Cytotherapy, 2010; 12: 538–546

informa
healthcare

Yield and characterization of subcutaneous human adipose-derived stem cells by flow cytometric and adipogenic mRNA analyses

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Abstract

Background aims. Adipose-derived stromal/stem cells (ASC) capable of multipotential differentiation can be isolated with high yields from human subcutaneous lipoaspirates. This study reports our recent experience of isolating and immunophenotypically characterizing ASC from >60 human patients with a mean age of 43.6 and body mass index (BMI) of 27. *Methods*. We examined the ASC yield per unit volume of lipoaspirate tissue, the surface antigen profile based on flow cytometry, histochemical differentiation potential along the adipogenic and osteogenic pathways, and expression of adipogenic mRNA by transcriptomic microarray and reverse transcription (RT)–polymerase chain reaction (PCR). *Results*. The population ($n = 64$) of predominantly Caucasian (84.3%) female (90.6%) donors had a mean age of 43.6 ± 11.1 years and a mean BMI of 27.0 ± 3.8. A yield of 375 ± 142 × 10^3 ASC was obtained per milliliter of lipoaspirate within a 4.1 ± 0.7-day culture period ($n = 62$). The ASC population was uniformly $CD29^+$ $CD34^+$ $CD44^{lo}$ $CD45^{lo}$ $CD73^+$ $CD90^+$ $CD105^+$ and capable of undergoing both adipogenesis and osteogenesis *in vitro* based on Oil Red O and Alizarin Red staining, respectively. Adipogenic differentiation was associated with a significant induction of multiple mRNA associated with lipid storage and synthesis based on microarray analysis of $n = 3$ donors. During an adipogenic differentiation time–course, representative mRNA (adiponectin, C/EBPα, leptin and LPL) displayed increases of several orders of magnitude. *Conclusions*. These findings demonstrate the reproducibility of subcutaneous lipoaspirates as a consistent and abundant source of functional ASC from donors across a spectrum of ages and BMI. These results have relevance for regenerative medical applications exploiting autologous and allogeneic ASC for soft and hard tissue engineering.

Key Words: *adipogenesis, adipose-derived stem cells, cell yield, differentiation, flow cytometry, human, transcriptome*

Introduction

Since their original identification by Friedenstein *et al.* (1) in bone marrow (BM), mesenchymal stromal cells (MSC) have been found in multiple tissues (2–4). In contrast to BM, placenta, umbilical cord, skeletal muscle and other sites, adipose tissue offers advantages in terms of its accessibility, abundance and regenerative capacity, as well as the willingness of many individuals to consent to its donation for tissue engineering and regenerative medical applications (5–7). The potential utility of adipose-derived stromal/stem cells (ASC) has been demonstrated in multiple pre-clinical animal models for orthopedic, soft tissue and ischemic injury (6,8–20). Similar to BM MSC, ASC have been characterized based on their immunophenotypic and differentiation properties (21–26). Because large volumes of adipose tissue can be obtained from individual donors, it is possible to obtain high yields of ASC within a single passage (21). We reviewed our experience in isolating and characterizing primary ASC from lipoaspirates in a cohort of >60 patients over a 2-year period between July 2007 and June 2009. In addition to quantification of cell yields, *in vitro* differentiation, flow cytometric immunophenotype and adipogenic mRNA data were investigated.

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(Received 16 October 2009; accepted 20 January 2010)

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653241003649528

Methods

Materials

All reagents were purchased from Fisher Scientific (Dallas, TX, USA) or Sigma-Aldrich (St Louis, MO, USA) unless otherwise noted.

Donor selection and informed consent

All protocols were reviewed and approved by the Pennington Biomedical Research Center (Baton Rouge, LA, USA) Institutional Review Board prior to tissue collection. All tissue was obtained from patients undergoing elective liposuction surgery. A signed consent agreement was obtained by the plastic surgeon and tissues were provided to the investigators in an anonymous manner. The demographic data on each patient were limited to age, ethnicity, gender, height, weight and history of metabolic illness.

Donor demographics

Adipose tissue specimens were obtained with informed consent from patients undergoing elective liposuction or abdominoplasty surgery ($n = 64$). The majority of patients were women (female 90.6%, male 4.7%, not recorded 4.7%). Ethnically, the majority of patients were Caucasian (84.3%), with the rest comprising African Americans (9.4%), Asians (1.6%) and not recorded (4.7%). Patient ages ranged from 18 to 66 years (mean 43.6 $\pm$ 11.1 years) and body mass index (BMI) from 18.3 to 37.2 (mean 27.0 $\pm$ 3.8).

Isolation and culture of ASC

ASC were isolated from fresh human subcutaneous adipose lipoaspirate according to published methods, with some minor modifications (21,27). The lipoaspirate tissue was washed extensively with warm phosphate-buffered solution (PBS) to remove erythrocytes and then digested in PBS supplemented with 0.1% collagenase type I (Worthington Biochemical Corporation, Lakewood, NJ, USA), 1% bovine serum albumin (BSA) and 2 mM $CaCl_2$ for 1 h at 37°C. Following room temperature centrifugation at 300 ***g*** and resuspension in stromal medium [Dulbecco's modified Eagle medium (DMEM)/Hams F-12 medium supplemented with 10% FBS (Hyclone, Logan, UT, USA) and 1% antibiotic/antimycotic], the stromal vascular pellet was plated at a density of 35 mL lipoaspirate digest/T175 flask (0.2 mL/cm^2). After 24 h of incubation at 37°C, 5% CO_2, the adherent cells were washed with warm PBS and maintained in stromal medium until 80–90% confluent. The adherent population was harvested by digestion with trypsin (0.05%)/ethylene diamine tetra acetic acid (EDTA; 1 mM) at 37°C for 5 min, washed in stromal medium and replated at 5×10^3 ASC/cm^2 (passage 1; P1) or used in flow cytometric analyzes (below) or cryopreserved (28) for future use.

Flow cytometry of ASC

Trypsin-harvested ASC were washed with PBS three times and aliquots of 10^5 cells were incubated with phycoerythrin (PE)-conjugated monoclonal antibodies directed against CD29 (catalog number 12–0297; eBioscience, San Diego, CA, USA), CD34 (catalog number 348057; Becton Dickinson, Franklin Lakes, NJ, USA), CD45 (catalog number 12-0459; eBioscience), CD73 (catalog number 550257; BD Pharmingen, BD Bioscience, San Jose, CA, USA), CD90 (catalog number 55596; BD Pharmingen) and IgG1κ control (catalog number 555749; BD Pharmingen), or fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies directed against CD44 (catalog number 348057; BD) and IgG1κ control (catalog number 554679; BD Pharmingen), for 20 min on ice, before being washed with PBS supplemented with 1% BSA three times and fixed in 1% formaldehyde overnight at 4°C. For each sample, 10^5 events were collected on a Becton Dickinson FACScaliber flow cytometer using CELLQuest acquisition software (Becton Dickinson) and analyzed using Flow Jo software (Tree Star). This antibody panel was selected, in part, based on the International Society for Cellular Therapy (ISCT) position paper on the criteria for defining MSC (29).

Adipogenic and osteogenic differentiation of ASC

Confluent cultures of ASC (P1) were induced with adipogenic differentiation medium [DMEM/Hams F-12, 3% FBS, 1% antibiotic/antimycotic, 0.5 mM isobutylmethylxanthine, 33 μM biotin, 17 μM pantothenate, 5 μM rosiglitazone (AK Scientific, Mountain View, CA, USA), 1 μM dexamethasone, 1 μM insulin] for 3 days before being converted to adipocyte maintenance medium (identical to adipogenic differentiation medium without isobutylmethylxanthine and rosiglitazone). Cells were maintained for 9 days before fixation and Oil Red O staining. In selected experiments, samples of uninduced and adipogenic-induced ASC were harvested for total RNA isolation at days 0, 3, 6 and 9 of differentiation. Confluent cultures of ASC were converted to osteogenic medium (DMEM/Hams F-12 or DMEM, 10% FBS, 1% antibiotic/antimycotic, 10 mM β-glycerophosphate, 50 μg/mL sodium 2-phosphate ascorbate and 10^{-8} M dexamethasone) and maintained in culture for 9–12 days, with medium changes every third day. The cultures were rinsed three times

with 150 mM NaCl, fixed in 70% ethanol, and stained with Alizarin Red.

Total RNA isolation, reverse transcription–polymerase chain reaction methods and primers

Total RNA was isolated from ASC cultures using TriReagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. The total RNA (2 μg) was incubated in a 20-μL volume with Moloney murine leukemia virus reverse transcriptase, dNTP and oligdT. Reverse transcription (RT)–polymerase chain reaction (PCR) was performed using SybrGreen PCR master mix (Applied Biosystems, Foster City, CA, USA) using an ABI 7900 real-time PCR system with primers for the following genes of interest: adiponectin (NM_004797) (for) GGCCGTGATGGCAGAGAT (rev) TTTCACCGATGTCTCCCTTAGG; CAAT/enhancer binding protein α (C/EBPα) (NM_004364.2) (for) GGGTCTGAGACTCCCTTTCCTT (rev) CTCATTGGTCCCCCAGGAT; leptin (NM_000230.1) (for) GGTTGCAAGGCCCAAGAA (rev) ACATAGAAAAGATAGGGCCAAAGC; lipoprotein lipase (LPL) (NM_000237.2) (for) TCCGCGTGATTGCAGAGA (rev) CGCTCGTGGGAGCACTTC; and 18S RNA (NR_003286.1) (for) AAACGGCTACCACATCCAAG; (rev) CCTCCAATGGATCCTCGTTA; with the following cycling conditions, 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 1 min.

Transcriptomic analysis

Total RNA was isolated from ASC obtained from three individual donors and maintained under uninduced or adipogenic-induced culture conditions for 9 days following confluence. The RNA was assessed with an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and cDNA synthesized using a Superscript cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) and a T7-$(dT)_{24}$ primer. Biotinylated cRNA was transcribed *in vitro* using a GeneChip IVT labeling kit (Affymetrix, Santa Clara, CA, USA), purified using a GeneChip sample cleanup module, incubated in fragmentation buffer (200 mM Tris acetate, pH 8.1, 500 mM potassium acetate and 150 mM magnesium acetate) at 94°C for 35 min, chilled on ice, and hybridized to a Human U133 Plus 2.0 Array of *c.* 47 400 transcripts and variants, including 38 500 well-characterized genes. Arrays were incubated at 45°C for 16 h rotating at 60 r.p.m., washed, stained at 25°C for 10 min with 10 μg/mL streptavidin R phycoerythrin (Vector Laboratories, Burlingame, CA, USA), stained at 25°C for 10 min (×2) with 3 μg/mL biotinylated goat anti-streptavidin antibody (Vector Laboratories), washed, and scanned using a GeneChip scanner 3000. Pixel intensities were measured, expression signals globally scaled to a target intensity value of 2500, and features extracted using GeneChip Operating Software v1.2 (Affymetrix). Data mining and statistical analyzes were performed with Data Mining Tool v.3 (Affymetrix) algorithms. The absolute call (present, marginal, absent) of each gene expression, the direction of change and the fold change were identified with the above software. The raw Affymetrix CHP data have been stored in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database under accession numbers GSM458608 (uninduced), GSM458609 (adipogenic-induced) and GSE18391 (attributes). Additional analyzes were performed with GeneGo (St Joseph, MI, USA) and Ingenuity (Redwood City, CA, USA) software programs.

Statistical methods

Values are presented as the mean ± SD. A Student's *t*-test was used to evaluate the significance ($P < 0.05$). Linear regression was used to determine the correlation coefficient between ASC yield and donor age and BMI. Experiments were performed a minimum of $n = 3$ times, with the exception of the microarray analyzes, where a single evaluation was performed on total RNA from uninduced and adipogenic-induced ASC of $n = 3$ biologic donors.

Results

ASC yield and growth time

The isolated stromal vascular fraction cells were plated immediately upon isolation at a density of 0.2 mL processed lipoaspirate tissue/cm^2. The adherent cell population, termed ASC, reached 80–90% confluence based on visual inspection within 4.1 ± 0.7 days ($n = 62$). Following trypsin digestion and harvest of the P0 ASC, the mean yield achieved was 375 ± 147 × 10^3 cell/mL lipoaspirate tissue. The yield displayed a positive correlation with both donor age ($r = 0.30$) and BMI ($r = 0.26$); however, as the stromal vascular fraction (SVF) cell plating density at the initiation of passage was not assessed, the significance of these values was open to question.

Flow cytometric analyzes

The immunophenotype of ASC at P0 was determined by flow cytometric analysis using a panel of monoclonal antibodies (Figure 1). The ASC displayed the following mean (± SD) percentage positive cells ($n = 64$ donors) for the indicated surface antigens: CD29 (β_1 integrin), 99.0 ± 1.7%; CD34,

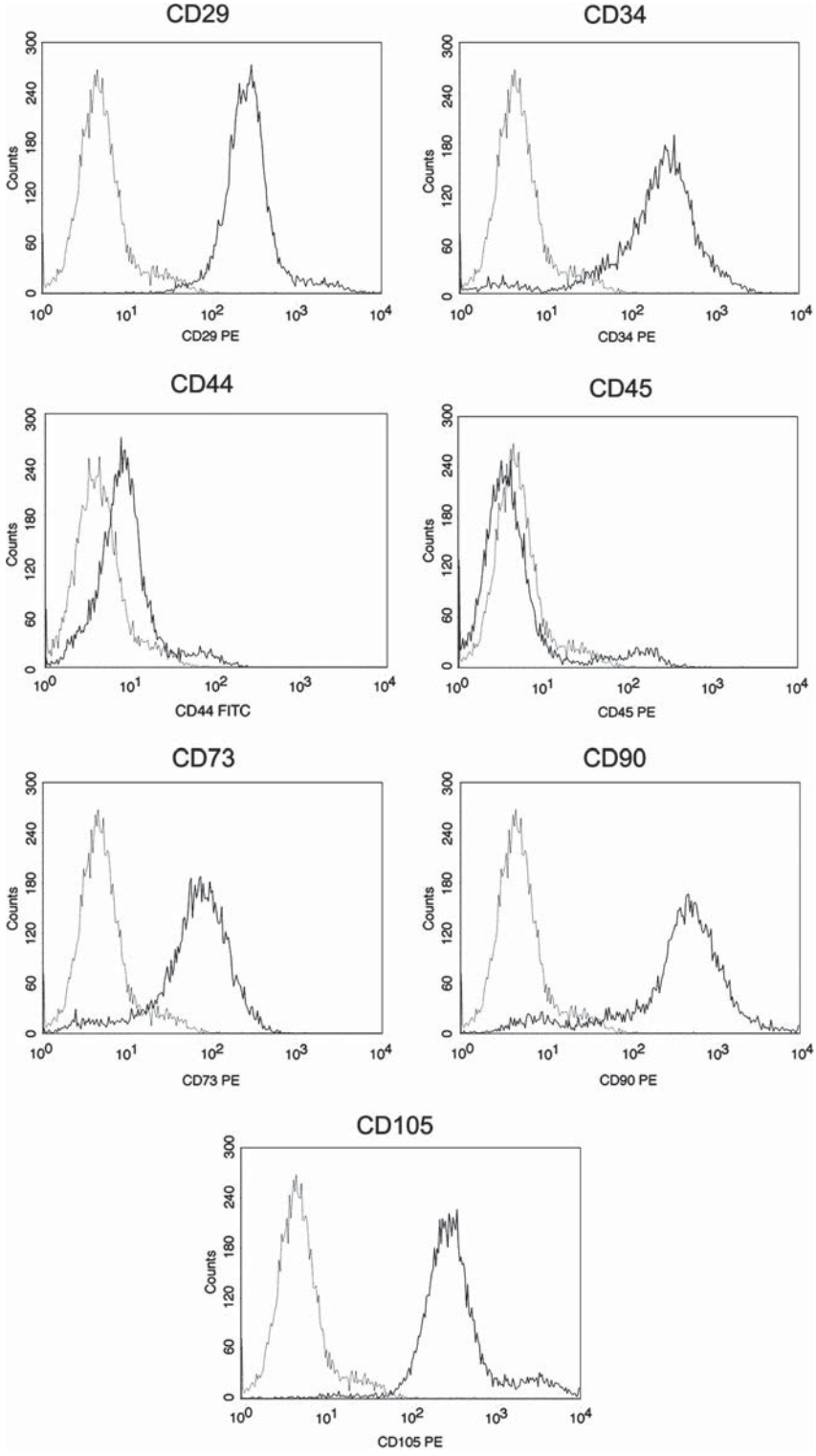

Figure 1. Flow cytometric histograms. The fluorescence-activated cell sorting analysis of human subcutaneous ASC isolated from a representative donor is displayed using antibodies directed against CD29, CD34, CD44, CD45, CD73, CD90 and CD105 (solid line); all are conjugated to PE except for CD44 (FITC). Negative controls conjugated to FITC or PE are displayed to the left of each panel (dotted line).

90 ± 9.5%; CD44 (hyaluronate receptor), 15.5 ± 8.5%; CD45 (leukocyte common antigen), 12.0 ± 6.0%; CD73 (5′ ecto-nucleotidase), 86.2 ± 7.2%; CD90 (Thy1), 90.0 ± 6.0%; and CD105 (endoglin), 97.8 ± 1.5%. In contrast, ASC stained with isotype control antibodies conjugated to PE or FITC displayed background fluorescent intensities of 6.1 ± 3.2% and 4.8 ± 3.3%, respectively.

Differentiation analysis

The isolated P0 ASC routinely displayed adipogenic and osteogenic differentiation potential *in vitro*. Figure 2 displays a representative photomicrograph of ASC maintained for 9 days under uninduced (Toluidine Blue), adipogenic (Oil Red O) and osteogenic (Alizarin Red) conditions.

Transcriptomic analysis of genes induced ≥2-fold with adipogenesis

Transcriptomic microarray comparison of total mRNA from ASC (n = 3 donors) identified a set of 429 probe sets induced by ≥2-fold under adipogenic relative to undifferentiated conditions (Supplementary Table I) (Supplementary Tables to be found at http://informahealthcare.com/cyt. DOI number: 10.3109/14653241003649528); the raw Affymetrix datasets have been entered into the NCBI GEO database. The provisional set of potential differentially expressed genes was created using a 2-fold selection threshold. Pathways analysis based on Fisher's exact test identified gene categories (such as canonic pathways, genes interconnected by regulation and molecular function) significantly over-represented in the provisional list. Discovered pathways were adjusted for potential false discovery rate (FDR) estimated by Benjamini–Hochberg algorithm (30). Both over-representation and FDR tests were implemented within the pathway analysis software. Our interpretation of gene expression pattern was based on functional groups (pathways). This approach, unlike stringent selection of microarray probes, allowed for a better accounting of interactive genes that may not be statistically significant considered separately but add strength to each other as a group. Pathway analysis has been proven effective for the analysis of metastasis biomarker association with cancer (31,32) and for signal transduction relationships with infectious disease (32). Ingenuity pathway analysis (Ingenuity Systems Inc., Redwood City, CA, USA) of the induced microarray dataset identified specific metabolic pathways that could be associated with between 16 and 32 individual genes. Among the general metabolic pathways detected were those associated with cancer and cell proliferation, immunity, metabolism (amino acid, lipid, carbohydrate), extracellular matrix formation and cell assembly/structure (Table I and Supplementary Figure 1) (Supplementary Figures to be found online at http://informahealthcare.com/cyt. DOI number: 10.3109/14653241003649528). Analyzes of protein classes determined that significant enrichment (P-value < 0.05) was observed for the categories of enzymes, ligands and proteases (Table II and Supplementary Figure 2). Among these were enzymes involved in fatty acid metabolism that were induced with adipogenesis (Supplementary

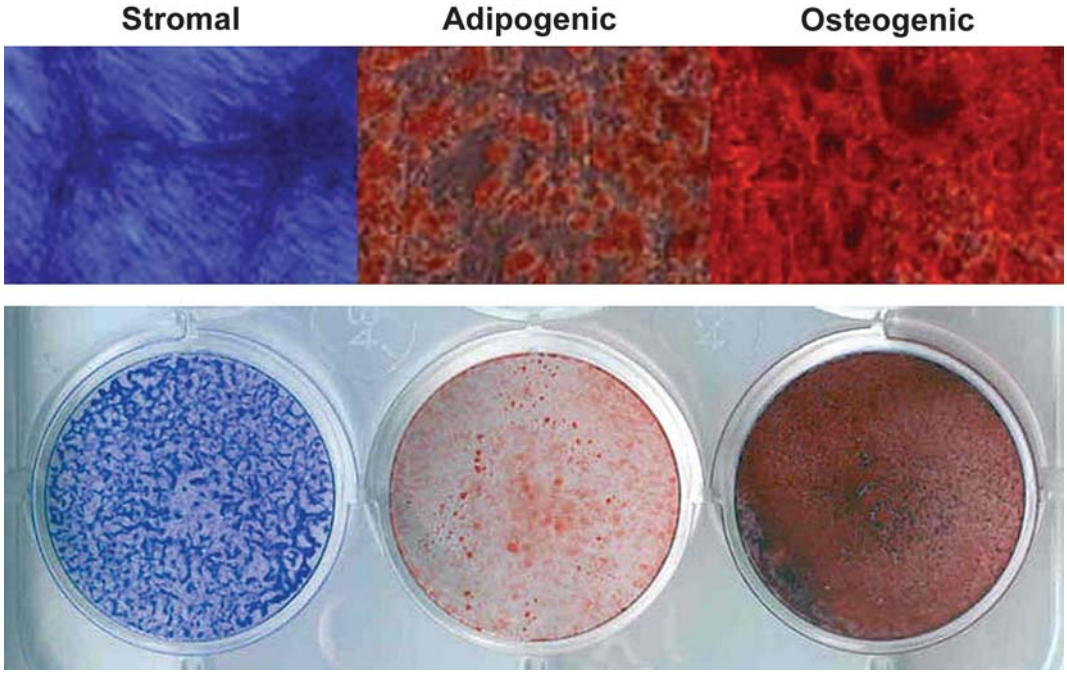

Figure 2. Histochemical differentiation. The human subcutaneous ASC isolated from a representative donor were cultured to confluence and then maintained under control conditions in the presence of stromal medium (left panel), adipogenic conditions (middle panel) or osteogenic conditions (right panel) for 9 days. At the conclusion of these culture periods, the cells were fixed and stained with Toluidine Blue (control), Oil Red O (adipogenic) or Alizarin Red (osteogenic). Photomicrographs (×20 magnification) of the wells taken at ×20 magnification (top) and scans of the entire well (bottom) are presented.

Table I. Ingenuity analysis of transcriptomic analysis of mRNA induced ≥2-fold under adipogenic conditions.

Metabolic pathway	Number of times identified
Cancer	16
Immune, inflammatory, infection, hematopoietic	9
Development/morphology	9
Lipid metabolism	8
Fibrosis/connective tissue/dermatology	3
Gastrointestinal disease	3
Amino acid metabolism	2
Cell assembly	2
Carbohydrate metabolism	1

Figure 3). While there was no significant enrichment for transcription factors as an overall class, a subset of 59 transcription factors was identified that displayed highly significant interactions with *Z*-scores >2.8 (Supplementary Table II). Among these were factors that had been previously associated with adipogenesis, including members of the nuclear hormone receptor family, PAS domain proteins, CCAAT/enhancer binding proteins (C/EBP), sterol receptor enhancer binding protein (SREBP) and signal transducers and activators of transcription (STAT). The latter transcription factors mediate signal transduction via the gp130 protein receptor for interleukin(IL)-6; adipogenic-associated changes in this pathway are highlighted in Supplementary Figures 4 and 5.

The time-dependent expression of representative adipogenic genes was determined by RT-PCR (Figure 3) ($n = 4$ donors, BMI 23.1 ± 1.4, age 39 ± 6 years). The adipogenic transcription factor C/EBPα, the adipokines adiponectin and leptin, and lipoprotein lipase (LPL) all displayed a time-dependent increase with adipogenesis, increasing between 3 and 7 orders of magnitude over a 9-day period of induction; however, the greatest percentage of this increase occurred within the initial 3-day period.

Table II. Enrichment by protein class.

Protein class	*r*	*R*	*P*-value	*Z*-score
Enzymes	261	2659	1.52E-09	6.245
Ligands	51	525	0.009621	2.527
Proteases	49	547	0.0404	1.876
Kinases	51	603	0.08348	1.482
Receptors	89	1431	0.1421	–1.122
Transcription factors	65	951	0.4775	–0.1387
Phosphatase	15	226	0.4939	–0.184
Other	822	13255	7.79E-09	–5.755

Values are based on a total of 1403 network objects in the activated dataset and a total of 20197 objects in the complete dataset. The value *r* is the number of network objects in the activated dataset that interact with a chosen object, while *R* is the number of network objects in the complete dataset that interact with the chosen object.

Further studies were conducted evaluating mRNA levels at day 3 of adipogenic conditions in ASC isolated from female, Caucasian donors with lean (mean BMI 22.82 ± 1.19, $n = 6$, range 21.63–24.93), overweight (mean BMI 27.53 ± 1.37, $n = 6$, range 25.81–29.4), and obese (mean BMI 32.04 ± 2.53, $n = 6$, range 30.65–37.15) BMI. The mean ages of these cohorts (lean 37 ± 9.6 years, overweight 41 ± 7.7 years, obese 42 ± 10.4 years) were not significantly different ($P > 0.4$, paired two-tailed *t*-test). The expression levels for adiponectin, aP2 (adipocyte myelin P2 protein homolog, also known as fatty acid binding protein 4), LPL and peroxisome proliferator activated receptor γ2 (PPARγ2) were not significantly different between the lean, overweight and obese cohorts (Figure 4).

Discussion

The results demonstrate that lipoaspirate tissue from donors across a wide age range yields substantial numbers of ASC per unit volume that display a consistent immunophenotype. Based on the experience reported in >60 patients, approximately 75 million cells can be recovered from 200 mL processed lipoaspirate within a 4–5 day culture period. In light of the fact that many liposuction procedures remove liter volumes of tissue, it is feasible to produce upwards of 375 million ASC/donor in less than 1 week. Calculations by Muschler & Midura (33) estimate that a cubic centimeter of bone contains 70 million cells. Thus the yield from adipose tissue will approach or exceed the quantities of stem cells that are required for clinical regenerative medical applications. Based on the current study, donor ages up to 66 do not present an obstacle to recovering sufficient cell yields. This is consistent with recent work by Zhu *et al.* (34), which did not find a significant effect of donor age on the proliferation rates of ASC isolated from a cohort of women ($n = 15$) between the ages of 20 and 58. While Zhu *et al.* (34) did not find any effect of donor age on adipogenic potential of the ASC, advancing age did significantly reduce their osteogenic potential (34). De Girolamo *et al.* (35) have reported similar age-dependent results with respect to osteogenesis in $n = 26$ women. Gender may also play a role as ASC from male, compared with female, donors display increased osteogenic potential *in vitro* (36). As seen with ASC, advancing donor age has been correlated with reduced osteogenic activity in human BM-derived MSC by some (37), but not all (38,39), studies. Consistent with this and in contrast to the work of Zhu *et al.* (34), Schipper *et al.* (40) have reported age-dependent differences in ASC adipogenic and proliferative capacity. They found that ASC isolated from multiple

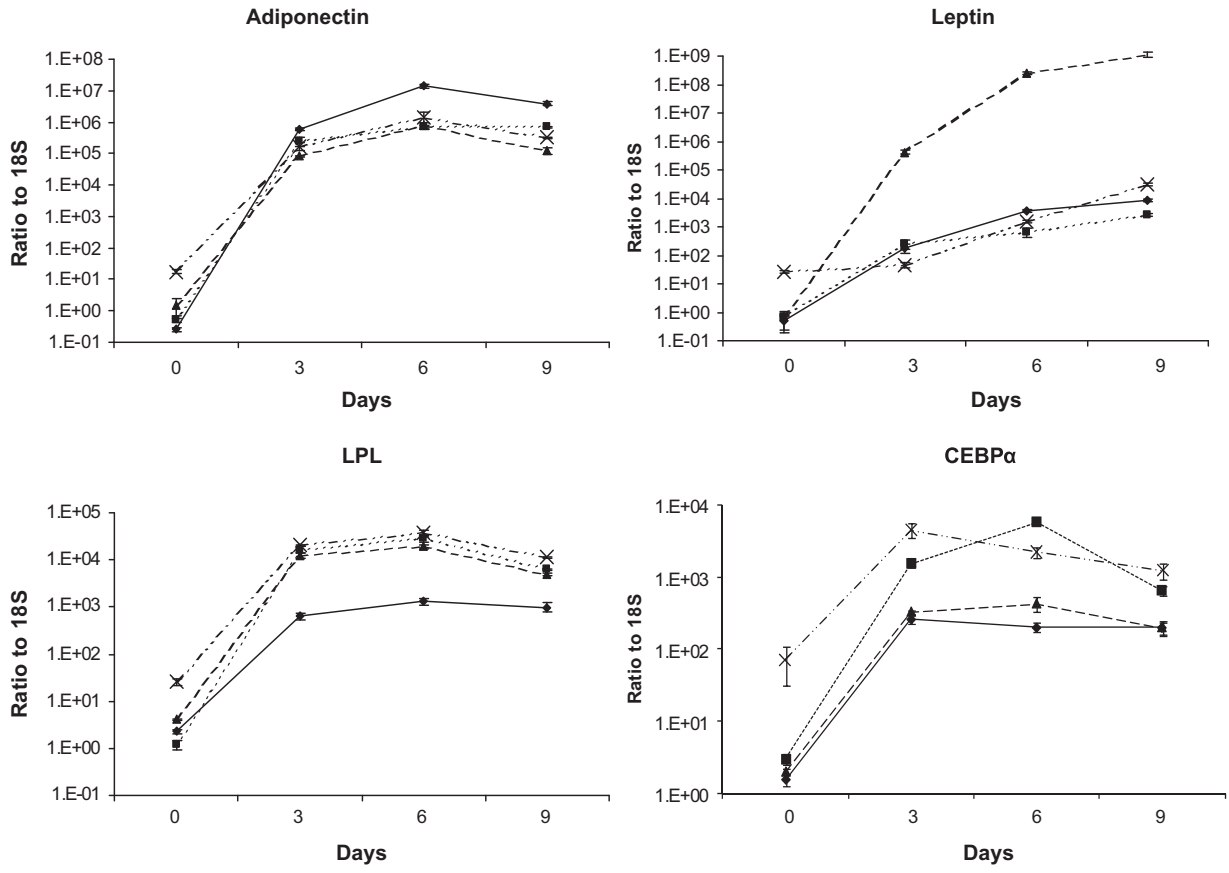

Figure 3. Adipogenic time–course by RT-PCR of selected mRNA. Human subcutaneous ASC isolated from $n = 4$ donors were cultured to confluence and induced to undergo adipogenesis at day 0. Individual wells for each donor were harvested at 0, 3, 6 and 9 days after induction. RT-PCR was performed for the following adipogenic genes: adiponectin, C/EBPα, leptin and LPL. The levels of each were normalized relative to that of 18S RNA.

anatomical sites in young (25–30 year old) donors proliferated more rapidly and expressed higher levels of PPARγ protein compared with older (55–60-year-old) donors (40). The current study suggests that, while donor BMI was associated with increased ASC yields, it had no significant effect on the expression of representative mRNA on day 3 of adipogenesis (Figure 4). These reports suggest that further investigation should be conducted relating the impact of age and BMI on ASC functionality.

With respect to immunophenotype, the predominance of the current findings confirm and extend the existing literature. As expected of an MSC (29), the ASC isolated from >60 patients consistently expressed high levels of CD29, CD73, CD90 and CD105 and low levels of CD45. However, in contrast to BM MSC (29), early passage ASC have been shown here and in multiple other reports (17,18,20,22,23,41) to be $CD34^+$. It has been established that the expression of CD34 by early passage ASC is transient and decreases significantly with successive expansion to levels comparable to those found on BM MSC (42). Thus the timing of the flow cytometry measurements relative to the culture period is a critical determinant of CD34 expression level. It remains to be determined whether CD34 constitutes a stem cell marker for the ASC lineage.

One unexpected finding in the current report was the low level of CD44 expression on ASC. Past analyzes by our group (22,23) and others (24) have detected CD44 on >60% of ASC using the same monoclonal (L178) or related fluorochrome-conjugated monoclonal antibodies. The detection of CD44 on *c.* 16% of the ASC P0 population is puzzling. As the CD44 gene is composed of 10 exons (43), it is possible that unique ASC splice variants are expressed, and future work will explore the use of alternative epitope-specific anti-CD44 monoclonal antibodies for immunophenotyping of the cells.

The ASC isolated from multiple donors had the ability to undergo both adipogenic and osteogenic differentiation *in vitro*. Under adipogenic conditions, the ASC mRNA expression profile based on a microarray analysis identified a subset of genes and metabolic pathways that were significantly induced (Table I and Supplementary Table I). These included

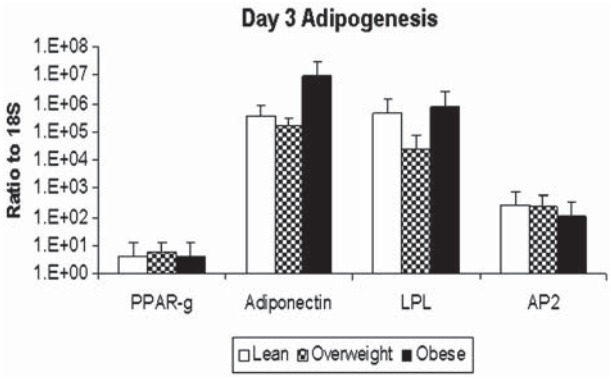

Figure 4. Adipogenic induction of mRNA in donors of varied BMI. Human subcutaneous ASC isolated from lean (BMI < 25), overweight (BMI 25 to 30) and obese (BMI > 30) female, Caucasian donors ($n = 6$ per cohort) were induced to undergo adipogenesis and total RNA harvested on day 3. RT-PCR was performed for the following genes: adiponectin, aP2, LPL and PPARγ2. The levels of each were normalized relative to that of 18S RNA. Statistical comparisons were conducted with a 2-tailed paired Student's *t*-test.

multiple genes related to lipid synthesis and storage that have been detected previously in related studies of adipogenesis in human and murine cell models (44,45). The RT-PCR analysis of four mRNA on the list confirmed their robust induction under adipogenic conditions (Figure 3). In contrast, the undifferentiated ASC expressed significantly higher levels of mRNA associated with the extracellular matrix and Wnt signaling pathways (Supplementary Table I), consistent with published reports that have demonstrated adipogenic inhibition through Wnt mechanisms (44,45).

In conclusion, these findings document the ability to obtain consistent yields of relatively homogeneous ASC from human subcutaneous lipoaspirates harvested from donors across a wide range of ages and BMI based on flow cytometric immunophenotype and *in vitro* differentiation potential. These results have relevance with respect to the use of autologous and allogeneic ASC for regenerative medical applications in the future.

Acknowledgments

The investigators express their appreciation to Elizabeth Clubb MD, James Wade MD, and their office staff; patients for providing the human lipoaspirate tissue used in this study; and Laura Dallam for administrative assistant supporting this work.

This work was partially supported by a CNRU Center Grant, number 1P30 DK072476, entitled 'Nutritional programming: environmental and molecular interactions' sponsored by NIDDK.

Disclosure of interest: JMG has been a consultant with companies pursuing the use of ASC for therapeutic purposes, including Cognate Bioservices, Toucan Capital, Vesta Therapeutics, VetStem and Zen-Bio.

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Supplementary material available online

Supplementary Figures S1–S5

Table showing collated results