Persistent High Glucose Concentrations Alter the Regenerative Potential of Mesenchymal Stem Cells

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Type 2 diabetes is associated with numerous long-term complications. This study aims to investigate whether impaired function of tissue-resident multipotent cells play role in pathogenesis of allied complications. Adipose-tissue-derived mesenchymal stem cells (ASCs) derived from nondiabetic (nASCs) and diabetic (dASCs) donors were compared with regard to glucose metabolism, cell replication, apoptosis, and differentiation potential. The data evidenced that elevation of glucose reduces proliferative capacity of both dASCs and nASCs, but impacts dASCs more significantly. Incorporation of insulin enhanced cell replication especially in nASCs. dASCs show higher levels of cellular senescence and apoptosis than nASCs. Unlike nASCs, apoptosis is induced via intrinsic pathway in dASCs. Data also evidenced that high glucose concentrations cause prominent disparities in nASCs and dASCs in expression of genes involved in insulin resistance such as adiponectin and resistin. Some changes in gene expression were irreversible in dASCs when treated with insulin. Additionally, high glucose concentrations reduce osteogenic and chondrogenic potential of ASCs, but enhance adipogenic potential. These results indicate that in addition to involvement in insulin resistance, impaired function of mesenchymal stem cells that reside in adipose tissue as one of the major sources of adult stem cells might be responsible for complications related to diabetes type 2.

Introduction

Type 2 diabetes affects more than 170 million individuals worldwide [1]. This disease develops from insulin resistance, which leads to hyperglycemia and hyperinsulinemia. Invariably, dyslipidemia, hypertension, cardiovascular and atherosclerotic complications are associated with type 2 diabetes. Diabetics have a propensity for delayed wound healing and development of nephropathy, retinopathy, and neuropathy [2].

Mesenchymal stem cells are tissue-resident multipotent cells that display a capacity for self-renewal and differentiation potential into several cell lineages, including adipocytes, osteocytes, chondrocytes, myocytes, and cells of neuronal lineages [3–7]. These cells are of crucial importance for routine repair processes. Adipose-tissue-derived mesenchymal stem cells (ASCs) are largely found in a quiescent state, and it is believed that upon stimulation by either internal or external stimuli, they re-enter the cell cycle for self-renewal and differentiation [8]. Stem cells are capable of restoring damaged tissues in chronic wounds [9], neurodegenerative diseases [10], and cardiovascular complications [11].

Prolonged hyperglycemia damages several organs, including heart, kidneys, and eyes, apart from affecting blood vessels and nerves. High glucose concentration is associated with deleterious effects on the function and proliferation capacity of endothelial and mesangial cells [12–14]. In the present study, we aim to investigate and compare ASCs obtained from nondiabetic (nASCs) and diabetic (dASCs) patients when exposed to chronic excess of glucose. Functional impairments of ASCs such as on their multilineage potential and proliferative capacity are a direct result of prolonged exposure to high glucose concentrations.

Procurement and comparison of nASC and dASC populations, in addition to investigating the roles of insulin-like growth factor-1 (IGF-1) and tumor necrosis factor-alpha (TNF-α), provide a better understanding regarding the biology of ASCs and their impaired functions associated with diabetic complications. Additionally, differential effects that elevated glucose and insulin treatment have on self-renewal and multilineage potential of nASCs and dASCs were evaluated. By focusing on the impact of continuous glucose abundance on the biology of adult
stem cells at the molecular level, this study intends to elucidate the role of stem cells in diabetic-related complications and the possible relevance for future cell therapies in diabetic patients.

Materials and Methods

Isolation and expansion of ASCs

Adipose tissue specimens were obtained under a protocol approved by the Institutional Review Board of the Tulane University Health Sciences Center. nASCs and dASCs were derived from 43 donors (34 nondiabetic and 9 type 2 diabetic patients) with age ranging from 32 to 64 (average age, 43 years). The average HbA1c for the diabetic donors was 10.75 [standard error (SE) ± 2.65]. ASCs were isolated from gross specimens from each donor using previously described methods [15,16]. Fifty grams of each tissue specimens was minced and digested with Collagenase Type I (Gibco, Invitrogen) for 60 min at 37°C. After being treated with RBC lysis buffer (BioWhittaker), cells were plated at a fixed density in α-minimum essential medium, supplemented with 20% fetal bovine serum (Atlanta Biological), 1% penicillin/streptomycin (Cellgro), and 1% penicillin/streptomycin (Cellgro) at 37°C with a 5% CO2 atmosphere. Cells were passaged upon reaching 70% confluence.

Cell replication in glucose-enriched medium

nASCs and dASCs were plated at a density of 1,000 cells/cm² after initial passage and counted after 48, 96, and 120 h. nASCs and dASCs were cultured in a medium supplemented with D-Glucose (Sigma) of 100 mg/dL (5.56 mmol/L), 250 mg/dL (13.9 mmol/L), 500 mg/dL (27.8 mmol/L), and 1,000 mg/dL (55.6 mmol/L) for 120 h. The medium was changed on alternate days and 600 pmol/L (log10 N = 0) is the number of seeded cells [18].

Colony-forming unit assay

nASCs and dASCs were plated at densities of 1,000, 500, 250, 100, 50, and 25 cells/cm². Three different glucose concentrations (100, 250, and 500 mg/dL) were used with or without daily addition of 600 pmol/L (100 μU/mL) insulin. After 10 days, cultures were fixed and stained with 1% crystal violet in 100% methanol (Sigma) and the formed colonies were counted [16].

Cellular senescence

nASCs and dASCs were grown in 100, 250, and 500 mg/dL glucose with or without insulin (100 μU/mL). An IGF-1 ASC cell line [19,20] was also treated in a similar manner. In similar experimental settings, 5 ng/mL TNF-α (Invitrogen) was added to the cell cultures every day. After culturing for 120 h, β-galactosidase-reactive cells were counted (Sigma) [21].

Apoptosis assay

Flow cytometry analysis was performed on nASCs and dASCs stained with 7-Amino-Actinomycin D (7-AAD) and Annexin V (BD Pharmingen) according to the protocol described by the manufacturer. 7-AAD was used to differentiate between early and late stages of apoptosis.

Quantitative real-time polymerase chain reaction analysis

Total cellular RNA was isolated from nASC and dASC cultures (100, 250, and 500 mg/dL glucose treated with or without insulin) after culturing for 120 h using an RNeasy mini kit (Qiagen). cDNA was obtained by the High Capacity Reverse Transcription Kit (Applied Biosystems) according to the manufacturer’s instructions. The following primers were used: GAPDH, 5′ CGAGATCCCCTCCAAATAACA 3′ and 5′ GGTTGAATACCAGTTTGGT 3′; caspase 3, 5′ CCCCCAGTCTAC 3′ and 5′ TGTCGTGCCGTCATCCC 3′; caspase 8, 5′ AACCTCGGGAATCTGACGTG 3′ and 5′ CCTGTCATCACTGAAATAGGCT 3′; TP53, 5′ TCTACCTCTCCCCGACAATAA 3′ and 5′ CTCCCTCCTACACAAACAAAAC 3′; BCL2, 5′ AGATGGGAACACTTGTTGAG 3′ and 5′ CTTCCCCAAAAGAAATCCTGCAA 3′; adiponectin, 5′ TGCCTCCTGTGCTCTTC 3′ and 5′ GAGCAATGAGATGCAAGGTT 3′; adiponectin receptor 1, 5′ CCGACTGCTCAAAAGGACGA 3′ and 5′ ATCCCAAAAAACCTTCCCTC 3′; adiponectin receptor 2, 5′ GCCATCCATAACATCTTTCG 3′; leptin (LEP), 5′ GAAGGTGCCATCTCCTCTGCAA 3′ and 5′ TTGGTTTCAGGCAAAGGAG 3′; CCAAT/enhancer binding protein alpha (CEBPA), 5′ CTAGGAAACAGGAACGCAGAT 3′ and 5′ ATGGTGTTTAGCAAGACGACC 3′; BMP-5, 5′ GCCATCTCCTAGCTCCTG 3′ and 5′ ATGCCTGAGAAGACCAATCACTGTCG 3′; and IL6, 5′ ATGCCTGAGAAGACCAATCACTGTCG 3′ and 5′ ATGCCTGAGAAGACCAATCACTGTCG 3′. Every reaction was performed in triplicates.

Multilineage potential of nASCs and dASCs

Adipogenic differentiation was assessed on cultures of nASCs and dASCs in different glucose concentrations with or without insulin after culturing for 120 h using adipogenic differentiation kit (Invitrogen). Multilineage potential of nASCs and dASCs was assessed based on previously described methods [22]. Osteogenic differentiation was induced based on previously described methods [22]. Chondrogenic differentiation was accomplished by the Stempro chondrogenesis differentiation kit (Invitrogen). About 100,000 cells were spun down in a 15-mL conical tube.
and grown in various glucose concentrations with or without insulin for 21 days. Micromasses were fixed and stained with toluidine blue.

**RT² qPCR gene expression analysis**

Gene expression was analyzed using total cellular RNA on PCR array kits (SABiosciences) that profile expression of 84 genes related to the onset, development, and progression of diabetes. nASC and dASC donors (n = 3 per group) were cultured for 120 h and each group consisted of 3 different glucose conditions (100 and 250 mg/dL with and without insulin). Data was analyzed using the software provided by SABiosciences (www.sabiosciences.com). Differential gene expression was evaluated for statistical significance (P < 0.05). A cut-off fold change of 1.5 was applied to consider only genes whose expression was perturbed in magnitude as well as in a statistically significant manner.

**Statistical analysis**

Data from quantitative experiments is presented as the mean ± SE. Statistical differences between the mean of ASC groups were analyzed using unpaired Student’s t-test.

**Results**

**nASC and dASC replication**

Cumulative population doublings were quantified in cultures containing nASCs and dASCs treated with 100, 250, 500, and 1,000 mg/dL glucose. Population doublings were comparable in both nASCs and dASCs at all glucose concentrations. A significant decrease in population doublings after 120 h of culture was observed in glucose concentrations of 500 and 1,000 mg/dL (3.62 at 100 mg/dL and 2.62 at 1,000 mg/dL for nASCs; 3.60 at 100 mg/dL and 2.59 at 1,000 mg/dL for dASCs) (P < 0.05) (Fig. 1A).

Insulin treatment increased population doublings in both nASC and dASC cultures. The effect of insulin became more pronounced in nASCs than in dASCs as glucose concentration was increased (nASCs: 8.16% and 18.2% vs. dASCs: 8.56% and 10.03% in 500 and 1,000 mg/dL, respectively).

Colony-forming units (CFUs) decreased with increasing glucose concentration for both nASCs and dASCs (nASCs: 21.33 and 10.0 vs. dASCs: 21.5 and 9.75 in 250 and 500 mg/dL, respectively). Insulin treatment increased CFU ability in both nASCs and dASCs. There was a 70% increase in colonies (17.0 vs. 10.0) in nASCs and a 50.35% increase in colonies of dASCs in 500 mg/dL + insulin (P < 0.05) (Fig. 1B). In addition to having less population doublings and CFUs, nASCs and dASCs exhibited altered cell morphology from spindle-shaped to flat and larger cells with more cytoplasmic area in higher glucose concentrations. Insulin treatment partially reversed this change in morphology.

Senescent rates in dASCs were significantly higher than in nASCs, and increased as glucose concentration was elevated. Insulin attenuated this response by decreasing senescent rates in both nASCs and dASCs. Insulin exerted its greatest effects at higher glucose concentrations in dASCs (3.36%, 7.73%, and 13.90% in nASCs, and 4.73%, 11.95%, and 13.95% in dASCs in 100, 250, and 500 mg/dL glucose, respectively) (Fig. 2A; Supplementary Figs. S1 and S2, available online at www.liebertonline.com/scd).

TNF-α is often elevated in obese and diabetic patients, and plays an important role in insulin resistance and cell survival [23,24]. Thus, addition of TNF-α dramatically increased cellular senescence in both nASCs and dASCs, but had its greatest effect on dASCs at higher glucose concentrations as well (24.61% nASCs vs. 34.17% in dASCs in 500 mg/dL glucose). To study the role of IGF-1 in ASC replication, an IGF-1 knockout ASC line (IGF-1−) was used. With exception to 500 mg/dL glucose and 250 mg/dL glucose + TNF-α groups, senescence rates were higher in IGF-1− cells (7.50% in 100 mg/dL) than in nASCs and dASCs (Fig. 2A). Senescence was lower in IGF-1− ASCs in 2 conditions of high glucose when treated with postprandial amounts of insulin (12.41% in 250 mg/dL and 15.15% in 500 mg/dL) than in dASCs cultured in the same glucose concentrations. Insulin and TNF-α had opposing effects on IGF-1− ASCs, with TNF-α having stimulatory but insulin having inhibitory influence on senescence.
dASCs exhibited a higher percentage of apoptosis as indicated by Annexin-V-positive (Annexin V+) cells (4.35%) than those of nASCs, indicating a higher rate of apoptosis (Fig. 2B). In addition, an increase in Annexin V+ cells correlated with higher glucose concentrations. Both insulin and TNF-α had similar effects on apoptotic activity as they did on senescence in nASCs and dASCs. Insulin, a potent inhibitor of apoptosis, decreased dASC Annexin V+ rate to 2.75% and 3.5% in 100 and 500 mg/dL, respectively. TNF-α dramatically increased apoptotic activity in both nASCs and dASCs, but had the greatest pro-apoptotic effect on dASCs (6.2% and 7.1% in nASCs vs. 10.9% and 12.3% in dASCs in 100 and 500 mg/dL, respectively). Insulin also attenuated the pro-apoptotic effect of TNF-α significantly on dASCs at 500 mg/dL glucose (8.1%).

IGF-1-ASCs showed increased apoptotic activity and a nearly 2-fold increase in Annexin V+ expression between 100 and 500 mg/dL glucose (5.8% and 10%, respectively). Insulin reduced apoptotic levels in IGF-1-ASCs (4.1% in 100 mg/dL and 7.7% in 500 mg/dL) (Fig. 2B).

Analysis of pro-apoptotic genes determined higher expression of caspase 3 and p53 in dASCs than in nASCs (Fig. 2C). Administration of insulin decreased levels of caspase 3, caspase 8, and p53 in both nASCs and dASCs, though its greatest effect was exerted on dASCs and, most notably, at 250 mg/dL glucose (caspase 3 = 1.93-fold, caspase 8 = 2.09-fold, and p53 = 3.79-fold). Caspase 3 showed an exceptionally high upregulation in 250 mg/dL glucose in dASCs (11.6-fold in dASCs vs. 1.59-fold in nASCs). Insulin treatment resulted in downregulation of 3 pro-apoptotic genes particularly in dASCs at 250 mg/dL glucose. Expression of caspase 8 remained higher in 250 mg/dL + insulin versus 100 mg/dL (1.41-fold). Elevation in expression levels of BCL-2, an antiapoptotic gene, was higher in dASCs, which may partially explain higher expression of p53 in dASCs (data not shown).

Analysis of pathways mediated by insulin signaling in ASCs revealed that expression of AdipoR1 was sensitive to both insulin and glucose. Overall expression of AdipoR1 was lower in dASCs than in nASCs. In both nASCs and dASCs, elevated glucose concentration downregulates AdipoR1 expression. Nonetheless, unlike dASCs, insulin proved to be a potent upregulator of AdipoR1 in nASCs treated with 100 and 250 mg/dL glucose. APPL1 responded to insulin only at 100 mg/dL glucose in both nASCs and dASCs (Fig. 2D).
Differentiation potential of nASCs and dASCs

ALP activity, an indicator of osteogenic differentiation potential of ASCs, declined with increasing glucose concentration in both nASCs and dASCs (Fig. 3A). ALP was significantly lower in dASCs, exhibiting <20% of the ALP activity of nASCs at all tested glucose concentrations. For example, nASCs expressed 5.34-fold more ALP activity than did dASCs in 100 mg/dL glucose. The most significant decrease in ALP activity was seen in dASCs at higher glucose concentrations. Insulin increased ALP activity only marginally in both nASCs and dASCs, but exerting its greatest effect on dASCs as glucose levels increased.

For chondrogenic differentiation, the pellet culture system was used. The average diameters of chondrogenic-differentiated nASC and dASC micromasses are shown in Fig. 3B. Micromass diameters decreased as glucose concentration increased from 100 mg/dL to 250 and 500 mg/dL, indicating a decrease in chondrogenic potential. dASCs yielded micromasses that were much smaller than those of nASCs, and also exhibited dependence on glucose concentration (Fig. 3B; Supplementary Figs. S3 and S4, available online at www.liebertonline.com/scd). Expression of lineage-specific genes (BMP-6, COL2A1, COL10A1, and Sox6) in dASCs was lower than nASCs (Supplementary Fig. S5, available online at www.liebertonline.com/scd).

dASCs displayed higher adipogenic differentiation potential. Insulin did not cause any significant difference in adipogenic potential of dASCs (Fig. 3C; Supplementary Figs. S6 and S7, available online at www.liebertonline.com/scd). Real-time PCR analysis of lineage-specific genes indicated differences between nASCs and dASCs (Fig. 3D). Adiponectin expression was highly glucose sensitive in nASCs treated with the adipogenic medium. However, adiponectin expression was undetectable in dASCs. Expression of AdipoR1 and APPL1 decreased with increasing glucose in both adipogenic-differentiated nASCs and dASCs (Fig. 3D). LEP and CEBPA were both significantly upregulated several fold in dASCs compared in nASCs. However, glucose was a major negative regulator of both LEP and CEBPA in nASCs.

FIG. 3. (A) Effect of high glucose concentration of differentiation potential of nASCs and dASCs. Levels of alkaline phosphatase in nASCs and dASCs cultured in the osteogenesis induction medium for 12 days (n = 5). Insulin treatment acted as a slight stimulator toward osteogenesis. The differences between nASCs and dASCs are statistically significant (P < 0.01), (P < 0.05) for 100/500 nASCs and 100/500 dASCs. (B) Average diameter of micromass from nASCs/dASCs that underwent chondrogenic differentiation. Differences between nASCs versus dASCs are statistically significant (P < 0.01). Error bars are given as the mean ± standard error. (C) Percentage of positively stained adipogenic-differentiated cultures of nASCs and dASCs. Cells were incubated in the adipogenic induction medium for 12 days and stained with Oil Red O, and absolute cell numbers were counted (n = 5). *Significance compared to 100 mg/dL nASCs/dASCs (P < 0.05). (D) Representative experiment of 4 genes expressed in adipogenic-differentiated cells. All values were normalized to 100 nASCs (x-axis). dASC, diabetic adipose-tissue-derived mesenchymal stem cell; Ins, insulin; nASC, nondiabetic adipose-tissue-derived mesenchymal stem cell.
RT²-based PCR array

A series of arrays were carried out to determine selected transcriptomic alterations in gene expression patterns in ASCs affected by elevated glucose and insulin. These experiments were performed using an RT²-based gene array (SABioscience Corp.) and the results are expressed as a function of either glucose or insulin. Hierarchical clustering is used to display genes that were subjected to change in dASCs, various glucose concentrations, and insulin. The initial analysis revealed that high glucose concentration and insulin significantly perturbed expression of genes involved in receptors/transporters/channels, nuclear receptors, metabolic enzymes, secreted factors, signal transduction, and transcription factor pathways, all of which have been reported to be affected by diabetes and obesity (Fig. 4; Supplementary Figs. S8–S12, available online at www.liebertonline.com/scd; Supplementary Table SI, available online at www.liebertonline.com/scd). Clustering of nASCs and dASCs in each experimental condition changed in each pathway, suggesting that the impact of glucose and insulin results in significant modification of gene expression (Fig. 4).

Gene expression analysis identified a cluster of 28 genes that were differentially expressed (≥1.5-folds; n = 6) between nASCs and dASCs (100 mg/dL). Expression of genes associated with insulin resistance changed in dASCs, for example, downregulation of GPD1, CEACAM1, IL10, IL12, and PPAR family genes and upregulation of IL6. Altered expression of these genes in insulin-resistant type 2 diabetic patients has been reported [25]. Several genes related to glucose metabolism also changed in dASCs (GCG, GCK, INSR, FBPI, CEBPA, and IGFBI) [26]. In addition, expression of PRKCB1, a gene responsible for the onset of diabetic nephropathy, was elevated (2.08-fold) [27]. The data in dASCs indicated an upregulation of FOXG1 and FOXP3H and downregulation of HNF4A, genes that are mainly associated with pathogenesis of type 1 diabetes [28].

In nASCs, expression of 42 genes changed (>1.5-fold) as a function of glucose (250 mg/dL). These changes were more pronounced in dASCs as an effect of glucose. Pathway analysis revealed that higher glucose concentrations significantly altered expression of genes associated with insulin resistance. For example, expression of IFNG, IL6, TNF, resistin (RETN), and PPAR family genes decreased, whereas expression of ADRB3, ENPP1, and GPD1 increased. In addition, on further analysis, a considerable increase in expression of genes linked to glucose pathways, such as CEBPA, was seen (Fig. 5).

Insulin treatment of nASCs in 250 mg/dL glucose modified expression of 26 genes (Supplementary Table S2, available online at www.liebertonline.com/scd). Specifically, insulin changed expression of genes associated with insulin resistance (ADRB3, IL10, TNF, and RETN). At the same time, expression of genes responsible for glucose metabolism was upregulated. However, IL10 expression decreased (−2.87 fold).

Selected pathway analysis revealed that 36 genes were impacted in dASCs treated with high glucose. Further, changes in the regulation of genes linked to insulin resistance and glucose metabolism were observed, including expres-
The present study exclusively identified the fundamental differences between nASCs and dASCs. Long-term complications that occur in patients with type 2 diabetes can be associated with impaired ASC function. In addition,
increasing glucose concentrations cause significant changes in genes involved in intermediary metabolism, which can be partially reversed by insulin. Previous studies have demonstrated that proliferation of endothelial cells, mesenchymal stem cells, and mesangial cells was dependent on glucose concentration [12–14,29]. Insulin treatment reversed the affected CFU in nASCs and dASCs. However, the differentiation potential could only be elicited in nASCs upon addition of insulin to cultures. Our data shows that although high glucose conditions reduced cell replication and increased cell senescence in both nASCs and dASCs, these alterations are more pronounced in dASCs.

Using an IGF-1 ASC revealed the important role of IGF-1 in ASC replication, particularly in the presence of TNF-α. We observed higher levels of senescence/apoptosis in the IGF-1 cell line, implying that expression of IGF-1 reduces senescence and apoptotic potential. Insulin treatment increased cell replication and decreased cell senescence and apoptosis indices. In addition, insulin activates cell growth by stimulating PI3-kinase/Akt pathway [30]. Moreover, insulin serves as an inhibitor of caspase 3 expression [31], while high glucose mainly exhibits its pro-apoptotic effect through the intrinsic caspase pathway [32]. High glucose concentration induces apoptosis via activation of caspase genes. This effect was observed as early as 120 h after culturing ASCs in high glucose concentrations. These observations are in line with previous studies where increased apoptotic activity of cells after exposure to high glucose concentrations was exhibited [29]. Insulin treatment caused significant decrease in expression of caspase 3, caspase 8, and p53. Thus, our data indicates that apoptosis was induced by glucose via intrinsic caspase pathway (via activation of caspase 3 and caspase 9) in dASCs, whereas in nASCs, glucose affects apoptosis by means of extrinsic caspase pathway (via caspase 8 activation). These results are in line with earlier reports, in which fibroblasts from diabetics exhibited higher expression of pro-apoptotic genes [33]. TNF may play important role in cell death as well; as shown in Fig. 2A and B, the effect of TNF-α is more pronounced in high glucose concentration. Recent reports also suggest that TNF-α enhances caspase activities [34]. This data helps to explain a variety of complications associated with type 2 diabetes, given that apoptotic and senescent levels were significantly higher in dASCs, especially in elevated levels of glucose.

Additionally, our data indicates that high glucose concentrations have drastic effects on the differentiation potential of ASCs. Advanced glycation end (AGE) products interfere with differentiation of mesenchymal stem cells into adipogenic, chondrogenic, and osteogenic lineages [35]. In this study, we observed considerable differences in differentiation capacity between nASCs and dASCs, as dASCs displayed significantly lower osteogenic and chondrogenic differentiation potential than nASCs. The dASCs, however, exhibit a greater tendency to differentiate into the adipogenic lineage. High glucose concentration is a potent inhibitor of osteogenesis, but insulin can increase ALP activity [36], which suggests that prolonged exposure to high glucose concentrations permanently changes dASCs’ osteogenic and chondrogenic potential. A significantly higher adipogenesis in dASCs corroborated with similar effects as observed for dASCs under high concentrations of glucose. Expression of adiponectin, an important factor involved in insulin signaling, was invariably lower in dASCs [37] and was downregulated in adipogenic-differentiated ASCs at higher glucose concentrations. Adiponectin initially exerts its antidiabetic, anti-inflammatory, and antiatherogenic effects through AdipoR1 and AdipoR2, and subsequently through its effector protein PPAR [38]. Consequently, this axis in its entirety was expressed at lower levels in differentiated dASCs, indicating lower insulin sensitivity in these cells.

Transcriptome analysis revealed that expression of numerous genes involved in pathogenesis of diabetes [39], including insulin signaling and resistance, was changed in dASCs, especially at high glucose concentrations. This suggests that increased glucose concentration changes expression of genes involved in insulin resistance, and most significantly in dASCs. The reported changes in gene expression were a result of ASCs being exposed to high glucose concentrations for only 5 days, a relatively short period compared with these cells’ exposure patterns in vivo. Further, a correlation between the incidence of diabetes and cardiovascular diseases is well documented. Our data indicates that at least some genes related to cardiovascular disease such as ACE and AGE (involved in blood pressure regulation) and insulin resistance were upregulated with higher prevalence in dASCs [40]. It has been shown that treatment with ACE inhibitors improves insulin sensitivity [41]. In support of such information our data demonstrates a downregulation of this family of genes upon insulin treatment.

These findings may explain some of the clinical consequences of diabetes such as vascular, skin, and foot complications, neuropathy, and delayed wound healing. These results also suggest that restoring the function of tissue-resident stem cells in diabetic patients is essential and helps in alleviating the intensity of type 2 diabetes complications. It is apparent that in type 2 diabetes, there is a compromise in the integrity of ASC function, which is likely to be regulated at least in part by insulin. It is important to take these aspects of diabetic pathologic into consideration when designing new treatment plans and cell therapies for type 2 diabetes.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

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