**SUPPLEMENTAL MATERIAL**

# Multi-omic analysis of the cardiac cellulome defines a vascular contribution to cardiac diastolic dysfunction in obese female mice

Malathi S. I. Dona[1#], Ian Hsu[1#], Alex I. Meuth[2,4], Scott M. Brown[2,4], Chastidy Bailey[2,4], Christian G. Aragonez[2,4], Jacob J. Russell[2,4], Crisdion Krstevski[1], Annayya R. Aroor[4,5], Bysani Chandrasekar[3-5], Luis A. Martinez-Lemus[3,6], Vincent G. DeMarco[4,5], Laurel A. Grisanti[2], Iris Z. Jaffe[7], Alexander R. Pinto[1,8*], Shawn B. Bender[2-4*]

[1]Baker Heart and Diabetes Research Institute, Melbourne, Victoria, Australia

[2]Biomedical Sciences and [3]Dalton Cardiovascular Research Center; University of Missouri, Columbia, Missouri, USA

[4]Research Service, Harry S Truman Memorial Veterans Hospital, Columbia, Missouri, USA

[5]Medicine and [6]Medical Pharmacology & Physiology, University of Missouri School of Medicine, Columbia, Missouri, USA

[7]Molecular Cardiology Research Institute, Tufts Medical Center, Boston, Massachusetts, USA

[8]Centre for Cardiovascular Biology and Disease Research, La Trobe University, Melbourne, Australia
[#]Equal contribution

*Running title:* Vascular role in cardiac dysfunction in obesity

***Correspondence:**

Shawn B. Bender, Ph.D.
Harry S. Truman Memorial Veterans' Hospital
and University of Missouri
Biomedical Sciences
E102 Vet Med Bldg
Columbia, MO 65211
Phone:573-814-6000 x53731
Fax:573-884-6890
Email: benders@missouri.edu

Alexander R. Pinto, Ph.D.
Baker Heart and Diabetes Institute
75 Commercial Rd
Prahran, Victoria
Australia 3004
Phone: +61 8532 1111
Email: alex.pinto@baker.edu.au

*Online Resource 1.* Primer sequences for real-time quantitative PCR

| Gene Name | Primer Sequence (5' → 3') | |
|---|---|---|
| | **Forward** | **Reverse** |
| **ITGAX (CD11c)** | CTGGATAGCCTTTCTTCTGCTG | GCACACTGTGTCCGAACTC |
| **ADGRE1 (F4/80)** | CTTTGGCTATGGGCTTCCAGTC | GCAAGGAGGACAGAGTTTATCGTG |
| **GAPDH** | TCACCACCATGGAGAAGGC | GCGAAGCAGTTGGTGGTGCA |
| **ICAM-1** | AACCGCCAGAGAAAGATCAG | TGTGACAGCCAGAGGAAGTG |
| **PECAM-1** | GAGCCCAATCACGTTTCAGTTT | TCCTTCCTGCTTCTTGCTAGCT |
| **TNF-α** | GCCTCTTCTCATTCCTGCTTG | CTGATGAGAGGGAGGCCATT |
| **VCAM-1** | CTTCATCCCCACCATTGAAG | TGAGCAGGTCAGGTTCACAG |

*Online Resource 2.* Phenotypic characteristics of study animals by group

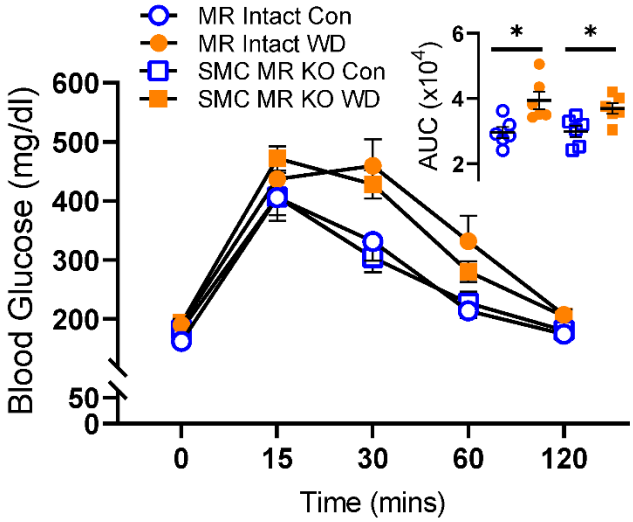
| | **Control** | **Western** | **Western + Spiro** |
|---|---|---|---|
| Body Weight (g) | 22.0 ± 0.7 | 25.7 ± 1.0* | 25.0 ± 0.9* |
| Periovarian Fat Weight (mg) | 431 ± 50 | 834 ± 186* | 790 ± 113* |

Values are mean ± SE, n=7/group; *p<0.05 vs Control.

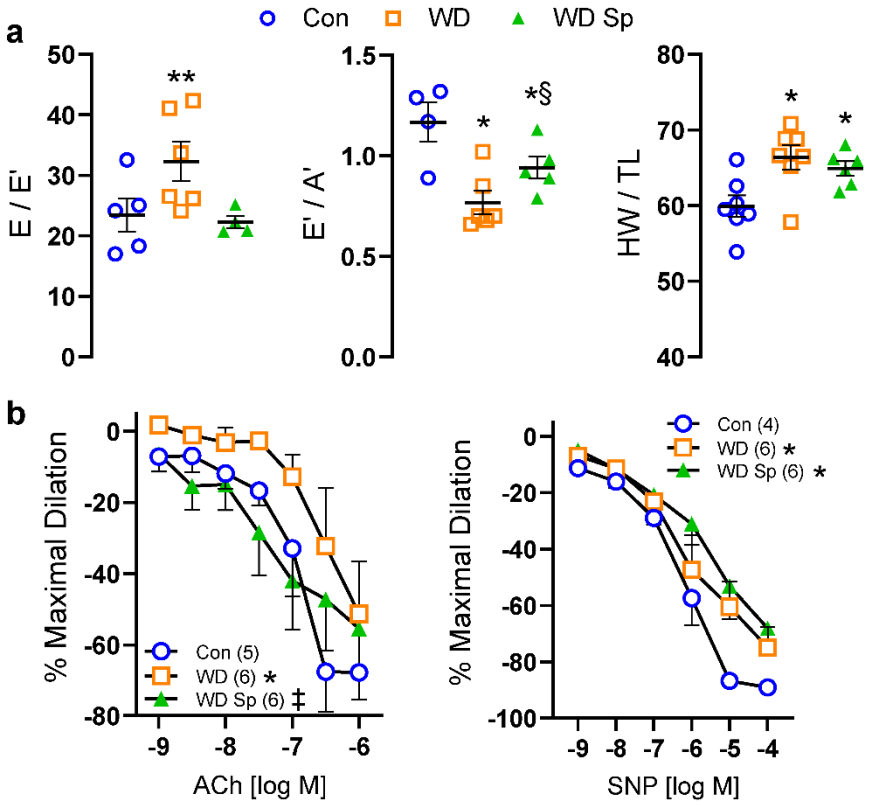
*Online Resource 3.* Phenotypic characteristics of study animals by group

| | MR-Intact Control | MR-Intact Western | SMC MR KO Control | SMC MR KO Western |
|---|---|---|---|---|
| Body Weight (g) | 22.3 ± 0.5 | 29.9 ± 1.0* | 21.5 ± 0.3 | 26.8 ± 1.0*† |
| Heart Weight (mg) | 114 ± 3 | 123 ± 4* | 113 ± 2 | 121 ± 3* |
| HW/TL | 64 ± 2 | 69 ± 2* | 63 ± 1 | 67 ± 1* |
| Periovarian Fat Weight (mg) | 740 ± 82 | 2104 ± 147* | 676 ± 57 | 1540 ± 153*† |
| Systolic Blood Pressure (mmHg) | 120 ± 7 | 119 ± 7 | 117 ± 8 | 124 ± 7 |
| Pulse Wave Velocity (cm/s) | 3.8 ± 0.4 | 3.8 ± 0.3 | 3.5 ± 0.2 | 3.9 ± 0.2 |
| *Plasma Parameters* | | | | |
| Blood Glucose (mg/dl) | 156 ± 11 | 183 ± 10* | 137 ± 7 | 191 ± 8* |
| Plasma Insulin (pg/ml) | 172 ± 25 | 266 ± 50** | 116 ± 24 | 319 ± 35* |
| Plasma Cholesterol (mg/dl) | 75 ± 4 | 115 ± 7* | 70 ± 5 | 110 ± 7* |
| Plasma Triglycerides (mg/dl) | 53 ± 6 | 56 ± 2 | 47 ± 5 | 51 ± 9 |
| Plasma Potassium (mEq/ml) | 4.0 ± 0.2 | 3.8 ± 0.1 | 4.0 ± 0.2 | 4.0 ± 0.2 |
| Plasma Aldosterone (pg/ml) | 763 ± 70 | 1159 ± 160* | 800 ± 61 | 1315 ± 152* |
| *Urine Parameters* | | | | |
| Proteinuria (mg/mg creatinine) | 1.3 ± 0.2 | 2.2 ± 0.2* | 1.1 ± 0.1 | 2.3 ± 0.3* |
| BUN (mg/dl) | 2916 ± 398.2 | 4184 ± 538* | 2149 ± 117 | 4222 ± 422* |

Values are mean ± SE, n=5-23; *p<0.05 vs genotype control, **p=0.08 vs genotype control, †p<0.05 vs MR-intact Western




**Online Resource 4.** *Smooth muscle cell mineralocorticoid receptor knockout (SMC-MR-KO) does not prevent western diet (WD)-induced glucose intolerance in female mice.* Glucose excursions over time during a glucose tolerance test (ip, 1 g/kg) and glucose area under the curve (AUC; inset) in MR-intact and SMC-MR-KO mice fed control (Con) and WD for 16 weeks. Values are mean±SE with individual data points shown (inset), n=6/group, *p<0.05 for indicated comparison.




**Online Resource 5.** *Systemic mineralocorticoid blockade with spironolactone (Sp) prevents western diet (WD)-induced cardiac and coronary vascular dysfunction in female mice.* (**A**) Indices of cardiac diastolic function, specifically estimated left ventricular filling pressure (E/E') and early-to-late diastolic septal annulus motion ratio (E'/A'), and cardiac weights (heart weight-to-tibia length ratio; HW/TL) in Con and WD fed mice. (**B**) Vasodilator responses of isolated coronary arteries to endothelium-dependent (acetylcholine, ACh) and -independent (sodium nitroprusside, SNP) agonists. Values are mean±SE with individual data points shown (A) and sample size indicated in parentheses (B); *p<0.05 versus Con, **p<0.05 versus all other groups, ‡p<0.05 versus WD ,§p=0.08 versus WD.

*Online Resource 6.* Cardiac function outcomes by experimental group

| Parameter | **Control** | **Western** | **Western + Spiro** |
|---|---|---|---|
| Heart Rate (bpm) | 542 ± 32 | 512 ± 27 | 525 ± 9 |
| *Morphological parameters* | | | |
| SWTd (mm) | 0.82 ± 0.03 | 0.89 ± 0.09 | 0.86 ± 0.04 |
| PWTd (mm) | 0.71 ± 0.03 | 0.81 ± 0.03§ | 0.73 ± 0.05 |
| LVIDd (mm) | 3.58 ± 0.07 | 3.98 ± 0.13* | 3.55 ± 0.12† |
| LVIDs (mm) | 1.98 ± 0.16 | 2.38 ± 0.16 | 2.01 ± 0.16 |
| RWT | 0.43 ± 0.01 | 0.43 ± 0.04 | 0.45 ± 0.02 |
| LA (mm) | 1.65 ± 0.09 | 2.02 ± 0.05* | 1.97 ± 0.12* |
| Ao (mm) | 1.40 ± 0.04 | 1.49 ± 0.09 | 1.48 ± 0.04 |
| LA/Ao | 1.18 ± 0.06 | 1.39 ± 0.10§ | 1.33 ± 0.07 |
| *Diastolic parameters* | | | |
| E ($m \cdot s^{-1}$) | 531.4 ± 28.7 | 602.3 ± 14.8 | 639.2 ± 33.8 |
| E' ($m \cdot s^{-1}$) | 23.7 ± 2.5 | 19.6 ± 1.9 | 26.4 ± 2.9 |
| A' ($m \cdot s^{-1}$) | 21.5 ± 2.0 | 25.5 ± 1.9 | 27.8 ± 2.0 |
| E/E' | 23.4 ± 2.8 | 32.3 ± 3.3* | 22.2 ± 1.0† |
| E'/A' | 1.17 ± 0.10 | 0.77 ± 0.06* | 0.94 ± 0.06*‡ |
| IVRT (ms) | 12.7 ± 0.9 | 12.7 ± 0.9 | 13.0 ± 0.9 |
| *Systolic parameters* | | | |
| EF (%) | 75 ± 4 | 71 ± 3 | 76 ± 3 |
| FS (%) | 44 ± 3 | 40 ± 2 | 45 ± 3 |

SWTd, septal wall thickness-diastole; PWTd, posterior wall thickness-diastole; RWT, relative wall thickness; LVIDd, LV inner dimension-diastole; LVIDs, LV inner dimension-systole; EF, ejection fraction; FS, fractional shortening; LA, left atrial diameter; Ao, aortic diameter; A′, peak late septal annular velocity; E′, early peak septal annular velocity; IVRT, isovolumic relaxation time; E, velocity of early mitral inflow; E/E' index of LA filling pressure; MPI, myocardial performance index. Values are mean ± SE, N=4-6. *p<0.05 versus Control; †p<0.05 versus Western Diet; ‡p=0.08 versus Western Diet; §p=0.07 versus Control.

*Online Resource 7.* Cardiac function outcomes by experimental group

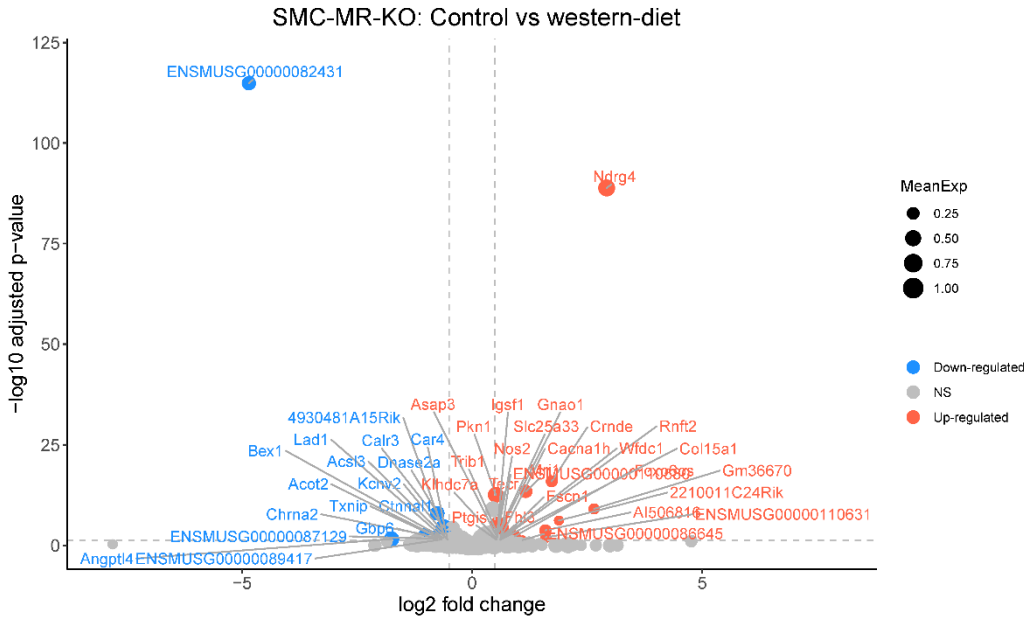
| Parameter | MR-Intact Control | MR-Intact Western | SMC MR KO Control | SMC MR KO Western |
|---|---|---|---|---|
| Heart Rate (bpm) | 423 ± 12 | 424 ± 14 | 406 ± 18 | 434 ± 16 |
| *Morphological parameters* | | | | |
| SWTd (mm) | 0.77 ± 0.03 | 0.91 ± 0.03* | 0.70 ± 0.03 | 0.81 ± 0.03‡ |
| PWTd (mm) | 0.68 ± 0.02 | 0.77 ± 0.03* | 0.70 ± 0.04 | 0.74 ± 0.03 |
| LVIDd (mm) | 3.51 ± 0.09 | 3.47 ± 0.08 | 3.49 ± 0.10 | 3.49 ± 0.08 |
| LVIDs (mm) | 2.37 ± 0.12 | 2.23 ± 0.07 | 2.41 ± 0.10 | 2.30 ± 0.10 |
| RWT | 0.42 ± 0.02 | 0.49 ± 0.01* | 0.40 ± 0.02 | 0.45 ± 0.02‡ |
| LA (mm) | 1.59 ± 0.06 | 1.74 ± 0.09 | 1.40 ± 0.07 | 1.53 ± 0.06† |
| Ao (mm) | 1.43 ± 0.09 | 1.32 ± 0.03 | 1.35 ± 0.09 | 1.31 ± 0.03 |
| LA/Ao | 1.14 ± 0.08 | 1.33 ± 0.07* | 1.05 ± 0.05 | 1.18 ± 0.04† |
| *Diastolic parameters* | | | | |
| E ($m \cdot s^{-1}$) | 477.1 ± 18.2 | 551.9 ± 16.9* | 539.2 ± 17.4 | 571.6 ± 22.9 |
| E' ($m \cdot s^{-1}$) | 19.5 ± 0.8 | 18.5 ± 1.4 | 21.0 ± 1.4 | 23.1 ± 1.9† |
| A' ($m \cdot s^{-1}$) | 16.9 ± 0.8 | 19.9 ± 1.1§ | 19.0 ± 0.9 | 20.1 ± 1.3 |
| E/E' | 25.2 ± 0.8 | 30.6 ± 1.9* | 26.3 ± 1.5 | 24.9 ± 1.6† |
| E'/A' | 1.20 ± 0.04 | 0.86 ± 0.05* | 1.12 ± 0.07 | 1.17 ± 0.08† |
| IVRT (ms) | 15.4 ± 0.7 | 16.8 ± 1.0 | 15.1 ± 1.1 | 14.2 ± 0.8 |
| *Systolic parameters* | | | | |
| EF (%) | 61 ± 2 | 66 ± 2§ | 60 ± 2 | 62 ± 2 |
| FS (%) | 33 ± 2 | 35 ± 2 | 31 ± 1 | 33 ± 1 |

SWTd, septal wall thickness-diastole; PWTd, posterior wall thickness-diastole; RWT, relative wall thickness; LVIDd, LV inner dimension-diastole; LVIDs, LV inner dimension-systole; EF, ejection fraction; FS, fractional shortening; LA, left atrial diameter; Ao, aortic diameter; A′, peak late septal annular velocity; E′, early peak septal annular velocity; IVRT, isovolumic relaxation time; E, velocity of early mitral inflow; E/E' index of LA filling pressure; MPI, myocardial performance index. Values are mean ± SE, N=9-14. *p<0.05 versus MR-Intact Control; †p<0.05 versus MR-Intact Western; ‡p<0.05 versus SMC MR KO Control; §p=0.07 versus MR-Intact Control.

*Online Resource 8.* Diameters of isolated coronary vessels by experimental group

| | Control | Western | Western + Spiro | |
|---|---|---|---|---|
| Diameter (μm) | 243 ± 4 | 257 ± 9 | 256 ± 6 | |
| | **MR-Intact Control** | **MR-Intact Western** | **SMC MR KO Control** | **SMC MR KO Western** |
| Diameter (μm) | 201 ± 10 | 226 ± 8 | 214 ± 7 | 212 ± 8 |

Values are mean ± SE, n=5-14; Diameter calculated from circumference following the normalization procedure.




**Online Resource 9.** *Western diet (WD) feeding altered cardiac gene expression in smooth muscle cell mineralocorticoid receptor knockout (SMC-MR-KO) mice.* Analysis of cardiac transcripts (13,565 transcripts) revealed differential expression (log2 fold change >0.5, corrected p<0.05) of 43 transcripts induced by WD feeding in SMC-MR-KO mice (17 downregulated, blue dots; 26 upregulated, red dots).