Effects of Obesity on Reparative Function of Human Adipose Tissue-Derived Mesenchymal Stem Cells on Ischemic Murine Kidneys

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Abstract

Introduction: Obesity is a health burden that impairs cellular processes. Mesenchymal stem/stromal cells (MSCs) are endowed with reparative properties and can ameliorate renal injury. Obesity impairs human MSC function *in-vitro*, but its effect on their *in-vivo* reparative potency remains unknown.

Subjects and Methods: Abdominal adipose tissue-derived MSC were harvested from patients without ('lean') or with obesity ('obese') (body mass index<30 or ≥30kg/m^2, respectively) during kidney donation or bariatric surgery, respectively. MSC (5×10^5/200μL) or vehicle were then injected into 129S1 mice 2 weeks after renal artery stenosis (RAS) or sham surgery (n=8/group). Two weeks later, mice underwent magnetic resonance imaging to assess renal perfusion and oxygenation *in-vivo*, and kidneys then harvested for *ex-vivo* studies.

Results: Similar numbers of lean and obese-MSCs engrafted in stenotic mouse kidneys. Vehicle-treated RAS mice had reduced stenotic-kidney cortical and medullary perfusion and oxygenation. Lean (but not obese) MSC normalized ischemic kidney cortical perfusion, whereas both effectively mitigated renal hypoxia. Serum creatinine and blood pressure were elevated in RAS mice and lowered only by lean-MSC. Both types of MSCs alleviated stenotic-kidney fibrosis, but lean-MSC more effectively than obese-MSC. MSC senescence-associated beta-gal activity, and gene expression of p16, p21, and vascular endothelial growth factor correlated with recipient

kidney perfusion and tissue injury, linking MSC characteristics with their *in-vivo* reparative capacity.

Discussion: Human obesity impairs the reparative properties of adipose-tissue-derived MSCs, possibly by inducing cellular senescence. Dysfunction and senescence of the endogenous MSC repair system in patients with obesity may warrant targeting interventions to restore MSC vitality.

Keywords

cellular senescence; mesenchymal stem cells; obesity; renal artery obstruction; renal artery stenosis

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Introduction

Since their first isolation, mesenchymal stem/stromal cells (MSC) have been extensively studied due to their potential reparative capacity. These multipotent stem cells reside in tissues such as bone marrow, synovium, human umbilical cord, and fat.[1] Of these, adipose tissue has become an important source of MSCs due to its wide availability and accessibility.[2] MSCs can differentiate into various mesenchymal cell types (e.g., osteocytes, chondrocytes and adipocytes), and exert beneficial effects including immunomodulation, anti-inflammation, and angiogenesis, primarily through paracrine effects.[3] MSCs also release extravesicular vesicles (EVs) containing mRNA, microRNA and cytokines, which locally and systemically promote tissue healing.[3] In light of their potential benefits, MSCs have been extensively studied as a potential therapeutic approach in many conditions, including acute and chronic kidney disease (CKD). Our group has demonstrated several benefits of MSC delivery on injured kidneys of animals[4–7] and patients[8] with renal artery stenosis (RAS). Due to their special immunomodulatory characteristics, MSCs are considered hypoimmunogenic and have been applied as allogeneic and xenotransplantation tools.[9]

Obesity is a major health burden associated with 1.5-folds increased risk of mortality.[10] Furthermore, obesity often co-exists with and aggravates risks imposed by cardiometabolic conditions such as diabetes, coronary heart disease, hyperlipidemia, hypertension, and CKD. At the cellular level, excess calories and fatty acids can lead to cellular stress, which in turns promotes a pro-inflammatory state, inducing release of inflammatory markers, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and plasminogen activator inhibitor-1 (PAI-1).[11] Moreover, obesity can induce cellular senescence, a state of permanent cellular growth arrest.[12] Senescence cells are metabolically active, albeit growth-stagnant, and able to secrete an array of cytokines and chemokines together known as senescence-associated secretory phenotype (SASP).[13] Conceivably, MSC from individuals with obesity may adversely be affected, culminating in impaired reparative functions. Several studies have demonstrated an adverse impact of obesity on MSCs and their EVs functions in vitro.[14–17] EVs from obese swine harbor proinflammatory constituents and less reparative protein content than lean-EVs, and induce inflammation in co-cultured renal tubular cells.[16] Obese swine MSCs also have increased expression of proteins, mRNAs, and microRNAs associated with mitochondrial dysfunction and inflammation.[17] Recently, we identified greater senescence and inflammatory gene expression in adipose-derived MSC

from patients with obesity compared to lean individuals. P16, p21, and IL-6 gene expression also correlated with the patients' body mass index (BMI), suggesting a dose-response relationship. Furthermore, obese-MSC co-cultured with injured human umbilical vein endothelial cell (HUVEC) failed to restore their angiogenic potential, indicating impaired reparative capacity *in-vitro*.[14]

Despite a growing body of evidence describing obesity-induced MSC impairment *in-vitro*, whether this in fact translates into *in-vivo* dysfunction remains incompletely understood. Therefore, our study aimed to explore the effect of obesity on reparative capacity of MSCs in repairing injured kidney of RAS mice. We hypothesized that MSCs harvested from individuals with obesity would fail to restore renal perfusion, oxygenation, function, and tissue injury in murine stenotic kidneys (STKs).

Methods

We first harvested and characterized human MSCs, followed by *in-vivo* studies of their effects when injected into RAS mice. The human study was approved by the Mayo Clinic Institutional Review Board, and informed consents were obtained. The MSCs were characterized and studied *in-vitro* for senescence, as previously reported.[14] The animal study was approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Subject recruitment

We obtained MSC from patients with and without obesity between 18–80 years old.[14] Patients with obesity ("obese") were defined as those with body mass index (BMI)   30 kg/m^2, whereas "lean" patients had BMI <30 kg/m^2. The patients were evaluated in nephrology, endocrine, general bariatric and obesity clinics at the Mayo Clinic Rochester between October 2017 and March 2019. "Lean" patients were recruited from potential kidney donors who had BMI<30kg/m^2. Exclusion criteria included pregnancy, chronic inflammatory disease (e.g., rheumatoid arthritis), active malignancy, recent stroke or myocardial infarction, solid organ transplant recipients, treatment with immunosuppression, or anticoagulation therapy.

MSC harvesting and preparation

Subcutaneous abdominal adipose tissue samples (0.5-2.0g) were collected during the gastric bypass surgery (patients with obesity) or donor nephrectomy (lean patients), and prepared per standard protocols.[14] Briefly, adipose tissue was minced and digested by collagenase-H at 37°C for 45 minutes. Serum-containing media was added to the suspension that was then filtered through a 100μm cell strainer. The cellular suspension was centrifuged, and the cellular pellet re-suspended. Subsequently, MSCs were expanded and cultured for 3 passages for the experiments.[14]

MSC characterization

Third-passaged MSCs were characterized by imaging flow cytometry to ascertain their expression of MSC-specific surface markers, which are positive for CD73, CD90, and CD105 and negative for CD14 or CD14. We further characterized MSC by their capability

for tri-lineage differentiation into adipocytes, chondrocytes, and osteocytes, as described previously.[18]

MSC gene expression

Gene expression was assessed in human MSC by real-time polymerase chain reaction (RT-PCR). We homogenized $0.5\text{-}1\times10^6$ human MSC in 350μL of ice-cold lysis buffer (mirVana PARIS). Total RNAs were then isolated from homogenized samples using a total RNA isolation kit (ThermoFisher, Cat# AM1556). Total RNA concentrations were measured by a Spectrophotometer (NanoDrop). We then treated 50μL RNA samples with DNase (ThermoFisher, Cat# AM1906) to remove possible DNA contamination during RNA isolation. First-strand cDNA was produced from 800ng of total RNA using SuperScript VILO master mix (ThermoFisher Scientific, Cat#11755-050). Relative quantitative PCR were performed using TaqMan assays, containing 16ng of cDNA products. All primers were from ThermoFisher Scientific, and included p16 (Cat#Hs00923894), p21 (Cat#Hs00355782), p53 (Cat#Hs01034249), activin-A (Cat#Hs01081598), IL-6 (Cat#Hs00174131), TNF-α (Cat#Hs00174128), monocyte chemoattractant protein-1 (MCP-1) (Cat#Hs00234140), PAI-1 (Cat#Hs00167155), galactosidase-β1 (GLB-1) (Cat#Hs01035168) and TATA box binding-protein (TBP) (Cat#Hs00427620) as a reference control. Negative controls with no cDNA were cycled in parallel with each run. PCR analysis was done on Applied Biosystems Quantstudio-7 using the following conditions: 50°C for 2 minutes, 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Fold-changes of gene expressions were calculated using $2^{-\Delta\Delta CT}$ method.

Beta-galactosidase assay

Senescence-associated β-galactosidase (SA-Beta-gal) was assessed in human MSCs using an assay (Enzo, Farmingdale, NY; Cat#ENZ-KIT129-0120).

Mice studies

Eleven-weeks-old male 129-S1 mice (Jackson Lab, Bar Harbor, ME) were initially acclimatized 2-3 days prior to the surgery and fed with Picolab Rodent Diet 20-5053. Mice were randomly divided into 6 groups based on types of surgery and treatments: sham+vehicle, sham+lean-MSC, sham+obese-MSC, RAS+vehicle, RAS+lean-MSC, and RAS+obese-MSC (n=8 mice/group).

RAS surgery was conducted by experienced animal technologists. Mice were surgically implanted with a 0.15mm diameter of plastic cuff around the right renal artery, whereas sham surgery included the same process but without cuff placement.[19, 20] Mice were deemed to have a successful RAS by right/left kidney volume or weight ratio <0.9.

Two weeks following surgery, mice were anesthetized (1.5-2.0% isoflurane inhalation) underwent a left internal carotid artery cannulation and a small plastic tube inserted caudally for aortic cell or vehicle injections. Phosphate buffered solution (PBS) or far-red dye (Life Technologies) pre-labelled MSC (5×10^5 cells in 200μL PBS) from either obese or lean subjects were slowly injected into the aorta, and the mice then allowed to recover. Two weeks following the infusion, mice underwent *in-vivo* studies, were subsequently

euthanized by exsanguination (blood collection), and kidneys harvested for *ex-vivo* studies (Supplementary figure 1). Urine was collected via bladder puncture prior to euthanasia. Blood pressures (BP, tail-cuff), heart rate, and body weight were recorded at baseline, 2 weeks (before infusion) and 4 weeks (before euthanasia).

Imaging studies

Two weeks after MSC injection, mice underwent magnetic resonance imaging (MRI) studies of the kidneys per previous protocol.[21] Renal perfusion was assessed using arterial spinning label (ASL), and oxygenation with blood oxygen dependent (BOLD)-MRI in both cortex and medulla as previously described.[21] All images were analyzed and quantified using MATLAB R2015-a (MathWorks, INC). Kidney volume was assessed using Analyze software (version 12.0;Biomedical Imaging Resource, Mayo Clinic, MN).[21] Image analyst was not blinded to the group allocation.

Serum creatinine was analyzed (Arbor Assays, Cat#KB02-H1) following manufacturer's protocol.

Histological studies

Five-μm kidney cross-sections were used for histological studies. Acute tubular injury was assessed in H&E-stained slides, as described.[22] Interstitial fibrosis was assessed by Masson trichrome (MT) with the fibrosis area quantified using MATLAB in 10 random 20X fields. Glomerulosclerosis was assessed in 50 randomly selected glomeruli. Vascular remodeling was evaluated by α-SMA immunohistochemistry staining (Abcam, Cat#7817), with media/lumen ratio averaged in 5 vessels per kidney. To assess microvascular loss, we performed CD31 stain (Cell Signaling, Cat#77699) to identify endothelial cells and quantify peritubular capillaries (PTC) per tubule under 100X, averaged for 10-12 fields. We assessed MSC retention by counting and averaging the pre-labelled MSC under fluorescence microscopy at 20X in 5 fields. We also assessed potential rejection by CD3+ (Abcam, cat#ab16669) and MSC colocalization. M1 and M2 macrophages were assessed by immunofluorescence staining. Double-positive F4/80 (1:100, Abcam, cat#ab6640) and iNOS (1:100, Santa-Cruz, cat#SC7271) cells were considered M1 macrophages, whereas double-positive F4/80 and MRC (mannose receptor-C) 1(1:100, Abcam, cat#ab8918) identified M2 macrophages. Tissue oxidative stress was assessed by immunoreactivity of 8-hydroxy-2′-deoxyguanosine (8-OHdG) (1:500, Abcam, cat#62623).

Stenotic kidneys gene expression

RT-PCR was used to assess gene expression of frozen mice STK homogenized (10mg) in 400μL of ice-cold lysis buffer (mirVana PARIS). We used p16 (Cat#mm00494449), p21 (Cat#mm00432448), p53 (Cat#mm01731290), activin-A (Cat#mm00434339), IL-6 (Cat#mm00446190), TNF-α (Cat#mm00443258), MCP-1 (Cat#mm00441242), PAI-1 (Cat#mm00435858), GLB-1 (Cat#mm00515342), fms-related tyrosine kinase-1 (FLK-1) (Cat#mm01222421), transforming growth factor-β (TGF-β)(Cat#mm01178820), vascular endothelial growth factor (VEGF) (Cat#mm00437306), and TBP (Cat#mm01277042) as reference control. Detailed RT-PCR methods were as described for MSC gene expression.

Statistical Analysis

Gaussian distribution was assessed in each variable by Shapiro-Wilk test. Data are reported as mean±SD or median (Inter-quartile range) for continuous variables and percentage in categorical variables. Differences among groups were assessed by one-way ANOVA or Kruskal-Wallis for continuous variables depending on the data distribution and variance among groups. Pair-wise analyses were subsequently calculated between each pair. Differences among categorical variables were assessed with Chi-square or Fischer Exact tests. Correlation between MSC senescence, inflammatory and angiogenic markers with murine renal perfusion, oxygenation and tissue findings were assessed using Pearson correlation. We used JMP 14.0 (SAS Institute, INC.) for statistical analyses and defined statistical significance as p 0.05.

Results

Animal Characteristics

Two weeks after surgery, all RAS mice have significantly higher systolic BP (SBP) than sham groups (Figure 1), suggesting successful RAS models, with no difference among them. SBP at 4 weeks was elevated in RAS+vehicle and reduced by lean-MSC to levels comparable to sham. Contrarily, RAS+Obese-MSC and RAS+vehicle mice remained hypertensive. Moreover, the absolute change in SBP from baseline to 4 weeks was lower in RAS+lean-MSC compared to RAS+vehicle and RAS+obese-MSC. RAS+vehicle had higher serum creatinine (SCr) at 4 weeks than Sham+vehicle and was lowered in RAS+lean-MSC but not RAS+obese-MSC mice. STK volume at 4 weeks was reduced in RAS+vehicle and significantly improved only in RAS+Lean-MSC compared to RAS+vehicle but not in RAS+obese-MSC, although it remained lower than normal in both MSC-treated RAS groups. Plasma renin content was elevated in RAS+vehicle mice and similarly normalized in both lean- and obese-MSC-treated RAS mice (Figure 1).

Lean-MSCs improve STK perfusion and oxygenation

RAS mice demonstrated lower cortical and medullary STK perfusion than sham groups (Figure 2AB). RAS+lean-MSC showed improved cortical but not medullary perfusion compared to RAS+vehicle, whereas in RAS+obese-MSC both cortical and medullary STK perfusion remained similar to RAS+vehicle. Contrarily, both lean- and obese-MSC improved cortical oxygenation compared to RAS+vehicle, and medullary hypoxia was no longer greater than controls (Figure 2CD).

Lean-MSCs mitigate kidney tissue injury

Lean and obese-MSCs were detected at similar numbers in STK parenchyma, with no CD3+ T-cells and MSC co-localization, suggesting no cellular-mediated immune rejection (Supplementary Figure 2).

Both lean and obese-MSCs ameliorated STK glomerulosclerosis, interstitial fibrosis, and tubular injury compared to RAS+vehicle and sham controls (Figure 3). However, lean-MSC ameliorated interstitial fibrosis more effectively than obese-MSCs (p=0.017) (Figure 3A).

Interestingly, both lean-and obese-MSCs slightly increased glomerulosclerosis and tubular injury in sham mice (Figure 3BC).

STKs of RAS+vehicle mice exhibited marked decreased PTC density (Figure 4) that both obese- and lean-MSC successfully mitigated, but again, lean-MSC more effectively than obese-MSC (Figure 4). Vascular remodeling (M/L) was also improved in RAS+lean-MSC compared to obese-MSC and vehicle-treated groups. Contrarily, obese-MSC did not alter STK M/L ratio compared to RAS+vehicle (Figure 4). Cortical and medullary oxidative stress denoted by 8-OHdG staining was highest in RAS+vehicle mice but diminished in RAS by both lean-MSC and obese-MSC; however, lean-MSC further ameliorated it compared to obese-MSC (Figure 5). Renal M1 and M2 macrophage numbers were both elevated in RAS+vehicle STK compared to Sham+vehicle. RAS+Lean-MSC exhibited reduced M1, but not M2, macrophages compared to RAS+vehicle and RAS+obese-MSC, but reduced M1/M2 ratio only compared to sham+obese-MSC, which was in turn higher than control.

In sham mice, both lean- and obese-MSCs slightly increased the numbers of M1 macrophages but only lean-MSC increased M2 macrophages as well. Hence, the M1/M2 ratio was elevated compared to Sham+vehicle, suggesting a shift in macrophages toward inflammatory phenotypes, in Sham+obese-MSC but not in Sham+Lean-MSC (Supplementary figure 3).

MSC senescence, inflammatory, and angiogenic markers correlat6 with STK perfusion and histological changes

There was no difference between the age, sex, and race of patients with and without obesity. BMI was significantly higher among patients with than without obesity, as were rates of diabetes and hypertension (Supplementary table 1). In-vitro, obese-MSC had lower VEGF gene expression and higher SA-Beta-gal activity than lean-MSC, whereas p53 and Activin-A expression tended to be lower. There was no difference in the remaining gene expressions between them (Supplementary table 2).

We then correlated these in-vitro MSC characteristics with renal perfusion, oxygenation, and histological changes in recipient mouse kidneys. SA-Beta-gal activity in human MSC inversely correlated with medullary STK perfusion (r_s=−0.352, p=0.035) and tended to inversely correlate with cortical STK perfusion (r_s=−0.325, p=0.053) 2 weeks after delivery. MSC VEGF gene expression directly correlated with both cortical (r_s=0.486, p=0.026) and medullary (r_s=0.477, p=0.029) STK perfusion. SA-Beta-gal activity in delivered MSC also directly correlated with M/L (r_s=0.641, p=0.004), interstitial fibrosis (r_s=0.472, p=0.048), and cortical oxidative stress (r_s=0.563, p=0.015) in recipient STKs, but inversely with PTC/tubule (r_s=−0.572, p=0.013) (Figure 6). Additionally, both log p16 and p21 gene expression in MSC directly correlated with interstitial fibrosis and tubular injury, and inversely with PTC/tubule, although only log MSC-p21 gene expression exhibited direct correlation with M/L (r_s=0.472, p=0.048) (Figure 6).

We also found direct correlation between TNF-α (r_s=0.653, p=0.033) and tubular injury, but no correlation between the remaining senescence, inflammatory, or angiogenic markers with renal perfusion, oxygenation, or histological changes.

Lean-MSC-treated stenotic kidneys demonstrated higher angiogenic and lower inflammatory and senescence markers

Gene expression of FLK-1 was lower than shams in RAS+vehicle STKs, whereas p21, Activin-A, IL-1α, MCP-1, IL-6 and PAI-1 were upregulated. Lean- and obese-MSC both restored FLK-1 expression. P21 expression was lower in RAS+lean-MSC compared to RAS+obese-MSC and RAS+vehicle. Several inflammatory marker gene expressions, comprising Activin-A, MCP-1, IL-6, and PAI-1, were also downregulated in RAS+lean-MSC, and comparable to sham groups (Supplementary figure 4). Furthermore, both lean- and obese-MSC-treated STKs manifested similarly lower gene expressions of p53, p16, TNF-α, TGF-β and VEGF than RAS+vehicle (data not shown).

Discussion

This study demonstrates that adipose tissue-derived MSC from lean patients possess a capacity to improve cortical STK perfusion superior to MSC from patients with obesity. Lean-MSC also showed superiority over obese-MSC in blood pressure reduction, preserving kidney function, ameliorating PTC loss and vascular wall remodeling. Contrarily, both lean- and obese-MSCs improved STK medullary perfusion and cortical and medullary oxygenation compared to vehicle-treated RAS mice. Furthermore, the attenuated repair potency of obese-MSC in recipient mice STKs correlated with obesity-induced senescence in donor MSC, implicating obesity in impairing the reparative function of MSC. These observations might have important ramifications for self-healing capacity of patients with obesity.

Obesity is an important and ubiquitous clinical entity that promotes kidney cellular damage. Obesity may predispose patients to tissue damage, interferes with wound healing, and is associated with post-surgical complications,[23] which might indicate impaired self-repair capacity.

MSCs are non-embryonic stem cells capable of trans-differentiation into mesenchymal cell lineages and exert their effects mainly via paracrine mechanisms.[3] Pre-clinical studies suggested their efficacy in several kidney diseases such as diabetic nephropathy, renovascular disease, and CKD.[3, 24] MSCs secrete growth factors, cytokines, as well as extracellular vesicles (EVs)[25], all of which can affect neighboring cells. We have previously shown the efficacy of adipose-derived MSCs in improving renal perfusion, hypoxia, and function, ameliorating oxidative stress and inflammation.[5, 6, 26] However, MSC function can be affected by donor characteristics, which might modulate their efficacy. For example, MSCs from patients with obesity demonstrate lower proliferation[14] and higher expression of pro-fibrotic genes.[27] Obesity also impairs mitochondrial function and protein uptake in swine MSC, culminating in increased oxidative stress and impaired energy production[28–30], and is associated with senescence and inflammatory phenotype. MSCs are susceptible to either replicative or stress-induced premature senescence,[31] and our group has shown direct

correlations between BMI and senescence/SASP gene expressions of p16, p21 and IL-6 in human adipose-derived MSC.[14]

The current study extends our previous observations and demonstrates a potential association between expressions of inflammatory, angiogenic, and senescence genes in harvested MSCs, and the functional and tissue changes that they ultimately induce in mice STKs in-vivo. While we did not detect a difference in senescence gene expressions between the relatively small number of donor lean- and obese-MSCs, obese-MSC showed greater beta-gal activity, indicating higher propensity for senescence. Moreover, beta-gal activity, p16, and p21 gene expressions (indices of senescence)[31, 32] in MSCs correlated with the ultimate renal perfusion and histology in recipient mice. Additionally, beta-gal activity correlated with renal vascular remodeling, interstitial fibrosis, oxidative stress, and PTC loss. P16 and p21 gene expressions correlated with tubular injury, interstitial fibrosis, and PTC loss, and p21 also correlated with the degree of vascular remodeling. Contrarily, while obese-MSC develop senescence, they did not overtly increase it in the STK, possibly because RAS induces prominent endogenous STK senescence greater than that provoked by exogenous obese-MSC.[33, 34] Collectively, these findings suggest a link between MSC senescence and their ability to avert STK tissue injury and underscore the notion that in-vitro characteristics of MSCs are maintained in culture and functionally consequential in-vivo. Senescent cells secrete SASP, consisting of wide array of cytokines and signaling factors[35] that can adversely affect neighboring cells in a paracrine fashion and evoke inflammation[36]. These can also impair the inherent proliferation, migration and homing ability of MSCs, although lean and obsess-MSCs were similarly engrafted in murine STKs, suggesting this particular function was unaffected in our MSCs.[13] We previously reported that approximately 8% of injected MSCs engrafted in mouse STKs.[7]

Compared to obese-MSC, lean-MSC also harbored greater expression of VEGF gene, which correlated directly with recipient STK cortical and medullary perfusion. We also found that STKs of RAS+lean-MSC mice upregulated FLK-1 gene expression to a greater extent than RAS+obese-MSC, possibly attributed to higher VEGF expression in lean-MSC, leading to microvascular preservation or neovascularization.[4, 37] Collectively, increased angiogenic activity augments the ability of lean-MSC to ameliorate PTC loss and promote renal perfusion more effectively than obese-MSC. This may also be secondary to senescence in obese-MSC, which may hinder their angiogenic potential, and thereby reparative functions.[38] In addition, a hypoxic milieu in the STK, underscored by BOLD-MRI, can amplify oxidative stress, which can in turn degrade angiogenic factors like VEGF, perpetuating microvascular loss.[39, 40] MSC has been shown to upregulate VEGF and its receptors in the swine STK, and in turn improve renal perfusion and hypoxia.[5] Indeed, lean-MSCs restore VEGF expression and tube formation by injured HUVEC in-vitro more potently in comparison to obese-MSC.[14]

Both lean- and obese-MSCs ameliorated STK tubular injury, glomerulosclerosis, and interstitial fibrosis to a similar extent, yet MSC gene expression of TNF-α correlated with the degree of tubular injury in recipient mice. Hypoxia and oxidative stress upregulate inflammatory markers like MCP-1, TNF-α, and IL-1α,[39, 41] which promote glomerular and tubulo-interstitial injury, resulting in glomerulosclerosis, interstitial fibrosis, and ultimately

loss of renal function.[39] Furthermore, these proinflammatory cytokines are part of the SASP profile and upregulated in cellular senescence[13] that was observed in the STK, perpetuating kidney injury.[34] Interestingly, expression of most inflammatory genes was unchanged in obese-MSC in-vitro. Indeed, MSCs ameliorate kidney inflammation in various settings[4–6, 8, 42]. Possibly, because obesity did not significantly impact MSC inflammatory gene expression, renal tubulo-interstitial damage was blunted in RAS+obese-MSC. Interestingly, we observed a small but measurable increase in glomerulosclerosis and tubular injury in sham mice treated with both lean- and obese-MSCs, the mechanism of which can be speculated. While cellular embolization is a possibility, we did not observe any histological findings suggesting renal mini-infarctions, and the numbers of injected and engrafted cells were small. Therefore, the mechanism might be paracrine. For example, xenotransplanted MSCs might incite some minimal immune response, as suggested by increased M1 macrophages numbers in sham+lean-MSCs and sham+obese-MSCs, which can contribute to a small degree of kidney injury.

Via immunomodulatory effects, MSCs modify macrophage phenotypes from inflammatory (M1) to reparative (M2), and reduce monocyte infiltration into injured kidneys.[7, 43] However, obesity increases preponderance of M1 macrophages,[44] hampers the ability of MSCs and their EVs to attenuate inflammation[15, 29], and thereby impairs MSCs reparative function. MSCs from obese swine release EVs containing proinflammatory proteins[16] and fewer TGF-β-genes, leading to downregulation of regulatory T-cells[45] and failure to restore microvasculature in the swine STK.[46] In RAS mice, both lean- and obese-MSCs upregulated M1 and M2 macrophages, resulting in unaltered M1/M2 ratio in comparison to RAS+vehicle. In sham groups, M1 were upregulated in both sham+lean- and obese-MSCs, whereas only lean-MSC upregulated M2 macrophages, so that the M1/M2 ratio increased only in sham+obese-MSC. This implies that both lean and obese-MSC can modulate macrophage phenotypes, yet obese-MSC may tilt the balance toward a more inflammatory phenotype.

Interestingly, Lean-MSCs lowered blood pressure in RAS mice. While this might be partly attributable to an observed reduction in renin production, renin was lowered similarly in RAS mice receiving lean- or obese-MSC compared to RAS+vehicle. A fall in plasma renin levels despite lingering vascular obstruction in MSC-treated RAS mice might be secondary to resolution of renal hypoxia, improved microvascular density, or altered intrarenal signaling.[47] Nonetheless, blood pressures remained elevated in RAS+obeste-MSC, possibly because obese-MSC also did not fully ameliorate renal injury.

Cellular senescence can be triggered by factors like aging, stress (oxidative stress, hypoxia), and hypertension.[36, 48] Lean-MSCs lowered p21 and Activin-A gene expression in STKs, implying lowered cellular senescence, consistent with greater reparative potency. Additionally, expression of the inflammatory markers IL-1α, MCP-1, IL-6, and PAI-1, which belong to the SASP repertoire, was downregulated in RAS+lean-MSC STKs. Overall, decreased inflammation, fibrosis, and oxidative stress in RAS+lean-MSC imposed less stress on the kidneys, and thus less cellular senescence than obese-MSCs.

We have not observed in the STK any histological features resembling rejection of either lean- or obese-MSCs, as assessed by their co-localization with CD3+ T-cells; neither did we observe rejection of swine MSCs transplanted into mice.[49, 50] MSC are relatively immune-privileged, and the reaction to transplanted allogenic MSC is usually minimal[9] thanks to low level of MHC class-I expression, and absence of MHC class-II and other costimulatory molecules.[51] While MSC engraftment notably wanes over time,[9] their beneficial effect may persist, suggesting long-term benefits of transient engraftment.[52]

Our study has some limitations. First, our model included young mice with relatively short duration of RAS than humans, yet demonstrates similar features to human STK.[7, 34] Second, xenotransplantation may induce rejection and hence reduce MSC engraftment, but we did not detect MSC rejection. Third, we elected to use 3rd passage of MSC to avoid replicative senescent,[53] but some MSC properties may still be lost during culture. In addition, we cannot exclude the contribution of hypertension or diabetes to obese-MSC dysfunction, and our population was largely white, and the findings therefore cannot be generalized.

In summary, obesity may impair reparative MSC function via several mechanisms, including promoting senescence, depletion of angiogenic cytokines, and enhancing inflammatory markers. In-vitro MSCs senescence correlated with their functional and tissue impact in the STK, warranting further studies testing strategies to restore MSC function.[38] The donor-dependent MSC function observed in our study may explain why some stem cell studies yield conflicting results, and may warrant caution during autologous MSC delivery. Moreover, our findings have important implications for self-healing capacity of individuals with obesity, given an undermined endogenous cellular repair system. Future studies are needed to explore MSC characteristics in different settings, and to identify measures, such as senolytic pre-conditioning, to preserve MSC function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Competing interest

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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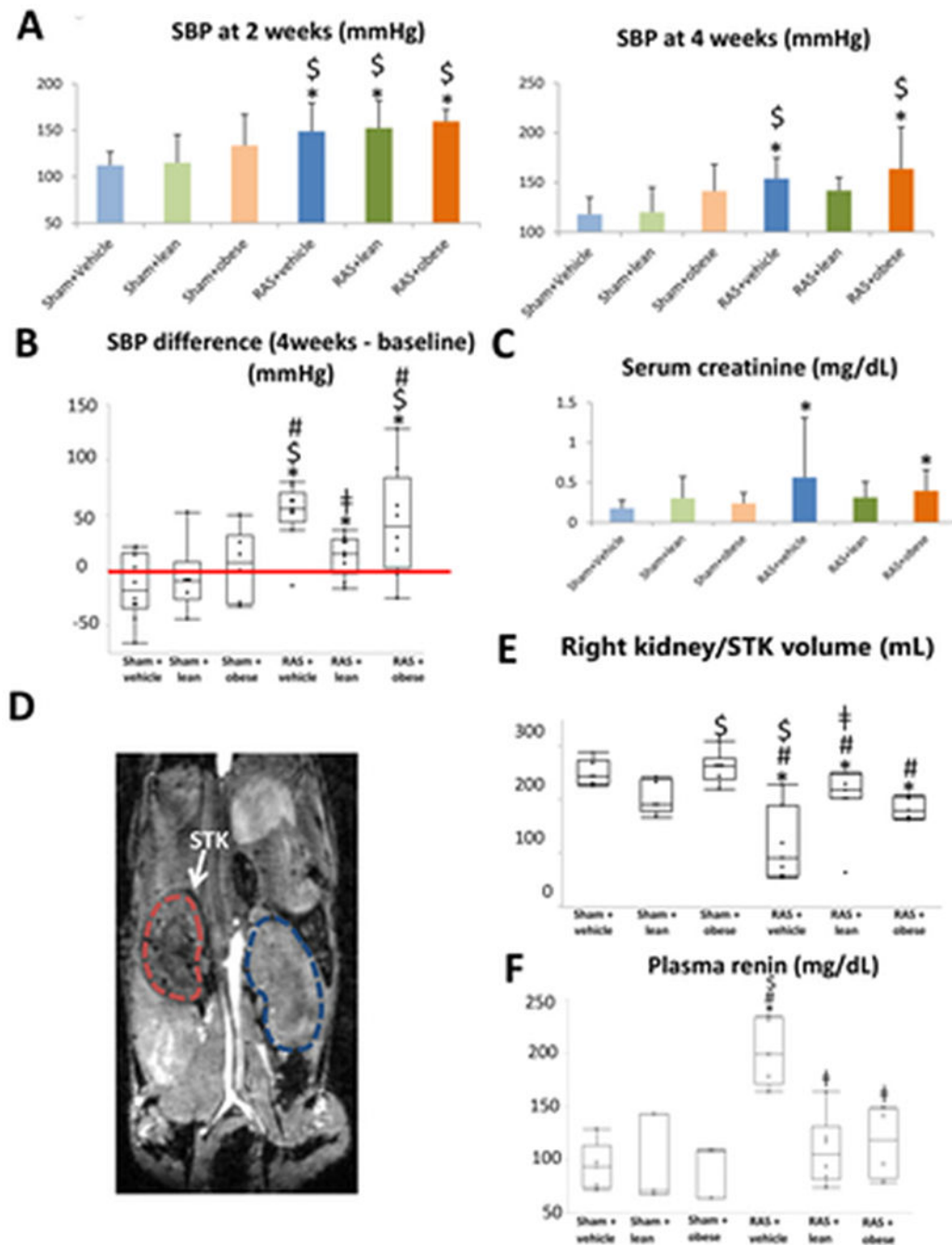
Figure 1: Lean-MSC improves blood pressure and kidney function.
A: All RAS mice had elevated systolic blood pressure (SBP) 2 weeks after surgery. At 4
weeks, lean-MSC improved SBP in RAS mice compared to obese-MSC. B: At 4 weeks,
RAS+lean-MSC had the smallest increase in SBP compared to baseline whereas there was
no difference between RAS+vehicle vs RAS+obese-MSC. C: Lean-MSC improved serum
creatinine in mice with renal artery stenosis (RAS) to levels comparable to shams D: An
MRI image depicting the smaller stenotic kidney (STK) compared to contralateral kidney.
E: Lean-MSC improved STK size compared to RAS+vehicle, whereas obese-MSC did not.

F: Both lean and obese-MSCs reduced renin level compared to RAS+vehicle. *p<0.05 vs sham+vehicle, $p<0.05 vs sham+lean-MSC, #p<0.05 vs sham+obese-MSC, ǂp<0.05 vs RAS+vehicle, &p<0.05 vs RAS+obese-MSC.
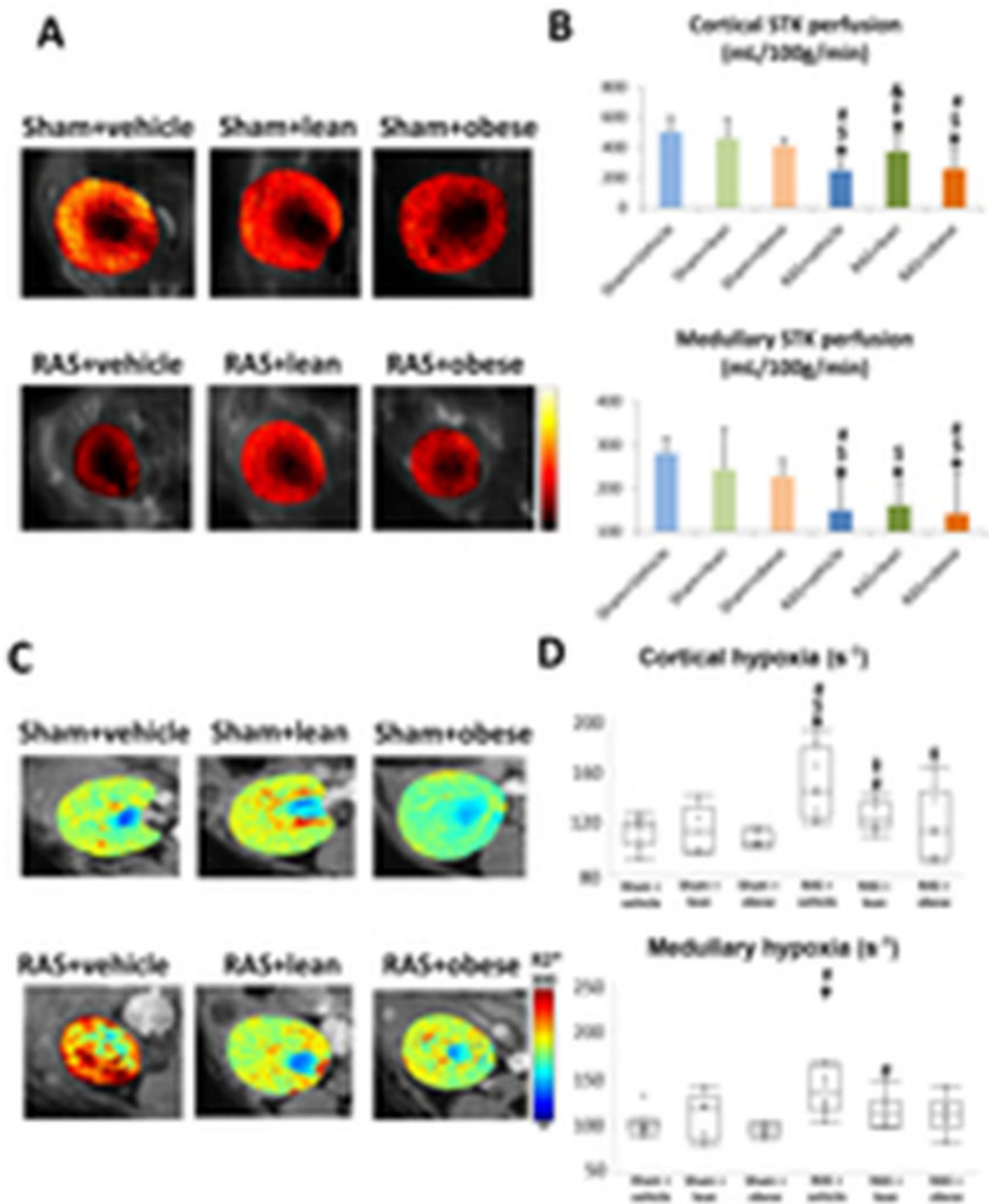
Figure 2: Lean-MSC improves stenotic kidney (STK) cortical perfusion more than obese-MSC.
A&B: Lean-MSC improved cortical STK perfusion compared to obese-MSC, although
it remained lower than sham. Neither lean- nor obese-MSCs affected STK medullary
perfusion. C&D: Both lean- and obese-MSCs improved STK cortical and medullary
oxygenation compared to RAS+vehicle. *p<0.05 vs sham+vehicle, $p<0.05 vs sham+lean-
MSC, #p<0.05 vs sham+obese-MSC, ǂp<0.05 vs RAS+vehicle, &p<0.05 vs RAS+obese-
MSC.
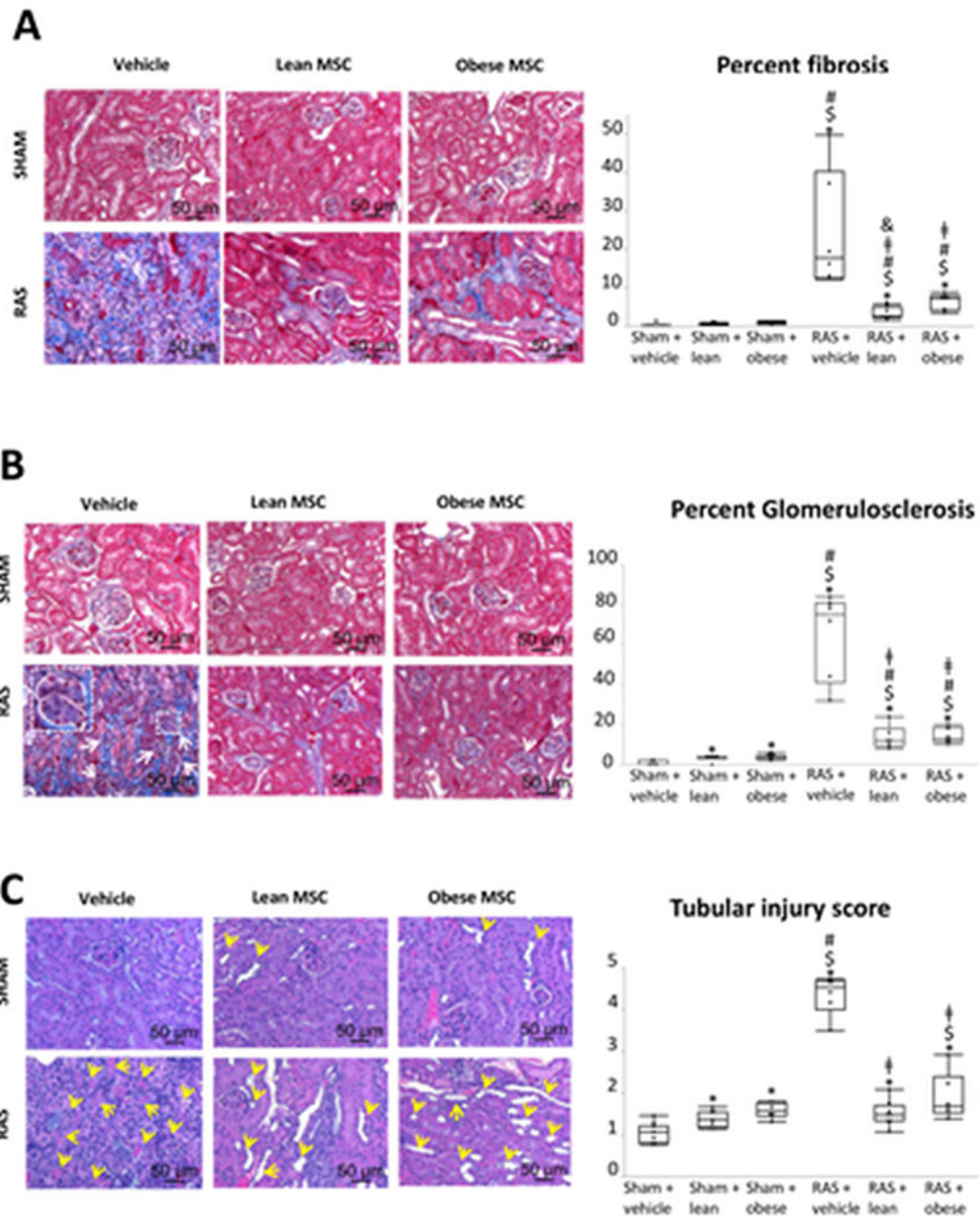
Figure 3: Tissue changes in STKs.
A: Both lean- and obese-MSCs improved interstitial fibrosis compared to RAS+vehicle, but lean-MSC was more effective (Masson Trichrome, 200X). B: Both lean and obese-MSCs improved, but not normalized glomerulosclerosis (Masson Trichrome, 200X). C: Both lean and obese-MSCs similarly improved tubular injury in stenotic kidneys compared to RAS+vehicle (Hematoxylin and eosin, 200X). *p<0.05 vs sham+vehicle, $p<0.05 vs sham+lean-MSC, #p<0.05 vs sham+obese-MSC, ‡p<0.05 vs RAS+vehicle, &p<0.05 vs

RAS+obese-MSC. Images were obtained via Zeiss microscopy and processed by Zeiss ZEN 2 (blue edition), Jena, Germany.
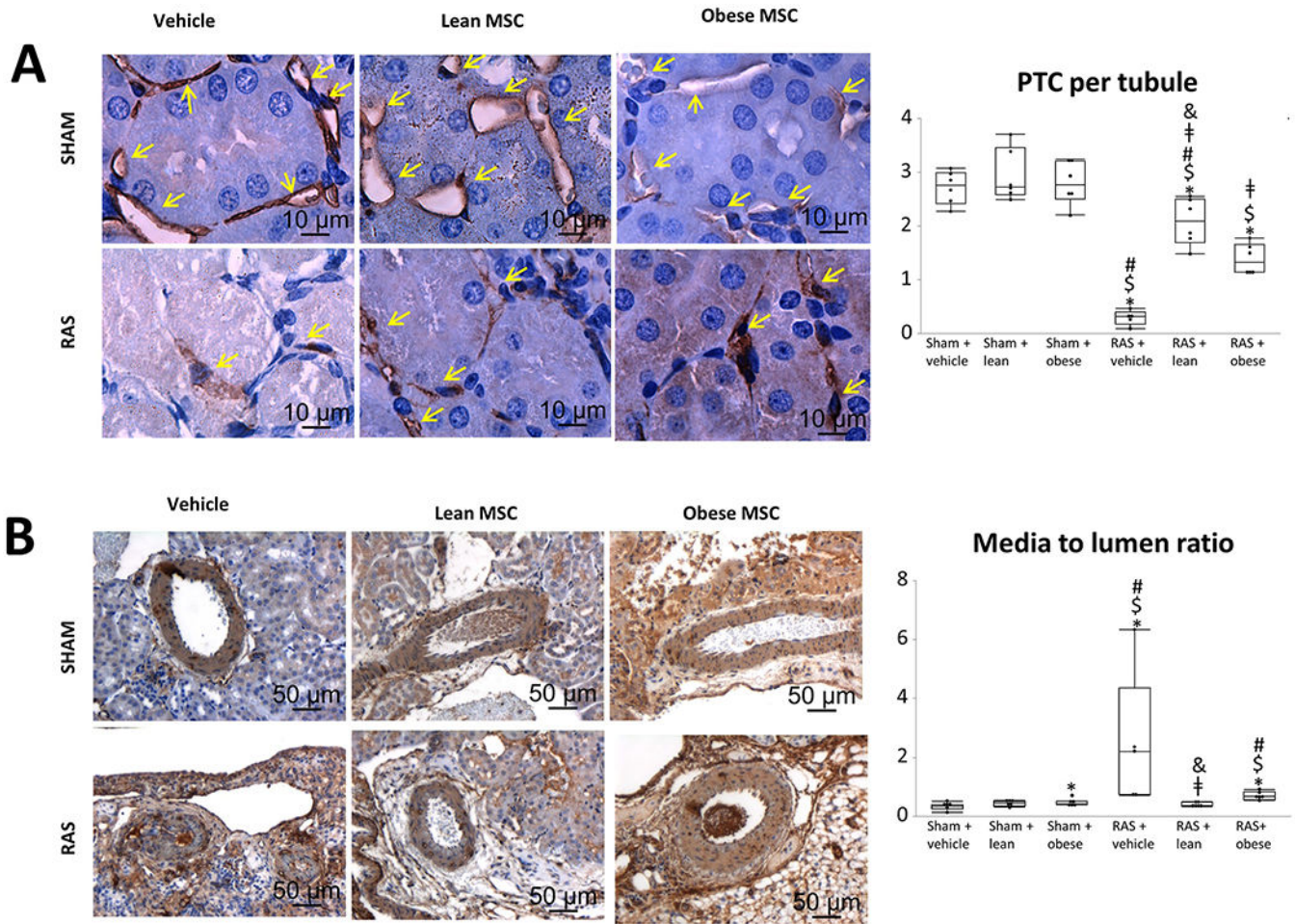
Figure 4: Peritubular capillary loss and vascular wall remodeling in the stenotic kidneys.
A: Both lean- and obese-MSCs ameliorated peritubular capillary (PTC) (yellow arrow)
loss, but lean MSC further reduced PTC loss compared to RAS+obese-MSC. (CD31
immunohistochemistry, 400X) B: Lean MSC more markedly mitigated vascular remodeling
compared to obese MSC [α-smooth muscle actin (SMA) immunohistochemistry, 200X].
*$p < 0.05$ vs sham+vehicle, \$$p < 0.05$ vs sham+lean-MSC, #$p < 0.05$ vs sham+obese-MSC,
‡$p < 0.05$ vs RAS+vehicle, &$p < 0.05$ vs RAS+obese-MSC. Images were obtained via Zeiss
microscopy and processed by Zeiss ZEN 2 (blue edition), Jena, Germany.
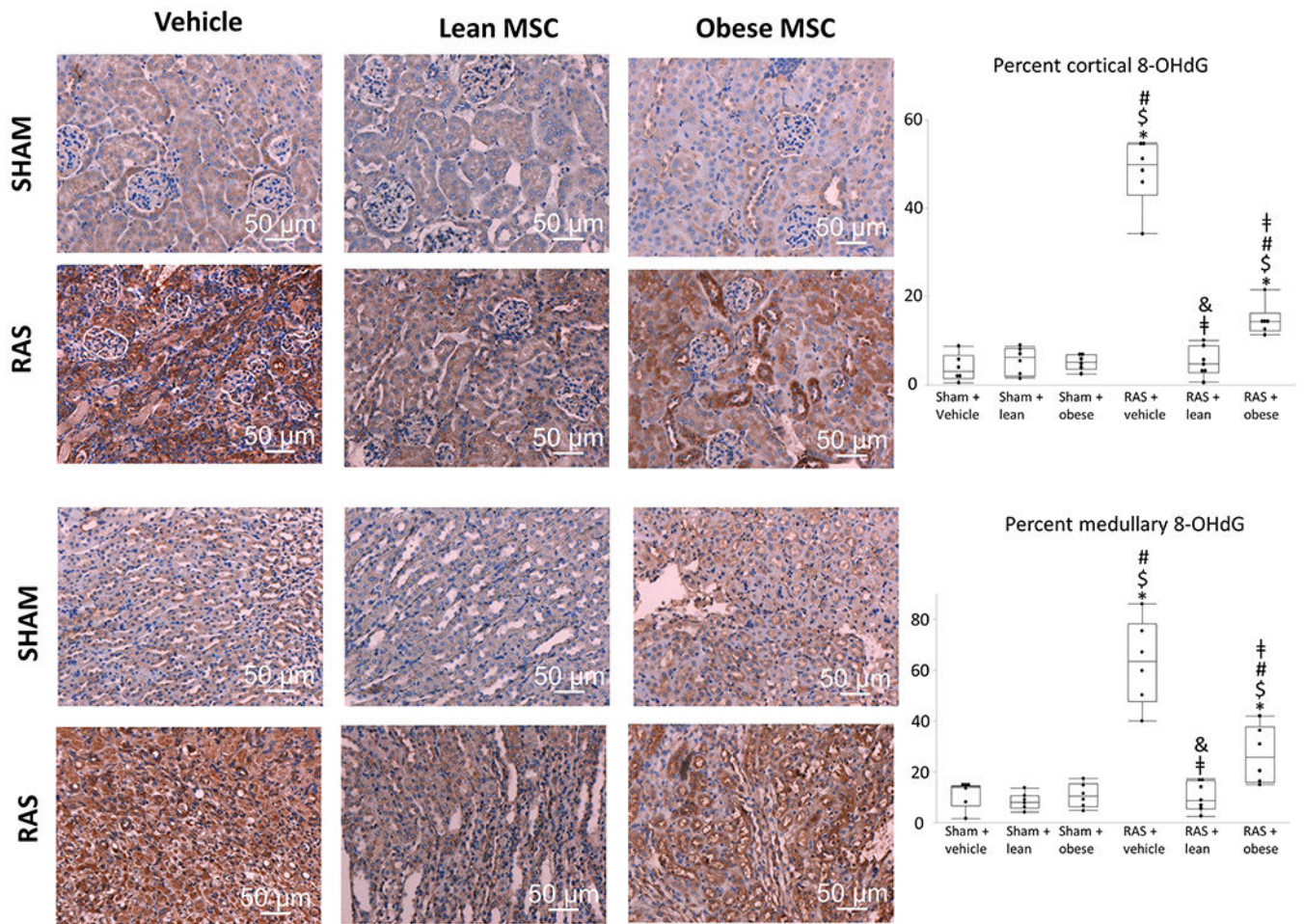
**Figure 5: Oxidative stress in the stenotic kidneys.**

Lean-MSC ameliorated oxidative stress, demonstrated by 8-hydroxy-2'-deoxyguanosine (8-OHdG) (brown color) in both cortex and medulla, more effectively than obese-MSC [8-hydroxy-2'-deoxyguanosine (8-OHdG), 200X]. *p<0.05 vs sham+vehicle, $p<0.05 vs sham+lean-MSC, #p<0.05 vs sham+obese-MSC, ‡p<0.05 vs RAS+vehicle, &p<0.05 vs RAS+obese-MSC. Images were obtained via Zeiss microscopy and processed by Zeiss ZEN 2 (blue edition), Jena, Germany.
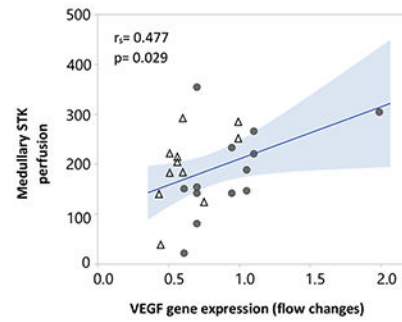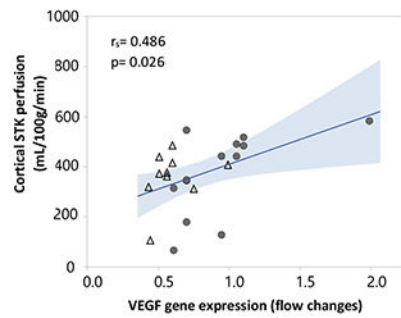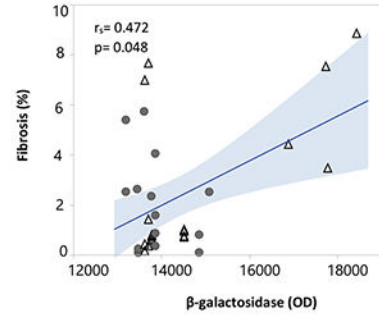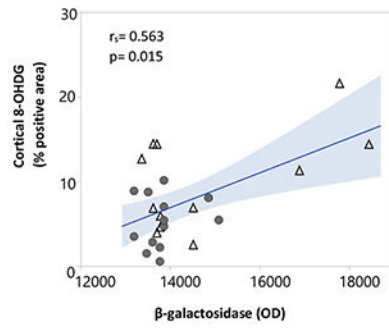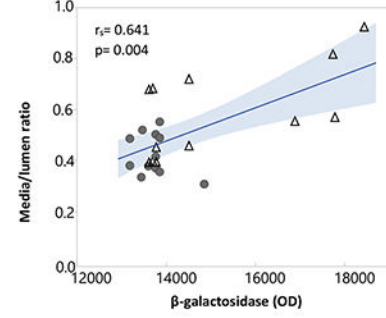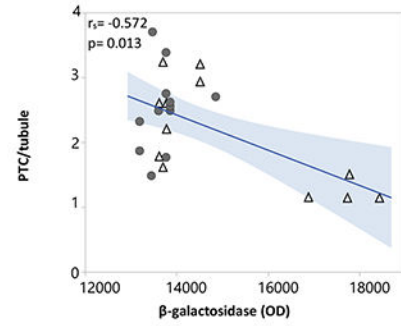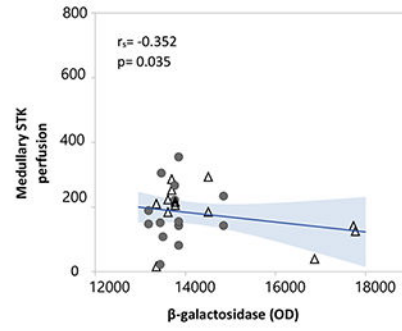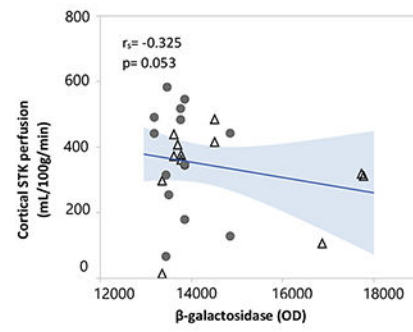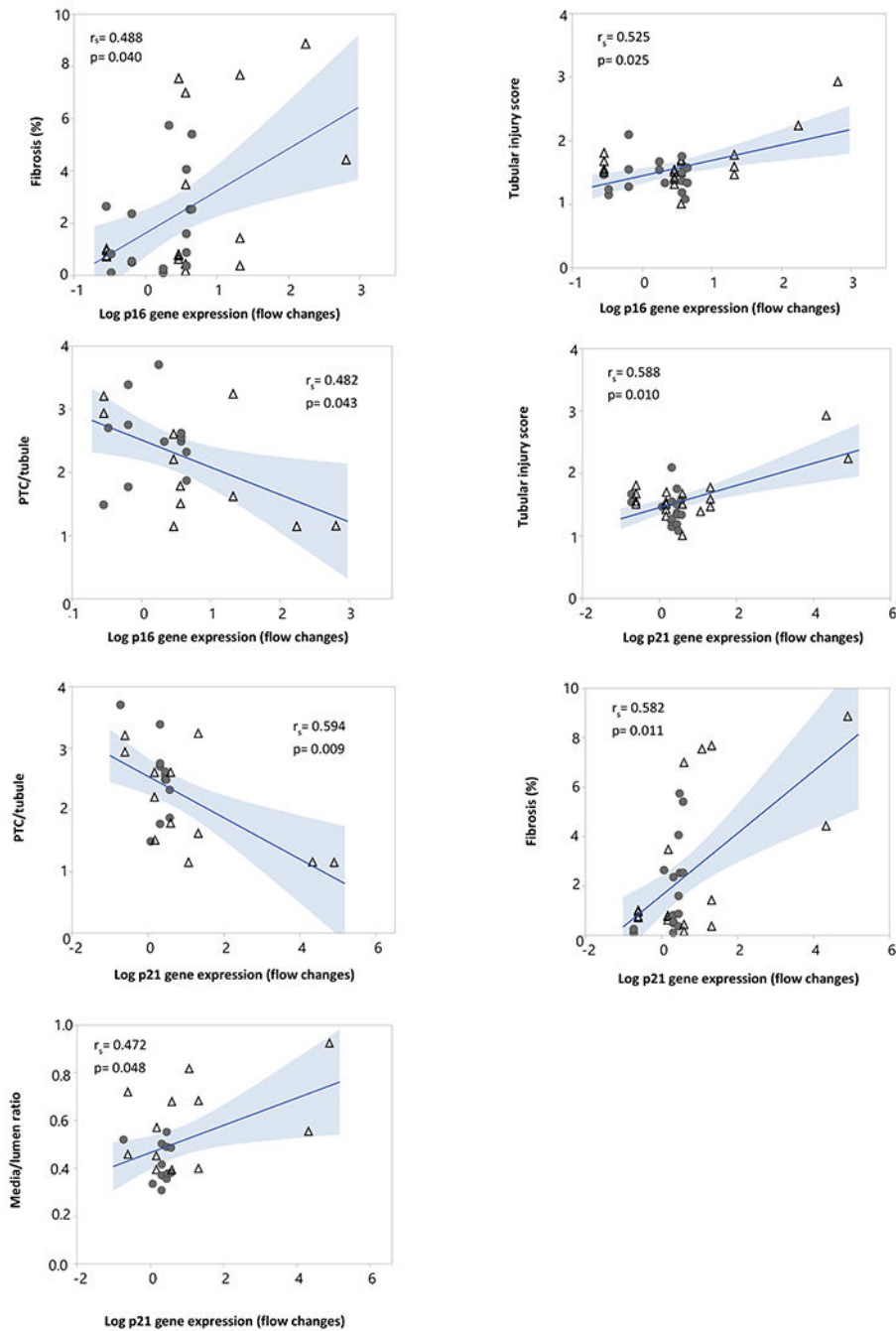
Figure 6: Correlation between MSC senescence, inflammatory and proangiogenic gene expression with renal perfusion and histological changes.

β-galactosidase activity of human MSC correlated inversely with cortical and medullary stenotic kidneys (STK) perfusion as well as the number of peritubular capillaries (PTC) per tubule, but correlated directly with the degree of cortical and medullary oxidative stress (8-OHdG: 8-hydroxy-2'-deoxyguanosine; 8-OHdG) and fibrosis. Proangiogenic gene expression (vascular endothelial growth factor; VEGF) in human-MSC directly correlated with cortical and medullary STK perfusion. Log MSC-p16 directly correlated with fibrosis and tubular injury, and inversely with the number of PTC per tubule. Similar to log MSC-

p16, log MSC-p21 also showed similar correlations with fibrosis, tubular injury, and the number of PTC per tubule. In addition, log MSC-p21 showed a direct correlation with media/lumen ratio. **Black dot**: lean-MSC, **triangle**: obese-MSC. OD: optical density.