Rapid Publication

Human Preadipocytes Display a Depot-Specific Susceptibility to Apoptosis

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Adipose tissue mass is determined by both the number and volume of adipose cells. Adipose cell number reflects the balance of cell acquisition and cell loss, whereas adipose cell volume represents the balance of lipolysis and lipogenesis. It is well recognized that insulin resistance, NIDDM, and other metabolic disorders are associated more strongly with increased omental adiposity than with subcutaneous adiposity. Depot-related differences exist in adipocyte responses to lipolytic and lipogenic stimuli, in adipocyte apoptosis, and in the potential for preadipocyte replication and differentiation. In the present study, we address the question of whether there might also be a site-specific difference in the susceptibility of human preadipocytes to apoptosis. Paired samples of human omental and subcutaneous preadipocytes from 12 individuals were cultured, and apoptosis was induced by serum deprivation or treatment with tumor necrosis factor (TNF)-α for 4 h. Cells were then stained with acridine orange, and apoptotic indices were calculated as the fraction of cells showing nuclear condensation. Under both conditions, in 9 of 11 subjects, apoptotic indices were substantially greater in preadipocytes from the omental depot than in those from the subcutaneous depot, and mean apoptotic indices were more than twofold higher in omental cells (serum-free medium: $P < 0.05$; TNF-α: $P < 0.02$; paired t test). Omental preadipocytes are therefore more susceptible to two different apoptotic stimuli than subcutaneous preadipocytes, demonstrating another intrinsic site-specific difference between human adipose cells of the two depots. These results suggest that the regulation of adipose tissue distribution in humans could involve depot-specific differences in rates of preadipocyte apoptosis. *Diabetes* 47:1365–1368, 1998

Obesity is characterized by an increased fat mass and occurs when the intake of food exceeds the energy requirement of the body for a sustained period (1). The degree of adiposity is determined by both the volume (lipid content) and the number of adipose cells (2). Adipocyte volume depends on the balance of lipolysis and lipogenesis, whereas adipose cell number is determined by the rate of cell acquisition versus cell loss (2). Adipose cell acquisition occurs by proliferation and differentiation of preadipocytes and cell loss occurs via apoptosis of preadipocytes and adipocytes, and possibly by other processes such as adipocyte dedifferentiation (2).

It is now well recognized that increased omental adiposity is more deleterious to health than subcutaneous adiposity. Numerous observations indicate that the metabolic syndromes closely associated with obesity, such as NIDDM, hyperlipidemia, cardiovascular disease, and syndrome X, are more tightly correlated with markers of central adiposity than with the degree of adiposity per se (3–5). Omental and subcutaneous adipose cells are known to display differences in various basal metabolic properties, pointing to an intrinsic difference between cells from the two sites. In vivo, omental fat cells have a higher rate of lipid turnover than subcutaneous cells (4,6,7). In vitro, adipocytes isolated from the omental depot have higher basal levels of cAMP, a greater number of glucocorticoid receptors, greater sensitivity to the lipolytic effect of catecholamines, and decreased sensitivity to the antilipolytic effect of insulin (4,8–12).

A further mechanism whereby the level of adiposity within each depot could be controlled is by depot-specific differences between cell acquisition and cell loss. There is evidence suggesting that preadipocytes from the omental depot display greater replication rates than their subcutaneous counterparts (13) and that subcutaneous preadipocytes differentiate more readily in response to thiazolidinediones than their omental counterparts (14). We have previously reported that human preadipocytes and adipocytes undergo apoptosis in vitro in response to tumor necrosis factor (TNF) and serum deprivation (15,16). We have also shown that human adipocyte apoptosis occurs in vivo and that rates of adipocyte apoptosis appear higher in omental cells than in subcutaneous cells in patients with malignancy (17). The in vitro effects of TNF-α and the observation that adipocyte apoptosis rates are higher in malignancy (when TNF-α levels are often elevated) support the likelihood that TNF-α–induced weight loss in vivo involves adipose cell apoptosis. This has not been investigated, but it has recently been reported that administration of leptin and thiazolidinedione induces adipocyte apoptosis in rodents (18,19). In this study, we investigate whether human preadipocytes display a depot-specific sensitivity to the induction of apoptosis by serum deprivation or the addition of TNF-α.

RESEARCH DESIGN AND METHODS

Patients and sample acquisition. Patients undergoing elective surgery at the Addenbrooke's Hospital (Cambridge, U.K.) were recruited for the study. The experimental protocols were approved by the institution's ethics committee, and all patients gave their informed consent to the procedure. Patients' characteristics are outlined in Table 1. No patients were on medications known to affect adipose tissue mass, distribution, or metabolism, and none had severe systemic illness or known malignancy. All patients fasted for at least 8 h before surgery, and

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Received for publication 16 January 1998 and accepted in revised form 6 May 1998.

PBS, phosphate-buffered saline; SFM, serum-free medium; TNF, tumor necrosis factor.

all underwent general anesthesia. Subcutaneous and omental adipose tissue biopsy specimens were obtained at the time of surgery and immediately transported to the laboratory in sterile normal saline (transport time, 5–10 min). Paired biopsy specimens were obtained from 12 patients, and cells from 10 of these patients (samples 1–10 in Figs. 1*A* and 2*A*) were used for both serum deprivation and TNF-α treatments. Cells from patient 11 were used for serum deprivation, and those from patient 12 were used for TNF-α studies.

Preadipocyte isolation and culture. Human preadipocytes were isolated and cultured as previously described (16). When 80–90% confluent, the cells were trypsinized and cultured in six-well plates in serum-containing medium comprising Dulbecco's modified Eagle's medium/Ham's F12 medium with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2 mmol/l glutamine. To induce apoptosis, cells were washed twice in phosphate-buffered saline (PBS) and incubated in serum-free medium (SFM) with or without recombinant human TNF-α (25 nmol/l) for 4 h. These conditions had been determined in our previous study (16). All experiments were performed in duplicate.

Acridine orange assay for apoptosis. Cells were stained with acridine orange, a fluorescent nuclear binding dye, allowing clear distinction of apoptotic from healthy cells on the basis of nuclear morphology (20,21). A 500 μg/ml stock solution of acridine orange was prepared in PBS. Immediately before cell counting, acridine orange stock was added to the culture medium to produce a final concentration of 10 μg/ml. Cell counting was undertaken within 5 min of staining, with a minimum of 200 cells counted in at least four fields of view in each well to derive an apoptotic index (number of apoptotic cells per total number of cells).

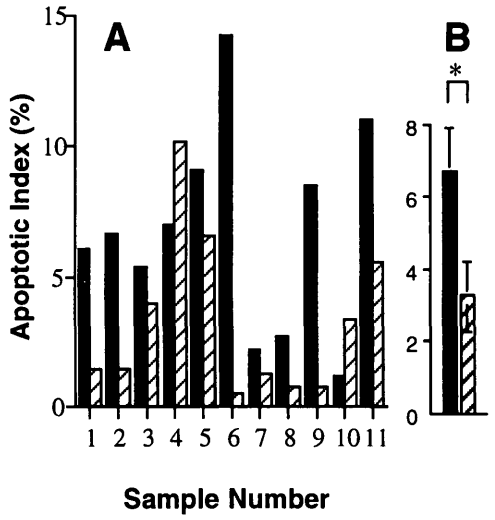

FIG. 1. Omental and subcutaneous preadipocyte apoptosis induced by serum deprivation. Samples of omental (■) and subcutaneous (▨) preadipocytes were obtained from 11 subjects. Cells were incubated in SFM for 4 h before acridine orange staining and calculation of apoptotic indices. *A*: Paired responses in individual subjects. *B*: Mean response of all 11 subjects. *$P < 0.05$ (paired Student's *t* test).

RESULTS

Apoptotic indices of preadipocytes cultured in serum-containing medium for the 4-h incubation time were 0.6 ± 0.2. No difference was found in this basal apoptotic index between subcutaneous and omental preadipocytes.

Serum deprivation. In preadipocytes from 9 of 11 individuals, the apoptotic index was greater in the omental depot than in the subcutaneous depot (Fig. 1*A*). Omental preadipocytes displayed a mean apoptotic index of 6.7 ± 1.2, whereas subcutaneous preadipocytes demonstrated a mean index of 3.3 ± 0.9 (Fig. 1*B*; $P < 0.05$ by both paired and unpaired *t* test).

TNF-α. In cells from 9 of 11 individuals, the apoptotic index was greater in the omental depot than in the subcutaneous depot (Fig. 2*A*). The mean apoptotic index of the omental samples was 8.5 ± 1.3, whereas the index of the subcutaneous samples was 4.0 ± 0.8 (Fig. 2*B*; $P < 0.02$ by both paired and unpaired *t* tests). As shown in our previous study (16), TNF-α increased the level of apoptosis beyond that observed in serum-free medium (SFM) alone. This increase was greater in the omental depot than in the subcutaneous depot (Fig. 3).

DISCUSSION

We have recently demonstrated that human adipose cells undergo apoptosis in response to serum deprivation and that this is increased by TNF-α (15,16). These two pro-apoptotic conditions involve distinct mechanisms for inducing apoptosis. Serum deprivation causes increased apoptosis by the removal of trophic factors essential for the survival of the cell (22), whereas TNF-α induces apoptosis by ligating its cell surface receptors and activating a cascade of cellular proteases known collectively as the caspases (23).

In the present study, we have demonstrated for the first time depot-specific differences in preadipocyte susceptibility to apoptosis. Preadipocytes from the omental depot are more susceptible to apoptosis induced by both serum deprivation (Fig. 1) and the combined effect of TNF-α and serum deprivation (Fig. 2). The increment in apoptosis produced by TNF-α also appeared greater in omental than in subcutaneous cells (Fig. 3). Thus, omental preadipocytes display a greater apoptotic response to two different apoptotic inducers, which strongly suggests an intrinsic difference between human preadipocytes from the two depots. We were not able to detect any difference in basal apoptosis between preadipocytes of the two depots, although basal apoptotic indices (<1%) were too low for precise quantification and comparison.

However, although these findings were significant, they were not consistent in all patients studied. Patient 5 showed a greater response in the omental cells when treated with SFM but did not show a depot-specific difference in apoptotic response to SFM + TNF-α. Patients 4 and 10 demonstrated a different response, with the subcutaneous depot showing a greater susceptibility to both SFM and SFM + TNF-α. Although patients 4 and 10 represented the oldest, and patient 5 the youngest, of those studied, they did not represent a subgroup of the patients with respect to diagnosis, sex, or BMI (Table 1). Therefore, we could not identify any obvious clinical features associated with the observed differences in depot-specific rates of preadipocyte apoptosis.

It is recognized that loss of weight (adipose tissue) involves a decrease in the number of adipose cells (24). This decrease could occur by two mechanisms. The first involves a reduction in the rate of production of differentiated adipose cells, which can occur through either impairment of preadipocyte replication or differentiation (as has been reported with TNF-α [25]), or by preadipocyte apoptosis (16). The second mechanism involves apoptosis of mature adipocytes, as occurs in human malignancy (17) and in response to leptin (18) or thiazolidinedione (19) administration in murine studies. Alterations in adipose tissue distribution could therefore be brought about by a site-specific difference in the regulation of these processes. Differences in replication rates of preadipocytes from different depots have been reported (13), and more recently, it has been shown that thiazolidinediones (acting as synthetic ligands for peroxisome proliferator activated receptor-γ) induce differentiation of human preadipocytes from the subcutaneous depot to a greater extent than those from the omental depot (14). Furthermore, there is evidence that in malignancy, human

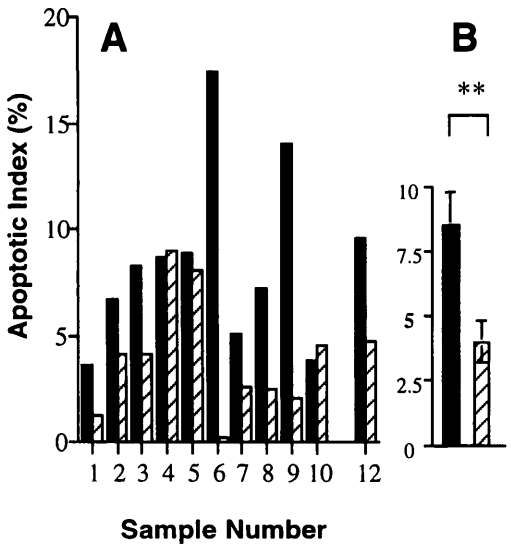

FIG. 2. Omental and subcutaneous preadipocyte apoptosis induced by TNF-α. Samples of omental (■) and subcutaneous (▨) preadipocytes were obtained from 11 subjects, 10 of whom were represented in Fig. 1. Cells were incubated in SFM containing TNF-α (25 nmol/l) for 4 h before acridine orange staining and calculation of apoptotic indices. *A*: Paired responses in individual subjects. *B*: Mean response of all 11 subjects. $^{**}P < 0.02$ (paired Student's *t* test).

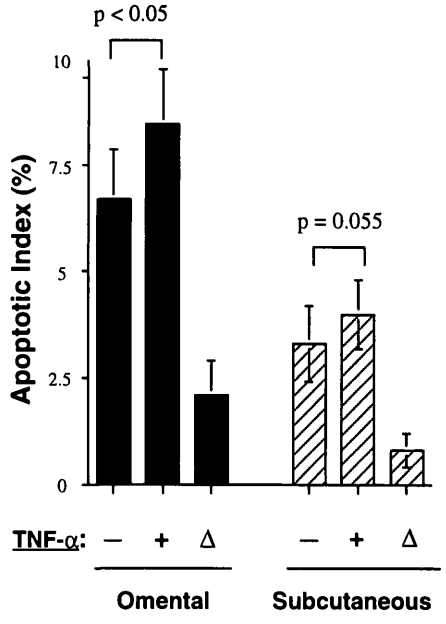

FIG. 3. Comparison of apoptosis under different experimental conditions. Mean apoptotic indices were calculated for the 10 subjects common to all experiments. Values shown are for apoptosis induced by serum deprivation without (−) or with (+) the addition of TNF-α (25 nmol/l), and for the increment of apoptosis attributable to TNF-α alone (Δ). Significance of differences was calculated by paired Student's *t* test.

adipocytes from the omental depot display greater rates of apoptosis than do those from the subcutaneous depot (17).

Our observations suggest that depot-specific susceptibility to preadipocyte apoptosis may be a further mechanism for regulation of adipose tissue distribution. The molecular basis for the depot-specific sensitivity of preadipocytes to apoptosis requires investigation, but it may reflect differences in relative levels of various apoptotic regulators within the cell, such as members of the Bcl-2 family, or differences in the production of paracrine/autocrine factors such as IGF-I. The difference in the cells from the two depots could be important in the regulation of number and distribution of adipose cells in vivo.

In addition to depot-specific differences in the regulation of fat cell number, there are differences in the regulation of adipocyte volume. Fat cells of the omentum possess a greater rate of lipid turnover than fat cells of the subcutaneous depot (6,7), and they exhibit differential responsiveness to various exogenous stimuli (4,8,9,26). Various factors have been reported to alter the distribution of fat mass by affecting adipose cell volume (by influencing lipolysis and lipogenesis), cell acquisition (by influencing replication and differentiation), or cell loss (by apoptosis). Stimuli such as steroid hormones,

TABLE 1
Patient characteristics

Patient	Sex	BMI	Age (years)	Apoptotic index (Omental/subcutaneous) TNF-α	SFM	Operation:diagnosis
1	F	33	50	2.8	4.1	Total abdominal hysterectomy: menorrhagia
2	F	17.3	70	1.6	4.5	Esophagogastrectomy: Barrett's esophagus with carcinoma in situ
3	F	23.9	45	2.0	1.4	Total abdominal hysterectomy: menorrhagia
4	M	20.7	82	1.0	0.7	Aortic graft: abdominal aortic aneurysm
5	F	23.3	33	1.1	1.4	Total abdominal hysterectomy: menorrhagia
6	M	19.8	63	87	28	Splenectomy: idiopathic thrombocytopenic purpura
7	F	29.7	43	2.0	1.7	Total abdominal hysterectomy: menorrhagia
8	F	25	70	2.9	3.4	Total abdominal hysterectomy: menorrhagia
9	M	23.3	70	10.7	6.7	Laparotomy: bowel obstruction (band adhesion)
10	F	29.3	72	0.4	0.8	Aortic graft: abdominal aortic aneurysm
11	F	27.9	52	ND	2.0	Total abdominal hysterectomy: leiomyomata
12	F	27.6	35	2.0	ND	Total abdominal hysterectomy: menorrhagia

Patient numbers correspond to sample numbers in Figs. 1 and 2. Diagnosis is based on clinical features, operative findings, and histology reports.

catecholamines, and insulin are actively involved in the regulation of regional fat distribution. Sex steroid hormones are capable of enhancing adiposity in either the visceral (androgen) or gluteofemoral (estrogen) depots (4). Supraphysiological levels of corticosteroids promote adiposity in the visceral depot, and at times, adipose tissue loss in the subcutaneous regions (2). Earlier work has shown that catecholamines induce lipolysis to a greater extent in the omental fat depot (26,27), whereas the antilipolytic action of insulin is greater in the subcutaneous depot (4,10).

The depot-specific difference in apoptotic susceptibility of both preadipocytes (reported here) and adipocytes (17), along with the reported differential capacity for preadipocyte differentiation and regulation of lipolysis and lipogenesis, would contribute to preferential loss of adipose tissue from the omental depot. Under conditions of weight loss (e.g., negative energy balance, sepsis, or malignancy), mobilization of energy from this depot would result in the supply of nutrients directly to the liver, which could be advantageous in the short term (as nutrient supply), but possibly deleterious in the long term (high concentrations of free fatty acids inhibit hepatic insulin binding and extraction [28]). On the other hand, relative preservation of subcutaneous fat would be beneficial because of the insulative properties of this depot. Increased knowledge of the underlying differences between adipose cells of these depots will provide insights into the molecular mechanisms regulating adipose distribution.

ACKNOWLEDGMENTS

K.S. thanks the British Diabetic Association for financial support. C.U.N. holds a studentship from the Commonwealth Trust, and J.B.P. is a Wellcome International Research Fellow. We are grateful to Peter Friend and other members of the Department of Surgery, Addenbrooke's Hospital, for provision of adipose samples for these studies.

We also thank members of Prof. S. O'Rahilly's laboratory—in particular, Ciaran Sewter, Fiona Blows, and Jan Digby—for assistance with the isolation of preadipocytes.

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