Proximal to Middle Left Coronary Artery Flow Velocity Ratio, As Assessed Using Color Doppler Echocardiography, Predicts Coronary Artery Atherosclerosis in Mice

Julia Grönlöws, Johannes Wikström, Ulrika Hägg, Birger Wandt, Li-ming Gan

Background—We aimed to establish a completely noninvasive technique to assess coronary artery atherosclerosis in living mice using proximal to middle left coronary artery (LCA) velocity ratio as assessed with color Doppler echocardiography (CDE).

Methods and Results—Three groups of apolipoprotein E/low-density lipoprotein receptor double-knockout (apoE/LDLr dko) mice 10, 40, and 80 weeks of age and 3 additional age-matched groups of C57BL/6 mice were examined under anesthesia. Coronary flow velocity in proximal (V_{prox}) and middle part (V_{mid}) of LCA was measured using CDE. A 40-MHz ultrasound biomicroscope (UBM) was used to visualize lumen and outer vessel diameter in the proximal LCA. Flow velocity in the proximal LCA increased significantly with age and remained constant in the middle part in the apoE/LDLr dko mice, whereas velocities at both the sites remained unchanged in C57 mice. CDE-assessed flow velocity ratio (V_{prox}/V_{mid}) increased significantly with age in apoE/LDLr dko mice (P = 0.0055) and correlated significantly to percentage wall thickness, as assessed by UBM (P = 0.0044; r = 0.65) and histology (P = 0.0002; r = 0.78). Wall thickness increased with age in the apoE/LDLr dko mice as measured with UBM (P = 0.0093; r = 0.49), which was also confirmed with histology (P < 0.0001; r = 0.73).

Conclusions—CDE and UBM are useful noninvasive tools to quantify mouse coronary artery atherosclerosis in vivo. (Arterioscler Thromb Vasc Biol. 2006;26:1126-1131.)

Key Words: coronary artery • velocity ratio • atherosclerosis • mouse • color Doppler echocardiography • ultrasound biomicroscopy

Genetically modified hyperlipidemic mice that develop human-like atherosclerotic lesions have now been available for more than a decade. However, to what extent these transgenic mice develop hemodynamically significant coronary artery atherosclerosis has still not been fully investigated, mainly because of methodological difficulties.

In addition to the invasive "gold standard" coronary angiogram, there is an emerging need for noninvasive techniques for imaging of coronary artery atherosclerosis in humans. Multislice CT and high-energy MRI have been shown to be powerful methods in assessing coronary artery morphology in the clinic settings. Color Doppler echocardiography (CDE) with its high time and spatial resolution, has been used successfully in recent years for imaging the left anterior descending coronary artery (LAD). Further, the technique has been applied recently to noninvasively determine the degree of coronary artery stenosis using velocity ratio between prestenotic and in-stenotic flow velocity in humans. Recently, Gradus-Pizlo et al demonstrated good feasibility and accuracy in imaging of LAD wall morphology in man using transthoracic high-resolution B-mode echocardiography. In mice with small vascular structures and extremely high heart rate, there are so far no established noninvasive methods for quantification of mouse coronary artery stenosis. Recently, we developed a technique to assess mouse coronary artery flow in vivo using transthoracic CDE. Using this method, flow velocity in mouse left coronary artery (LCA) can be measured reproducibly and with great success rate. Lately, a high-frequency ultrasound biomicroscope (UBM) method has been developed and shown to be capable of visualizing peripheral as well as coronary artery morphology in vivo. Using CDE-based coronary flow velocity reserve (CFVR) approach and UBM, we showed recently that mouse LCA minimal lumen diameter after lumen occlusive plaque growth, can be measured in a quantitative manner. The purpose of the present study was to investigate whether mouse coronary artery stenosis could be studied noninvasively using CDE and UBM. We hypothesized that flow velocity ratio between the proximal and middle LCA
predicted the degree of potential LCA stenosis, which was also assessed by direct in vivo UBM-assisted measurement of lumen diameter and proximal LCA wall thickness (WT). Finally, all the imaging data were confirmed with histology.

Methods

For an extended version of the Methods, please see online supplement I, available at http://atvb.ahajournals.org.

Animals

Eighteen male apolipoprotein E and low-density lipoprotein receptor double-knockout (apoE/LDLr dko) mice 10, 40, and 80 weeks of age and 15 age-matched C57BL/6 were purchased from M&B (Ejby, Denmark). Additionally, 12 apoE/LDLr dko mice 50 to 70 weeks of age were included for evaluation of UBM intraobserver and interobserver variability. The regional animal ethics committee of Göteborg University approved the experimental protocol.

Cardiac Ultrasound

An Acuson Sequoia 512 echocardiograph was used with a Microson 15L8 transducer with color Doppler frequencies of 7 to 14 MHz and pulsed wave spectral Doppler frequency of 7 MHz. All measurements were made off-line using an image analysis program (MedArch Viewer 2.1; Secure Archive) in accordance with guidelines from the American Society of Echocardiography.

Flow Velocity Measurement by CDE

Mean diastolic flow velocity at the proximal ($V_{\text{prox}}$) to the middle site ($V_{\text{mid}}$) in LCA was measured after outlining the Doppler signal (Figure 1). Coronary flow velocity ratio was calculated off-line according to mean diastolic velocity in proximal LCA/mean diastolic velocity in middle LCA ($V_{\text{prox}}/V_{\text{mid}}$) similar to previously described formulas.

Imaging of Proximal LCA Morphology by UBM

A UBM (Vevo 600; Visualsonics) with a transducer frequency of 40 MHz (providing theoretical resolution of 40 μm in a frame rate of 32 Hz) was used to visualize the proximal LCA (Figure 2). Degree of proximal stenosis measured with UBM was calculated in long-axis views using ECST grading method for stenosis according to European Carotid Surgery Trial: [(total vessel diameter−lumen diameter)/total vessel diameter]×100%.

Methodological Validation

Six 50- and six 70-week-old apoE/LDLr dko mice were used for UBM validation of intraobserver and interobserver variability.

Analysis of Plasma Lipids

Plasma cholesterol and triglycerides were both analyzed in a Cobas Mira Plus (Roche) with enzymatic spectrophotometric methods (Roche Diagnostics; Catalog No. 12016630 and 12146029, respectively).

Histology

Serial sections with 30-μm intervals were performed from the level of the mitral valve to the aortic root to obtain cross-sectional histological preparations of the middle and proximal LCA, respectively. Both PWT and WT in proximal LCA measured with histology were calculated on cross-sections in a similar way as with UBM.

Data Analysis and Statistics

All image analysis was performed off-line using an image analysis software (Image pro plus 5.0; Media Cybernetics, Inc.). Data are presented as means±SEM. For statistical evaluation, Prism 4 software (GraphPad Inc.) was used. The differences between the groups were evaluated by nonparametric 1-way ANOVA. Spearman’s correlation test was used to analyze all correlations. A $P$ value ≤0.05 was considered statistically significant. Coefficient of variation, SD ($x$−$y$)/mean ($x$, $y$)×100 was used to determine interobserver and intraobserver variability.

Results

Cardiac Ultrasound

For basic cardiac data, please see supplemental Table Ia and Ib in online supplement II.
Flow Velocity Measurement by CDE
Mean diastolic flow velocity in the proximal part of LCA increased significantly with age in the apoE/LDLr dko mice \((P=0.0059; \text{Table 1})\). No difference was found in the C57 animals in proximal LCA (Table 2). Flow velocity in middle LCA remained constant with age in both animal groups. The velocity ratio \(V_{\text{prox}}/V_{\text{mid}}\) increased by age in the apoE/LDLr dko \((P=0.0055; \text{Table 1})\) but remained unaffected in the C57 animals.

Imaging of Proximal LCA Morphology by UBM
PWT increased significantly with age in apoE/LDLr dko mice: \(36.6\pm3.6\%\) (10 weeks of age), \(47.6\pm2.5\%\) (40 weeks of age), \(64.7\pm2.1\%\) (80 weeks of age) of total vessel diameter \((P<0.0001)\). Further, UBM-assessed PWT is significantly correlated with CDE velocity ratios in apoE/LDLr dko mice \((P=0.0044; \ r=0.65; \text{Figure 3})\). WT increased with age \(115\pm18\ \mu\text{m} (10 \text{ weeks of age}), 195\pm28\ \mu\text{m} (40 \text{ weeks of age})\) and \(310\pm30\ \mu\text{m} (80 \text{ weeks of age}; P=0.0093)\).

Methodological Validation
Intraobserver coefficient of variation for total vessel diameter, lumen diameter, and stenosis were \(4.5\%, 8.9\%\), and \(3.2\%\), respectively. Interobserver coefficient of variation for total vessel diameter, lumen diameter, and stenosis were \(4.8\%, 16.1\%\), and \(9.8\%\), respectively.

Analysis of Plasma Lipids
Triglyceride levels decreased with age in apoE/LDLr dko mice: \(2.7\pm0.40 \text{ mmol/L} (10 \text{ weeks of age}), 1.8\pm0.39 \text{ mmol/L} (40 \text{ weeks of age})\), and \(0.7\pm0.17 \text{ mmol/L} (80 \text{ weeks of age}; P=0.0123)\), whereas cholesterol levels increased with age: \(14.4\pm0.92 \text{ mmol/L} (10 \text{ weeks of age}), 21.6\pm1.56 \text{ mmol/L} (40 \text{ weeks of age})\), and \(23.2\pm0.99 \text{ mmol/L} (80 \text{ weeks of age}; P=0.0046)\). Similar to previously published data, the C57BL/6 mouse had a normal cholesterol level \(\approx2 \text{ mmol/L}\) and triglyceride \(<1 \text{ mmol/L}.^{12}\)

Histology
For comparison between CDE, UBM, and histology results, sections with the narrowest lumen from the proximal LCA were chosen for histological evaluation. Histology images reveal proximal coronary artery stenosis in aged apoE/LDLr dko mice that confirms previous results from both of the imaging techniques (Figure 4). Histological assessed PWT increased significantly by age in apoE/LDLr dko mice with \(25.6\pm2.6\%\) (10 weeks of age), \(44.7\pm7.4\%\) (40 weeks of age), and \(63.0\pm10.4\%\) (80 weeks of age; \(P=0.0164\)). As a nonstenotic comparison, there was no significant change of PWT by age in C57 with \(22.9\pm1.0\%\) (10 weeks of age), \(25.2\pm3.5\%\) (40 weeks of age), and \(30.0\pm4.1\%\) (80 weeks of age; \(P=\text{NS}\)).

Discussion
Genetically modified mice lacking the apoE lipoprotein and LDL receptor develop coronary atherosclerosis in the proximal part of LCA with age.13 Based on our previously established mouse coronary artery imaging protocols, we show in this study that flow velocity ratio between the proximal and middle LCA is a useful parameter, correlating

---

**TABLE 1. Mean Flow Velocity Measurements With CDE in the 10-Week-Old, 40-Week-Old, and 80-Week-Old ApoE/LDLr dko Mice**

<table>
<thead>
<tr>
<th></th>
<th>10-Week-Old dko</th>
<th>40-Week-Old dko</th>
<th>80-Week-Old dko</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_{\text{prox}})</td>
<td>16.1±4.5</td>
<td>29.7±7.3</td>
<td>65.0±12.0</td>
<td>0.0059</td>
</tr>
<tr>
<td>(V_{\text{mid}})</td>
<td>19.4±3.9</td>
<td>25.5±5.3</td>
<td>28.2±6.0</td>
<td>NS</td>
</tr>
<tr>
<td>(V_{\text{prox}}/V_{\text{mid}})</td>
<td>0.90±0.095</td>
<td>1.15±0.17</td>
<td>2.58±0.44</td>
<td>0.0055</td>
</tr>
</tbody>
</table>

Data are presented as means±SEM. \(P<0.05\) is considered significant. \(V_{\text{prox}}/V_{\text{mid}}\) indicates velocity ratio between maximal flow velocity proximal part/maximal flow velocity middle part.

**TABLE 2. Mean Flow Velocity Measurements With CDE in the Age-Matched C57BL/6 Mice**

<table>
<thead>
<tr>
<th></th>
<th>10-Week-Old C57</th>
<th>40-Week-Old C57</th>
<th>80-Week-Old C57</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_{\text{prox}})</td>
<td>37.2±7.0</td>
<td>42.4±5.7</td>
<td>53.1±3.2</td>
<td>NS</td>
</tr>
<tr>
<td>(V_{\text{mid}})</td>
<td>27.2±4.0</td>
<td>35.4±5.8</td>
<td>38.3±4.6</td>
<td>NS</td>
</tr>
<tr>
<td>(V_{\text{prox}}/V_{\text{mid}})</td>
<td>1.32±0.14</td>
<td>1.29±0.14</td>
<td>1.44±0.09</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as means±SEM. \(P<0.05\) is considered significant. \(V_{\text{prox}}/V_{\text{mid}}\) indicates velocity ratio between maximal flow velocity proximal part/maximal flow velocity middle part.
to the percentage WT in the proximal LCA, assessed using UBM as well as histology. Also, UBM-measured WT in proximal LCA reflects directly the proximal LCA atherosclerosis burden (ie, histological plaque area).

Lumen-narrowing plaque growth is associated with locally increased flow velocity, a hemodynamic phenomenon, which is widely used in, for example, measurement of carotid artery stenosis. Interestingly, this observation also holds true in the aortic roots in atherosclerotic mouse models. Recently, velocity ratio between prestenotic and stenotic flow using CDE was applied to determine the degree of restenosis in patients undergoing angioplasty. Our present findings reveal that flow velocity in the proximal LCA increases with age in atherosclerotic mice, although it remains unaffected in the middle part of LCA, a phenomenon not evident in the nonatherosclerotic C57 control animals. Thus, the flow velocity ratio between the proximal and middle part (Vprox/Vmid) of LCA, appear to be useful to assess mouse proximal coronary artery atherosclerosis.

Although C57 mice are not always appropriate controls to the atherosclerotic mice, despite similar background, the C57 mice were used to illuminate the normal age-dependent changes in cardiac function. With age, left ventricular mass, stroke volume, and cardiac output increased in a similar manner in both C57 and apoE/LDLr dko mice, whereas LCA proximal/middle flow velocity ratio increased only in apoE/LDLr dko mice. Indeed, 80-week-old apoE/LDLr dko mice demonstrated lower end-diastolic volume, cardiac output, as well as stroke volume compared with age-matched C57 mice. The increased flow velocity in the proximal LCA can thus not be attributable to a hyperkinetic circulatory status. These basic cardiac data therefore support that the observed increased flow velocity ratio in aged apoE/LDLr dko mice is primarily attributable to proximal coronary artery stenosis rather than other cardiac mechanisms. Although previous studies have shown that left ventricular mass increases secondary to coronary occlusion, the observed relative increase of weight-adjusted left ventricular mass in 80-week-old apoE/LDLr dko seems to be secondary to the retarded body weight increase.

In our experience, the baseline coronary flow does differ substantially between different strains, as seen in Tables 1 and 2. Strain-related difference in coronary artery diameter, myocardial oxygen demand, body temperature, as well as influence of anesthesia might be responsible for the baseline coronary flow differences. The precise underlying mechanism is still under investigation. However, the strain-dependent baseline velocities may have minor importance when calculating degree of coronary artery stenosis using the velocity ratio approach as presented here.

Using the coronary artery imaging windows validated by us, we show in this study that UBM-assessed WT did reflect proximal coronary artery plaque area. This technique provides us with a quantitative tool to assess coronary artery atherosclerosis in mice in real-time with a resolution down to 40 μm. Contrary to the more distal part of the LCA, the proximal portion of LCA, which is epicardially localized, can be easily visualized. The outer and inner lumen diameter was measured in diastole, when LCA is maximally dilated. The ratio between the WT and the outer vessel diameter was calculated to assess the degree of stenosis in diseased animals according to ECST grading method. Using the approach described above, feasibility and reproducibility are high and indeed comparable with the human setting, in which Gradus-Pizlo et al showed recently that WT and lumen diameter of the proximal LAD could be measured in a very similar imaging window. Gradus-Pizlo et al showed also that the transthoracically assessed LAD WT correlated well with that measured during open-chest surgery and comprised both IMT as well as adventitia. It is conceivable that asymmetrical plaque growth in the proximal LCA is unfairly illustrated because of the 2D nature of UBM, which only visualizes plaques at the tangential epicardial plane at the aortic root. Despite this, we found a significant correlation between flow velocity ratio and proximal LCA stenosis degree, as assessed by UBM.
Several imaging techniques are available today for assessment of in vivo cardiovascular morphology and function in various animal models, as well as in human. Echocardiography has become one of the most frequently used implements for investigation of cardiac function in human and animal models. It has the advantage of being noninvasive and less time-consuming compared with other ex vivo and in vivo methods used in animal models.\(^{13,18,19}\) Our group and others have shown previously that mouse coronary arteries could be visualized using either CDE or UBM.\(^{7,20}\) In a comparative study with MRI by Zhou et al, UBM could accurately visualize the mouse cardiovascular systems.\(^{20}\) Recently, we showed that UBM is also a powerful and accurate tool to image atherosclerosis-related minimal lumen diameter in mouse coronary arteries. Further, by using adenosine- or hypoxia-induced CFVR approach, we showed that the minimal lumen diameter could be functionally predicted using CDE.\(^9\)

In man, CFVR has been shown to reflect hemodynamically significant coronary artery stenosis, as well as coronary endothelial dysfunction.\(^{21}\) Hypercholesterolemia, in absence of coronary artery stenosis, is associated with decreased CFVR both in man and animals, probably because of endothelial as well as smooth muscle cell dysfunction.\(^{22,23}\) However, in a general population of patients with coronary artery disease and multiple cardiovascular risk factors, a CFVR value <2.0 has been shown to comprise high sensitivity and specificity for prediction of hemodynamically significant coronary artery stenosis irrespective of other risk factors.\(^{21}\) However, in mouse, because of the species-specific coronary anatomy and physiology, as well as the exaggerated lipid disturbance in the transgenic mice, any CFVR cutoff values for significant stenosis still remain to be determined. In the current study, the apoE/LDLr dko mice showed slightly age-dependent increase in total cholesterol and decrease in triglyceride levels. This could be attributable to the continuous enrichment in cholesterol particles in lipoproteins with age, whereas the latter could also be associated with the methodological difficulty in triglyceride measurement of the mouse plasma. Nevertheless, the strain-specific lipid profiles could be a potential confounding factor when studying mouse coronary artery function using the CFVR approach. This was one of the major reasons prompted us to develop the current direct stenosis measurement based on velocity ratio approach to distinguish between coronary microvascular dysfunction and flow-limiting stenosis.

Several mouse models that develop atherosclerosis have been available for the last decade, and these mice demonstrate fatty streaks to fibrous plaques throughout the whole vascular tree with or without an atherogenic diet.\(^{1,2,4,25}\) Nakashima et al showed that peripheral artery atherosclerosis often preceded the development of coronary lesions in apoE-deficient mice. Feeding 40-week-old mice with a Western diet for 20 weeks significantly narrowed lumen of both left and right arteries.\(^{25}\) These observations confirm the current morphological findings that in apoE/LDLr dko mice, coronary artery atherosclerosis starts in the proximal part of LCA and proceeds in the distal direction, which could be further validated in vivo using CDE and UBM.

Coronary artery atherosclerosis is the leading cause underlying acute coronary syndrome. Caligiuri et al succeeded in inducing myocardial infarction in aged apoE/LDLr dko mice using mental and hypoxic stress in combination with an atherogenic diet. In that model, myocardial infarction has been suggested to be a consequence of hypoxia-induced coronary artery spasm in the atherosclerotic vessels.\(^{13}\) However, in unstressed animals aged between 46 and 88 weeks of age, only a small number of spontaneous infarctions were evident, as validated at AstraZeneca by histological serial sections of the entire cardiac tissue. These minor and infrequent histological changes did not seem to impact on the cardiac performance in the current study. With the spatial resolution of the cardiac ultrasound used, no apparent regional cardiac motion defects were detected. Thus, despite advanced coronary artery atherosclerosis, spontaneous myocardial infarction is still scarce in this mouse model. It is increasingly evident that acute coronary syndrome involves a complex array of molecular mechanisms, and dyslipidemia is only partially responsible. These observations may further emphasize the acute need of a relevant noninvasive tool to perform adequate screening of the mouse cardiovascular system to select mouse models relevant for human coronary artery disease. By specifically studying mice with well-defined coronary artery atherosclerosis, potential mechanisms involved in acute coronary syndromes may be identified in future studies.

**Study Limitations**

The velocity ratio approach seems to be useful to identify critical coronary artery stenosis. Also, using color Doppler guidance and a relatively large gate size of 1 mm, it is possible to map potential stenosis along the major part of the mouse LCA. However, this technique is only applicable in cases of severe lumen narrowing. UBM, on the other hand, provides detailed information about not only lumen narrowing, but also vascular wall structure, even in the early stages of the atherosclerotic process. However, because of the limited spatial resolution of 30 μm of UBM compared with the mouse coronary artery dimension of 300 μm in lumen diameter, quantitative morphological measurement may be associated with larger methodological variations. Also, interindividual anatomic variation of the mice as well as the potential operator-dependent variations in projection techniques may contribute to a considerable interobserver variability. To improve this direct morphological imaging technique, a semiautomatic edge-detection software can probably be useful as in the context of intima-media thickness measurement in human, which makes it possible to measure an average value of, for example, lumen diameter along the proximal segment of the LCA. Using the present UBM protocol, the relatively large intraobserver and interobserver variability in coronary lumen measurement should be taken into consideration when the technique is used in, for example, intervention studies.

**Conclusions**

In summary, genetically modified mice lacking the apoE lipoprotein and low-density lipoprotein receptor develop
coronary stenosis in the proximal part of LCA with age. Using CDE and UBM technique, mouse coronary artery atherosclerosis can be accurately studied in real time with great feasibility. These completely noninvasive imaging techniques may be useful tools for in vivo phenotyping of mice with specific focus on the coronary artery disease.

Acknowledgments
This work was supported by grants from the Swedish Medical Research Council, the Swedish Heart-Lung Foundation, the Åke Wiberg Foundation, the Memorial Foundation of Lars Hierta, the Magnus Bergvall Foundation, and AstraZeneca R&D Mölndal. We thank Ulla Brandt-Eliasson for excellent technical assistance.

References
Proximal to Middle Left Coronary Artery Flow Velocity Ratio, As Assessed Using Color Doppler Echocardiography, Predicts Coronary Artery Atherosclerosis in Mice
Julia Grönros, Johannes Wikström, Ulrika Hägg, Birger Wandt and Li-ming Gan

Arterioscler Thromb Vasc Biol. 2006;26:1126-1131; originally published online March 2, 2006; doi: 10.1161/01.ATV.0000216121.17190.a5
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/5/1126

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2006/03/06/01.ATV.0000216121.17190.a5.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/
Table Ia and Ib

Cardiac functions measured with echocardiography in a) the 10w, 40w and 80w of ApoE/LDLr dko mice and in b) the age-matched C57BL/6 mice. Data is presented as means ± SEM. P ≤ 0.05 considered significant. Left ventricle mass (LVM), mg; weight-adjusted left ventricle mass (LVM/BW), mg/g; end diastolic volume (EDV), µl; ejection fraction (EF), %; stroke volume (SV), µl; cardiac output (CO), ml/min; body weight (BW), g.
### Table 1a

<table>
<thead>
<tr>
<th></th>
<th>10w dko</th>
<th>40w dko</th>
<th>80w dko</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVM (mg)</td>
<td>80.2 ± 5.1</td>
<td>111.1 ± 5.2</td>
<td>117.3 ± 5.5</td>
<td>0.0043</td>
</tr>
<tr>
<td>LVM/BW (mg/g)</td>
<td>3.03 ± 0.02</td>
<td>3.12 ± 0.18</td>
<td>4.18 ± 0.30</td>
<td>0.0271</td>
</tr>
<tr>
<td>EDV (µl)</td>
<td>67.4 ± 5.2</td>
<td>84.4 ± 4.5</td>
<td>78.9 ± 3.8</td>
<td>ns</td>
</tr>
<tr>
<td>EF (%)</td>
<td>54.9 ± 4.2</td>
<td>58.1 ± 5.7</td>
<td>75.0 ± 6.7</td>
<td>ns</td>
</tr>
<tr>
<td>SV (µl)</td>
<td>37.4 ± 4.6</td>
<td>48.6 ± 4.5</td>
<td>48.7 ± 5.0</td>
<td>0.0446</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>11.9 ± 1.6</td>
<td>17.6 ± 1.4</td>
<td>18.6 ± 1.7</td>
<td>0.0342</td>
</tr>
<tr>
<td>BW (g)</td>
<td>26.0 ± 1.2</td>
<td>35.5 ± 2.5</td>
<td>28.7 ± 3.0</td>
<td>0.0120</td>
</tr>
</tbody>
</table>

### Table 1b

<table>
<thead>
<tr>
<th></th>
<th>10w C57</th>
<th>40w C57</th>
<th>80w C57</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVM (mg)</td>
<td>88.3 ± 2.3</td>
<td>98.1 ± 6.7</td>
<td>138.3 ± 6.4</td>
<td>0.0227</td>
</tr>
<tr>
<td>LVM/BW (mg/g)</td>
<td>3.58 ± 0.11</td>
<td>3.10 ± 0.20</td>
<td>2.47 ± 0.09</td>
<td>0.0134</td>
</tr>
<tr>
<td>EDV (µl)</td>
<td>66.9 ± 3.1</td>
<td>83.0 ± 7.0</td>
<td>116.1 ± 9.5</td>
<td>0.0164</td>
</tr>
<tr>
<td>EF (%)</td>
<td>58.9 ± 6.3</td>
<td>58.6 ± 6.1</td>
<td>69.3 ± 6.3</td>
<td>ns</td>
</tr>
<tr>
<td>SV (µl)</td>
<td>39.1 ± 4.1</td>
<td>47.5 ± 4.9</td>
<td>79.8 ± 6.4</td>
<td>0.0253</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>11.9 ± 1.8</td>
<td>18.3 ± 2.6</td>
<td>34.5 ± 3.4</td>
<td>0.0103</td>
</tr>
<tr>
<td>BW (g)</td>
<td>24.8 ± 1.6</td>
<td>31.7 ± 2.2</td>
<td>56.0 ± 0.8</td>
<td>0.0023</td>
</tr>
</tbody>
</table>