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HUMAN ADIPOCYTE APOPTOSIS OCCURS IN MALIGNANCY

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Summary. Rapid weight loss is frequently seen in malignancy. This weight loss is considered to result from enhanced lipolysis. Here, we show that adipocyte deletion by apoptosis, demonstrated by electron microscopy and DNA gel electrophoresis, occurs in some patients. Adipocyte apoptosis could not be demonstrated in patients without malignancy. These findings suggest that fat cell loss by apoptosis plays a role in malignancy-associated weight loss.

Changes in the size of fat stores reflect alteration in either volume or number of component adipocytes, or both. Large increases in fat stores are thought to involve both an increase in adipocyte volume (by lipogenesis) and number (1). Increase in adipocyte number occurs by recruitment, replication and differentiation of preadipocytes (1,2). Reduction in fat store size is thought to occur primarily by reduction in adipocyte volume (3). Whilst the mechanism by which adipocyte

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volume may decrease is well established (lipolysis), there has, until recently, been no reported mechanism by which adipocyte deletion may occur. Indeed, adipocyte acquisition has been widely regarded as permanent (1,4), despite reports of adipocyte "dedifferentiation" (to preadipocytes) (1,5) *in vitro*, and a report of reduction in adipose tissue mass, including loss of DNA, in rats rendered diabetic (6). We have recently shown the occurrence of adipocyte apoptosis *in vitro* (7), and now report its occurrence *in vivo.*

Clinically, dramatic loss of adipose tissue is observed in malignancy and severe infection. It is in these patients that we postulated that adipocyte apoptosis may occur, as it is likely that the profound loss of adipose tissue results from both decrease in adipocyte volume (by lipolysis), and decrease in adipocyte number.

METHODS

Adipose tissue samples were obtained (with informed consent) from nine patients with known malignancy, and variable degrees of weight change (Table 1). No patients had gastrointestinal obstruction symptomatically at the time of study, although patient 9 had oesophageal obstruction prior to treatment with combined chemo/radiotherapy. Resultant relief of obstruction led to the documented weight gain prior to this study. In eight patients adipose tissue was obtained from both the omental and abdominal subcutaneous depots. For comparison, six patients with no known malignancy were also studied, one with diet-induced weight loss of 10 kg (120 → 110 kg), and five who were weight stable.

The tissue samples were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 hrs, then transferred to buffer pending processing for light and electron microscopy. Semithin (0.5-1 μm) resin (EPON/ARALDITE) sections stained with toluidine blue were used for light microscopy. Ultrathin (50-70 nm) sections stained with lead citrate were observed in a JEOL 1200 EXII electron microscope.

DNA was extracted from the tissue samples using a standard phenol/chloroform/isoamyl alcohol technique (8). Electrophoretic gels were run using 1.8% agarose with TAE (0.04 M Tris-acetate, 0.001 M EDTA) as the running buffer. Gels were run for approximately 45 min, stained with ethidium bromide and photographed under ultraviolet light.

RESULTS

Of the nine patients studied with malignancy, six had evidence of adipocyte apoptosis (Table 1). The occurrence of apoptosis did not appear to correlate with

Table 1. Presence (+) or absence (-) of adipocyte apoptosis in abdominal subcutaneous (S/C) and omental fat from nine patients with malignancy. (SCC - squamous cell carcinoma, TCC - transitional cell carcinoma.)

Patient	Malignancy	Weight change (kilograms)	Apoptosis	
			S/C	Omental
1	Dissem. SCC Oesophagus	stable (58)	+	+
2	Adenocarcinoma Oesophagus	82 → 80	+	+
3	Recurrent cholangiocarcinoma	72 → 68	+	+
4	Gastric carcinoma	75 → 70	+	+
5	Dissem. TCC ureter	stable (78)		+
6	Adenocarcinoma rectum	72 → 63	+	-
7	Dissem. duodenal carcinoid	stable (80)	-	-
8	Oesophageal SCC *in situ*	stable (75)	-	-
9	Dissem. SCC oesophagus	72 → 52 → 63	-	-

degree of weight loss, type or extent of malignancy, age or sex. Evidence included characteristic ultrastructural changes (9) including convolution of the nuclear envelope, chromatin condensation, presence of a large nucleolar remnant and preservation of cytoplasmic organelles and cellular membranes (Fig. 1a). Apoptotic cells were seen interspersed with ultrastructurally normal cells (As in Fig. 1b). Numerous fat-laden interstitial macrophages were observed in these tissue samples, suggesting a possible fate for the lipid from the apoptotic cells. DNA extracted from the tissue displayed a "ladder" pattern after gel electrophoresis, confirming internucleosomal cleavage by endonucleases - a characteristic of apoptosis (10) (Fig. 1c). In Patients 1-4, more (2-20 fold) subcutaneous than omental DNA was needed to give comparable density of the "ladder" pattern after electrophoresis, suggesting greater rates of apoptosis in the omental depot. The reverse pattern was seen in Patient 6, with no demonstrable apoptosis in the omental depot. In the six subjects without malignancy, including the individual with dietary induced weight loss, no evidence of apoptosis was obtained using similar methods.

DISCUSSION

It is likely that under normal circumstances, adipocyte turnover occurs at a low rate, but the apoptosis is undetectable using the methods outlined above. Adipocytes have a very low nuclear - cytoplasmic ratio which enables, on average, only 0-2

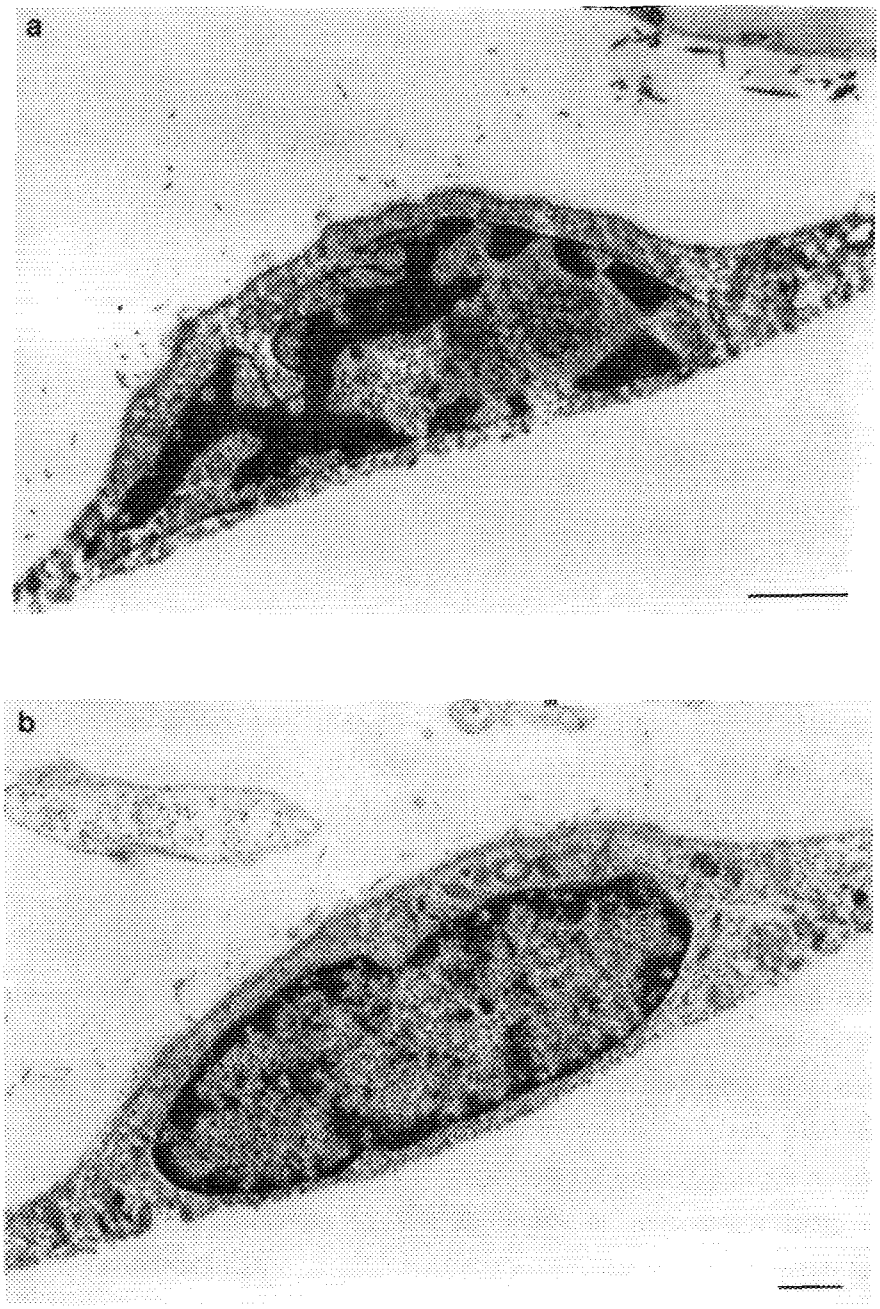

Fig. 1. **(a)** Human adipocyte nucleus in subcutaneous fat from a patient with a gastric carcinoma and 5 kg weight loss. Note regular, well-defined clumping of chromatin beneath the nuclear envelope, convolution of the nuclear outline and the large nucleolar remnant, all characteristic of apoptosis. Cytoplasmic organelles and cell membranes have remained intact. **(b)** Normal control human adipocyte nucleus, from a weight stable patient with no known malignancy. (Bars = 1 μm.) **(c)** Electrophoretic gel of human adipocyte DNA. Lane 1, molecular weight marker (ϕX174 RF DNA–Hae III Digest. New England Biolabs). Lane 2, control adipose tissue. Lane 3, omental adipose tissue from a patient with oesophageal adenocarcinoma and 2 kg weight loss.

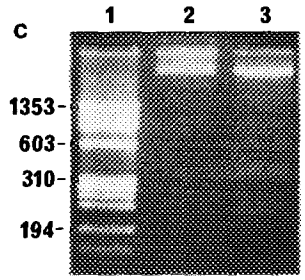

Fig. 1. - continued

nuclei to be observed per EM section of 250 x 250 μm. This severely restricts the number of cells available for examination, making difficult identification of small numbers of apoptotic cells, and comparison of apoptotic rates. The demonstration of adipocyte apoptosis in Patients 1 & 5, despite the documented absence of weight loss, suggests that malignancy *per se* may induce (or increase the rate of) adipocyte apoptosis. The mechanism of malignancy-induced adipocyte apoptosis remains to be determined, but a possible explanation is our observation that tumour necrosis factor-α is a potent inducer of adipocyte apoptosis *in vitro* (manuscript in preparation).

We have demonstrated that adipocyte apoptosis occurs in some patients with malignancy. This indicates that weight loss in patients with malignancy may involve an overall reduction in adipocyte number. Further study is indicated to delineate the role of adipocyte apoptosis in physiological and pathological situations.

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