fatty acid and triacylglycerol content, along with lipoprotein metabolism disruption and decreased lipoprotein lipase activity, are among the alterations reported (3-5).

The various depots of adipose tissue are heterogeneous in terms of biochemical properties, response to hormones and amount of fat stored (6,7). Lipolysis is known to differ markedly according to the depot of adipose tissue, and even among adipocytes of the same depot, as a consequence of different adrenergic receptor types and density (7,8).

The purpose of the present study was to investigate the response of different white adipose tissue depots - epididymal, retroperitoneal and mesenteric - to cancer cachexia. Walker 256 carcinosarcoma, a well-established model for the study of cachexia (9), was inoculated into Wistar rats, and the fat and protein content, the lipid composition and morphometric and ultrastructural aspects of the three fat depots were studied.

Material and Methods

Male Wistar rats (200-250 g) obtained

from the Animal House of the Institute of Biomedical Sciences, USP, were kept under conditions of constant photoperiod (12-h/12-h light-dark cycle) and temperature, and received food (commercial chow) and water *ad libitum*. Weight and food intake were assessed daily, always in the afternoon. Walker 256 tumor cells (2 x 10^7 cells) were injected subcutaneously into the right flank of the animals. All experiments were carried on the 14th day post-inoculation, when the rats were killed by decapitation. Plasma insulin concentration was determined with a radioimmunoassay kit (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA, USA).

For the assessment of total fat content in the tissues (epididymal adipose tissue - EAT, retroperitoneal adipose tissue - RPAT, mesenteric adipose tissue - MES) the lipid fraction was extracted three times with petroleum ether, as described by Stansbie et al. (10). Protein content in the same adipose tissues was evaluated by the method of Lowry et al. (11).

For the analysis of morphometric aspects as described by Hirsch and Gallian (12), after the removal of the adipose depots, approximately 100 mg of each tissue was incubated for 48 h at 37°C in collidine buffer (0.2 M collidine, 0.1 M HCl, 0.15 M NaCl, pH 7.4), to which 2% osmium tetroxide was added. The cells were washed with 0.9% NaCl and distributed on slides. The area, approximate diameter, longest axis and shortest axis, perimeter and shape factor were measured in 100 adipocytes (3 slides for each tissue from one rat, 3 rats per group). Therefore, a total of 900 cells per group were studied. Analysis of the results was performed with the Sigma ScanPro4 program.

The ultrastructure of the different adipose tissues was studied after sample fixation with 3% glutaraldehyde, followed by postfixation with 1% osmium tetroxide. Successive dehydration with ethanol (70°, 95°, 100°) and acetone was followed by embedding in Spurr. Ultrathin slices were contrasted with uranyl acetate and lead citrate for 5 min and the samples were observed with a transmission electron microscope (Jeol 1010). A minimum of 100 cells were studied for each tissue.

Tissue fatty acid composition was assessed after homogenization in chloroform/methanol (2:1) (13). Twice-distilled water was added and the samples were dried. Ethanol was then added and the samples were dried and treated with methanol (5 parts) and sulfuric acid (1 part). One microliter from the lower phase was injected into a polyethyleneglycol column (DBWAX, J & W Scientific, Folsom, CA, USA) of the mass spectrophotometer (Shimadzu GCMS-QP5050) for chromatographic separation (14) of fatty acids, and compared with standard fatty acid methyl esters (Sigma, St. Louis, MO, USA). After warming to 250°C, the samples were arrested with helium (134 kPa) through the column and detector.

Statistical analysis was performed by ANOVA and the Tukey test, followed by the Student *t*-test, as recommended by the Department of Statistics of the Faculty of Mathematics and Statistics, University of São Paulo. The level of significance was set at $P<0.05$.

Results

The caloric intake (control: 70.20 ± 3.51 kcal; tumor-bearing: 71.75 ± 3.59 kcal) and the absolute weight gain (control: 49.67 ± 6.90 g; tumor-bearing: 41.54 ± 16.40 g) of tumor-bearing rats did not differ from control rats. However, the total weight gain after 14 days was reduced in the tumor-bearing rats in comparison with control, since the mass of the tumor corresponded to approximately 20% of the final body weight. The relative weight of the adipose depots is presented in Table 1, with tumor-bearing rats showing decreased EAT mass (41%) in rela-

tion to control. The fat content of the tissues studied was not significantly modified by the presence of the tumor (Table 2), although absolute values were always lower in tumor-bearing rats. Protein content (Table 2) in the tissues was not changed. Plasma insulin concentration differed between control and tumor-bearing rats (30.07 + 4.9, N = 22, and 17.62 + 3.34 µIU/ml, N = 22, respectively).

The fatty acid composition of the various white adipose tissue depots was not very different in the control animals. Therefore, a pattern where the proportions of the various fatty acids was 16:0>18:2>18:1>18:0>16:1, 14:0, with the absence of 12:0, was common to the three adipose depots studied (Table 3). Cachexia, however, had a distinct effect on each of them. For MES, a decrease (13%) in the percentage of total fatty acids represented by stearic acid and the appearance of 16:1 were found in tumor-bearing rats, compared with control. For EAT, no difference in the amount of 18:0 was verified, while that of 18:2 was enhanced (30%) in the cachectic animals. RPAT fatty acid composition did not change in response to cachexia.

The results of the morphometric analysis are presented in Table 4. Adipocyte area was different for control rats in the three unilocular adipose tissue depots studied (EAT> RPAT>MES). MES adipocytes showed reduced diameter, perimeter and major and minor axis in comparison with RPAT and EAT. Shape factor was not significantly different between tissues. In tumor-bearing rats, the adipocytes of MES showed increased area (2.5-fold), approximate diameter (1.6-fold), perimeter (1.6-fold) and major (1.6-fold) and minor (1.7-fold) axis in comparison with control. The shape factor was not altered. For the RPAT of tumor-bearing animals, a 2.9-fold increase in adipocyte area was observed, accompanied by an increase in the values of the other parameters studied. None of the studied morphometric parameters was altered in the cells obtained from the EAT of tumor-bearing rats compared to control.

The ultrastructural analysis of MES showed no noteworthy alterations in the aspect of the adipocytes in tumor-bearing rats compared with control. RPAT cells from tumor-bearing rats, however, when compared to control (Figure 1), showed alterations in membrane conformation and in the aspect of mitochondria, which were more electron-dense and presented tubular cristae (Figure 2). Some of the adipocytes obtained from the EAT of tumor-bearing rats, unlike the controls (Figure 3), presented structures resembling nuclear bodies (Figure 4) - an aspect common to neoplastic cells (15), this being the only aspect differing from control.

Table 1. Relative weight (percentage of total body mass) of adipose depots of control (C) and tumor-bearing (TB) rats.

Depot	Relative weight (%)	
	C	TB
MES	2.54 ± 0.53	3.47 ± 0.74
RPAT	3.45 ± 0.48	3.16 ± 0.43
EAT	3.46 ± 0.40	2.02 ± 0.49*

Data are reported as the mean ± SEM of tissues from 14 rats. MES, mesenteric adipose tissue; RPAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue.
*P<0.05 compared to control (ANOVA and Tukey test).

Table 2. Percentage of total tissue weight represented by protein in adipose tissue depots and mg fat/g tissue in control (C) and tumor-bearing (TB) rats.

	MES	RPAT	EAT
Protein content (%)			
C	5.20 ± 0.50	3.62 ± 0.17	3.77 ± 0.55
TB	4.70 ± 0.85	3.60 ± 0.60	3.20 ± 0.55
Fat content (mg fat/g tissue)			
C	719.55 ± 49.06	802.16 ± 48.98	814.16 ± 53.80
TB	684.75 ± 8.57	793.25 ± 4.79	775.76 ± 7.69

Data are reported as the mean ± SEM of tissues from 6 rats. MES, mesenteric adipose tissue; RPAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue.

Table 3. Percentage of different fatty acids present in the adipose tissue depots obtained from control (C) and tumor-bearing (TB) rats.

Fatty acids	MES (%)		RPAT (%)		EAT (%)	
	C	TB	C	TB	C	TB
14:0	2.03 ± 0.45	1.59 ± 0.33	2.37 ± 0.24	1.55 ± 0.07	1.99 ± 0.21	1.72 ± 0.08
16:0	45.70 ± 4.11	39.99 ± 3.3	43.49 ± 2.39	43.00 ± 10.6	45.53 ± 5.76	41.35 ± 8.77
16:1	-	0.55 ± 0.01*	1.17 ± 0.64	0.76 ± 0.81	1.91 ± 0.66	3.82 ± 1.24
18:0	10.11 ± 0.65	8.79 ± 0.50*	7.37 ± 1.53	2.68 ± 2.57	4.44 ± 1.49	1.00 ± 0.74
18:1	16.81 ± 2.54	21.19 ± 0.51	18.38 ± 0.80	22.01 ± 3.79	19.63 ± 2.85	16.17 ± 4.58
18:2	24.24 ± 1.60	27.20 ± 3.27	27.06 ± 3.99	37.67 ± 4.68	26.98 ± 2.06	36.27 ± 2.0*

Data are reported as the mean ± SEM of tissues from 24 rats. MES, mesenteric adipose tissue; RPAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue.
*P<0.05 compared to control (ANOVA and Tukey test).

Table 4. Morphometric analysis of the adipocytes obtained from the adipose depots of control (C) and tumor-bearing (TB) rats.

Morphometric data	MES (%)		RPAT (%)		EAT (%)	
	C	TB	C	TB	C	TB
Area (μm^2)	261.2 ± 10.85	647.9 ± 12.8*	641 ± 21.15	1918 ± 63*	858 ± 52.2	829 ± 41.45
Diameter (µm)	17.4 ± 0.32	28.37 ± 0.27*	27.42 ± 0.45	48.04 ± 0.75*	28.15 ± 0.94	29.01 ± 0.75
Perimeter (µm)	61.6 ± 1.44	99.9 ± 1.0*	96.36 ± 1.88	170.4 ± 2.67*	100.50 ± 3.3	107.2 ± 3.78
Shape factor	0.83 ± 0.009	0.80 ± 0.006*	0.82 ± 0.01	0.79 ± 0.005*	0.80 ± 0.006	0.79 ± 0.006
Major axis (µm)	19.85 ± 0.44	32.86 ± 0.36*	31.65 ± 0.58	57.24 ± 0.9*	33.41 ± 0.10	34.7 ± 0.98
Minor axis (µm)	13.4 ± 0.32	23.66 ± 0.3*	22.55 ± 0.44	40.5 ± 0.75*	23.83 ± 0.84	24.25 ± 0.66

Data are reported as the mean ± SEM of 900 cells. MES, mesenteric adipose tissue; RPAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue.
*P<0.05 compared to control (ANOVA and Tukey test).

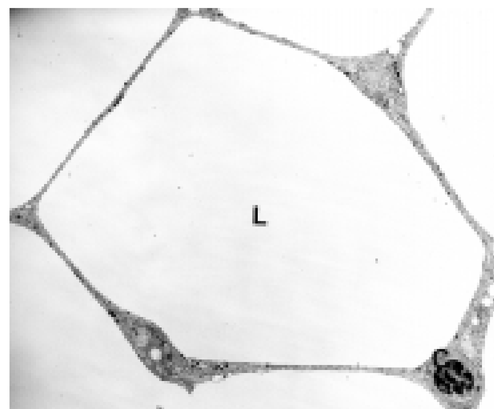

Figure 1. Aspect of a retroperitoneal adipose tissue adipocyte of a control rat. The polygonal cell shows segregation of nucleus and cytoplasm due to the volume of the lipid droplet (L). Capillary (C). (3,000X).

Discussion

Since the 1930's there has been no reduction in the death rate among cancer patients caused by cachexia, a paraneoplastic syndrome that induces great body mass waste (16,17). The mechanisms underlying the syndrome are unknown, and clinical care for cancer patients is generally directed towards the suppression of tumor growth. Since about 70% of hospitalized cancer patients will die of cachexia, it seems reasonable that there should be more emphasis on the study of this syndrome.

The loss of fat mass accounts for a large part of the dramatic weight loss observed both in humans and in animal models, and lipid metabolism is markedly altered (5,18). Since it has been well established that different white adipose tissue depots present distinct metabolism in response to hormonal stimuli, and that cancer cachexia induces great changes in plasma hormone concentration (19), we investigated the response of different unilocular fat depots to the syndrome. The fact that interleukin-1, tumor necrosis factor-α and prostaglandin E_2, factors whose concentration is increased in cachexia (2), are known to regulate lipolysis, showing different actions according to the adipose tissue depot studied (7), indicates a possible origin for the heterogeneity of the response to cachexia.

Indeed, the relative weight (percentage of total body mass) of the fat depots studied here showed a specific response to cachexia. While EAT mass was decreased, the mass of the other adipose depots did not differ from control. In another study by our group (5), fat uptake was reduced in the RPAT and EAT of Walker 256 tumor-bearing rats, as measured on the 14th day post-inoculation (after which animals spontaneously die due to cachexia). These previously obtained results demonstrated that even if variations in fat content and adipose tissue mass did not reach statistical significance at the final stage of the

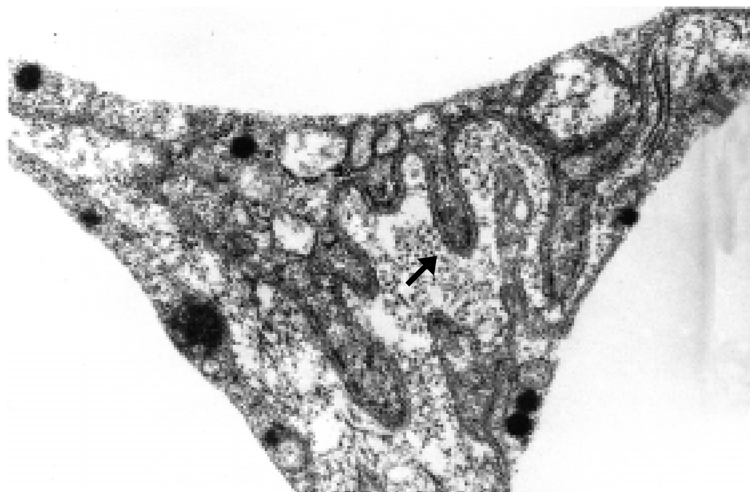

Figure 2. Membrane projections observed in the retroperitoneal adipose tissue of tumor-bearing rats. Note the basal lamina (arrow). (40,000X).

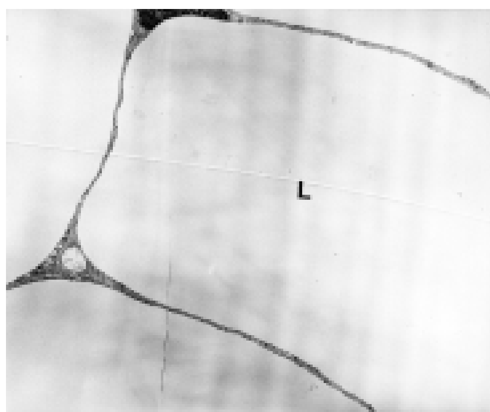

Figure 3. Aspect of a control rat epididymal adipose tissue adipocyte. Note the lipid droplet (L). (5,000X).

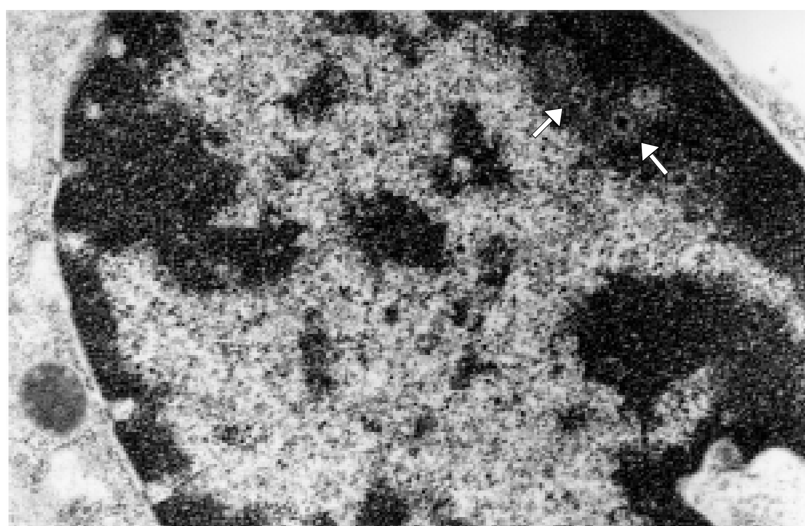

Figure 4. Detail of the nucleus of an adipocyte obtained from the epididymal adipose tissue of tumor-bearing rats. Observe the presence of nuclear bodies (arrows). (50,000X).

condition, the various adipose regions clearly presented different fat uptake capacity in response to terminal cachexia. The decrease of plasma insulin concentration observed in tumor-bearing rats, which has been also reported before (19) could, *per se*, modulate alterations in lipid metabolism, possibly causing diminished fatty acid uptake capacity, since this hormone regulates, for instance, lipoprotein lipase activity in a heterogeneous manner in adipose tissue depots (20). Further demonstrating a regional response of adipose tissue to cancer cachexia, the fatty acid composition of the depots studied, while not being statistically significantly different from that of control rats, was modified by the cachectic state in a region-related pattern. RPAT composition did not change, but MES (appearance of 16:1, decrease in the percentage of stearic acid) and EAT (increase of 18:2) were diversely affected by cachexia.

Morphometric adipocyte data also changed in cachexia. In control rats the cells were found to be larger in EAT and RPAT compared to MES, a result also reported by DiGirolamo et al. (21), who measured cell volume. Cachexia, however, affected the depots in a more specific way: while it induced an increase of the dimensions of MES and RPAT cells, it caused no significant changes in EAT adipocytes. Rat MES has been shown (21) to grow mainly by hypertrophy and normally demonstrates a higher percentage of water than RPAT and EAT. RPAT expansion under physiological conditions is predominantly by hyperplasia (21), but the results obtained suggest that cachexia is able to induce hypertrophy of this tissue. Since cachexia promotes an increase in body water content (3), we speculate that the enlargement of MES and RPAT adipocytes was caused at least in part by water retention, as opposed to an increase in lipid. Endotoxin administration, which induces cachexia, was found to promote adipocyte enlargement (22). The shape factor was modified in MES and RPAT of tumor-bearing animals, but not in EAT. Along with these alterations, ultrastructural changes in EAT and RPAT were observed.

It is therefore clear that the white adipose tissue of tumor-bearing rats responds in a depot-dependent manner to the cachexia syndrome, showing heterogeneous patterns of fatty acid uptake and deposition, and alteration of cell size and shape. Changes in adipocyte volume are related to altered secretion of leptin and tumor necrosis factor (22), factors which interfere with the general status of the organism.

Based on the results herein presented, we suggest that cachexia affects the white adipose tissue in a qualitative manner, in addition to any quantitative alterations observed in different experimental tumor models. These regional responses are probably related to the degree of innervation, vascularization and tissue-specific concentration of hormones and cytokines in the depot.

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