

1 **Statins reverse postpartum cardiovascular dysfunction in a rat model of**  
2 **preeclampsia.**

3

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45 **Abstract**

46 Preeclampsia (PE) is associated with increased cardiovascular long-term risk;  
47 however, the underlying functional and structural mechanisms are unknown. We  
48 investigated maternal cardiac alterations after PE. Female rats harboring the human  
49 angiotensinogen gene [TGR(hAogen)L1623] develop a preeclamptic phenotype with  
50 hypertension and albuminuria during pregnancy when mated with male rats bearing  
51 the human renin gene [TGR(hRen)L10J], but behave physiologically normal before  
52 and after pregnancy. Furthermore, rats were treated with pravastatin. We tested the  
53 hypothesis that statins are a potential therapeutic intervention to reduce cardiovascular  
54 alterations due to simulated preeclamptic pregnancy. Although hypertension persists  
55 for only 8 days in pregnancy, former PE rats exhibit significant cardiac hypertrophy  
56 28 days after pregnancy observed in both speckle tracking echocardiography and  
57 histological staining. In addition, fibrosis and capillary rarefaction was evident.  
58 Pravastatin treatment ameliorated the remodeling and improved cardiac output  
59 postpartum. Preeclamptic pregnancy induces irreversible structural changes of cardiac  
60 hypertrophy and fibrosis, which can be moderated by pravastatin treatment. This  
61 pathological cardiac remodeling might be involved in increased cardiovascular risk in  
62 later life.

63 **Key words:** preeclampsia, pregnancy, cardiovascular risk, remodeling, pravastatin

64

65 **Introduction**

66 In 2011, the American Heart Association recognized pathological pregnancy, including  
67 gestational diabetes, preterm birth and preeclampsia as the first gender specific  
68 cardiovascular risk factor<sup>1</sup>. PE is a disorder with clinical symptoms in the last half of

69 pregnancy characterized by the onset of high blood pressure and signs of damage to  
70 another organ system (CNS with eclampsia, hematologic with thrombocytopenia),  
71 mostly relating to the liver and kidneys<sup>2</sup>. It was considered as a temporary condition as  
72 preeclamptic symptoms are generally resolved by placental birth. However, even if  
73 symptoms disappear, the higher risk for long-term renal and cardiovascular disease  
74 remains<sup>3</sup>. It is still unclear whether the increased risk is related to preeclamptic  
75 pregnancy or to predisposing factors, which already existed before pregnancy<sup>4</sup>. We  
76 have shown earlier that the mating of female Sprague-Dawley rats harboring the  
77 human angiotensinogen gene [TGR(hAogen)L1623] with [TGR(hRen)L10J] males  
78 leads to a preeclamptic phenotype with increased blood pressure starting on day 13 of  
79 pregnancy and albuminuria<sup>5, 6</sup>. In addition, fetal offspring are growth restricted<sup>6</sup>. The  
80 non-pregnant female [TGR(hAogen)L1623] rat show no noticeable phenotype, only  
81 when mated with [TGR(hRen)L10J] males, both transgene products interact in the  
82 uteroplacental unit resulting in high Angiotensin II (Ang II) levels and inducing the  
83 preeclamptic phenotype<sup>6</sup>.

84 Statins are crucial in the prevention and treatment of cardiovascular disease<sup>7</sup>. Besides  
85 the established cholesterol-lowering effect, pleiotropic effects including modulation of  
86 immune function and inflammatory processes as well as endothelial protection are  
87 important<sup>7</sup>. Currently the use of statins in pregnancy is not recommended; however,  
88 the topic is under intensive experimental and clinical research. In an initial case series  
89 a teratogenic risk was reported<sup>8</sup>, however, multiple recent meta-analysis failed to  
90 confirm this. Several potentially beneficial applications of statins in pregnant women,  
91 including PE<sup>9</sup> and anti-phospholipid antibody syndrome warrant further evaluation<sup>10</sup>.  
92 From all the different statins, pravastatin is most suitable for the application in  
93 pregnancy due to its unique pharmacokinetic and physiochemical properties.

94 Pravastatin has a limited ability to cross the placenta and is known to be one of the  
95 most hepatoselective and hydrophilic (polar) statins<sup>11</sup>. Thus, pravastatin has been  
96 shown to improve maternal and fetal impacts of PE in multiple mouse models<sup>12, 13</sup> as  
97 well as in human pilot studies<sup>9, 10</sup>. Furthermore, it is known that statins have various  
98 protective effects in the cardiovascular system<sup>7</sup>. We tested the hypothesis that  
99 pravastatin has a beneficial effect on the remodeling of the maternal heart after  
100 preeclamptic pregnancy and therefore lowers the long-term cardiovascular risk.

101

## 102 **Methods**

103 *The authors declare that all supporting data are available within the article (and its*  
104 *online supplementary files).*

105 *Animals:* 12-week-old virgin female Sprague-Dawley rats harboring the human  
106 angiotensinogen gene [TGR(hAogen)L1623] were mated with male rats bearing the  
107 human renin gene [TGR(hRen)L10J]. Daily, females were inspected for vaginal plugs.  
108 The first day that a plug was noticed was termed as first day (d1) of pregnancy.  
109 Pregnant transgenic rats developed typical preeclamptic phenotype with hypertension  
110 starting on d13 and albuminuria in later pregnancy<sup>5</sup>. Rats were housed in a  
111 temperature-controlled environment of 22±2°C, a humidity of 55±15% and 12:12-  
112 hour light/dark cycle. The animals had access to food (Sniff V1324-300) and water ad  
113 libitum. Rats were sacrificed at the end of pregnancy (d21) and 28 days postpartum by  
114 decapitation with prior isoflurane anesthesia or due to predefined stopping criteria  
115 according with the European law for animal protection. Local authorities approved the  
116 studies (State Office of Health and Social Affairs Berlin).

117 *Experimental design:* [TGR(hAogen)L1623] female rats impregnated by  
118 [TGR(hRen)L10J] form the preeclamptic group (PE). Age- and body weight-matched

119 wild-type Sprague-Dawley rats constituted the control group (WT). For potential  
120 intervention, one group of PE rats were treated daily with pravastatin (5 mg/kg/day,  
121 Sigma Aldrich)<sup>14</sup> via drinking water until 28 days postpartum (PE pp + prava) which is  
122 equivalent to two years of life in human<sup>15</sup>. Treatment started on day 15 of gestation.  
123 WT + prava was not included in the study because of the low clinical need.

124 *Blood pressure measurement:* DSI telemetry devices (DSI, HD-T11) for blood pressure  
125 monitoring were implanted 14 days before mating as described before<sup>6</sup>.  
126 Measurements were taken before, at the end of pregnancy (d21) and 28 days  
127 postpartum in a 5-minute distance.

128 *Echocardiography:* Transthoracic echocardiography was performed in anesthetized  
129 animals (1.5% isoflurane via an oxygen mask) at the end of pregnancy (d21) and 28  
130 days postpartum. ECG, respiration and temperature were monitored. Rectal  
131 temperature was maintained at 36°C by heated platform. All of the hair was removed  
132 from the abdomen by depilatory cream and pre-warmed gel was used as an  
133 ultrasound-coupling medium. A Vevo 3100 high-resolution imaging system (Fujifilm,  
134 VisualSonics Inc.) with a 21 MHz transducer (MS250) mounted on an integrated rail  
135 system was used. All images were acquired and stored for offline analysis by blinded  
136 observer using VisualSonics VevoStrain software (Version 2.2.0, Toronto, Canada). B-  
137 Mode cine loops were used in parasternal long and short axis view to assess basic  
138 parameters for systolic function and more sensitive speckle tracking analysis. Images  
139 were checked for quality with regard to differentiation of wall borders and absence of  
140 artefacts. The endocardium of the left ventricle was traced manually in parasternal  
141 short- and long-axis views in end-diastole. Analysis was performed on three  
142 consecutive cardiac cycles; mean values from three measurements were calculated.  
143 Global strain values were obtained from the average of the six segments of the left

144 ventricle. M-mode was obtained to measure cardiac wall and chamber dimensions.  
145 Relative wall thickness was calculated by the formula  $(2*PWd)/LVEDD$ .

146 *mRNA isolation and qRT-PCR:* Snap frozen cardiac tissue of the left ventricle was  
147 homogenized by ceramic beads. Total RNA was extracted using commercial Kits (lysis  
148 reagent and RNeasy mini kit, Qiagen) and protocols provided by the manufacturers. 2  
149  $\mu$ g of mRNA was reverse transcribed into cDNA using High Capacity cDNA Reverse  
150 Transcription Kit (Applied Biosystems). Relative quantification of gene expression was  
151 performed by real-time polymerase chain reaction (PCR) using an ABI 7500 Fast  
152 Sequence Detection System (Applied Biosystems) and analyzed by 7500 Fast System  
153 Software (Applied Biosystems). Primers and probes (Supplementary Table S1) were  
154 designed with Primer Express 3.0 (Applied Biosystems) and synthesized by Biotex,  
155 Germany. Quantitative analysis of target mRNA expression was performed with real-  
156 time PCR using the relative standard curve method. 36B4 was used as housekeeping  
157 gene. The primer for all target were validated by blasting.

158 *Circulatory and urinary factors:* The expression of circulating BNP and sFlt1 was  
159 analysed in venous blood plasma by using ELISA kits (#ab108816 BNP45, abcam:  
160 #MBS725733 sFlt1, MyBioSouce). Urinary albumin was detected by the company  
161 CellTrend, Luckenwalde Germany.

162 *Immunohistochemistry:* Hearts were harvested, fixed with formalin and embedded in  
163 paraffin. Samples were cut through the short axis into 2  $\mu$ m thick sections and stained  
164 with antibodies for wheat germ agglutinin (WGA, #FL-1021 Vector Laboratories),  
165 collagen type I (#1310-01, SouthernBiotech), fibronectin (#ab23751, abcam), CD68  
166 (#MCA341R, Bio-Rad) and CD31 (#AF3628, R&D) followed by Cy3-labeled secondary  
167 antibody to detect cardiac remodeling. Vectashield mounting medium with DAPI (#H-  
168 1200, Vector Laboratories) was used to stain nuclei. The stained sections were imaged

169 by fluorescence slide scanner Panoramic MIDI II BF/FL high speed (3DHISTECH  
170 Ltd., Budapest, Hungary), saved and offline evaluated using CaseViewer analysis  
171 software (3DHISTECH Ltd., Budapest, Hungary). To quantify the perimeter of  
172 cardiomyocytes, 100 randomly selected cells per section were framed manually in  
173 WGA staining. Collagen type I staining was used to determine the content of  
174 perivascular fibrosis. All vessels were assessed with regard to internal diameter, media  
175 and fibrotic boarder. Fibrotic area in relation to media area was compared. To quantify  
176 interstitial fibrosis, 16 representative microscope fields per section without vascular  
177 content were determined with regard to percentage of fibrosis (fibronectin staining)  
178 using ImageJ (NIH). CD68 staining was applied to clarify inflammation status. 20  
179 representative microscope fields per section without vascular content were manually  
180 counted for positive cells. To clarify capillary density in the hearts, CD31 staining of  
181 endothelial cells was exerted<sup>16</sup>. 10 representative microscope fields per section were  
182 manually counted for positive signals. For all staining, mean score for each animal was  
183 calculated by blinded observer and used to deduce a group mean score. To test  
184 specific binding sites negative controls were used without primary antibody to make  
185 sure that the secondary antibody do not bind unspecific. No isotype control was used.

186 *Statistics:* Statistical analyses were performed by using Prism 7.0 software (GraphPad  
187 Software Inc.). ROUT method was performed for outlier identification with an average  
188 false discovery rate less than 1%.  $P < 0.05$  was considered statistically significant.  
189 After testing for normal distribution group differences were analyzed by 2-tailed  
190 unpaired t test, Mann-Whitney U test, one-way ANOVA with Tukey post hoc test for  
191 multiple comparison or 2-way ANOVA with Bonferroni post hoc test, as appropriate.  
192 All data is presented as means $\pm$ SD.

193



194 **Results**

195 *Evaluation of cardiac function by Speckle Tracking Echocardiography (STE)*

196 Detailed echocardiography including STE was performed to evaluate alterations in  
197 cardiac function (Tab. 1). Ejection fraction is mildly but significantly decreased  
198 postpartum in the PE rats, but remains within normal limits. In these rats, an increase  
199 in heartrate is also observed. Left ventricular mass is more than 10 % higher in former  
200 PE rats compared to wild-type controls (WT pp) and goes closely together with an  
201 increased posterior wall thickness and a higher relative wall thickness. Values of inner  
202 diameter are unaltered. Pravastatin treatment (PE pp + prava) improves the mentioned  
203 parameter, but not completely to the level of unaffected control pregnancy. Global  
204 longitudinal strain, global radial strain, and global circumferential strain are significantly  
205 reduced postpartum in the PE rats. Similar results are observed for the corresponding  
206 global strain rates. Former preeclamptic rats who were treated with statins  
207 demonstrate better values than untreated ones. The detected echocardiographic  
208 changes are already partly seen at the end of pregnancy (data not shown), but  
209 becoming more pronounced postpartum. Figure 1 summarizes the relative postpartum  
210 changes due to a preeclampsia simulating pregnancy and the benefits of statin  
211 treatment. As previously published, blood pressure and proteinuria are increased  
212 during pregnancy in this transgenic rat model for PE<sup>6</sup>. We confirm higher mean arterial  
213 pressure (MAP) in the PE rats on day 18 of pregnancy, independent of statin treatment  
214 (Suppl. Fig. S1A-B). Importantly, no differences in MAP are observed postpartum  
215 (Suppl. Fig. S1C-D). Increased levels of urinary albumin are detected during PE and  
216 could be reduced by pravastatin (data not shown).

217

218 *Pathological cardiac remodeling*

219 Replacement fibrosis in the myocardium is the hallmark of myocardial remodeling,  
220 which can be found in pathological hypertrophy<sup>17</sup>. Hearts after a preeclamptic  
221 pregnancy (PE pp) show larger cardiomyocytes. This enlargement can be avoided by  
222 pravastatin (Fig. 2A). Immunohistochemical studies show increased levels of  
223 fibronectin postpartum. Pravastatin can reduce this interstitial fibrosis (Fig. 2B). In  
224 addition, we detect fibrotic spots in 7 out of 11 preeclamptic hearts postpartum. Hearts  
225 in the control group were unaffected. Treated PE rats show these spots only in 3 out  
226 of 9 hearts (supplemental Fig. S2A). In addition, preeclamptic hearts also show a  
227 higher perivascular fibrosis compared to controls postpartum (Fig. 2C). Postpartum is  
228 no difference in CD68-positive cells noticed (Fig. 2D). Inflammation in maternal hearts  
229 is only detectable during PE by a higher number of CD68-positive cells (ED1 staining).  
230 Treatment with pravastatin reduces the number of macrophages (Suppl. Fig. S2B). To  
231 test the additional hypothesis that microvascular damage is sustained during  
232 preeclamptic pregnancy, we compare capillary density. Hearts of PE rats show a lower  
233 number of capillaries. This rarefaction could be avoided by pravastatin (Fig. 2E).

234 Brain natriuretic peptide (BNP) is still increased in blood plasma levels in PE rats pp.  
235 Animals treated with pravastatin show no increase compared to wild-type controls (Fig.  
236 3A). This is already detectable during pregnancy (Suppl. Fig. S3A). Soluble fms-like  
237 tyrosine kinase-1 (sFlt1) is a protein with antiangiogenic properties and is used as one  
238 of the first biomarker for PE<sup>18</sup>. With increased binding affinity to the vascular endothelial  
239 growth factor (VEGF), sFlt1 reduces blood vessel growth<sup>19</sup>. Preeclamptic animals  
240 show increased levels of blood sFlt1 not only at the end of pregnancy (Suppl. Fig. 3B),  
241 but also postpartum this factor is still increased. Pravastatin treatment reduces levels  
242 of sFlt-1 (Fig. 3B). Treated groups show no increase in sFlt-1 compared to wild-type  
243 controls.

244

245 *Alteration of gene expression levels*

246 Predominantly at the end of pregnancy but also postpartum, we could detect altered  
247 gene expression related to hypertrophy, fibrotic remodeling, inflammation and  
248 disturbed angiogenesis (Fig. 4). Four weeks after delivery most dysregulated genes  
249 are comparable to controls. PE rats show higher levels of atrial natriuretic protein (*Anp*)  
250 and a borderline significant increase in *Bnp*. Fibrotic marker genes like fibronectin (*Fn*)  
251 or collagen type I (*Col1*) are also increased in PE rats. Matrix metalloproteinase 2  
252 (*Mmp2*) shows no difference. Connective tissue growth factor (*Ctgf*) is only elevated  
253 postpartum, with no effect of treatment. Tissue growth factor  $\beta$  (*Tgf  $\beta$* ), tumor necrosis  
254 factor  $\alpha$  (*Tnf  $\alpha$* ) and interleukin-6 (*Il-6*) are not changed. Monocyte chemoattractant  
255 protein 1 (*Mcp1*) and the cluster of differentiation 68 (*CD68*), as well as lipocalin (*Ngal*)  
256 are increased during PE. The CCAAT/enhancer binding protein  $\beta$  (*Cebpb*) is  
257 unchanged and the genes for myosin heavy chain  $\alpha$  (*Myh 6*) and  $\beta$  (*Myh 7*) only reach  
258 borderline significance. The Angiotensin II receptor type 1 (*At-1*), Phospholamban (*Pln*)  
259 and the hypoxia inducible factor 1 (*Hif1*) are reduced in preeclamptic animals during  
260 pregnancy. The *sFlt-1* is not altered in heart tissue. Matrix metalloproteinase 12  
261 (*Mmp12*) is highly upregulated in PE rats. These changes could be improved by statins  
262 and do not persist. Postpartum persistent and unaffected by treatment is the  
263 expression of endothelin-1 (*Et-1*) and platelet endothelial cell adhesion molecule  
264 (*Pecam-1*). Vascular endothelial growth factor A (*Vegfa*) and matrix metalloproteinase 9  
265 (*Mmp9*) seem downregulated. The Rho associated coiled-coil containing protein  
266 kinase 1 (*Rock1*) is reduced during PE and higher expressed when the animals are  
267 treated. Total values of gene expression are given in supplemental table S3.

268

269 *Safety of fetal outcome*

270 Pravastatin did not influence the numbers of fetuses, implantations and resorptions in  
271 regard to untreated PE rats or WT controls (Suppl. Fig. 4A-C). PE offspring often suffer  
272 from placental insufficiency and growth restriction<sup>20</sup>. Pravastatin show no negative  
273 effect in weight of the uteroplacental unit (Fig. 5A) or weight the fetus (Fig. 5B). Fetal  
274 heart weight (Fig. 5C), heart body ratio (Fig. 5D), brain liver ratio (Fig. 5E) or fetal  
275 kidney weight (Fig. 5F) are not altered.

276

277 **Discussion**

278 The major finding in our study is that PE leads to persistent structural remodeling and  
279 functional changes in the maternal heart, which can be attenuated by pravastatin  
280 treatment. The effect of pravastatin was independent of blood pressure. Cardiac  
281 hypertrophy and fibrosis were present during PE and persisted postpartum. Interstitial  
282 fibrosis is regarded as replacement fibrosis and we speculate that this process might  
283 be persistent. Hypertrophy and fibrosis are major causes for the observed diastolic  
284 dysfunction, described by several parameters in the echocardiographic data. In  
285 addition, we used speckle tracking analysis to analyze the degree of myocardial  
286 deformation and to detect even subclinical changes already described in humans<sup>21</sup>.  
287 Preeclampsia is not a mono-causal disease as it is in the used animal model. However,  
288 the transgenic animal model resembles many important features of the human  
289 syndrome, such as placenta induced pathology<sup>22</sup> with high blood pressure, proteinuria  
290 and IUGR<sup>6</sup>, increased sFlt-1 levels<sup>23</sup> or existing AT1 auto-antibodies<sup>24</sup>. Furthermore  
291 the female is healthy before pregnancy and blood pressure resolves after delivery. We  
292 hypothesize that the observed findings leading to heart failure stage B are  
293 pathophysiological mechanisms leading to the observed significantly higher risk for

294 long-term cardiovascular disease in these patients. Women with PE and their offspring<sup>3</sup>  
295 have an increased risk for later cardiovascular disease (CVD). Two theories speculate  
296 about the causality that link PE and CVD. The first is that PE and atherosclerosis share  
297 risk factors for systemic inflammation and endothelial dysfunction, which are  
298 unmasked by the “stress” of pregnancy<sup>25</sup>. It is also possible that pregnancy, and  
299 especially PE, may induce permanent arterial changes, mediating risk for future CVD  
300 through the pro-atherogenic stress of PE that could activate arterial wall inflammation  
301 that fails to resolve after delivery.

302 The effect of PE on the cardiovascular microvasculature is striking. A large number of  
303 human studies have documented that healthy women with a history of PE have  
304 elevated vasoconstrictor responses<sup>26</sup>, increased arterial stiffness<sup>27</sup> and show retinal  
305 microvascular dysfunction<sup>28</sup>. This goes closely together with our findings regarding  
306 rarefaction of myocardial capillaries and might be the cause for healthy former  
307 preeclamptic women having an increased long-term risk for cardiovascular disease.  
308 Statins are able to ameliorate the decrease in capillary density in the heart shown in a  
309 study with pigs<sup>29</sup>. *Paulus et al.* recently introduced a novel concept for heart failure with  
310 preserved ejection fraction<sup>30</sup>. They propose that the cause of myocardial structural and  
311 functional alterations is a systemic pro-inflammatory state leading to microvascular  
312 endothelial dysfunction. We already could show that the cardiovascular biomarker  
313 midregional proatrial natriuretic peptide (MR-proANP) could also be a suitable marker  
314 for PE and speculated that it reflects a cardiovascular hemodynamic stress<sup>31</sup>. There is  
315 an increasing understanding that CVDs are generally progressive disorders that  
316 proceed through asymptomatic to symptomatic stages<sup>32</sup>. Women are much more likely  
317 to suffer from Ischemia and Non Obstructive Coronary Artery Disease (INOCA)<sup>33</sup>.  
318 These patients have elevated risk for a cardiovascular event and appear to be at higher

319 risk for the development of heart failure with preserved ejection fraction. It is  
320 remarkable that the preeclamptic phenotype which lasts in the animal model for only 8  
321 days has such a profound long-term effect. Recently a novel concept has been  
322 proposed indicating that the pathological remodeling process induced by cardiac  
323 stressors such as angiotensin II (Ang II) depends on the context and condition of the  
324 organism. Ang II induces dramatic vasoconstriction, collapse and regression of  
325 immature capillaries in the developing organ of 8 weeks old mice, whereas in an adult  
326 mouse the same dosage has a far less profound effect on the retinal vasculature<sup>34</sup>. We  
327 speculate that pregnancy, with its pro-inflammatory milieu and the extraordinary  
328 metabolic demands and cardiovascular adaptation, represents a potentially unstable  
329 situation where cardiac stressors such as Ang II or preeclamptic mediators, such as  
330 sFlt1 or activating autoantibodies against the AT1 receptor are capable of inducing  
331 long-term cardiovascular consequences.

332 At the moment, the only “cure” for PE is delivery. Treatment during pregnancy to  
333 improve maternal and fetal outcome is limited. Methyldopa or nifedipine, the drugs of  
334 first choice, lower blood pressure and help to extend wearing time<sup>35</sup>. However, no  
335 treatment has yet been shown to reduce long-term cardiovascular risk in preeclamptic  
336 patients. The beneficial effect of pravastatin treatment is described by enhanced  
337 cardiac function, less cardiac hypertrophy and diminished cardiac fibrosis. Even if there  
338 was no change in systolic blood pressure, our results indicate a reduction in structural  
339 remodeling which goes hand in hand with an improvement in cardiac function.  
340 Reversion of fibrosis in the heart by statins was shown in other disease models  
341 before<sup>36, 37</sup>. *Hermida et al.* demonstrated that statin treatment reverses myocardial  
342 remodeling and improves ventricular relaxation through AMPK-mediated anti-fibrotic  
343 effects<sup>37</sup>. Another study showed that simvastatin inhibited the fibrosis around coronary

344 arteries in endogenous adrenomedullin heterozygous knockout mice treated with  
345 angiotensin (Ang) II and high salt loading<sup>36</sup>. Taken together, pravastatin attenuates  
346 hypertrophic and pro-fibrotic stimuli and thereby reduces the long-term risk of  
347 cardiovascular diseases after preeclamptic pregnancy. All positive effects seen by  
348 pravastatin were cholesterol-independent due to the fact that statins do not lower  
349 serum cholesterol in rats because of compensatory increases in hepatic enzyme  
350 production<sup>38</sup>. Similar to the findings of *Garrett et al.*<sup>12</sup> and *Bauer et al.*<sup>39</sup>, we postulate  
351 a beneficial impact of pravastatin treatment with no detectable harm to the fetal  
352 outcome. In comparison with other disease models<sup>40</sup> which need much more duration  
353 of high blood pressure than this short period during pregnancy, the advantageous  
354 effect of pravastatin could be explained. Mice exposed to Ang II overexpression for  
355 four weeks, developed high blood pressure in combination with cardiac hypertrophy  
356 and fibrosis<sup>40</sup>. Daily treatment with pravastatin had no effect on systolic blood pressure  
357 but improved cardiac function and reduced left ventricular hypertrophy and fibrosis.  
358 Pleiotropic effects of statins restored endothelial function and decreased vascular  
359 inflammation. Many of these effects are mediated by the localization and function of  
360 intracellular signaling molecules like small GTP-binding proteins, Rho, Ras and Rac<sup>41</sup>.  
361 We showed that Rock1 which is the intermediate downstream target of RhoA is  
362 dysregulated in the heart in preeclamptic rats at the end of pregnancy compared to  
363 control rats. Pravastatin ameliorates the downregulation of Rock1. For this reason, the  
364 pathway of Rho-kinase seems to have a crucial influence on the effects of pravastatin  
365 in lowering long-term cardiovascular risk after preeclampsia<sup>42</sup>. Additionally, it was  
366 shown in Hemoxygenase 1 deficient mice that pravastatin increases HO-1 activity in  
367 liver and placenta and improves survival of the fetus<sup>43</sup>.

## 368 **Perspectives**

369 Preeclampsia currently has no effective pharmacological treatment to reduce long-  
370 term cardiovascular risk. In the transgenic rat model with preeclampsia-like symptoms,  
371 we found that pravastatin has a high potential to benefit maternal outcome with no  
372 harm to the offspring. Statins have a well-established role in the prevention of  
373 cardiovascular disease in general population<sup>44</sup> and there are counting indications that  
374 statins may have similar cardiovascular gain in preeclampsia<sup>45</sup>. Indeed, a human pilot  
375 study detected no identifiable safety risk<sup>9</sup>.

376

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387

### 388 **Disclosures**

389 The authors have declared that no conflict of interest exists.

390



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541

542 **Novelty and Significance**

543 **What Is New?**

- 544 - PE leads to persistent structural remodeling of the heart
- 545 - Preeclamptic mothers may benefit from pravastatin treatment to prevent cardiac  
546 changes
- 547 - Pravastatin treatment in the last trimester does not harm the fetus of a  
548 preeclamptic pregnancy

549 **What Is Relevant?**

550 PE leads to increased cardiovascular long-term risk, however, the underlying  
551 functional and structural mechanisms are unknown. The present study investigated  
552 persistent structural alterations as presumed cause and showed the benefit of statin  
553 treatment for maternal long-term health.

554 **Summary:**

555 With advanced echocardiography we demonstrate alterations in the transgenic rat  
556 model for simulated preeclampsia. Former preeclamptic rats exhibit significant cardiac  
557 hypertrophy postpartum in combination with fibrosis and capillary rarefaction.  
558 Pravastatin treatment ameliorated the remodeling and improved cardiac output  
559 postpartum.

560

561 **Figure Legends**

562 **Table 1.** *Functional and structural changes after PE were detected by advanced*  
563 *echocardiography and improved by statin treatment.* Data shown as mean±SD; WT  
564 n=8, PE n=10, PE+prava n=7; pp postpartum, LV left ventricle; One-way ANOVA, \*  
565 significant to WT, # significant to PE.

566 **Figure 1.** *Pravastatin improves cardiovascular dysfunction.* Relative values of treated  
567 and untreated preeclamptic animals in comparison to the healthy wild-type group are  
568 shown. Healthy controls were normalized to 1. Pp postpartum.

569 **Figure 2.** *Preeclampsia led to persistent hypertrophy, increased fibrosis and capillary*  
570 *rarefaction.* Cardiac hypertrophy (A), interstitial (B) and perivascular fibrosis (C), CD68-  
571 positive cells (D) and cardiac capillaries (E) are shown. Data shown as mean±SD; WT  
572 n=8, PE n=8, PE +prava n=8; pp postpartum; Scale WGA 20µm, Fn 50µm, Col1 50µm,  
573 CD31 20µm, ED1 20µm. One-way ANOVA, ns. not significant, \*\*\*p<0.001,  
574 \*\*\*\*p<0.0001.

575 **Figure 3.** *Pravastatin reduced persistent elevation of plasma BNP and sFlt1 levels*  
576 *after preeclamptic pregnancy.* Levels of brain natriuretic peptide (A) and soluble fms-  
577 like tyrosine kinase-1 (B) are shown. Data shown as mean±SD; WT n=6, PE n=7, PE  
578 +prava n=6; pp postpartum; One-way ANOVA, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

579 **Figure 4.** *Alterations in gene expression levels were detectable predominantly at the*  
580 *end of PE.* Heat maps of gene expression levels during pregnancy and postpartum.  
581 Data given as median. Total values are given in Tab. S2. WT d21 n=6, PE d21 n=5,  
582 PE d21 +prava n=5, WT pp n=8, PE pp n=8, PE pp +prava n=8; d21 day 21 of  
583 pregnancy, pp postpartum; One-way ANOVA \*p<0.05, \*\*p<0.01, \*\*\*p<0.001,  
584 \*\*\*\*p<0.0001, #p<0.05 significant to PE.



585 **Figure 5.** *Pravastatin has no harmful effect on the offspring.* Placental weight (A), fetal  
586 body weight (B) and heart weight (C), as well as fetal heart body ratio (D), fetal brain  
587 liver ratio (E) and fetal kidney weight (F) are shown. Data shown as mean±SD; mean  
588 values per dam are shown; WT n=3, PE n=7, PE + prava n=7; One-way ANOVA, ns.  
589 not significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.01, \*\*\*\*p<0.0001.