INTRODUCTION: Obesity is characterized by chronic low-grade systemic inflammation, which is believed to contribute to a variety of musculoskeletal diseases, such as osteoarthritis (OA) and impaired tissue healing. Tissue adiposity and the production of pro-inflammatory cytokines due to obesity are also associated with elevated levels of free fatty acids (FFAs) [1]. Such changes to the tissue environment may induce cell dysfunction. Mesenchymal stem cells harvested from bone marrow (BM-MSCs) are multipotent cells that are able to differentiate into specific lineage and play a significant role in tissue repair and remodeling. Cells with multilineage potential can also be isolated from the infrapatellar fat pad (IFP) [2]. In obese patients, both bone marrow and IFP may undergo tissue remodeling. However, it is unknown whether the stem cells that reside in these tissues are affected by obesity. We hypothesized that obesity alters the multipotent capacity of BM-MSC and IFP cells. The objective of this study was to evaluate the effect of obesity on multilineage potential of BM-MSCs and IFP cells.

METHODS: Male C57BL/6j mice fed either a 60 kcal% high-fat diet (n = 12) or a standard chow containing 10 kcal% fat (n = 12) for 14 weeks were obtained from Jackson Laboratory. Mice were sacrificed at 20 weeks of age in accordance with an IACUC approved protocol. Bones (femurs and tibia) and IFP were collected and digested with collagenase type I. BM-MSCs were purified for Scα-1 PDGFRα CD45+ TER119 from the digested bone by fluorescent activated cell sorting [3]. Cells from the collagenase digested fat pad were plated directly to derive IFP cells. Both BM-MSCs and IFP cells were expanded in hypoxic conditions (2% O2). Passage 3 cells were differentiated into adipose, osteo-, and chondrogenic lineages to evaluate their multipotency under normoxic conditions. For adipogenesis, cells were cultured with adipogenic medium for 14 days. Lipid droplets were stained by Oil Red O, which was released and quantified by absorbance at 535 nm and normalized to DNA. For osteogenesis, cells were cultured with osteogenic medium supplemented with 50 ng/ml BMP-2 for 21 days. Mineral deposits were stained by Alizarin Red and the released stain was quantified by absorbance at 405 nm and normalized to DNA. For chondrogenesis, 250,000 cells were centrifuged in 1 mL polypropylene tubes to form pellets and maintained in chondrogenic medium supplemented with 10 ng/ml TGF-β3 and 500 ng/ml BMP-6. After 28 days, pellets were analyzed for their glycosaminoglycan (GAG) and DNA content by DMB and PicoGreen assay, respectively. Some pellets were also processed for immunohistochemical labeling for collagen type II. The immunophenotype of the cells was analyzed by flow cytometry. A two-factor ANOVA and the post hoc test were performed to determine significant differences (p < 0.05). Values are expressed as mean±SEM.

RESULTS: Multilineage potential: For adipogenesis, obese BM-MSCs exhibited significantly decreased adipogenesis compared to lean BM-MSCs (Fig. 1A; p < 0.001), while obese IFP cells showed the strongest adipogenic capacity compared to all groups (p < 0.001). For osteogenesis, obese IFP cells had significantly enhanced osteogenesis, while obese BM-MSCs showed decreased osteogenic potential as compared to lean BM-MSCs (Fig. 1B; p < 0.05). For chondrogenesis, both obese BM-MSCs and IFP cells had a significantly lower GAG/DNA ratio compared to the corresponding cells from lean mice (Fig. 2A; p < 0.05). Immunohistochemistry against type II collagen showed less staining intensity in obese pellets as compared to lean pellets (Fig. 2B).

Immunophenotype: Passage 3 cells were analyzed by flow cytometry for their phenotype. All the cells were negative for hematopoietic cell markers including CD11b, TER119 and CD45 (all ≤ 0.1%) but positive for Scα-1 and CD44 (all ≥ 99%). In vitro expansion of BM-MSCs did not downregulate expression of PDGFRα (Fig. 3A; > 68%), the marker used for initial sorting, and a high percentage of lean and obese IFP cells also expressed PDGFRα (≥ 90%). Both lean and obese IFP cells showed high CD105 expression (Fig. 3B; ≥ 80%). Interestingly, obese BM-MSCs showed a trend toward decreased CD105 expression as compared to lean BM-MSCs (68.87±11.54% of lean vs. 31.35±10.57% of obese; p = 0.06).

DISCUSSION: The results of this study indicate that obesity alters the multipotency of adult stem cells in a manner that depends on the tissue source of the cells. Obese IFP cells showed increased adipogenesis compared to lean IFP cells. This finding implies that the joint fat pad may have increased fat-storing capacity during weight gain. Interestingly, obese BM-MSCs showed significantly decreased adipogenic and osteogenic potential compared to lean BM-MSCs. The effect of obesity on bone metabolism is not fully elucidated, as increased body mass seems to encourage bone formation but inflammation due to excessive fat tissues may be detrimental to bone [4-5]. Our result is consistent with the finding that BM-MSCs showed decreased adipogenesis when cultured in a simulated obese environment containing the conditioned medium of FFA-treated adipocytes [6]. Several approaches for cartilage repair and regeneration rely on chondrogenesis of stem cells. For example, microfracture is a procedure to stimulate BM-MSC migration directly from bone marrow into focal cartilage defects. Scaffolds seeded with culture-expanded autologous adult stem cells for cartilage repair are also currently undergoing intensive investigation [7]. However, the potential impact of obesity on the intrinsic chondrogenic ability of these cells is not well understood. Our data showed that both obese BM-MSC and IFP cells exhibit significantly decreased chondrogenesis. The reduced chondrogenesis of obese BM-MSCs is potentially due to reduced expression of CD105, an accessory protein mediating signaling of TGF-β superfamily [8]. An improved understanding of the mechanisms by which obesity alters the chondrogenesis of adult stem cells will provide new insights for improving the success of various cartilage repair procedures.

SIGNIFICANCE: Our data suggest that obesity alters the multilineage capacity of adult stem cells. This finding is significant for the development of autologous stem cell therapy for obese patients as well as the mechanisms underlying the altered repair and remodeling of musculoskeletal tissues with obesity.


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