Original Article

Fat Depot–Specific Characteristics Are Retained in Strains Derived From Single Human Preadipocytes

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Fat depots vary in size, function, and potential contribution to disease. Since fat tissue turns over throughout life, preadipocyte characteristics could contribute to this regional variation. To address whether preadipocytes from different depots are distinct, we produced preadipocyte strains from single abdominal subcutaneous, mesenteric, and omental human preadipocytes by stably expressing human telomere reverse transcriptase (hTERT). These strains could be subcultured repeatedly and retained capacity for differentiation, while primary preadipocyte adipogenesis and replication declined with subculturing. Primary omental preadipocytes, in which telomeres were longest, replicated more slowly than mesenteric or abdominal subcutaneous preadipocytes. Even after 40 population doublings, replication, abundance of the rapidly replicating preadipocyte subtype, and resistance to tumor necrosis factor α–induced apoptosis were highest in subcutaneous, intermediate in mesenteric, and lowest in omental hTERT-expressing strains, as in primary preadipocytes. Subcutaneous hTERT-expressing strains accumulated more lipid and expressed more adipocyte fatty acid–binding protein (aP2), peroxisome proliferator–activated receptor γ2, and CCAAT/enhancer-binding protein α than omental cells, as in primary preadipocytes, while hTERT abundance was similar. Thus, despite dividing 40 population doublings, hTERT strains derived from single preadipocytes retained fat depot–specific cell dynamic characteristics, consistent with heritable processes contributing to regional variation in fat tissue function. *Diabetes* 55:2571–2578, 2006

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C/EBPα, CCAAT/enhancer-binding protein α; FBS, fetal bovine serum; G3PD, glycerol-3-phosphate dehydrogenase; hTERT, human telomere reverse transcriptase; mRNA, messenger RNA; PPAR, peroxisome proliferator–activated receptor; TNF, tumor necrosis factor.

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Fat distribution varies considerably, even among individuals with similar total body fat content. Increased central adiposity is associated with the metabolic syndrome and associated risks for diabetes, atherosclerosis, dyslipidemia, hypertension, and malignancies (1). Surgical removal of visceral fat is associated with increased hepatic insulin sensitivity and decreased hepatic glucose production in rats (2). Surgical removal of subcutaneous fat from hamsters results in insulin resistance (3). These observations led us to ask whether variation in fat distribution and function is solely a result of influences extrinsic to adipose cells (including hormonal and paracrine microenvironment, local nutrient availability, innervation, and anatomic constraints) or whether regional differences in inherent properties of adipose cells also contribute.

Fat tissue turns over throughout life. In rats, new fat cells and preadipocytes continue to appear in advanced old age (4). Preadipocytes, the progenitors that differentiate into new fat cells, are an important cell type in their own right, accounting for 15–50% of cells in fat (4). Some investigators have reported that preadipocytes cultured under identical conditions originating from different depots from the same individuals vary in capacities for replication, differentiation, and apoptosis, consistent with the view that inherent preadipocyte characteristics contribute to distinct features of different depots (5–12). Others, however, have not found regional variation in some of these preadipocyte characteristics (13–15). A complication in interpreting studies of primary cultured preadipocytes is potential contamination with macrophages, mesothelial cells, or other cell types, as regional variation in their relative abundance might occur. One approach for addressing whether there are depot-specific, inherent differences would be to study cultures originating from single preadipocytes. However, the capacity of primary human preadipocytes to differentiate begins to decline after a few subcultures (16), as does capacity to replicate (17), precluding this approach.

As cells divide, shortening of telomeres, nucleoprotein structures comprising repeated TTAGGG sequences and associated proteins at ends of chromosomes, correlates with loss of replicative potential and function (18,19). Telomeres prevent chromosomal degradation, recombination, and exposure of DNA ends to the intracellular environment that could otherwise activate DNA damage checkpoints and cellular stress responses (20). Telomeric DNA is lost during successive cell divisions unless telome-

rase, a ribonucleoprotein complex that adds blocks of telomere repeats, is active (21). Under most conditions, somatic cells do not express human telomerase reverse transcriptase (hTERT), the rate-limiting component of the telomerase complex (22). hTERT overexpression permits continued replication and retention of functional characteristics of serially passaged endothelial, adrenocortical, myoblast, and osteoblastic cells (23–27), as well as fat cell progenitors (17,28,29).

To test the hypothesis that preadipocytes from different depots are inherently distinct, we made preadipocyte strains stably expressing hTERT from single abdominal subcutaneous, mesenteric, and omental human preadipocytes. We found that regional variation in capacities for replication and differentiation, susceptibility to apoptosis, and abundance of preadipocyte subtypes were retained despite repeated divisions.

RESEARCH DESIGN AND METHODS

Fat tissue was resected during gastric bypass surgery for management of obesity from 31 subjects (29 female) who had given informed consent. The protocol was approved by the Boston University Medical Center Institutional Review Board for Human Research. All subjects had fasted at least 10 h. Subjects were aged (means ±SE) 42 ± 3 years (range 18–69). Mean BMI was 51 ± 2 kg/m^2 (27–81). Tissue from obese subjects was included because of clinical relevance of defining mechanisms of fat development in this population. Subjects with malignancies or on thiazolidinediones or steroids were excluded. None had fasting plasma glucose levels >120 mg/dl. One-half to 10 g of midline abdominal subcutaneous (external to the fascia superficialis), mesenteric (colonic epiploices), and greater omental fat was obtained from each subject.

Preadipocyte culture. Fat tissue was minced and digested in Hank's balanced salt solution containing 1 mg/ml collagenase and 7.5% fetal bovine serum (FBS) in a 37°C shaking water bath until fragments were no longer visible and digests had a milky appearance. Digests were filtered and centrifuged at 800*g* for 10 min. Digests were treated with an erythrocyte lysis buffer to enhance subsequent differentiation (30,31). Cells were plated in medium (1:1 Dulbecco's modified Eagle's medium; Ham's F12 that contained 10% bovine serum and antibiotics) at 4 × 10^4 cells/cm^2. After 18 h, a time during which no replication occurs as assessed by ^{3}H thymidine incorporation, ~95% of cells were trypsinized and replated. This facilitates accurate plating density and leaves relatively trypsin-resistant macrophages behind. Macrophages were rare (less than five per 10^6 cells as assessed by phase-contrast microscopy) in the replated cultures. Medium was changed every 2 days until confluence. Cultures were serially passaged by replating confluent cells at half their confluent density, growing the cells to confluence, and replating again at half their confluent density. Abundance of macrophage, endothelial, and stem cell marker mRNA was determined in undifferentiated subcutaneous and omental preadipocytes with Affymetrix U133A arrays (GEO database accession no. GSE1657 [available at http://www.ncbi.nlm.nih.gov/geo/]). Expression of macrophage (ADAM8 [a disintegrin and metalloproteinase domain 8], CD11b, CD68, F4/80, MIP-1α, and MCP-1), endothelial cell (PECAM1, CD46, VEGF receptor 2, von Willebrand, Tie-1, and Tie-2), and stem cell (including Nanog, CD117, Sox2, and ABCG2 [ATP-binding cassette, subfamily G, member 2]) markers did not differ systematically among preadipocytes isolated from different depots. Indeed, ADAM8, CD11b, F4/80, MIP-1α, von Willebrand, Tie-1, Nanog, and CD117 were not detectible in preadipocyte cultures from any depot. Thus, bias due to potential contamination by nonpreadipocyte cell types in our cultures does not explain observed regional differences.

Preadipocyte differentiation. From confluence, cells were either held in an undifferentiated state using plating medium without serum, serially passaged, or differentiated. For differentiation, a previously published method (32) was used with modifications that included the following. Cultures were treated for 7–30 days (as indicated in RESULTS and figure legends) with plating medium (without serum) enriched with 100 nmol/l dexamethasone, 500 nmol/l human insulin, 200 pmol/l triiodothyronine, 0.5 μmol/l rosiglitazone, antibiotics, and 540 μmol/l methylisobutylxanthine (removed after 2 days). In preliminary studies, higher rosiglitazone and insulin concentrations did not further enhance differentiation. Medium was changed every 2 days.

Preadipocyte transduction with hTERT retrovirus. Primary preadipocytes that had undergone seven population doublings were infected with a retrovirus (33) containing the plasmid, pBABE-hTERT-Puro (34) that expresses hTERT driven by the long terminal repeat promoter. Polytropic RetroPack PT67 cells (35) were infected with 10 μg hTERT-Puro DNA using a calcium phosphate medium (36). Culture supernatants containing virus were collected 24–48 h after infection and filtered through a 0.45-μm cellulose acetate filter. Undifferentiated subcutaneous, mesenteric, and omental preadipocytes at 80% confluence were infected with supernatants in the presence of 4 μg/ml polybrene for 12 h, after which medium was replaced with α minimal essential Eagle's medium containing 10% FBS and antibiotics. After 48 h, cells were trypsinized, plated at a 1:15 split ratio, treated with 0.5 μg/ml puromycin for 15 days, and followed daily to ensure the colonies selected had arisen from single cells. The 9 abdominal subcutaneous, 6 mesenteric, and 7 omental puromycin-resistant clones capable of achieving confluence fastest were selected for amplification from a total of 40 subcutaneous, 32 mesenteric, and 42 omental clones. Strains S1.17 and S1.22 were from a 49-year-old female subject; S2.5, S2.11, S2.13, M2.5, M2.6, M2.10, Om2.2, Om2.5, and Om2.7 were from a 42-year-old female subject; and S3.1, S3.5, S3.6, S3.8, M3.1, M3.5, M3.8, Om3.1, Om3.5, Om3.6, and Om3.8 were from a 50-year-old female subject. Each of these strains accumulated lipid following induction of adipogenesis.

Preadipocyte cloning. Wild-type or hTERT-expressing preadipocytes were replated at 50 cells/96-well plate in plating medium. At this density, the probability of any one well being seeded by more than one cell is <2% (7,9). After 2 weeks, colonies were evident, and by 3 weeks, some hTERT clones were confluent.

Apoptotic index. Cells were stained with bisbenzamide and examined using fluorescence microscopy by observers unaware of depot origin or treatment of cells. Cells were classified as apoptotic if they exhibited irregular nuclear condensation (37). The apoptotic index is the percent of such nuclei as a function of all nuclei in a field.

Glycerol-3-phosphate dehydrogenase and DNA assays. Glycerol-3-phosphate dehydrogenase (G3PD) was measured in supernatants of cell homogenates by following NADH disappearance spectrophotometrically (38). G3PD activity was not detectable in undifferentiated preadipocytes. DNA was measured in homogenates using a fluorimetric intercalating dye reaction (39). Cell numbers in confluent cultures estimated by this method agreed within 3% of directly counted cell numbers.

RNA analysis. RNA was isolated from preadipocytes using the guanidinium thiocyanate-phenol method (40). RNA integrity was verified using 1% formaldehyde-containing denaturing agarose gels. Messenger RNA (mRNA) was measured by relative quantitative RT-PCR in which target genes were coamplified with an internal 18S rRNA control sequence. Analysis of mRNA expression was carried out during the exponential phase of the amplification, which was assessed in preliminary experiments for each set of primers. Amplified product reproducibility was confirmed in two PCR rounds. The ratios of intensity of target to internal control bands were used to indicate relative abundance of message. 18S rRNA amplification was titrated to match that of adipocyte fatty acid–binding protein (aP2; a differentiation-dependent target of peroxisome proliferator–activated receptor [PPAR]γ2 and CCAAT/enhancer-binding protein α [C/EBPα]), C/EBPα, and PPARγ2 mRNA by adding competitive primers (Ambion, Austin, TX) that modulate extension of the 18S cDNA. The quantitative nature of this approach was confirmed by measuring aP2, PPARγ2, and C/EBPα mRNAs in serially diluted samples. RNA preparations were checked for DNA contamination by amplifying control aliquots that had not been reverse transcribed. The following primers were used: for aP2, sense, GGCCAGGAATTGACGAAGTC, antisense, ACAGAATG TTGTAGAGTTCAATGCGA (41); for PPARγ2, sense, GCGATTCCTTCACTGA TAC, antisense, GCATTATGAGACATCCCCAC (42); for C/EBPα, sense, GACACGCTGCGGGGCATCT, antisense, CTGCTCCCCTTCCTTCTCTCA (43); and for hTERT, sense CACCTCACCCACGCGAAAA, antisense, CCAAAGAGT TTGCGACGCATGTT (24). Ratios are shown of expression of each gene to 18S rRNA, which is similar in preadipocytes among depots and with differentiation (11).

Telomere length and karyotyping. To measure telomere length, samples were assessed for DNA concentration and quality by agarose gel electrophoresis. Telomere length was determined as abundance of telomeric template relative to a single gene by quantitative real-time PCR (44) using TelA: CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT and TelB: GGCTT GCCTTACCCTTAC CCTTACCCTTACCCTTACCCT as forward and reverse primers, respectively. Measurements were performed in quadruplicate. Three DNA samples with known telomere lengths (3.0, 5.5, and 9.5 kb) were run as internal standards. For karyotyping, 0.5 μl colcimide/ml were added to 60–70% confluent preadipocytes for 2 h at 37°C. Cells were washed with Hanks' balanced salt solution, trypsinized, and washed again with plating medium. Plating medium was removed, and 20 ml of 0.075 mol/l KCl were added for 20 min at 37°C. The hypotonic KCl solution was removed and cells fixed in methanol and acetic acid.

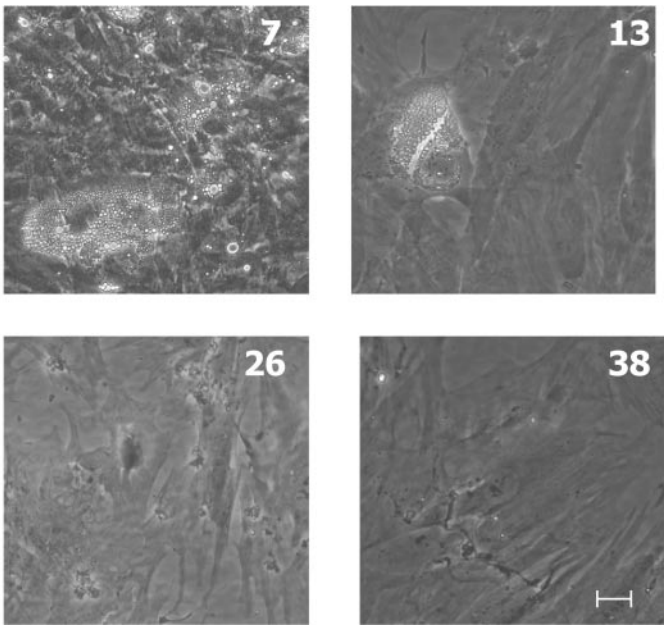

FIG. 1. Capacity to accumulate lipids declines as preadipocytes undergo increasing cell divisions. Abdominal subcutaneous preadipocytes that had undergone 7, 13, 26, or 38 population doublings were exposed to differentiation medium for 10 days. The bars represent 20 μm. Representative of three experiments.

Statistical analysis. Results are means ± SE, and significance determination was by unpaired t tests or ANOVA with post hoc comparisons by Duncan's multiple range test (45). Two-tailed $P < 0.05$ was considered significant. A generalized estimating equation χ^2 test that accounts for correlation was used to compare telomere lengths among preadipocytes from different fat depots, since preadipocytes from different depots were grouped by subject (46).

RESULTS

Preadipocyte replication capacity and adipogenesis vary among depots and decrease with subculturing. We serially subcultured primary abdominal subcutaneous preadipocytes at a 1:2 split ratio for 2 years. The cells were no longer capable of reaching confluence after 36 ± 3 passages ($n = 3$ experiments using cells from different subjects), as noted by others (17). With increasing passage, telomeres shortened (by 112 ± 22 [$n = 6$] bp per population doubling) and cells became flattened with large nuclei and spindling, features characteristic of senescence. Serially passaged visceral preadipocytes took longer to achieve confluence than subcutaneous cells, precluding practical determination of maximum division potential. Tenth-passage abdominal subcutaneous preadipocytes took 9.8 ± 2.9 days to reach confluence, while omental cells took 21.3 ± 3.7 days ($n = 6$ subcutaneous and 5 omental serially passaged cultures from different subjects; $P < 0.05$; t test).

Lipid accumulation and G3PD activity decreased with passage following exposure to differentiation medium (Figs. 1 and 2; G3PD activity was 735 ± 110 vs. 360 ± 60 nmole/s per 10^6 cells in differentiating first- versus fourth-passage abdominal subcutaneous cells; $n = 5$; $P < 0.05$; Duncan's multiple range test). G3PD activity was higher in first-passage subcutaneous than mesenteric or omental cells ($P < 0.05$; Duncan's multiple range test), consistent with previous reports (10,11,47) of greater differentiation capacity in subcutaneous than mesenteric or omental preadipocytes. Thus, preadipocyte replication and adipogenesis vary among depots; preadipocytes at later passage undergo morphologic changes similar to those of senescent fibroblasts; and repeated divisions are associated with reduced replicative potential and decreased capacity for adipogenesis (particularly in visceral cells) and telomere shortening.

Telomeres are longer in omental than subcutaneous or mesenteric preadipocytes. Since omental preadipocytes have lower potential for replication and differentiation than subcutaneous or mesenteric preadipocytes and since telomere shortening is also associated with reduced capacities for replication and adipogenesis, we tested the hypothesis that telomeres are shorter in omental than subcutaneous or mesenteric preadipocytes. However, we found that telomeres were longer in the omental preadipocytes (5.6 ± 0.5 vs. 4.4 ± 0.6 and 4.5 ± 0.6 kb in omental versus subcutaneous and mesenteric preadipocytes, respectively; $n = 6$ subjects; $P < 0.01$ that omental are longer than subcutaneous or mesenteric telomeres; generalized estimating equation parameter intercept). This could be due to the omental preadipocytes having undergone fewer divisions in vivo before the cells were harvested (or less telomere shortening/division in vivo), consistent with lower replicative potential of omental preadipocytes in vitro (28). Thus, reduced replicative potential and limited adipogenesis in omental compared with subcutaneous or mesenteric preadipocytes are not due to shorter telomeres in omental cells.

Telomerase is expressed in transduced preadipocytes. After 40 population doublings, hTERT mRNA was similar in subcutaneous and omental strains made from single preadipocytes by retroviral hTERT transduction (Fig. 3; $n = 5$ strains [S2.11, S2.13, S3.1, S3.5, S3.8, Om2.2, Om2.5, Om2.7, Om3.1 and Om3.8]; $P = 0.98$; t test) and the strains had robust telomerase activity in a terminal repeat amplification protocol (TRAP) assay (data not shown), suggesting equivalent activity of mechanisms required for transcription from the stably incorporated LTR promoter across fat depots.

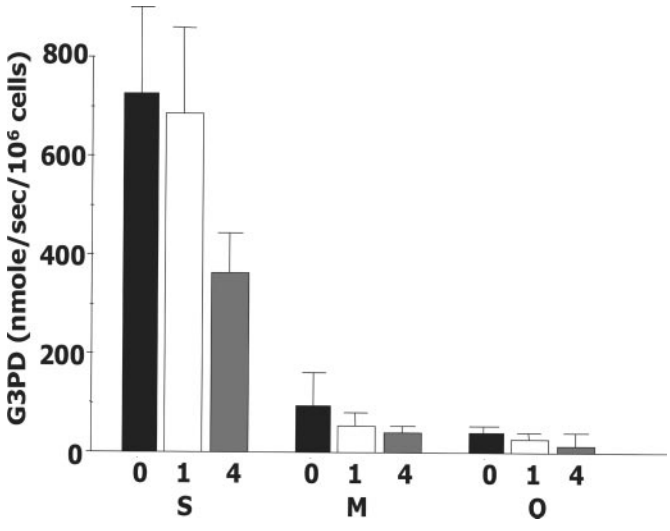

FIG. 2. G3PD activity is higher in subcutaneous than visceral preadipocytes and declines with increasing cell divisions. G3PD in homogenates from primary, first-, and fourth-passage abdominal subcutaneous (S), mesenteric (M), and omental (O) preadipocytes exposed to differentiation medium for 7 days was higher in S than M or O and in first- than fourth-passage S cells ($n = 5$; $P < 0.05$; ANOVA; Duncan's multiple range test).

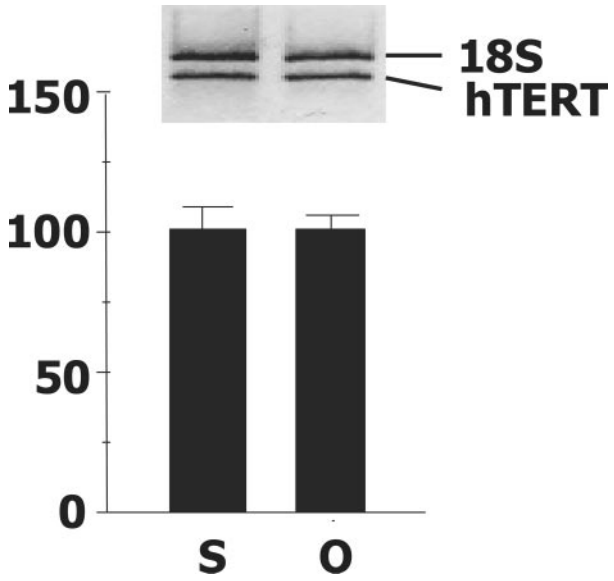

FIG. 3. hTERT expression is similar in subcutaneous and omental strains. hTERT mRNA was assayed by RT-PCR with 18S rRNA as an internal control in undifferentiated subcutaneous (S) and omental (O) strains that had undergone 40 population doublings. Representative gels are shown above. Below are means ± SE of densitometric analyses of hTERT mRNA, expressed as percentages of 18S rRNA and normalized within experiments (n = 5 strains from each depot; P = 0.98; t test).

hTERT expression enhances preadipocyte replicative potential and adipogenesis. hTERT-expressing and wild-type primary abdominal subcutaneous preadipocytes from the same subject were subcloned by plate dilution (S1.17 and S1.22). The hTERT cells had undergone 35 population doublings between initial isolation from fat tissue and subcloning. The primary cells had only undergone six population doublings. Despite this, the first colony arising from an hTERT cell achieved confluence 21 days following subcloning, while the first primary subclone took 38 days. Although they had been in culture for approximately half the time of the primary cells, the hTERT subclones contained more than twice as many cells as the primary clones ($16 \pm 2 \times 10^3$ cells in hTERT vs. $6 \pm 3 \times 10^3$ in primary clones; 105 hTERT and 57 primary clones; $P < 0.05$; t test). The proportion of hTERT cells that achieved 14 population doublings within 21 days was 2.5-fold higher than primary cells within 38 days (38 ± 6% of hTERT strains vs. 15 ± 6% of primary clones; $P < 0.05$; t test). Thus, hTERT preadipocytes are capable of more rapid replication than primary preadipocytes. The subcutaneous hTERT preadipocyte strain that we tested (S3.6) for capacity to be subcultured extensively could achieve 200 population doublings. None of 105 subclones prepared from the hTERT strain exhibited morphological features of transformation, such as development of cell islands or loss of contact inhibition, and they retained normal chromosome complement (online appendix Fig. 1 [available at http://diabetes.diabetesjournals.org]), as noted by others (17). Capacity for lipid accumulation was greater in subcultured hTERT than primary preadipocytes (Fig. 4). Although lipid accumulation was reduced in hTERT subcutaneous preadipocytes following exposure to differentiation medium after 140 population doublings compared with cells at 40 doublings, cells that accumulated lipids in large droplets could still be found (Fig. 4*C*).

hTERT preadipocyte replicative potential is fat depot dependent. Individual abdominal subcutaneous, mesenteric, and omental hTERT preadipocytes that had been subcultured for 40 population doublings were subcloned by plate dilution (Fig. 5*A*; 50 cells/96-well plate; n = 6 strains [S2.5, S2.11, S2.13, S3.1, S3.5, S3.8, M2.5, M2.6, M2.10, M3.1, M3.5, M3.8, Om2.2, Om2.5, Om2.7, Om3.1, Om3.5, and Om3.8], 10 plates/strain; 2,044 subcutaneous, 2,050 mesenteric, and 2,013 omental subclones were assayed). After 21 days, a higher proportion of subcutaneous hTERT subclones achieved ≥6 (maximum 11) population doublings than either mesenteric or omental subclones ($P < 0.01$; Duncan's multiple range test), the same depot-dependant pattern as in primary preadipocytes (28). Thus, regional differences in primary preadipocyte replicative potential are retained in the hTERT strains.

Regional variation in preadipocyte subtype abundance is retained in hTERT strains. There are two preadipocyte subtypes, the abundance of which varies among fat depots. One subtype is capable of more extensive replication and adipogenesis and more resistant to tumor necrosis factor (TNF)α-mediated apoptosis than the other (28,48). Each subtype can convert into the other. The slowly replicating, adipogenesis-resistant subtype may constitute a reserve pool of progenitors that permits retention of fat tissue regenerative capacity following

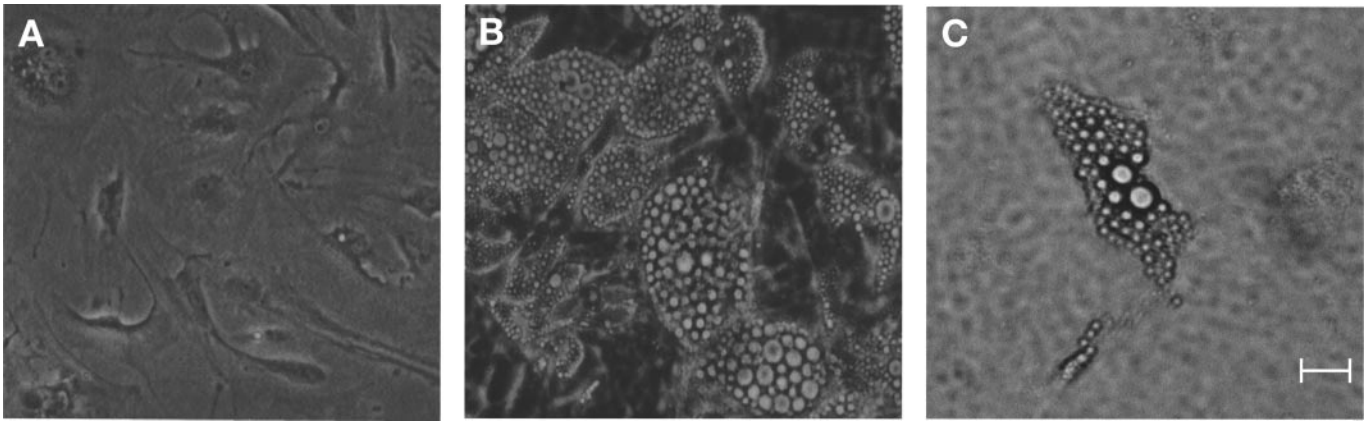

FIG. 4. Expression of hTERT prevents loss of adipogenic capacity following serial passage. Primary subcutaneous preadipocytes that had undergone 32 population doublings (*A*) and subcutaneous hTERT cells (strain S1.22) that had undergone 39 doublings (*B*) were exposed to differentiation medium for 30 days. hTERT cells retained capacity to accumulate lipid, while primary preadipocytes accumulated little lipid. Cells with lipid could be found in subcutaneous hTERT preadipocytes (S3.6) even after 140 population doublings (*C*). The bars represent 20 μm.

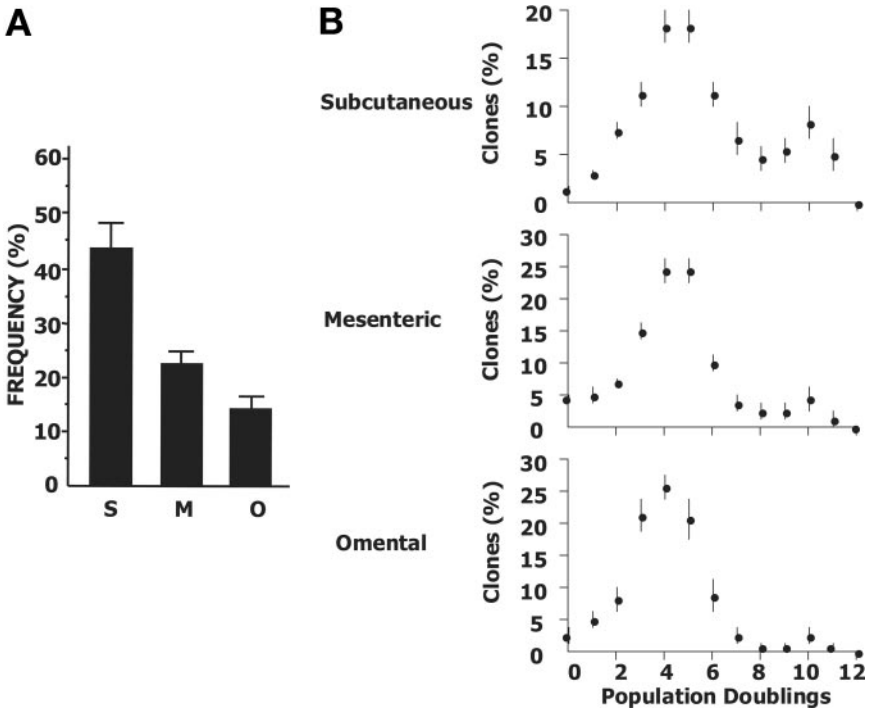

FIG. 5. Regional variation in replicative potential and subtype abundance are retained in hTERT strains. *A*: Individual subcutaneous (S), mesenteric (M), and omental (O) 40th-population doubling hTERT strains were subcloned by culturing individual cells for 21 days. Frequencies (means ± SE) of clones that achieved at least six doublings are shown. A greater proportion of S achieved six or more doublings than M or O subclones ($P < 0.01$; Duncan's multiple range test). *B*: Cell numbers in resulting clones were determined, and frequency distributions of doublings achieved are shown ($n = 6$ hTERT strains from each depot in parallel [S2.5, S2.11, S2.13, S3.1, S3.5, S3.8, M2.5, M2.6, M2.10, M3.1, M3.5, M3.8, Om2.2, Om2.5, Om2.7, Om3.1, Om3.5, and Om3.8]).

extensive recruitment of preadipocytes into fat cells under adipogenic conditions. The rapidly replicating preadipocyte subtype is more abundant in primary abdominal subcutaneous than mesenteric or omental preadipocyte populations. To test if this fat depot–dependent pattern of subtype abundance is heritable, individual abdominal subcutaneous, mesenteric, and omental 40th-population doubling hTERT cells were cultured in growth medium for 3 weeks. Both subtypes were found (Fig. 5*B*). The rapidly replicating subtype was more abundant in subcutaneous than mesenteric or omental hTERT strains (Table 1). The modal number of doublings achieved and colony-forming efficiency of each subtype were similar. Thus, fat depot–specific differences in abundance of preadipocyte subtypes are retained for at least 40 population doublings in hTERT strains derived from single preadipocytes.

Adipogenic capacity of subcutaneous is greater than visceral hTERT strains. Sixth-passage primary abdominal subcutaneous, mesenteric, and omental preadipocytes were grown to confluence then exposed to differentiation medium for 30 days, as were hTERT strains derived from these depots. Lipid accumulation was greater in subcutaneous than mesenteric or omental primary preadipocytes, as noted previously (10,11,47) (Fig. 6*A*). This pattern was retained in the hTERT strains, despite having been propagated for 40 population doublings. While more subcutaneous than visceral preadipocytes accumulated lipid, individual cells that contained large lipid droplets were found in subcutaneous and omental hTERT strains after 45 population doublings (Fig. 6*B*). Such cells are not seen in cells in skin fibroblasts grown under these conditions (9), confirming the adipocytic lineage of the hTERT strains. aP2, PPARγ2, and C/EBPα mRNA abundance was measured in the subcutaneous and omental strains following exposure to differentiation medium for 15 days (Fig. 6*C*). Expression was higher in the subcutaneous than omental cultures (aP2: $P < 0.05$; PPARγ2: $P < 0.05$; and C/EBPα: $P < 0.01$; $n = 5$ strains from each depot [S2.5, S2.13, S3.1,

TABLE 1
Colony-forming efficiency and replicative potential of hTERT preadipocyte subtypes

	Abdominal subcutaneous	Mesenteric	Omental
Colony-forming efficiency (%)	68.2 ± 2.2	68.4 ± 1.8	67.2 ± 3.0
Rapidly replicating subtype cells (%)	25 ± 5*	8 ± 3	4 ± 2
Slowly replicating subtype cells (%)	75 ± 5*	92 ± 3	96 ± 2
Modal number of doublings achieved by rapidly replicating subtype cells	9.7 ± 0.5	9.5 ± 0.2	9.3 ± 0.2
Modal number of doublings achieved by slowly replicating subtype cells	4.3 ± 0.2	4.5 ± 0.2	4.0 ± 0.3

Data are means ± SE. Abdominal subcutaneous, mesenteric, and omental hTERT strains (six strains from each depot [S2.5, S2.11, S2.13, S3.1, S3.5, S3.8, M2.5, M2.6, M2.10, M3.1, M3.5, M3.8, Om2.2, Om2.5, Om2.7, Om3.1, Om3.5, and Om3.8]) were cloned by plate dilution. Fifty cells from each depot were plated into each of 10 96-well plates. Twenty-one days after cloning, cell numbers were determined. Colony-forming efficiency is expressed as percent of cells plated that formed colonies. *Denotes significant difference from other values in the same row ($P < 0.05$; Duncan's multiple range test). Colony-forming efficiencies did not differ significantly among depots ($P = 0.92$; ANOVA).

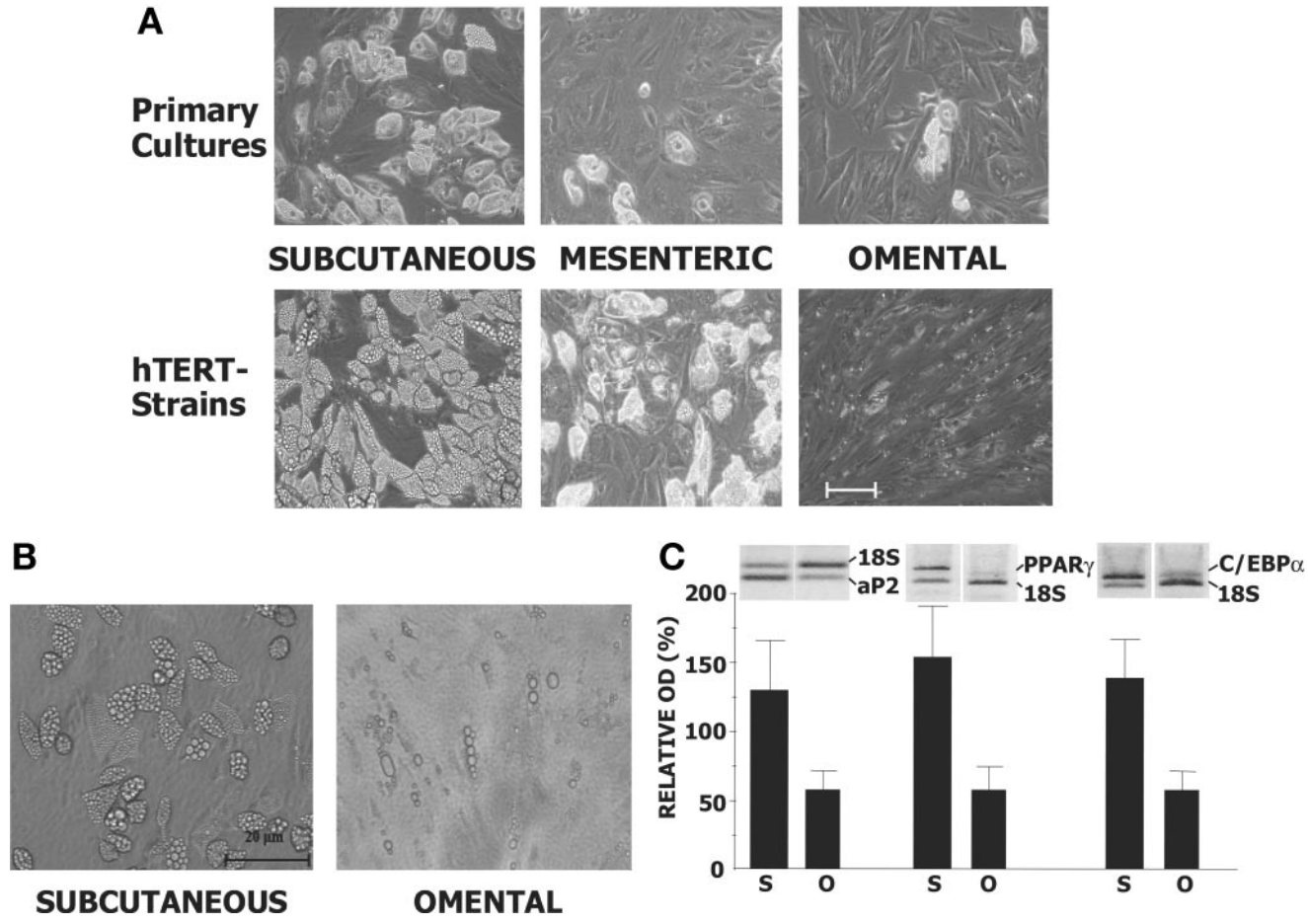

FIG. 6. Regional variation in adipogenesis is retained in serially passaged hTERT preadipocytes. *A*: Sixth-passage primary subcutaneous, mesenteric, and omental preadipocytes and hTERT strains (40 population doublings) derived from each depot were grown to confluence then treated with differentiation medium for 30 days. Each phase-contrast photomicrograph was taken at the same magnification (bar represents 20 μm; representative of appearance of sets of cultures from 11 subjects). *B*: 45th-population doubling subcutaneous and omental hTERT-expressing cells were treated with differentiation medium for 30 days. Although less abundant in omental than subcutaneous strains, cells with extensive lipid accumulation were still found in omental cultures, attesting to their adipocytic lineage. *C*: aP2, PPARγ2, and C/EBPα mRNA was assayed by RT-PCR (with 18S rRNA as internal control) in confluent cultures of subcutaneous (S) and omental (O) 40th-population doubling strains treated with differentiation medium for 15 days. Representative gels are shown above. Below are means ± SE of densitometric analyses of mRNA (*P < 0.05 and **P < 0.01; n = 5 subcutaneous and five omental strains [S2.5, S2.13, S3.1, S3.5, S3.8, Om2.2, Om2.7, Om3.1, Om3.5, and Om3.8]) expressed as a function of 18S rRNA.

S3.5, S3.8, Om2.2, Om2.7, Om3.1, Om3.5, and Om3.8]). These patterns recapitulate the higher aP2, PPARγ2, and C/EBPα in differentiating subcutaneous than visceral primary preadipocytes previously reported (10,11).

Regional differences in susceptibility to TNFα-induced apoptosis are retained in hTERT strains. Abdominal subcutaneous, mesenteric, and omental primary preadipocytes and hTERT strains were exposed to various concentrations of TNFα for 4 h (Fig. 7). Apoptotic indexes, determined by observers who were not aware of treatments the cells had received, were highest in the omental and lowest in the subcutaneous primary preadipocytes, consistent with the observation that omental preadipocytes and fat cells are more susceptible than subcutaneous cells to TNFα-induced apoptosis (12). The same fat depot–dependent differences in susceptibility to TNFα were evident in the 40th-population doubling hTERT clones, indicating that depot-dependent differences in cytokine responses are heritable.

DISCUSSION

Visceral and peripheral fat depots vary in size, cellularity, function, and association with disease, particularly the metabolic syndrome. Regional variation in innervation, circulation, nutrient supply, anatomic constraints, and abundance of cell types such as macrophages likely contributes to differences in function across fat depots. Since fat tissue turns over in adulthood (4), inherent, fat depot–dependent differences among the preadipocytes from which new fat cells arise could also contribute. Indeed, preadipocytes appear to vary in capacities for replication, adipogenesis, and apoptosis among fat depots, although there has been debate about this (5–15,28). Reasons for discrepant findings across studies may include differences in culture conditions, duration of exposure to agents such as those that induce adipogenesis, characteristics of subjects from whom preadipocytes were isolated, or contamination of primary cultures by other cell types potentially present in differing quantities among different fat depots. In studies where regional differences were found, subcutaneous preadipocytes tended to have greater capacity for replication, lipid accumulation, G3PD activity, expression of aP2, C/EBPα, and PPARγ and responsiveness to thiazolidinediones than visceral preadipocytes. Reduced susceptibility to TNFα-induced apoptosis (12) and a higher proportion of preadipocytes of the rapidly replicating

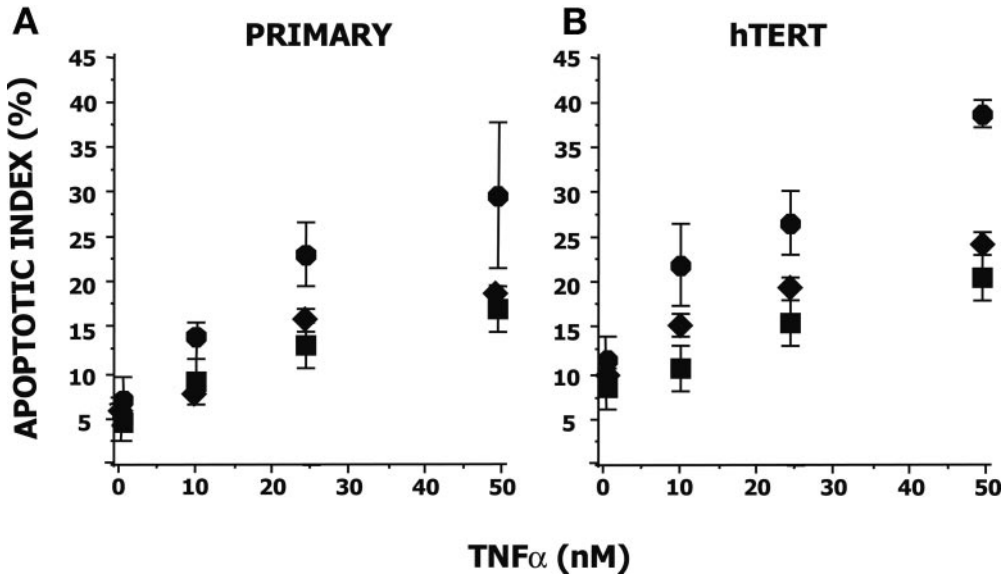

FIG. 7. Regional differences in susceptibility of preadipocytes to TNFα apoptosis are retained in hTERT strains. *A*: Confluent subcutaneous (squares), mesenteric (diamonds), and omental (circles) sixth-passage primary preadipocytes cultured in parallel in α minimum essential Eagle's medium with 10% FBS were washed and exposed to 0–50 nmol/l TNFα without FBS for 4 h. Observers not aware of treatments determined apoptotic indexes (means ± SE; $n = 3$ subjects). *B*: 40th-doubling hTERT strains were exposed to TNFα and apoptotic indexes determined ($n = 3$ hTERT strains in parallel from each depot [S3.1, S3.5, S3.8, M3.1, M5, M3.8, Om3.1, Om3.5, and Om3.8]).

subtype have also been noted in subcutaneous compared with visceral preadipocytes (28). Depot-dependent differences in capacity for adipogenesis, replication, and preadipocyte subtype abundance have been found in rats, even after prolonged culture (5,7,48), attesting to the evolutionary conservation of regional variation in inherent properties of preadipocytes.

We found that telomerase expression confers enhanced potential for replication and preserves adipogenic capacity in preadipocytes after repeated subcultures. This permitted us to test the hypothesis that preadipocytes have inherently distinct, depot-dependent properties by determining whether differences persist over many cell generations in preadipocytes from different fat depots cultured under identical conditions. Fat depot–dependent differences in replicative potential, adipogenesis, susceptibility to apoptosis, and preadipocyte subtype abundance remained evident in hTERT strains that reflect depot-dependent differences in these characteristics among primary preadipocytes. This supports the contention that preadipocytes from different fat depots are distinct. Since the hTERT strains were derived from single preadipocytes, regional differences in preadipocyte properties are not merely the result of presence of other cell types in primary cultures, such as macrophages, that are abundant in fat from obese subjects (49) and whose abundance could vary among fat depots. Generation of preadipocytes stably expressing hTERT shows promise for expanding the available repertoire of euploid human preadipocyte cell strains, especially for defining mechanisms responsible for regional differences in fat tissue function.

The regionally distinct preadipocyte characteristics that remained evident for 40 population doublings could be epigenetic, appearing during early development or be set later by the particular microenvironment of different fat depots or might result from different populations of early progenitors (e.g., resident versus nonresident progenitors [50]), giving rise to committed preadipocytes among depots. Additionally, BMI, sex, visceral compared with subcutaneous obesity, subject age, preadipocyte replicative history, and associated disease states might influence these properties through epigenetic, genomic, or progenitor subpopulation selection mechanisms. Since new fat cells continue to arise from preadipocytes in adulthood, regional differences in preadipocyte characteristics could contribute to differences in function among fat depots. Indeed, different fat depots appear to be distinct miniorgans. Since preadipocytes from different depots are distinct, it is conceivable that drugs could be developed to target specific fat depots.

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