Cardiac Metabolic Alterations in Hypertensive Obese Pigs

Xin Zhang,* Zi-Lun Li,* Alfonso Eirin, Behzad Ebrahimii, Aditya S. Pawar, Xiang-Yang Zhu, Amir Lerman, Lilach O. Lerman

Abstract—Obesity and hypertension are major risk factors for cardiovascular diseases, and their growing coexistence accounts for an increase in adverse cardiac events, but the mechanisms are yet to be determined. We hypothesized that obesity exacerbates mitochondrial dysregulation imposed by hypertension and augments left ventricular dysfunction. Obesity-prone Ossabaw pigs were randomized to lean (standard diet) and obese (high-fat diet), without (Lean-sham and Obese-sham) or with renovascular hypertension (Lean-hypertension and Obese-hypertension), induced after 12 weeks of diet (n=7 each). Cardiac function, myocardial perfusion and oxygenation, and microvascular remodeling were assessed 4 weeks later. Mitochondrial biogenesis signals and structural proteins, respiratory chain complex activities, and mitochondrial self-degradation were examined, as was fibrosis. Obesity alone exerted no apparent effect on mitochondrial dynamics, but aggravated in hypertensive hearts the reduction of mitochondrial proteins, deoxyribonucleic acid content, and respiratory chain complex IV subunits activity, and amplified mitochondrial self-degradation. Synergistic interaction of obesity with hypertension also exacerbated myocardial fibrosis and left ventricular diastolic dysfunction. Mitochondrial content, respiratory chain complex IV subunits activity, and mitophagy were correlated with myocardial fibrosis. These findings suggest that obesity aggravates in renovascular hypertension cardiac mitochondrial aberrations. Mitochondrial function may regulate the progression of cardiac injury and functional deterioration in hypertension concomitant with obesity. (Hypertension. 2015;66:430-436. DOI: 10.1161/HYPERTENSIONAHA.115.05478.) • Online Data Supplement

Key Words: hypertension ■ mitochondrial turnover ■ obesity ■ renal artery stenosis

Obesity remains prominent among public health concerns. According to recent national estimates, 16.9% of youth and 34.9% of adults are obese.1 Obesity has been shown to have adverse effects on the cardiovascular system, promote atherosclerotic plaques,2 and worsen outcomes in patients with coronary artery disease.3 Obesity can also induce cardiac remodeling,4 particularly left ventricular (LV) hypertrophy and diastolic dysfunction,5 which is directly related to mortality.6

The mechanisms by which obesity induces cardiac injury are yet to be fully determined. Inflammation and oxidative stress because of increased fatty acid substrates have been regarded as common pathogenic factors. Recent studies have also linked cardiac metabolism to obesity-related cardiac alterations. As a result of increases in circulating fatty acids and insulin resistance that commonly accompany obesity, the myocardium is exposed to excessive nutrient substrates. Nevertheless, energy production becomes less efficient, as overloaded mitochondria undergo stress and develop dysfunction.7,8 High-fat diet has been shown to decrease mitochondrial biogenesis, reduce mitochondrial coupling efficiency, and impair adenosine triphosphate synthesis.9-11 These findings suggest that mitochondrial homeostasis can be modulated by energy supply and potentially impact cardiac health and function.

Hypertension is one of the most common causes of LV hypertrophy, and more prevalent in obese individuals than in the lean population.12 Particularly, renovascular hypertension (RVH), a common cause of secondary hypertension because of renal artery stenosis, accelerates LV remodeling,13 driven by the activated inflammation and renin–angiotensin system in response to decreased renal blood supply distal to the stenosis.14 We have recently shown that the RVH heart is characterized by attenuated myocardial mitochondrial biogenesis and by enhanced mitophagy.15 However, whether concurrent obesity affects mitochondria integrity in the hypertensive heart remains unclear. We hypothesized that coexistence of obesity would exacerbate myocardial mitochondrial dysregulation and fibrosis, and magnify LV diastolic dysfunction in RVH pigs.

Methods
Littermate Ossabaw pigs were randomized to lean (standard chow) and obese (high-fat diet), without (Lean-sham and Obese-sham) or with
RVH secondary to unilateral renal artery stenosis (Lean-hypertension and Obese-hypertension), induced after 12 weeks of diet (n=7 each). Cardiac structure, function, and oxygenation were studied with multidetector computed tomography and blood-oxygen-level-dependent-magnetic resonance imaging. The myocardium was examined ex vivo for indices of mitochondrial biogenesis and function, and tissue damage (detailed descriptions of all experimental methods are included in the online-only Data Supplement).

**Results**

**Animal Systemic Characteristics**

Diet increased body weight, total cholesterol, and low-density lipoprotein in Obese pigs compared with Lean (Table 1), as well as intra-abdominal and pericardial fat deposition (Figure S1A–S1D in the online-only Data Supplement). Basal homeostasis model assessment–insulin resistance index was also increased in both the obese groups, indicating insulin resistance (Table 1). Renal artery stenosis was similarly established in hypertensive Lean and Obese pigs (87.9±4.9% versus 85.6±4.7%; P>0.10), increasing mean arterial pressure, which was further elevated in Obese-hypertension. Hypertension tended to increase and obesity increased plasma renin activity, which resulted in elevation of plasma renin activity only in Obese-hypertension (Table 1), suggesting additive effects by coexistence of obesity and hypertension. Hypertension-elevated plasma tumor necrosis factor-α level in the both groups, and interacted with diet to markedly increase sE-selectin levels only in Obese-hypertension (Table 1).

**Cardiac Function and Hemodynamics**

Hypertension raised LV muscle mass, but the increase reached statistical significance only in Obese-hypertension (Table 1). Obese-hypertension also suppressed E/A ratio, indicating exacerbating impact of early obesity on hypertension to induce LV diastolic dysfunction. Hypertension increased heart rate in both groups compared with their sham groups, and elevated cardiac output in Obese-hypertension compared with Obese-sham. Hypertension also increased rate pressure product in both groups and to a greater extent in Obese-hypertension (Table 1), indicating increased LV workload, whereas myocardial oxygenation was similarly decreased in both hypertension groups (elevated R2*, Figure 1A and 1D). Stroke volume and ejection fraction remained unchanged. Although basal myocardial perfusion was unaltered, its response to adenosine was blunted by diet and hypertension, and synergistically further suppressed in Obese-hypertension (Figure 1E and 1F). Diet also interacted with hypertension to increase media/lumen ratio and down-regulate endothelial nitric oxide synthase expression only in Obese-hypertension (Figure S2A, S2C, S2D, and S2F). These results suggest that coexistence of obesity and hypertension precipitates diastolic LV dysfunction, accompanied by microvascular remodeling and dysfunction, which were subtle in hypertension or diet alone.

**Mitochondrial Dynamics**

The mitochondrial phospholipid cardiolipin decreased in all experimental groups, particularly by the synergistic

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**Table 1. Characteristics (mean±SEM) of Lean or Obese Pigs With or Without HT (n=7 each group)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham</th>
<th>HT</th>
<th>P Value for 2-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight, kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>31.4±4.3</td>
<td>45.0±5.7*</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>35.7±2.1</td>
<td>45.3±1.1†</td>
<td></td>
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<tr>
<td></td>
<td>MAP, mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Lean</td>
<td>108.0±7.1</td>
<td>119.3±5.8</td>
<td>0.01 0.30 0.52</td>
</tr>
<tr>
<td>Obese</td>
<td>122.6±4.4*</td>
<td>137.2±5.5†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>61.3±2.6</td>
<td>67.0±7.0</td>
<td>0.54 0.018 0.87</td>
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<tr>
<td>Obese</td>
<td>76.8±5.4*</td>
<td>84.5±6.4*‡</td>
<td></td>
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<td></td>
<td>RPP, mmHg×bpm</td>
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<tr>
<td>Lean</td>
<td>83.4±7.5</td>
<td>91.9±10.6</td>
<td>0.24 0.002 0.017</td>
</tr>
<tr>
<td>Obese</td>
<td>115.7±8.3*</td>
<td>137.7±9.7†</td>
<td></td>
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<tr>
<td></td>
<td>Total cholesterol, mg/dL</td>
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<tr>
<td>Lean</td>
<td>97.0±2.9</td>
<td>388.0±48.1*</td>
<td>&lt;0.001 0.81 0.58</td>
</tr>
<tr>
<td>Obese</td>
<td>85.4±5.0</td>
<td>346.7±40.6†</td>
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<td>LDL, mg/dL</td>
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<td></td>
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<tr>
<td>Lean</td>
<td>38.3±2.9</td>
<td>243.0±21.9*</td>
<td>&lt;0.001 0.66 0.09</td>
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<tr>
<td>Obese</td>
<td>32.4±3.0</td>
<td>187.3±27.3†</td>
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<td></td>
<td>Triglycerides, mg/dL</td>
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<tr>
<td>Lean</td>
<td>26.0±5.6</td>
<td>37.6±13.9</td>
<td>0.16 0.77 0.10</td>
</tr>
<tr>
<td>Obese</td>
<td>17.4±2.9</td>
<td>34.6±5.6</td>
<td></td>
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<tr>
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<td>HOMA-IR, μU/mL×mg/dL</td>
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<tr>
<td>Lean</td>
<td>3.6±0.3</td>
<td>6.7±2.4*</td>
<td>0.005 0.63 0.006</td>
</tr>
<tr>
<td>Obese</td>
<td>2.6±0.5</td>
<td>10.5±2.9†</td>
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<tr>
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<td>TNF-α, pg/mL</td>
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<tr>
<td>Lean</td>
<td>38.1±15.4</td>
<td>55.0±11.8</td>
<td>0.77 0.029 0.52</td>
</tr>
<tr>
<td>Obese</td>
<td>138.9±49.7*</td>
<td>101.4±23.4*</td>
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<tr>
<td></td>
<td>sE-selectin, pg/mL</td>
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<tr>
<td>Lean</td>
<td>5.6±3.9</td>
<td>57.5±7.5</td>
<td>0.17 0.017 0.005</td>
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<tr>
<td>Obese</td>
<td>15.4±7.1</td>
<td>36.8±10.8†</td>
<td></td>
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<tr>
<td></td>
<td>8-epi-Isoprostane, pg/mL</td>
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<tr>
<td>Lean</td>
<td>126.9±24.4</td>
<td>142.2±27.4</td>
<td>0.27 0.49 0.73</td>
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<tr>
<td>Obese</td>
<td>97.9±8.0</td>
<td>139.5±12.8</td>
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<tr>
<td></td>
<td>PRA, ng/mL per h</td>
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<tr>
<td>Lean</td>
<td>0.02±0.01</td>
<td>0.10±0.03</td>
<td>0.005 0.07 0.029</td>
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<tr>
<td>Obese</td>
<td>0.07±0.02</td>
<td>0.16±0.06*</td>
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<tr>
<td></td>
<td>LVMM, g</td>
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<td>Lean</td>
<td>39.8±2.6</td>
<td>44.7±3.7</td>
<td>0.25 0.034 0.10</td>
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<tr>
<td>Obese</td>
<td>49.0±4.7</td>
<td>52.1±4.3*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>1.6±0.1</td>
<td>1.5±0.2</td>
<td>0.28 0.021 0.023</td>
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<tr>
<td>Obese</td>
<td>1.5±0.2</td>
<td>1.1±0.2‡</td>
<td></td>
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<tr>
<td></td>
<td>Stroke volume, mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>36.5±3.6</td>
<td>35.6±4.6</td>
<td>0.74 0.74 0.67</td>
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<tr>
<td>Obese</td>
<td>34.0±2.5</td>
<td>35.8±2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ejection fraction, %</td>
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<td></td>
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<tr>
<td>Lean</td>
<td>59.5±4.2</td>
<td>62.0±3.3</td>
<td>0.96 0.54 0.60</td>
</tr>
<tr>
<td>Obese</td>
<td>63.8±2.0</td>
<td>62.4±3.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cardiac output, mL×bpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>2.5±0.3</td>
<td>2.9±0.2†</td>
<td>0.40 0.15 0.023</td>
</tr>
<tr>
<td>Obese</td>
<td>2.5±0.3</td>
<td>2.9±0.2†</td>
<td></td>
</tr>
</tbody>
</table>

HOMA-IR indicates homeostasis model assessment insulin resistance; HT, hypertension; LDL, low-density lipoprotein; LVMM, left ventricular muscle mass; MAP, mean arterial pressure; PRA, plasma renin activity; RPP, rate pressure product; and TNF-α, tumor necrosis factor-α.

*P<0.05 vs Lean-sham.

†P<0.05 vs Lean-HT.

‡P<0.05 vs Obese-sham.
Obese-hypertension alone also reduced mitochondrial DNA-encoded NADH dehydrogenase 2 gene copy number in the myocardium (Figure 1H), and markedly blunted mitochondrial transcription factor-A immunoreactivity (Figure 1C and 1I), indicating decreased mitochondrial DNA content. In addition, Obese-hypertension inhibited the activities of mitochondrial respiratory chain complex IV (COX) subunits 1 and 4, suggesting compromised mitochondrial function, whereas Lean-hypertension only suppressed COX4 activity (Figure 2A and 2B). In concert, hypertension decreased the expression of the mitochondrial biogenesis regulator peroxisome proliferator–activated receptor-γ coactivator 1-α in both the groups, but its cofactor nuclear respiratory factor (NRF)-1 only in Obese-hypertension (Figure 2C–2E). Furthermore, hypertension increased circulating plasma levels of the mitochondrial DNA-encoded NADH dehydrogenase 1 and COX3, and diet increased COX3 and NADH dehydrogenase 2, consistent with release of mitochondrial components into the circulation because of cellular mitochondrial injury. Notably, Obese-hypertension elevated all 3 markers (Figure 2F–2H), suggesting more severe mitochondrial damage than obesity or hypertension alone, possibly mediated by their additive effects. Furthermore,
Obese-hypertension stimulated myocardial mitophagy, reflected by translocation of parkin to the mitochondrial outer membrane indicated by its marker Tom20, and upregulated expression of dynamin-related protein-1 (Figure S2B, S2C, S2E, and S2G). Collectively, Obese-hypertension amplified dysregulation of mitochondrial turnover and function compared with hypertension alone.

**Oxidative Stress and Fibrosis**

Systemic level of the oxidative stress marker 8-epi-isoprostane did not differ among the groups (Table 1), whereas myocardial oxidative stress (dihydroethidium staining) was enhanced only in Obese-hypertension (Figure S3A). The interaction of diet and hypertension also aggravated myocardial fibrosis in Obese-hypertension compared with either alone (Figure S3B). Notably, myocardial fibrosis was inversely associated with the content of mitochondrial cardiolipin and COX activity, and directly with the extent of mitophagy (Figure S3C–S3F).

**Discussion**

This study demonstrates that despite subtle cardiac alterations in obesity and hypertension alone, their coexistence markedly dysregulated mitochondrial turnover and function, including suppressed mitochondrial biogenesis and respiratory chain complex activities, and enhanced mitochondrial self-degradation. This might subserve myocardial fibrosis, and is associated with LV diastolic dysfunction. Therefore, coexisting obesity may facilitate the progression of myocardial injury in early RVH by enhancing mitochondrial dysregulation. We have
previously observed mitochondrial damage in the poststenotic kidney in RVH. This study extends our previous observations and shows that in the heart, superimposition of obesity on RVH leads to decreased levels of mitochondrial mtDNA, mitochondrial biogenesis regulators, and respiratory chain complex activities, and bolsters mitochondrial degradation and spill-over. These novel findings significantly suggest a wide-range of mitochondrial damage involving their quantity, quality, and dynamics in the RVH heart with coexisting obesity.

Studies have suggested impaired mitochondrial biogenesis in obesity. In this study, although pre-existing obesity alone had a minor impact on mitochondrial biogenesis, it was predominant in Obese-hypertension, where hypertension suppressed mitochondrial function most prominently, underscoring the detrimental interaction of obesity with hypertension. Although systemic oxidative stress remained unchanged, local oxidative stress (myocardial dihydroethidium) evoked by the coexistence of diet and hypertension can increase nitric oxide degradation or decrease its production, and, in turn, reduce mitochondrial biogenesis. Peroxisome proliferator–activated receptor-γ coactivator 1-α downregulated by hypertension also decreased mitochondrial turnover by its effector NRF-1. By binding to and coactivating the transcriptional function of its downstream NRF-1 on the promoter for mitochondrial transcription factor-A, peroxisome proliferator–activated receptor-γ coactivator 1-α decreases the activity of mitochondrial transcription factor-A, a direct regulator of replication/transcription of mitochondrial DNA, which encodes subunits of NADH dehydrogenase and complex IV, such as NADH dehydrogenase 2 and COX1, respectively. NRF-1 also regulates nucleus-encoded COX4. As a result, a decrease in these mitochondrial biogenic regulators impaired mitochondrial function. Importantly, spillover of circulating mtDNA markers and mitochondrial proteins because of cellular injury, as we observed, may also contribute to depletion of functional tissue mitochondria. Furthermore, in addition to the excessive nutrient abundance that stresses the mitochondria by increasing their workload, activated renin–angiotensin system involved in adiposity and pressure overload in Obese-hypertension might suppress mitochondrial biogenesis and magnify mitochondria. Increased mitochondrial degradation in Obese-hypertension could, in turn, magnify depletion of mitochondria. Consequently, dysregulated mitochondrial turnover and function ensue in Obese-hypertension, where the nutrient overload, cell injury, and pressure overload in concert magnify a fall in mitochondrial quantity and quality compared with either factor alone.

Interestingly, despite mitochondrial dysfunction and associated myocardial fibrosis (Figure S3), Obese-hypertension exhibited preserved cardiac systolic function (cardiac output and stroke volume). Tissue injury such as fibrosis often precedes and accompanies development of left ventricular hypertrophy. Similarly, we have observed that their coexistence amplified kidney injury and fibrosis, whereas renal function was relatively preserved. Possibly, increased insulin levels and extracellular volume may sustain or enhance renal and cardiac function in the early stages of obesity. Furthermore, mitochondrial dysfunction contributes to early stages of hypertensive heart remodeling, preceding systolic dysfunction. Decreased adenosine triphosphate production contributes to isolated LV diastolic dysfunction, and a fall in mitochondrial transcription factor-A impairs myocyte relaxation. Notably, the severity of mitochondrial dysfunction is important in the transition from hypertrophy to systolic dysfunction, as does its duration, and interventions that confer mitochondrial protection may ameliorate or reverse cardiac hypertrophy. Indeed, we have shown previously that stabilization of mitochondrial cardiolipin normalized LV E/A ratio in RVH. Collectively, these findings signify the adverse impact of mitochondrial dysfunction on diastolic function at the early stages of hypertensive heart disease. Nevertheless, more severe and prolonged mitochondrial damage might eventually lead to cardiac systolic dysfunction.

In this study, obesity alone did not increase blood pressure, but magnified its increment in hypertension (Table 1), underscoring their significant interaction. It has been shown that obese subjects have higher circulating angiotensinogen, renin, and aldosterone. Body weight control by 5% leads to a reduction of renin–angiotensin–aldosterone not only in the adipose tissue but also in the plasma, which correlates with the waist circumference decline. These observations link obesity with increased circulating renin–angiotensin levels, which possibly contributes to greater mean arterial pressure in Obese-hypertension compared with Lean-hypertension. Yet, although plasma renin activity was increased in Obese-hypertension compared with Lean-sham, it was not higher than Lean-hypertension, implicating additional factors in the higher mean arterial pressure in Obese-hypertension compared with Lean-hypertension. For example, increased renal sodium reabsorption because of renal artery stenosis can be enhanced in obesity as proximal tubules cells may undergo hypertrophy, leading to volume expansion. Adipokines released from adipose tissue in obesity are related to increased sympathetic nerve activity, and insulin resistance (reflected by homeostasis model assessment–insulin resistance index) may also promote hypertension. These factors working in concert might increase mean arterial pressure in Obese-hypertension compared with Lean-hypertension. Because of increased pressure load, cardiac oxygen demand (rate pressure product) rises. Alas, proliferating microvascular smooth muscle cells and decreased endothelial nitric oxide bioavailability in Obese-hypertension, resulting in diminished perfusion reserve, may restrict oxygen supply and lead to myocardial hypoxia. Interestingly, however, blood-oxygen-level-dependent $R^2$ in Obese-hypertension was comparable with that in Lean-hypertension and Obese-sham, suggesting relatively preserved oxygenation. Possibly, blunted mitochondrial COX1 and 4 activities and loss of mitochondrial genome may decrease oxygen use capacity and reduce oxygen consumption, leading to a paradoxical hypoxic-to-normoxic shift in the tissue.

This study is limited by our relatively young animals, which may show greater resistance to insults exerted by obesity and hypertension, yet this relatively short-term exposure provided with an opportunity to capture the initial pathophysiological changes that they induce. Our findings underlie the adverse effects of renovascular disease with coexisting obesity on myocardial mitochondrial quantity, quality, and dynamics. Further studies need to examine the potential alterations in myocardial adenosine triphosphate levels in obesity and pressure-overload, and the causal link between mitochondrial dynamics and cardiac outcomes. The potential protective

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effects of obesity management integrated with antihypertensive measures on mitochondrial homeostasis in individuals with obesity and hypertension also need to be investigated.

**Perspectives**

Our study demonstrates that coexistence of obesity and RVH impairs mitochondrial biogenesis and function, and enhances mitochondrial self-degradation. This is associated with magnified tissue remodeling and LV diastolic dysfunction. As the confluence of hypertension and obesity is becoming increasingly prevalent, greater understanding of cardiac mitochondrial metabolic dynamics and their potential roles as therapeutic targets in cardiac remodeling and dysfunction are in growing need.

**Acknowledgments**

Dr Li was supported by the China Scholarship Council under the authority of the Ministry of Education of the People’s Republic of China.

**Sources of Funding**

This study was partly supported by National Health Institutes grants numbers DK73608, HL12160, HL121561, DK104273, DK 102325, and C06-RR018898, and the American Heart Association.

**Disclosures**

None.

**References**


**Novelty and Significance**

**What Is New?**
- Our study implicates synergistic dysregulation of mitochondrial homeostasis as an important contributor in mediating myocardial injury in renovascular hypertension coexisting with obesity.

**What Is Relevant?**
- Renovascular hypertension and obesity often coexist and may, therefore, worsen cardiac outcomes, but the mechanisms are incompletely understood. Our study implicates mitochondrial dynamics in mediating cardiac alteration under renal vascular hypertension with concurrent obesity.

**Summary**

Synergistic mitochondrial loss and respiratory chain complex dysfunction may play important roles in mediating cardiac damage during coexisting renovascular hypertension and obesity.
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Hypertension. 2015;66:430-436; originally published online June 15, 2015;
doi: 10.1161/HYPERTENSIONAHA.115.05478

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/66/2/430

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CARDIAC METABOLIC ALTERATIONS IN HYPERTENSIVE OBESE PIGS

Xin Zhang¹,* MD; Zi-Lun Li¹,³,* MD, PhD; Alfonso Eirin¹, MD; Behzad Ebrahimi¹, PhD; Pawar Aditya¹, MD; Xiang-Yang Zhu¹, MD, PhD; Amir Lerman², MD; Lilach O. Lerman¹,², MD, PhD.

* These authors contributed equally

¹Division of Nephrology and Hypertension and ²Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN; ³Division of Vascular Surgery, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Correspondence:

Lilach O. Lerman, MD, PhD, Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. lerman.lilach@mayo.edu

Phone: (507)-266-9376; Fax: (507)-266-9316
METHODS

1. Experimental protocol

The study was approved by the Institutional Animal Care and Use Committee. Littermate Ossabaw pigs (Swine Resource, Indiana University) were randomized to 4 groups (n=7 each): Lean-sham, Obese-sham, Lean-hypertension (HT), and Obese-HT. Lean and obese pigs were fed starting at the age of 3-months 16 weeks of standard chow or an high-fat/high fructose diet (5B4L; Purina Test Diet, Richmond, Indiana)\(^1\), respectively. HT was induced by establishing unilateral renal artery stenosis at 12 weeks of diet.\(^2\) Four weeks later, cardiac structure, function and oxygenation were studied with multi-detector computed-tomography (MDCT) and blood-oxygenation-level-dependent (BOLD)-magnetic resonance imaging (MRI), and blood samples collected. For each in vivo study animals were weighed, induced with Telazol and xylazine (5 mg/kg and 2 mg/kg, respectively, intramuscular injection) and intubated. For MDCT, animals were anesthetized by continuous intravenous infusion of Ketamine and ventilated with room air. During MRI, pigs were maintained anesthetized by inhalation of 2% isoflurane-containing oxygen.

Three days following the completion of in vivo studies, pigs were euthanized by intravenous sodium pentobarbital (100mg/kg, Fatal Plus, Vortech Pharmaceuticals, Fort Washington, PA)\(^3\). Hearts were removed, preserved, and prepared for ex-vivo tissue studies.

2. Induction of renal artery stenosis and blood pressure measurement

Under fluoroscopic guidance, a percutaneous transluminal balloon 7.0 mm in diameter (Cordis, Miami, Fla) wrapped with a local-irritant coil was advanced through the femoral artery into the proximal middle section of the right renal artery. The balloon was inflated (6 atm) for 45 seconds, deflated, and removed, leaving the local-irritant copper coil embedded in the vascular wall.\(^4\) The procedure elicits gradual increase of blood pressure within the following 10 days, accompanying progressive luminal narrowing.\(^5,6\) The degree of renal artery stenosis was determined by angiography 4 weeks later. A telemetry transducer (TA-D70, Data Sciences International, MN)\(^5\) was implanted in the left femoral artery during renal artery stenosis induction\(^7,8\) to monitor and record systolic and diastolic blood pressure for the following 4 wks. Mean arterial pressure (MAP) was calculated using the formula: MAP=1/3 systolic pressure+2/3 diastolic pressure. MAP was averaged from the last 3 days prior to an in vivo experiment.

3. Systemic parameters

Blood samples collected during MDCT study were examined for total cholesterol, low-density lipoprotein, triglycerides and basal homeostasis model assessment insulin resistance. Plasma renin activity was assessed, and tumor necrosis factor-\(\alpha\) and sE-selectin, and 8-epi-Isoproterolane measured for systemic inflammatory and oxidative stress status.\(^1,8,9\)
4. LV function and oxygenation

Cardiac function and structure were assessed in vivo using 64-slice MDCT (Somatom Definition-64, Siemens Medical Solution, Forchheim, Germany).\(^8, 10, 11\) Two parallel 6-mm-thick mid-left ventricle (LV) levels were selected for evaluation of myocardial perfusion and LV function. A bolus injection of nonionic, low osmolar contrast medium (Isovue-370, 0.33 ml/Kg over 2 seconds) into the right atrium was followed by a 50-s flow study during respiratory suspension. After a 15-minutes interval, the same process was repeated during a 5-minute intravenous infusion of adenosine (400µg/kg/min).\(^8, 10, 12\) LV myocardial perfusion was measured at both baseline and after adenosine infusion to assess microvascular function. Subsequently, the entire LV was scanned 20 times throughout the cardiac cycle to obtain parameters of cardiac function, including LV end diastolic volume, E/A ratio, stroke volume, and ejection fraction. LV muscle mass (LVMM) was acquired at the end-diastole by tracing the LV endocardial and epicardial borders. The rate pressure product (RPP; systolic blood pressure x heart rate) served as an index of oxygen demand. The images were analyzed with the Analyze\(^\text{TM}\) software package (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN).

To assess LV myocardial oxygenation, pigs were positioned in the MRI scanner (Signa EXCITE 3T system, GE, Waukesha, WI) and BOLD images (4-5 axial-oblique) acquired along the cardiac short axis during suspended respiration.\(^10, 13\) Gated Fast Gradient Echo sequence was used with TR/TE/number of echoes/Matrix size/FOV/Slice thickness/Flip angle=6.8 ms/1.6-4.8 ms/8/128x128/35/0.5 cm/30°. In each slice on T2*-weighted images obtained, the BOLD index, R2*, was estimated in each voxel by fitting the MR signal intensity vs. echo times to a single exponential function and calculating the MR intensity decay rate. Images were subsequently analyzed using MATLAB 7.10 (MathWorks, Natick, MA).

In addition, myocardial microvascular remodeling and function were evaluated by media/lumen area fraction by α-smooth muscle actin (SMA, abcam) staining and endothelial nitric oxide synthase (eNOS, abcam 1:1000) by western blotting, respectively.

5. Myocardial mitochondrial metabolic dynamics and activity

Protein expression of the mitochondrial biogenesis regulators peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α (abcam 1:1000) and its coactivator nuclear respiratory factor (NRF)-1 (abcam 1:500) were examined by western blotting. The mitochondrial inner membrane phospholipid cardiolipin was quantified for mitochondrial content by immunofluorescence staining. Mitochondrial DNA regulator mitochondrial transcription factor A (TFAM, abcam 1:1000) and mitochondrial DNA-encoded-NADPH dehydrogenase (ND)2 gene copy number (Life Technology) were examined by Western blotting and real time quantitative polymerase chain reaction (PCR), respectively. S18 ribosome DNA was used as reference. In addition, circulating plasma levels of ND1, ND2, and respiratory chain complex IV (COX) subunit 3 (all Life Technology) were measured to indicate mitochondrial component spillover secondary to cellular mitochondrial injury.\(^14\) Their primers’ sequences are as follow:
ND2 (CCWR2L5):
Activities of mitochondrial respiratory chain complex IV subunits COX1 and 4 were determined by ELISA (both Abcam). Mitochondrial autophagy was assessed by protein expression of dynamin related protein-1 (Cell Signaling 1:1000) by western blotting, and co-localization of mitochondrial outer membrane marker Tom20 and Parkin (both Santa Cruz, 1:50, immunofluorescent staining), the latter translocating from the cell plasma to the mitochondrial outer membrane, mediating mitophagy.\textsuperscript{15, 16}

6. Myocardial oxidative stress and fibrosis

Dihydroethidium staining was performed to assess tissue oxidative stress. Fibrosis was examined by trichrome staining.

7. Adipose tissue assessment

Pericardial and abdominal fat volumes were quantified on MDCT images, as described previously.\textsuperscript{10}

8. Statistical analysis

Results are expressed as mean±SEM. JMP software package version 9.0 (SAS Institute, Cary, NC) was used to perform two-way ANOVA to analyze the individual and interactive effects of factors diet and HT, followed by Tukey’s test as appropriate. Significant factors are indicated following ♠; significance from post-hoc tests is indicated as *, $, and †. Paired Student’s t-test was performed for comparisons within groups (myocardial perfusion response to adenosine). The link of mitochondrial biogenesis and mitophagy to myocardial fibrosis was detected by Pearson correlation analysis. Results were considered significant for p<0.05.
REFERENCES FOR ONLINE SUPPLEMENT


Figure S1. Representative cross-sectional multi-detector computed tomography images for abdominal (A) and pericardial (B) fat (both yellow) from Lean and Obese Ossabaw pigs with or without hypertension (HT), and their quantification (C-D). ♠ Diet: significant effect of diet (Two-way ANOVA). *p<0.05 vs. Lean-sham, †p<0.05 vs. Lean-HT.

Figure S2. A, D: Representative images of microvascular α-smooth muscle actin (SMA) and media/lumen ratio. B, E: Representative images for co-localization of Tom20 with parkin and its quantification (Tom20 red, parkin green, co-localization orange, blue nuclei).
C, F-G: Expression of endothelial nitric oxide synthase (eNOS) and dynamin-related protein (DRP)-1. ♠ Diet: significant effect of diet. ♠ HT: significant effect of HT. ♠ DietxHT: significant interaction (Two-way ANOVA). *p<0.05 vs. Lean-sham, $p<0.05 vs. Obese-sham, †p<0.05 vs. Lean-HT.
Figure S2

A) Lean sham Obese sham Lean HT Obese HT

B) Tom20+ Parkin

C) Lean sham Obese sham Lean HT Obese HT

eNOS 133 KD
GAPDH 37 KD
DRP-1 97 KD
GAPDH 37 KD

D) DietxHT
HT

E) DietxHT
HT
Diet

F) DietxHT
HT

G) DietxHT
Figure S3. A: Representative images of dihydroethidium (DHE) staining and its quantification. B: Trichrome staining and quantification. C-F: Pearson correlation for association of myocardial fibrosis with mitochondrial content, respiratory chain complex IV enzyme activities, and extent of mitophagy. ♠ Diet: significant effect of diet. ♠ HT: significant effect of HT. ♠ DietxHT: significant interaction (Two-way ANOVA). *p<0.05 vs. Lean-sham, $p<0.05 vs. Obese-sham, †p<0.05 vs. Lean-HT.