Adiponectin deficiency: a model of pulmonary hypertension associated with pulmonary vascular disease

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**Abstract:** Adiponectin (APN) is an adipocyte-derived factor that exists at high concentrations in serum and has anti-inflammatory, and systemic vascular-protective properties. In this study, we investigated APN’s role in pulmonary vascular homeostasis. We found that APN localizes to the luminal side of blood vessels in lung and acts *in vitro* to block TNF-α-induced E-selectin upregulation in pulmonary artery endothelial cells. Targeted deletion of the APN gene in mice leads to a vascular phenotype in lung characterized by E-selectin upregulation and age-dependent increases in peri-vascular inflammatory cell infiltration and pulmonary arterial pressures. Taken together, these findings demonstrate an important role for APN in lung vascular homeostasis, and suggest that APN deficient states may contribute to the pathogenesis of inflammatory pulmonary vascular disease and to the development of pulmonary hypertension.
Introduction:

Pulmonary arterial hypertension (PAH) is a clinical condition associated with sustained elevations in pulmonary arterial pressure, eventually leading to right ventricular failure and death. PAH can develop as a primary disorder, but often occurs in association with other conditions such as congenital heart disease and liver disease, as well as with various inflammatory conditions, including connective tissue disease and HIV infection.³

Adiponectin (APN) is an adipocyte-derived protein that is abundantly present in plasma.⁵;¹⁷;²³ Circulating APN levels are high in lean healthy individuals, but levels are reduced in individuals with increased body mass index, insulin resistance and various cardiovascular disease states.¹ Interest in APN relates to its ability to regulate diverse biological processes in a wide range of tissues in experimental models.⁴;¹³;¹⁸;¹⁹;²² APN was initially recognized as an insulin sensitizing agent; however, recent findings support a role in vascular homeostasis and inflammation.¹⁷;¹⁸ For example, APN has been shown to activate nitric oxide synthase (eNOS) and upregulate prostaglandin I₂ synthase production, and downregulate expression of select adhesion molecules in endothelial cells.⁷;¹²;¹⁴;²⁰ Furthermore, APN appears important in the regulation of systemic vascular tone as APN -/- mice are more susceptible to salt-sensitive hypertension, and APN supplementation leads to a sustained decrease in blood pressure in these mice.¹³ Recent reports also demonstrate that APN protects tissues against ischemic injury. For example, APN -/- mice are more susceptible to ischemia-induced myocardial and cerebral injury, and over-expression of APN reduces tissue damage in these models.¹²;¹⁴ The protective affects of APN are mediated, at least in part, by its ability to block inflammation and
stimulate nitric oxide production via an AMPK-dependent pathway.\textsuperscript{20}

To date, studies investigating APN’s role in vascular homeostasis have largely been restricted to the systemic circulation; however, two recent reports indicate that APN deficient mice develop an abnormal pulmonary vascular phenotype when placed in chronic hypoxic conditions or exposed to chronic allergic airway inflammation.\textsuperscript{9,11} In this study, we show that APN protein localizes throughout the pulmonary vascular endothelium, and that deficiency in APN leads to upregulation of E-selectin in lung. Moreover, APN -/- mice develop a spontaneous pulmonary vascular phenotype that is characterized by an age-dependent increase in peri-vascular inflammatory cell infiltration and elevated pulmonary artery pressures. Together, these observations provide further support for APN’s role as a regulator of pulmonary vascular homeostasis, and suggest for the first time that chronically low levels of APN may be associated with development of pulmonary vascular disease.

Methods:

Mice: APN -/- mice were generated by targeted gene disruption as previously described.\textsuperscript{20} All studies were performed using male mice. Age-matched wild-type C57/BL6 mice were purchased from Jackson Laboratories (Bar Harbor, Maine). Mice were maintained in 12-hour light, 12-hour dark schedule and given food and water \textit{ad libitum}. Euthanasia was performed by isoflurane anesthesia followed by cervical dislocation. Heart, and body weight, and tibial length were recorded in all mice (Table 1). All animal studies were conducted according to protocols approved by the National
Lung fixation: Lung fixation was performed after flushing the vasculature with saline. A blunt 22-gauge needle inserted into the trachea, and perfused lungs were inflated using 4% paraformaldehyde. Tissue was dissected en-block from the thoracic cavity, and fixed overnight at 4 C. Lung tissue was dehydrated in graded alcohols and embedded in paraffin blocks. Five-micrometer tissue sections were cut, and placed on charged slides for immuno-histochemistry staining.

Histochemistry. Tissue sections were deparaffinized and re-hydrated through graded alcohol to water. Immuno-fluorescent staining for E-selectin and alpha smooth muscle actin was performed immediately after quenching tissue sections with sodium borohydride for 20 min and blocking with 1% goat serum. E-selectin antibody staining was performed using a rat polyclonal antibody directed against mouse E-selectin (Santa Cruz Biotech, Santa Cruz, CA) at dilution of 1:25. The primary anti–alpha smooth muscle actin mAb was obtained from Sigma-Aldrich and was already FITC conjugated. Secondary staining for E-selectin was performed using a biotin-conjugated goat anti-rat antibody (Vector Laboratories, Burlingame, CA) at dilution of 1:100 followed by a streptavidin-FITC (BD Pharmingen, San Jose, CA) step at a dilution of 1:100. In parallel, isotype control staining was performed. Upon completion of staining, slides were washed in PBS and counterstained using the nuclear dye 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma-Aldrich, St Louis, MO).
APN and CD45 staining were performed using DAB-substrate detection methods. Slides were initially quenched with 3% H2O2 and blocked with 1% goat serum. Staining for APN was performed using a monoclonal rat anti-mouse APN (R&D Systems, Minneapolis, MN) at dilution of 1:400. Staining for CD45 was performed using a monoclonal rat anti-mouse CD45 (BD Biosciences, San Jose, CA) at dilution of 1:40. Secondary staining was performed using a biotin-conjugated goat anti-rat antibody (Vector Laboratories, Burlingame, CA) at dilution of 1:100. Slides were then treated with ABC reagent (Vector Laboratories, Burlingame, CA) and developed using diaminobenzidine (DAB) for 1-3 minute (Vector Laboratories, Burlingame, CA). Upon completion of staining, slides were washed in PBS and counterstained.

*Morphometry and quantitative assessment of vascular inflammation:* Peri-vascular inflammation was assessed in tissue sections after staining for the pan-hematopoietic marker CD45. Images were captured at 40x using a Zeiss N HBO100 microscope (Thornwood, NY) fitted with an AxioCam MR CCD digital camera. A total of 80-100 blood vessels ranging from 150-300 micrometers were analyzed using the Axiovision 3.1 software program. The number of peri-vascular CD45 positive inflammatory cells was compared in aged matched WT and APN -/- mice. Peri-vascular inflammatory cells were defined as the number of CD45 expressing cells present outside the vascular lumen, but juxtaposition to the blood vessel wall.

Smooth muscle thickness was measured in fully muscularized blood vessels captured in cross-section after staining for alpha smooth muscle actin. The thickness of the smooth
muscle layer (the transverse diameter) was assessed at two sites along the blood vessel, and at least fifty blood vessels of similar size (150–200 μm) were analyzed per group.

**Cell culture:** Human pulmonary artery endothelial cells (HPAEC) were grown to confluence in EBM-2 media containing 10% FBS and growth supplements (Clonetics, San Diego, CA). All experiments were performed using cells at passage 6–10. E-selectin expression was determined in HPAEC pre-treated with APN (10 μg/ml) or vehicle for 18h followed by 6h-stimulation with TNF-α. E-selectin mRNA levels were quantified in RNA extracts. Recombinant human adiponectin was obtained from BioVendor (Candler, NC). Adiponectin concentration in supernatant was measured by ELISA (R&D Systems, Minneapolis, MN).

**Real-time PCR:** RNA was isolated from HPAEC. cDNA was generated from RNA extracts by using a reverse transcription kit (Promega, Madison, WI). Quantitative expression of E-selectin was performed with 0.5 mcg of starting total RNA. Values were calculated based on the ΔΔ CT method. Human E-selectin levels were expressed relative to internal control gene 36B4 levels.

**Western blot:** Lung tissue was homogenized with mortar and pestle and incubated in buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, and 150 mM NaCl in the presence of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin A) for 30 min at 4°C. Lysates were sheared by passing through a 21-gauge needle before centrifugation. Protein concentration was
measured in the supernatant using the Bradford assay. For analysis, 20 µg of protein was separated through a 10% SDS-polyacrylamide gel before electrophoretic transfer to a nitrocellulose membrane. Membranes were blocked with 2% bovine serum albumin (BSA) in Tris-buffered saline (TBS) for 1 h. Primary antibody staining (E-selectin, 1:2500) was performed overnight in TBS with 0.1% Tween 20 (TBST) and 2% BSA. Mixture was incubated overnight at 4°C. Secondary antibody staining [anti-rabbit-horse-radish peroxidase (HRP), dilution 1:6,000] was performed for 1 h at room temperature. Membranes were then washed three times with TBST. Chemiluminescence was performed with the Western Lighting detection reagent (Perkin Elmer, Waltham, MA).

**Hemodynamics:** 1 year-old mice were anesthetized with isoflurane (1.5% - 2.5%). The right jugular vein and left carotid artery were isolated by standard cutdown approach. Hemodynamic measurements were obtained from the right ventricle, ascending aorta and left ventricle using a 0.8 F catheter (Millar Instruments, Houston, TX). Measurements include heart rate (HR), systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, right and left ventricle end diastolic pressure, max dp/dt, min dp/dt and tau.

**Echocardiography:** Transthoracic echocardiography was performed using a Vevo 770 High Resolution Imaging System with 30-MHz RMV-707B scanning head (VisualSonics Inc. Toronto, Ontario, Canada). Mice were anesthetized using 2% isoflurane and heart rate was maintained at 400-500 beats per minute on a thermal heated plate. Left ventricle dimension, percent fractional shortening (%FS), left ventricular mass index (LVMI),
anterior wall thickness (AWT), posterior wall thickness (PWT), and relative wall thickness (RWT) were calculated as previously described. Right ventricular imaging was obtained by short-axis transverse view at the level of aortic valve, and right ventricle diameter was measured at end-diastole. The peak velocity and acceleration time (PA-ACT) were measured by pulse-wave Doppler at the level of pulmonary artery root. Early filling wave (E), late filling wave (A) and the ratio of E to A was calculated using transmitral pulse-wave doppler velocity. For measurement of transmitral flow velocity, heart rates were maintained at less than 400 beats per minute in order to separate the E and A waves.

Statistical analysis: All data are presented as mean +/- standard error. Statistical analysis was performed using 2-tailed unpaired Student’s t test. Statistical significance was achieved when p<0.05 at 95% confidence interval

Results:

*APN localizes to pulmonary vascular endothelium.* APN protein was examined in paraffin-embedded lungs sections using a polyclonal antibody directed against mouse APN. Findings demonstrate that APN was present at peri-vascular sites in lung (Figure 1 A,B). As expected, APN staining was not detected in lungs of APN +/- mice (Figure 1 C) or in tissue sections stained with isotype control antibody (Figure 1 D). Confocal
microscopy further localized APN protein expression to the luminal surface of the pulmonary vascular endothelium (Figure 1E).

Based on the peri-vascular distribution of APN we examined whether deficiency leads to a vascular phenotype in lung. Histological comparison of blood vessels in 3-month old WT and APN -/- mice revealed no significant structural differences; however, a mild increase in the number of peri-vascular inflammatory cells was visualized (Figure 2). To quantify differences, WT and APN -/- lungs were immuno-stained with an antibody directed against the pan-hematopoietic marker CD45. Statistical analysis confirmed that peri-vascular inflammatory cell number was increased in lungs of APN -/- mice (Figure 2E). Consistent with an inflammatory vascular phenotype, we found that E-selectin expression (Figure 3) was increased, and wet-to-dry ratios (Supplemental figure 1) were slightly, but significantly increased in lungs of APN -/- mice at this time point.

To corroborate the observation that APN regulates E-selectin expression in endothelial cells, HPAEC were cultured in presence or absence of physiological levels of APN protein (10 µg/ml) and stimulated with TNF-α (100 pg/ml) for 6 h. Of note, adiponectin was not detected in media of HPAEC in standard culture conditions (data not shown). As expected, TNF-α stimulation led to robust upregulation of E-selectin transcript in HPAEC. Pre-treatment with APN resulted in a marked reduction in TNF-α induced E-selectin expression (Figure 4).
APN deficiency leads to an age-dependent pulmonary vascular phenotype. To determine whether prolonged deficiency in APN leads to more noticeable phenotype detailed histological examination of the pulmonary vascular system was performed in 1-year old mice. In comparison to young mice, a progressive inflammatory vascular phenotype was observed at 1 year. As shown in Figure 5, peri-vascular mononuclear cells were observed in WT and APN -/- lungs at 1 year; however, inflammatory infiltrates were significantly more apparent in APN -/- mice. In addition, proteinaceous exudates were observed in the peri-vascular space of medium and large sized blood vessels in APN -/- mice, and E-selectin was noted to be upregulated. Proteinaceous exudates and E-selectin expression was not detected in lungs of aged matched WT mice. Together, these findings indicate that prolonged adiponectin deficiency leads to a progressive inflammatory vascular phenotype.

APN deficiency leads to an age-dependent increase in pulmonary artery pressures. Given the link between pulmonary vascular inflammation and PAH in certain patient populations, we speculated whether APN deficiency might lead to increased pulmonary artery pressures. Non-invasive screening for elevated pulmonary artery pressures was performed by transthoracic echocardiography in age-matched WT and APN -/- mice. Consistent with the mild histological phenotype observed at 3 months, no significant differences in right ventricular dimensions or pulmonary arterial hemodynamics were seen between WT and APN -/- mice at this time point (Supplemental table 1). In contrast, RV dimensions (Table 2) measured by transthoracic echocardiography were significantly increased in APN -/- mice (1.93+/−0.02 vs 2.25+/−0.03, p<0.001) at 1 year. Furthermore,
invasive hemodynamic measurements demonstrated that RVSP, RVDP, mean RVP, and max dp/dt were significantly increased in APN -/- mice at this time point (Table 3). These observations indicate that pulmonary artery pressure, as reflected by right ventricular dimensions and pressure, increases in the setting of chronic APN deficiency. In contrast, non-invasive and invasive measurements of left heart function (systemic blood pressure, cardiac output, and LV function) were not significantly different in WT and APN -/- mice at 1 yr; except for the observed increase in LVEDP in APN -/- mice.

Discussion:

The major finding of this study is that APN deficiency leads to an inflammatory vascular phenotype in lung. To our knowledge, this study is the first to indicate a direct role for APN in pulmonary vascular homeostasis, and the first to suggest that prolonged APN deficient states may contribute to the development of pulmonary vascular disease, including pulmonary hypertension. While it has recently been reported that APN deficiency can give rise to pulmonary hypertension in conjunction with hypoxia and chronic airway inflammation, our study shows that APN deficiency per se is sufficient to produce pulmonary hypertension in aged mice. Furthermore, the PAH that develops in 1 yr old APN deficient mice may be a consequence of the perivascular inflammation that appears in young mice and progresses over time.

Our findings show that mononuclear cells accumulate around pulmonary blood vessels in APN -/- mice by three months of age, and this phenotype is more notable when mice are 1 yr old. In addition, we demonstrate by invasive hemodynamic monitoring that
pulmonary artery pressure is elevated in older APN −/− mice. It is well-recognized that there is a link between several inflammatory conditions, such as HIV infection and connective tissue disease, and PAH. Furthermore, inflammation is commonly observed in pathological specimens of patients with other forms of PAH. For example, lymphocytes, macrophages, mast cells and antibody-complement deposits have been observed within plexiform lesions in patients with PAH. Moreover, auto-antibodies and elevated levels of various cytokines and chemokines have been observed in the plasma of patients with PAH. However, despite evidence supporting an association between immune dysregulation and PAH, a causal association has yet to be established. Based on our findings, we speculate that APN may be a contributory molecule in the linkage between inflammation and PAH.

APN was first identified as an insulin-sensitizing agent, but is now recognized for its anti-inflammatory activities. For example, epidemiological studies show that serum APN levels are decreased in patients with chronic inflammatory conditions, and that levels inversely correlate with circulating concentrations of pro-inflammatory markers like C-reactive protein (CRP), TNF-α, and interleukin 6 (IL-6). Consistent with these observations, APN deficiency in mice is associated with greater elevations in inflammatory cytokines and autoantibody production in a model of autoimmune disease.

APN has anti-inflammatory actions on a variety of cell types. We and others have demonstrated that APN blocks LPS-induced TNF-alpha production in macrophages, and
downregulates NF-κB activation. Furthermore, we recently showed that APN acts in lung to suppress alveolar macrophage activation, and that APN deficiency is associated with an emphysema-like phenotype. Emphysema develops in these mice, at least in part, from damage caused by excess alveolar macrophage pro-inflammatory cytokine production. Relevant to the current study, APN also suppresses inflammation in non-immune cell types. For example, APN blocks agonist-induced IL-8 production in endothelial cells, and down-regulate reactive oxygen species production. APN’s reported action on endothelial cell types further supports the findings observed in this study.

Another finding of the current study is that APN localizes to the luminal side of blood vessels in lung, and regulates E-selection expression in pulmonary vascular endothelial cells. These observations are consistent with published reports showing that APN acts on the systemic circulation to down-regulate E-selectin expression and inhibit leukocyte-endothelial cell interactions. E-selectin is normally expressed at very low levels in the pulmonary circulation, but increases in response to lung injury. Furthermore, increased expression has been reported in the vascular endothelium of patients with PAH. Thus, it is tempting to speculate that low APN levels may account for the observed increase in E-selectin expression in these patients.

Although our findings indicate that APN -/- mice develop PH, we recognize that some histological features were absent in this model. For example, pulmonary artery smooth muscle thickness was not significantly increased in 1 year old APN mice (Supplemental figure 2). We speculate that this relates to the fact that pulmonary artery pressures were
only modestly elevated, and that smooth muscle hyperplasia is not a prominent feature of murine models of PH. We acknowledge that histological evidence of smooth muscle hypertrophy might be present at later time points.

While our findings suggest that chronic inflammation leads to the development of PAH, we recognize that other mechanisms may be contributing. For example, we have previously reported that APN -/- mice develop an emphysema-like phenotype. Thus, it is possible that pulmonary artery hemodynamics could have been influenced by this phenotype. In addition, we observed an increase in LVEDP and left ventricular wall thickness in APN -/- mice. These findings suggest that diastolic left ventricular dysfunction may also spontaneously develop with age in APN -/- mice; however, hemodynamic (min dp/dt, tau) and echocardiogram (E/A) measurements do not support this alternative hypothesis.

In summary, our findings demonstrate a role for APN in lung vascular homeostasis, and suggest that APN deficiency may directly contribute to the pathogenesis of inflammatory pulmonary vascular disease. We believe these data provide further rationale for investigating APN’s role in animal models of pulmonary hypertension and in patients with pulmonary vascular disease.

**Acknowledgements:** This work was supported by National Institute of Health Grants K08 HL077138, AG15052, HL-59215 Gilead Sciences Research Scholars Program in Pulmonary Arterial Hypertension.
Figure Legend:

Figure 1: Adiponectin (APN) localizes to vascular sites in lung. A,B.) Representative sections showing that APN protein (brown stain) localizes to blood vessels (v) in lung. Staining is absent in proximal (AW) and distal airway structures. APN staining is not detected in lung sections of APN deficient mice (C), and in wild-type lung sections stained with isotype control antibody (D). E) Confocal microscopic analysis of APN protein localization was detected on the luminal side of blood vessels in lung where it co-localized with the vascular endothelium. APN (green) co-localizes to F-actin (red) on the endothelial side of the blood vessel wall.

Figure 2: Peri-vascular inflammation is present in lungs of 3 month old adiponectin (APN) deficient mice. CD45 staining (brown) was performed in wild-type (WT) and APN deficient lung sections. A,B) Low and high power images of WT lungs, respectively. C,D) Low and high power images of APN deficient lung sections. E) Bar graph showing the average number of perivascular CD45 positive cells/blood vessel (40x) in WT and APN deficient lungs (* p < 0.05).

Figure 3: E-selectin is upregulated in pulmonary vascular endothelium of 3 month old adiponectin (APN) deficient mice. E-selectin staining is not detected by immunohistochemistry in lungs of wild-type (WT) mice (A), but staining is present throughout the endothelium of APN deficient mice (B). Staining was not detected in APN deficient sections stained with isotype control antibody (data not shown). C) Western blot showing E-selectin concentration in WT and APN deficient lungs. D) Bar graph showing relative E-selectin concentration when controlled for GAPDH.

Figure 4: Adiponectin (APN) blocks TNF-induced E-selectin up-regulation in human pulmonary artery endothelial cells (HPAEC). HPAEC were pretreated with APN for 18 hours followed by stimulation with TNF-alpha for six hours. Data are expressed as E-selectin mRNA levels relative to internal control gene 36B4 levels (n = 5, * p < 0.05).

Figure 5: Peri-vascular inflammation increases with age. CD45 staining (brown) at 1 year demonstrates the presence of peri-vascular inflammatory cell infiltrates in wild-type (WT) and adiponectin (APN) deficient lungs. Mild inflammation is observed in lungs of WT mice. A.,B.) Low and high power images, respectively. Peri-vascular inflammation is readily apparent in lungs of APN deficient mice. C,D) Low and high power images, respectively. E) Bar graph showing the average number of perivascular CD45 positive cells/blood vessel (40x) in WT and APN deficient lungs (* p < 0.05).

Figure 6: Inflammatory vascular phenotype is present in lungs of 1 year old adiponectin (APN) deficient mice. E-selectin expression is detected in lungs of 1 year old APN deficient mice (B), but not in lungs of aged matched WT mice (A). Peri-vascular inflammation and proteinaceous exudates are readily apparent in lungs of 1 year old APN deficient mice (E, low power, F, high power view). Similar findings were not observed in lungs of 1 year old WT mice (C, low power, D, high power view).
Table 1: Morphometric data obtained from wild-type and adiponectin deficient mice at 1 year of age.

<table>
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<tr>
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<th>WT 1Y</th>
<th>APN -/- 1Y</th>
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<tr>
<td>Body weight (g)</td>
<td>37.5 ±1.5</td>
<td>47.7 ±1.5**</td>
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<td>Tibial length (mm)</td>
<td>17.7 ±0.1</td>
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<td>Whole heart weight (mg)</td>
<td>142.6 ±4.6</td>
<td>164.5 ±4.4**</td>
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<td>LV+SW (mg)</td>
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<td>RV weight (mg)</td>
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<td>33.6 ±1.05**</td>
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<td>RV/LV + S</td>
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<td>0.26 +/- 0.009*</td>
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<td>LV + Sept/TL (mg/mm)</td>
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<td>7.5 ±0.18**</td>
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<td>RV/TL (mg/mm)</td>
<td>1.40 ±0.03</td>
<td>1.85 ±0.11**</td>
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Values are mean ±SEM, *p<0.05, **p<0.01 vs control mice
LV left ventricle, SW septal weight, RV right ventricle, TL tibial length.
# Cardiac Echocardiographic Measurements

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<th>APN +/- 1Y</th>
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<tr>
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<tr>
<td>RVDd (mm)</td>
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<td>LVDD (mm)</td>
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<td>PA ACT (msec)</td>
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<td>AWT (mm)</td>
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<td>LVMI/TL</td>
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Values are mean±SEM, *p<0.05, **p<0.01 vs control

RVDd Right ventricular dimensions, LVDD Left ventricular dimensions, PA ACT Pulmonary artery acceleration time, AWT anterior wall thickness, PWT posterior wall thickness, FS fractional shortening, RWT relative wall thickness, TMF E/A transmirtal inflow peak early (E) and late (A) diastolic flow velocities, LVMI Left ventricular mass index, TL tibial length

Table 2: Echocardiographic measurements in 1yr wild-type and adiponectin-deficient mice. The numbers reported represent the mean and the standard error (S.E.), * p< 0.05, ** p< 0.01.
**Table 3**: Right and left heart catherization performed in 1yr wild-type and adiponectin-deficient mice. The numbers reported represent the mean and the standard error (S.E.), *p* < 0.05, **p** < 0.01.
Reference List


Figure 1

A

B

C

D

E

Vessel
lumen

F-actin
Adiponectin
Figure 2

A

B

C

D

E

CD45+ cells per blood vessel

0  10  20

WT  APN -/-
Figure 3

Wild type

APN -/-

A

B

C

D

WT APN -/-

0 1 2 3 4 5 Relative Concentration

Relative Concentration

WT APN -/-
Figure 4

Relative Expression E-selectin

Vehicle  TNF-a  TNF-a + APN
Figure 5
Figure 6