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25(OH) vitamin D suppresses macrophage adhesion and migration by downregulation of ER stress and scavenger receptor A1 in type 2 diabetes

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Abstract

Cardiovascular disease (CVD) is the leading cause of mortality in patients with type 2 diabetes mellitus (T2DM). Vitamin D deficiency is not only more prevalent in diabetics but also doubles the risk of developing CVD. However, it is unknown whether 25-hydroxy vitamin D [25(OH)D₃] replacement slows monocyte adhesion and migration, critical mechanisms involved in atherosclerosis progression. In this study, monocytes from vitamin D-deficient diabetic patients were cultured either in the patient's serum or in vitamin D-deficient media with or without 25(OH)D₃ treatment. Adding 25(OH)D₃ to monocytes cultured in vitamin D-deficient serum or media decreased monocyte adhesion to fibronectin and migration stimulated by monocyte chemotactic protein 1 (MCP-1). Accordingly, 25(OH)D₃ decreased adhesion marker β1- and β2-integrin expression and migration receptor chemokine (C-C motif) receptor 2 (CCR2) expression. 25(OH)D₃ treatment downregulated monocyte endoplasmic reticulum (ER) stress and scavenger receptor class A, type 1 (SR-A1) expression. The absence of SR-A1 prevented the increased macrophage adhesion and migration induced by vitamin D deficiency. Moreover, the absence of SR-A1 prevented the induction of adhesion and migration and expression of their associated membrane receptors by Thapsigargin, an ER stress inducer. These results identify cellular activation of monocyte/macrophage vitamin D signaling through 25(OH)D₃ as a potential mechanism that could modulate adhesion and migration in diabetic subjects.

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Keywords

Vitamin D; macrophage; adhesion; migration; SR-A1; diabetes

1. Introduction

Approximately 20 million Americans suffer from type 2 diabetes mellitus, a disease frequently associated with elevated blood pressure and characterized by an increased risk of cardiovascular disease [1]. The combination of these metabolic abnormalities is the most common cause of morbidity and mortality in Western populations [2]. Despite the notion that insulin resistance and chronic inflammation lead to accelerated vascular disease in patients with T2DM, very little is known about the mechanisms by which these risk factors promote vascular complications. Vascular inflammation is recognized as a major contributor to atherosclerotic plaque development [3–5]. Monocytes migrate from the circulation into the intima of the arterial wall where they differentiate into macrophages, which then take up modified lipoproteins thereby transforming into foam cells [6]. Monocyte-derived macrophages are abundantly present at all stages of the disease process and play a pivotal role in the development and progression of the disease [5, 7, 8]. Decreased circulating monocytes and reduced tissue macrophages resulting from the absence of macrophage colony-stimulating factor (M-CSF) in mice reduces high-fat-diet-induced atherosclerosis [9, 10]. Moreover, multiple genetically modified mouse models with the absence of monocyte chemokine receptors (CX3C chemokine receptor 1, CCR2, or CCR5) or deletion of adhesion molecules such as selectins or integrins are associated with decreased atherosclerosis development, suggesting that understanding monocyte behavior will be key to decreasing atherosclerosis progression [6].

The class A, type 1 scavenger receptor (SR-A1) belongs to a large family of scavenger receptors expressed primarily by monocytes/macrophages with critical roles in vascular inflammation and atherosclerosis progression. In early atherosclerosis, SR-A1 mediates macrophage adhesion [11], while SR-A1^{-/-} peritoneal macrophages display reduced adhesion and spreading in the first 24 hours after isolation [12]. Interestingly, SR-A1-dependent macrophage adhesion may only be of significance under environmental conditions such as diabetes when SR-A1 expression is increased or when the SR-A1 ligands are glycated. Macrophages with absence of the insulin receptor show increased ER stress and SR-A1 expression, suggesting a link between insulin signaling and macrophage stress signaling pathways [13]. In vitro, macrophages can adhere to surfaces coated with glucose-modified basement membrane collagen IV through their SR-As, highlighting the role of SR-A1 in the accelerated atherosclerosis in diabetes [14]. SR-A1 facilitates modified-cholesterol uptake in macrophages and works as an immune pattern recognition receptor, triggering apoptosis in endoplasmic-reticulum-stressed macrophages, promoting advancement of lesions and plaque necrosis [15]. Thus, identifying environmental conditions that modulate vascular SR-A ligands and monocyte SR-A expression could be key to the development of novel therapies to slow the accelerated atherosclerosis seen in patients with diabetes.

Vitamin D deficiency is a largely unacknowledged epidemic associated with incident T2DM and CVD [16–18]. Deficiency of 25(OH)D, the principal storage form of vitamin D, is 30%

more prevalent in diabetics than in control subjects and nearly doubles the relative risk of developing CVD compared to diabetic patients with normal 25(OH)D levels [19, 20]. The vitamin D receptor (VDR) and its converting enzyme, 25(OH)D₃-1 α -hydroxylase, are present in monocytes and macrophages, demonstrating the capability of these cells to generate active vitamin D and induce vitamin D cell signaling locally. 1,25-dihydroxy vitamin D [1,25(OH)₂D₃], the active form of vitamin D, facilitates adhesion in monocytic cell lines and stimulates differentiation of myeloid progenitors into macrophages in vitro through the VDR [21, 22]. During monocyte differentiation, the ER reorganizes both structurally and functionally in order to carry out new cell functions, leading to ER stress [23, 24]. We have shown that 1,25(OH)₂D₃ suppresses ER stress in monocytes and macrophages from diabetic patients, along with preventing monocyte adhesion and migration and macrophage cholesterol deposition, all indicating that active vitamin D modulates properties contributing to atherosclerosis progression [25–27]. Furthermore, cross-sectional analysis of T2DM patients with 25(OH)D >30 ng/mL demonstrated decreased monocyte adhesion and migration compared to those with 25OHD <20 ng/mL [26]. However, it is unknown whether 25(OH)D supplementation, more consistent with common clinical practice, may suppress monocyte adhesion and migration.

To determine whether 25(OH)D₃ decreases monocyte adhesion and migration and expression of membrane receptors implicated in these processes, we obtained monocytes from T2DM patients with vitamin D deficiency and incubated them briefly in culture in the original patient's serum with or without 25(OH)D₃ supplementation to mimic the normal in vivo environment. We also explored the effects of 25(OH)D₃ on ER homeostasis in the regulation of monocyte/macrophage adhesion and migration and assessed whether these effects are SR-A1- dependent.

2. Materials and Methods

2.1 Study design

We performed a cross-sectional analysis of 12 obese patients with T2DM with vitamin D deficiency [25(OH)D <30 ng/mL; mean 17 $\pm$ 7 ng/mL, BMI 36 $\pm$ 2.6]. Subjects were voluntarily recruited from the outpatient clinics at Washington University School of Medicine and Barnes-Jewish Hospital, St. Louis. Subjects underwent a single fasting venous blood draw for monocyte isolation and 25(OH)D level measured by liquid chromatography-tandem mass spectrometry (Mayo Medical Laboratories, Rochester, MN).

2.2 Isolation and preparation of human monocytes

Peripheral monocytes were isolated by standard Ficoll isolation techniques and selected by cluster of differentiation 14 (CD14) marker positivity (Miltenyi Biotec, Auburn, CA). To closely mimic in vivo conditions, monocytes were maintained in (vitamin D-deficient) 100% serum from the original patient with or without 25(OH)D₃ incubation at 150 nM (60 ng/mL) for 24 hours [28]. Monocytes were then also maintained in vitamin D-deficient media [deficient in both 25(OH)D and 1,25(OH)₂D; obtained by DMEM plus 10% charcoal/dextran-treated FBS] incubated with or without 25(OH)D₃ at 150 nM for 24 hours [26, 29].

In both monocyte conditions 25(OH)D₃ induced VDR signaling activation (Supplemental Figure 1).

2.3 Isolation of murine macrophages

Macrophages were isolated after peritoneal normal saline lavage of 16-week-old C57BL/6 mice lacking scavenger receptor A-1 (SR-A1^{-/-}) and WT C57BL/6 mice fed a chow diet. Macrophages were cultured in vitamin D-deficient media incubated with or without 25(OH)D₃ at 60ng/mL for 1 week. ER stress was induced by adding Thapsigargin (Sigma, St. Louis, MO) to macrophages for the final 18 hours of culture incubated in 25(OH)D₃ media [29, 30].

2.4 Adhesion assays

96-well-plates were coated with fibronectin (Sigma) overnight. Macrophages (0.1×10^5 cells/plate) were added to fibronectin-coated plates and incubated for 4 hours at 37°C. Adhered cells were then washed, fixed in formaldehyde, and stained with Crystal violet. Well absorbance was read at 585 nm [26].

2.5 Migration assays

Transwell migration assays were performed (Costar polycarbonate filters, 5 µm pore size) as we previously described [27]. Membranes and 12-well plates were coated with fibronectin (5µl/mL; Life Technologies, Grand Island, NY) overnight at 4 degrees. Macrophages (0.5×10^5 cells/well) were added to the upper chamber, and MCP-1 (100 ng/well; Sigma) in 0.8% agarose solution was added to the lower chamber to stimulate migration. Cells migrating into the lower chamber after 8 hours of incubation were manually counted.

2.6 Gene expression and western blot analysis

Quantitative reverse-transcription polymerase chain reaction (qPCR) analyses were performed by Sybgreen methodologies. Results were normalized to the housekeeping gene L32. Western blot analyses from macrophage protein extracts were normalized to β-actin expression.

2.7 Statistical Analysis

GraphPad Prism software was used for statistical calculations. Descriptive variables were expressed as mean ± SD for continuous data. Experiments were carried out with duplicate or triplicate samples, and all experiments were replicated in multiple sets of samples. Analytic data were expressed as mean ± SEM for continuous variables. Normal distribution of continuous variables was verified graphically. Statistical significance of differences was calculated using t-test for parametric data involving two groups or one-way analysis of variance (ANOVA) followed by Tukey's post-test for parametric data involving three or more groups. Differences were considered statistically significant if $p < 0.05$.

3. Results

3.1 25(OH) vitamin D decreases monocyte adhesion to fibronectin and adhesion receptor expression

To determine whether 25(OH) vitamin D regulates monocyte adhesion, we performed functional adhesion assays in monocytes from vitamin D-deficient diabetic patients. Monocytes from vitamin D-deficient diabetics were isolated and cultured for 24 hours in 100% serum from the original patient with or without incubation with 25(OH)D₃. Incubation with 25(OH)D₃ significantly decreased the adhesion to fibronectin compared to monocytes maintained in vitamin D-deficient patient serum, (Figure 1A, $p < 0.05$). Accordingly, incubation with 25(OH)D₃ also decreased mRNA expression levels of adhesion markers β_1 -integrin and β_2 -integrin by more than 50% (Figure 1B–C, $p < 0.001$ for both) compared to vitamin D-deficient cells. To confirm the influence of 25(OH)D₃ on monocyte adhesion, we also cultured monocytes for 24 hours in vitamin D-deficient media with or without incubation with 25(OH)D₃. Similarly to culture conditions with patient serum, 25(OH)D₃ significantly suppressed monocyte adhesion to fibronectin, and decreased β_1 -integrin and β_2 -integrin expression compared to cells cultured in vitamin D-deficient media (Figure 1D–F, $p < 0.01$ for all). In both monocyte models (incubated in serum or media) 25(OH)D₃ suppressed β_1 -integrin protein expression (Supplemental Figure 2). However, the difference between β_1 -integrin gene expression in serum monocyte models and the protein expression suggests a posttranslational defect induced by the serum of vitamin D deficient patients (supplemental Figure 2). These data suggest that monocytes from patients with diabetes exhibit increased atherogenic behaviors under vitamin D-deficient conditions that are reversible with 25(OH)D₃ supplementation.

3.2 25(OH) vitamin D decreases monocyte migration and migration receptor expression

To determine whether 25(OH)D₃ regulates monocyte migration, we performed functional assays in monocytes from T2DM cultured one of four conditions: 100% serum from the original patient or vitamin D-deficient media, both with and without incubation with 25(OH)D₃. Transwell assays demonstrated at least a 20% reduction in monocyte migration in response to MCP-1 in cells incubated with 25(OH)D₃ compared to vitamin D-deficient conditions, regardless of whether cells were cultured in media or patient serum (Figure 2A–B, $p < 0.005$ for both). To further investigate the correlations between vitamin D and monocyte migration, we assessed expression of cell surface migration molecules in monocytes from a subset of cells under each of the four conditions. We found that the mRNA and protein expression of migration receptor CCR2 was significantly suppressed in cells incubated with 25(OH)D₃ when compared to vitamin D-deficient conditions for both patient serum and media (Figure 2C–D, $p < 0.001$ for both, and Supplemental Figure 2), supporting our findings of suppression of monocyte adhesion and migration by 25(OH)D₃.

3.3 25(OH) vitamin D decreases monocyte ER stress and SR-A1 expression

We previously demonstrated that active vitamin D suppresses monocyte adhesion and migration by downregulation of monocyte ER stress [26, 27]. We also found that phenylbutyric acid, a chaperone that inhibits ER stress, suppresses adhesion and migration induced by vitamin D deficiency. To further characterize the mechanisms involved in the

regulation of adhesion and migration by 25(OH)D₃, we evaluated protein expression of SR-A1, a downstream target of ER stress involved in monocyte adhesion, and ER stress proteins in cultured monocytes under the same four conditions: 100% serum from the original patient or vitamin D-deficient media, both incubated with or without 25(OH)D₃. We found that incubation with 25(OH)D₃ was sufficient to suppress expression of SR-A1, as well as ER stress proteins phospho-protein kinase RNA-like endoplasmic reticulum kinase (p-PERK), phospho-inositol-requiring enzyme 1 alpha (p-IRE1α), and CEBP homologous protein (CHOP), compared to vitamin D-deficient conditions for both patient serum and media, suggesting that brief incubation with 25(OH)D₃ induces monocyte cellular vitamin D signaling and suppresses ER stress (Figure 3).

3.4 Absence of SR-A1 prevents increased monocyte adhesion and migration induced by ER stress

It is known that peritoneal macrophages from SR-A1^{-/-} mice display reduced adhesion and migration in vivo [31]. Furthermore, macrophage ER stress regulates SR-A expression [29]. To determine whether 25(OH)D₃ decreases monocyte adhesion and migration by downregulation of ER stress and SR-A1 expression, we obtained peritoneal macrophages from SR-A1^{-/-} or WT mice and incubated them in vitamin D-deficient media with or without 25(OH)D₃. One week of incubation with 25(OH)D₃ suppressed macrophage adhesion to fibronectin by 33% and migration by 28% in WT cells, but had no effect in SR-A1^{-/-} cells, which already showed low adhesion and migration (Figure 4A–B p<0.005 for both). To further determine whether the absence of SR-A1 prevents increased expression of adhesion and migration membrane receptors, we performed quantitative PCR of β₁- and β₂-integrin and CCR2, demonstrating that WT cells in vitamin D-deficient conditions showed at least 5-fold higher expression than WT cells in 25(OH)-treated conditions or SR-A1^{-/-} cells regardless of the incubation with 25(OH)D₃ (Figure 4C–E p<0.01 for all). To clarify whether ER stress stimulation of macrophage adhesion and migration is blunted by the absence of SR-A1, we obtained peritoneal macrophages from SR-A1^{-/-} and WT mice, cultured in 25(OH)D₃-treated media for one week, then induced ER stress by adding Thapsigargin for the final 18 hours of culture. Thapsigargin-treated WT macrophages had at least 49% increased adhesion (Figure 5A, p<0.001) and 25% increased migration (Figure 5B, p<0.001) when compared to non-Thapsigargin-treated WT and both groups of SR-A1^{-/-} macrophages, suggesting that the absence of SR-A1 blunts the induction of adhesion and migration by ER stress. However, the absence of SR-A1 did not completely prevent the induction of macrophage migration by Thapsigargin when compared to non-Thapsigargin-treated cells, suggesting possible other contributing mechanisms. Accordingly, Thapsigargin induced expression of adhesion membrane markers β₁- and β₂-integrin by at least 4-fold and expression of migration membrane receptor CCR2 by at least 6-fold in macrophages from obtained from WT mice when compared to non-Thapsigargin-treated WT or both groups of SR-A1^{-/-} macrophages (Figure 5C–E, p<0.005 for all). These data suggest that downregulation of ER stress by 25(OH)D₃ reduced SR-A1 expression and suppressed monocyte adhesion and migration.

4. Discussion

Multiple observational studies support the potential role of vitamin D in cardiovascular disease. Immune cells express the VDR and are also able to transform 25(OH)D into its active form, which functions as an autocrine/paracrine factor [32]. In vitro studies suggest that active vitamin D decreases adhesion, migration, and cholesterol deposition in macrophages [25–27]. However, the effects of 25(OH)D replacement on atherogenic properties of circulating monocytes in patients with type 2 diabetes are unknown. In this study, we found that 25(OH)D₃ treatment suppressed monocyte adhesion and migration by suppressing ER stress and SR-A1 in patients with diabetes. 25(OH)D₃ replacement also decreased expression of monocyte adherence proteins β 1- and β 2-integrin and migration receptor CCR2. The absence of SR-A1 prevented macrophage adhesion and migration induced by vitamin D deficiency by suppressing β 1- and β 2-integrin and CCR2 expression. Moreover, increased macrophage adhesion and migration induced by ER stress were prevented by the absence of SR-A1. These data suggest that local cellular activation of vitamin D signaling in monocyte/macrophages decreases adhesion and migration by downregulation of ER stress and suppression of SR-A1 expression in patients with diabetes.

Downregulation of macrophage ER stress by 1,25(OH)₂D exerts a pivotal role in atherosclerotic plaque development [30, 33]. The absence of vitamin D receptor signaling in mice increases blood pressure by increasing renal renin release and accelerates atherogenesis [33]. Peritoneal macrophages from these mice have higher expression of adhesion markers intercellular adhesion molecule 1 and P-selectin, suggesting potential effects in early atherosclerosis. In patients with diabetes, downregulation of ER stress by 1,25(OH)₂D₃ generates a monocyte/macrophage with anti-atherogenic properties, preventing monocyte adhesion and migration and suppressing macrophage modified cholesterol uptake and foam cell formation [25, 27, 29]. 25(OH)D deficiency has also been associated with monocyte/macrophage properties that contribute to vascular inflammation and atherosclerosis. In mice, diet-induced vitamin D deficiency increases macrophage ER stress and accelerates atherosclerosis [30]. Previously, we found that monocytes from T2DM patients with 25(OH)D levels below 20 ng/mL have increased adhesion when compared to those from diabetics with 25(OH)D levels >30 ng/mL [26]. However, this is the first study that has evaluated the effects of 25(OH)D₃ treatment on atherogenic monocyte/macrophage functions. We have shown that in monocytes from patients with diabetes, brief incubation with 25(OH)D₃ suppressed monocyte adhesion and migration and expression of their corresponding membrane receptors by downregulation of ER stress. Therefore, similarly to the effects of 1,25(OH)₂D₃ on monocyte/macrophage function, 25(OH)D₃ seems to prevent early events in the development of atherosclerosis, potentially through local conversion to 1,25(OH)₂D₃ or independent activation of the VDR.

Diabetes is a major risk factor for atherosclerotic disease. Insulin resistance and the resultant hyperinsulinemia are considered independent risk factors for macrovascular atherosclerotic disease [34]. Macrophages with absence of the insulin receptor have lower insulin-stimulated AKT phosphorylation, increased induction of SR-A1 expression by ER stress, and larger, more complex atherosclerotic lesions [13]. However, the contributions of SR-A1 to mouse models of diet-induced insulin resistance and atherosclerosis have been difficult to

reconcile. In the Apolipoprotein E (ApoE)^{-/-} background, mice with the complete absence of SR-A1 show significantly reduced lesion formation compared with ApoE^{-/-} controls [12], while in low density lipoprotein receptor-deficient (LDLR^{-/-}) mice, the absence of SR-A1 only results in marginal lesion reduction, suggesting that SR-A1 may function differently depending on an animal's genetic background [35]. However, transplant of hematopoietic progenitors from SR-A1^{-/-} mice into lethally irradiated C57BL/6 or LDLR^{-/-} mice reduced atherosclerosis formation by 60%, suggesting that SR-A1 expression in macrophages is a key to atherosclerosis prevention [36]. In this study, we found that circulating monocytes from vitamin D-deficient patients with T2DM have increased induction of SR-A1 expression by ER stress and acceleration of monocyte adhesion and migration. The absence of macrophage SR-A1 prevented adhesion and migration and expression of their associated membrane receptors by vitamin D deficiency. Furthermore, SR-A1 knockout suppressed macrophage adhesion and migration by ER stress, suggesting that the downregulation of ER stress and SR-A1 expression by 25(OH)D₃ may be a potential treatment to address the increased susceptibility to vascular inflammation seen in diabetics.

5. Conclusion

This study demonstrates that in monocytes from type 2 diabetics with vitamin D deficiency, 25(OH)D₃ works as a monocyte/macrophage ER stress reliever, suppressing SR-A1 expression and decreasing adhesion and migration similarly to active vitamin D supplementation. This data suggest that at a cellular level, local hydroxylation of 25(OH)D₃ to 1,25(OH)₂D₃ may be critical to the immunomodulatory and anti-atherogenic effects of vitamin D and lays the mechanistic groundwork for interventional trials of vitamin D replacement in patients with T2DM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations: Abbreviations used include

CVD	cardiovascular disease
T2DM	type 2 diabetes mellitus
25(OH)D	25-hydroxy vitamin D
MCP-1	monocyte chemotactic protein 1
CCR2	chemokine (C-C motif) receptor 2
ER	endoplasmic reticulum

SR-A1	scavenger receptor class A, type 1
M-CSF	macrophage colony-stimulating factor
VDR	vitamin D receptor
1,25(OH)₂D	1,25-dihydroxy vitamin D
CD14	cluster of differentiation 14
qPCR	quantitative RT-PCR
p-PERK	phospho-protein kinase RNA-like endoplasmic reticulum kinase
p-IRE1α	phospho-inositol-requiring enzyme 1 alpha
CHOP	CEBP homologous protein
ApoE	Apolipoprotein E
LDLR	low density lipoprotein receptor

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Highlights

- 25(OH)D suppresses monocyte/macrophage adhesion to fibronectin
- 25(OH)D reduces monocyte/macrophage migration induced by MCP-1
- 25(OH)D downregulates monocyte ER stress protein and SR-A1 expression
- SR-A1 knockout prevents vitamin D deficiency-induced macrophage adhesion/migration
- SR-A1 knockout suppresses ER-stress-induced macrophage adhesion/migration

Figure 1. 25(OH)D₃ suppresses monocyte adhesion in type 2 diabetics

For **A–C**, monocytes from vitamin D-deficient type 2 diabetics were cultured in vitamin D-deficient or 25(OH)D₃-treated patient serum. **A**. Adhesion assay showing absorbance of cells adhered to fibronectin (n=12, *p<0.05 vs. vitamin D-deficient). **B–C**. qPCR for mRNA of adhesion receptors **B**. $\beta 1$ -integrin and **C**. $\beta 2$ -integrin (n=6, *p<0.001 for both vs. vitamin D-deficient). For **D–F**, monocytes from vitamin D-deficient type 2 diabetics were cultured in vitamin D-deficient or 25(OH)D₃-treated media. **D**. Adhesion assay showing absorbance of cells adhered to fibronectin (n=12, *p<0.001 vs. vitamin D-deficient). **E–F**. qPCR for mRNA of adhesion receptors **E**. $\beta 1$ -integrin and **F**. $\beta 2$ -integrin (n=6, *p<0.01 for both vs. vitamin D-deficient).

Figure 2. 25(OH)D₃ suppresses monocyte migration in type 2 diabetics

Transwell migration assays in monocytes from vitamin D-deficient type 2 diabetics cultured in **A**, vitamin D-deficient or 25(OH)D₃-treated patient serum (n=12, *p<0.001 vs. vitamin D-deficient) and **B**, vitamin D-deficient or 25(OH)D₃-treated media (n=12, *p<0.005 vs. vitamin D-deficient). **C–D**, qPCR for mRNA of CCR2 from monocytes from vitamin D-deficient type 2 diabetics cultured in **C**, vitamin D-deficient or 25(OH)D₃-treated patient serum and **D**, vitamin D-deficient or 25(OH)D₃-treated media (n=6, *p<0.001 for both vs. vitamin D-deficient).

Figure 3. 25(OH)D₃ suppresses monocyte ER stress in type 2 diabetics

Monocytes from vitamin D-deficient type 2 diabetics were cultured in vitamin D-deficient or 25(OH)D₃-treated patient serum or media. Western blot for protein expression of scavenger receptor SR-A1 and ER stress proteins p-PERK, p-IRE1α, and CHOP.

Figure 4. SR-A1 knockout suppresses macrophage adhesion and migration induced by vitamin D deficiency

Peritoneal macrophages were isolated from WT and SR-A1^{-/-} mice after normal saline injection and cultured in vitamin D-deficient or 25(OH)D₃-treated media. **A.** Adhesion assay showing absorbance of cells adhered to fibronectin (n=4 per group, p<0.001 by ANOVA, *p<0.05 vs. all other groups by Tukey's post-test). **B.** Transwell migration assay (n=4 per group, p<0.005 by ANOVA, *p<0.05 vs. all other groups by Tukey's post-test). **C–E.** qPCR for mRNA of adhesion receptors **C.** β 1-integrin and **D.** β 2-integrin and **E.** migration receptor CCR2 (n=3 per group, p<0.01 by ANOVA for each, *p<0.05 vs. all other groups by Tukey's post-test).

Figure 5. SR-A1 knockout suppresses macrophage adhesion and migration induced by ER stress
 Peritoneal macrophages were isolated from WT and SR-A1^{-/-} mice after normal saline injection and cultured in 25(OH)D₃-treated media with or without Thapsigargin. **A.** Adhesion assay showing absorbance of cells adhered to fibronectin (n=4 per group, p<0.001 by ANOVA, *p<0.05 vs. all other groups by Tukey's post-test). **B.** Transwell migration assay (n=4 per group, p<0.001 by ANOVA, *p<0.05 vs. all other groups by Tukey's post-test, **p<0.05 between indicated groups by Tukey's post-test). **C–E.** qPCR for mRNA of adhesion receptors **C.** β1-integrin and **D.** β2-integrin and **E.** migration receptor CCR2 (n=3 per group, p<0.005 by ANOVA for each, *p<0.05 vs. all other groups by Tukey's post-test).