Supplementary information

Obesity-induced adipokine imbalance impairs mouse pulmonary vascular endothelial function and primes the lung for injury

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Running title: Obesity impairs lung endothelial cell function

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Supplementary Figure 1.  Obesity promotes endothelial cell activation in the lung.  (a) Enzyme-linked immunosorbent assay for E-selectin in the serum of lean and obese mice (n=6, *p<0.05 high fat diet (HFD) vs. normal control diet (NCD)) at baseline (BSL).  (b) Transcript levels for ICAM-1, VCAM-1 and E-selectin in the lungs of lean and obese DBA/2J mice (n=4, *p<0.05 vs. NCD group).  (c) Western blot analysis for ICAM-1, VCAM-1 and E-selectin in the lungs of lean and obese DBA/2J mice. Image is representative of two different blots. Densitometry analysis shown below (n=6, *p<0.05 vs. NCD group).  (d) Western blot analysis for VE-cadherin, β-Catenin and pSrc in the lungs of lean and obese DBA/2J mice. Image is representative of two different blots. Densitometry analysis shown on below (n=6, *p<0.05 vs. NCD group).  Data are expressed as mean ± SE. The statistical significance was assessed using a Student’s unpaired t test.
Supplementary Figure 2. Levels of circulatory factors promotes endothelial cell activation. (a-d) Serum free fatty acid, triglyceride, cholesterol, leptin and adiponectin levels in lean and obese DBA/2J mice (n=6, *p<0.05, **p<0.01 and ***p<0.001 vs. NCD group). Data are expressed as mean ± SE. The statistical significance was assessed using a Student’s unpaired t test. NCD stands for normal control diet while HFD stands for high fat diet.
Supplementary Figure 3. Obesity exacerbates LPS-induced lung inflammation and neutrophil influx. (a,b) Total and neutrophil cell counts in the BAL fluid of lean and obese DBA/2J mice at baseline and 24 h after it. LPS (n=6 each group, **p<0.01 and ***p<0.01 vs. NCD group). (c,d) Enzyme-linked Immunosorbent assay (ELISA) for TNF-α and IL-6 in lung homogenate from lean and obese DBA/2J mice at baseline and 24h after it. LPS (n=6 each group, *p<0.05, **p<0.01 and ***p<0.01 vs. NCD group). (e,f) ELISA for the chemokine KC and MIP-2 in lung homogenate from lean and obese DBA/2J mice at baseline and 24 h after it. LPS instillation (n=6 each group, *p<0.05 and ***p<0.01 vs. NCD group). Data are expressed as mean ± SE. The statistical significance was assessed using a Student’s unpaired t test and one-way analysis of variance (ANOVA). NCD stands for normal control diet while HFD stands for high fat diet.
Supplementary Figure 4. Obesity impairs endothelial junction and leads to vascular injury. (a) Transcript levels for ICAM-1, VCAM-1 and E-selectin in the lungs of lean and obese mice at 24 h after LPS administration (n=4, *p<0.05 and **p<0.01 high fat diet (HFD) vs. normal control diet (NCD) group). (b) Western blot analysis for ICAM-1, VCAM-1 and E-selectin in the lungs of lean and obese mice 24 h after LPS administration. Image is representative of two different blots. Densitometry analysis shown on right (n=6, *p<0.05 vs. NCD group). (c) Western blot analysis for VE-cadherin, β-Catenin and pSrc in the lungs of lean and obese mice 24 h after LPS administration. Image is representative of two different blots. Densitometry analysis shown on right (n=6, *p<0.05 vs. NCD group). (d,e) Total and IgM protein concentration in the BAL fluid of lean and obese DBA/2J mice (n=6 each group, **p<0.01 and ***p<0.01 vs. NCD group) at baseline (BSL) and 24 h after it. LPS. Data are expressed as mean ± SE. The statistical significance was assessed using a Student’s unpaired t test and one-way analysis of variance (ANOVA).
Supplementary Figure 5. Obese C57BL6/J mice are not more susceptible to developing ALI. (a) Intraperitoneal glucose tolerance test carried on C57BL6/J mice fed with high fat diet and normal control diet for 14 weeks. (b,c) Serum adiponectin and leptin levels in C57BL6/J mice after 14 weeks of normal control diet (NCD) or high fat diet (HFD). (d,e) Enzyme-linked Immunosorbent assay (ELISA) for TNF-α and KC in lungs of diabetic and lean mice at baseline (BSL) and 24 h after LPS administration. (f) Total cell counts in the BAL fluid of diabetic and lean C57BL6/J mice at BSL and 24h after LPS. (g,h) Total and IgM protein in the BAL fluid of diabetic and lean C57BL6/J mice at BSL and 24h after LPS. (i,j) Western blot analysis for ICAM-1, VCAM-1 and E-selectin in the lungs of lean and diabetic mice at BSL and 24 h after LPS administration. Data are expressed as mean ± SE (n=6 each group, **p<0.001 vs. NCD group). The statistical significance was assessed using a Student’s unpaired t test and one-way anova analysis.
Supplementary Figure 6.
Supplementary Figure 7.

- E selectin
- VCAM-1
- ICAM-1
- GAPDH
Supplementary Figure 8.

- p-Src
- β-catenin
- VE-cadherin
- GAPDH
Supplementary Figure 9.
Supplementary Figure 10.

- **p-Src**
  - NCD-serum
  - HFD-serum

- **β-Catenin**
  - NCD-serum
  - HFD-serum

- **VE-cadherin**
  - NCD-serum
  - HFD-serum

- **GAPDH**
  - NCD-serum
  - HFD-serum
Supplementary Figure 11.

- E-selectin
- VCAM1
- ICAM1
- GAPDH

NCD and HFD conditions are shown.
Supplementary Figure 12.
Supplementary Figure 13.

E-selectin
VCAM-1
ICAM1
GAPDH
HFD HFD
HFD +APN HFD +APN
HFD HFD
HFD +APN HFD +APN
HFD HFD
HFD +APN HFD +APN

GAPDH
Supplementary Figure 14.

- **p-SRC**
- **β-catenin**
- **VE-cadherin**
- **GAPDH**
Supplementary Figure 15.

E-selectin
VCAM1
GAPDH
ICAM1
Supplementary Figure 16.
**Supplementary Table 1.** Body weight and glucose tolerance results in AKR/J and DBA/2J mice fed a normal chow diet (NCD) or high-fat diet (HFD) for 14 weeks.

| Mice strain | Body weight | Glucose level (mg/dl) | Glucose level (mg/dl) |
|-------------|-------------|------------------------|------------------------|
|             | 0 weeks     | 14 weeks               | Fasting               | IGTT (120 mins) |
|             | NCD         | HFD                    | NCD                    | HFD           | NCD            | HFD           |
| AKR/J       | 9.31±0.45   | 9.69±0.62              | 27.32±1.84             | 42.84±1.84*** | 74.75±3.19    | 82.80±6.80    | 104 ± 5.56    | 130.2±5.17    |
| DBA/2J      | 10.67±0.37  | 10.66±0.49             | 28.55±0.57             | 41.84±1.77*** | 72.70±3.19    | 85.25±5.30    | 107.5±6.70    | 134.5±4.1     |

Data are expressed as mean ± SE. The statistical significance was assessed using a Student’s unpaired t test. (n=8, ***p<0.001 vs. NCD group)
### Supplementary Table 2. Morphometric and Echocardiographic measurement in lean and obese mice

|                        | Lean (Baseline) | Obese (Baseline) | Lean (LPS 4 h) | Obese (LPS 4 h) |
|------------------------|-----------------|------------------|----------------|-----------------|
| Body weight, g         | 28.50 ± 0.58    | 45.75 ± 0.36**   | 28.40 ± 0.50   | 46.15 ± 0.30**  |
| Whole heart weight, mg | 129.1 ± 2.4     | 142.8 ± 4.8*     | 129.4 ± 3.2    | 144.4 ± 5.21*   |
| Left ventricle, mg     | 92.68 ± 1.8     | 103.5 ± 3.1      | 94.5 ± 2.2     | 105.38 ± 3.6    |
| Right ventricle, mg    | 23.18 ± 1.1     | 33.88 ± 1.5*     | 26.33 ± 1.4    | 35.24 ± 2.4     |
| % EF                   | 69.73 ± 7.58    | 64.28 ± 8.20     | 62.72 ± 7.34   | 59.22 ± 7.77    |
| %FS                    | 44.70 ± 4.94    | 39.12 ± 5.97     | 40.46 ± 6.14   | 37.12 ± 5.10    |
| LV Mass (mg)/BW (g)    | 3.67 ± 1.46     | 2.78 ± 0.35      | 3.58 ± 1.52    | 3.28 ± 0.75     |

Data are expressed as mean ± SE. The statistical significance was assessed using a Student’s unpaired t test. (n=8, *p<0.05 and **p<0.01 vs. lean mice group stands for mice fed with normal control diet (NCD) and obese mice group stands for mice fed with high fat diet (HFD).
Supplementary Table 3. Right and left heart catheterization performed in baseline and 4h after LPS injection in lean and obese mice

|                        | Baseline                  | LPS 4 h                  |
|------------------------|---------------------------|--------------------------|
|                        | Right Heart               | Left Heart               | Right Heart | Left Heart |
|                        | lean          | obese | lean    | obese    | lean      | obese | lean | obese |
| Heart rate, beats/min  | 396±20        | 375±26 | 378±20 | 365±25   |
| CVP, mmHg              | 3.6±0.2       | 4.1±0.4 | 3.4±0.35 | 4.0±0.48 |
| Systolic BP, mmHg      | 27.2±1.7      | 31.1±1.1 | 107.1±1.9 | 108.9±4.3 |
| Diastolic BP, mmHg     | 2.9±0.2       | 3.4±0.3 | 4.6±0.5 | 5.4±0.5 |
| Aortic systolic, mmHg  | 105.9±4.1     | 97±0.5 | 95.5±3.7 | 93.3±6.4 |
| Aortic diastolic, mmHg | 69±5.8       | 63±1.3 | 66.4±4.1 | 63.7±5   |
| MAP mmHg               | 81.3±5.1      | 74.4±4.1 | 76.1±3.9 | 73.6±5.5 |
| Max dp/dt, mmHg/s      | 1,446±246     | 1,813±89 | 8,764±273 | 9,073±408 |
| Min dp/dt, mmHg/s      | 1,128±209     | 1,479±182 | 5,906±390 | 6,887±681 |

Data are expressed as mean ± SE. The statistical significance was assessed using a Student’s unpaired t-test.
**Supplementary Table 4.** Complete blood counts in AKR/J mice fed a normal chow diet (NCD) or high-fat diet (HFD) for 14 weeks.

| Parameters    | Lean                        | obese                  |
|---------------|-----------------------------|------------------------|
| **Leukocytes**|                             |                        |
| WBC (K/µl)    | 8.077±0.921                 | 11.47±1.074*           |
| NE (K/µl)     | 1.053±0.151                 | 2.085±0.296**          |
| LY (K/µl)     | 6.220±0.681                 | 8.328±0.545*           |
| MO (K/µl)     | 0.380±0.049                 | 0.490±0.049            |
| **Erythrocytes**|                            |                        |
| RBC (M/µl)    | 8.962±0.672                 | 9.963±0.554            |
| Hb (g/dl)     | 12.28±0.470                 | 13.02±0.449            |
| **Thrombocytes**|                            |                        |
| PLT (K/µl)    | 820.8±43.88                 | 905.3±44.93            |

Data are expressed as mean ± SE. The statistical significance was assessed using a Student’s unpaired t test. (n=8, *p<0.05 and **p<0.01 vs. lean group). WBC, NE, LY, MO, RBC, Hb, PLT stand for white blood cell, neutrophil, lymphocyte, monocyte, red blood cell, hemoglobin and platelets, respectively.