Renin-Angiotensin System Blockade Associated with Statin Improves Endothelial Function in Diabetics

Ronaldo Altenburg Gismondi, Ricardo Bedirian, Cesar Romaro Pozzobon, Márcia Cristina Ladeira, Wille Oigman, Mário Fritsch Neves
Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ – Brazil

Abstract

Background: Studies suggest that statins have pleiotropic effects, such as reduction in blood pressure, and improvement in endothelial function and vascular stiffness.

Objective: To analyze if prior statin use influences the effect of renin-angiotensin-aldosterone system inhibitors on blood pressure, endothelial function, and vascular stiffness.

Methods: Patients with diabetes and hypertension with office systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 80 mmHg had their antihypertensive medications replaced by amlodipine during 6 weeks. They were then randomized to either benazepril or losartan for 12 additional weeks while continuing on amlodipine. Blood pressure (assessed with ambulatory blood pressure monitoring), endothelial function (brachial artery flow-mediated dilation), and vascular stiffness (pulse wave velocity) were evaluated before and after the combined treatment. In this study, a post hoc analysis was performed to compare patients who were or were not on statins (SU and NSU groups, respectively).

Results: The SU group presented a greater reduction in the 24-hour systolic blood pressure (from 134 to 122 mmHg, p = 0.007), and in the brachial artery flow-mediated dilation (from 6.5 to 10.9%, p = 0.003) when compared with the NSU group (from 137 to 128 mmHg, p = 0.362, and from 7.5 to 8.3%, p = 0.820). There was no statistically significant difference in pulse wave velocity (SU group: from 9.95 to 9.90 m/s, p = 0.650; NSU group: from 10.65 to 11.05 m/s, p = 0.586).

Conclusion: Combined use of statins, amlodipine, and renin-angiotensin-aldosterone system inhibitors improves the antihypertensive response and endothelial function in patients with hypertension and diabetes. (Arq Bras Cardiol. 2015; 105(6):597-605)

Keywords: Hypertension; Renin-Angiotensin System/drug effects; Endothelium/physiopathology; Diabetes Mellitus.

Introduction

Statins are the main LDL-cholesterol lowering drugs. Studies show that these drugs have pleiotropic effects, such as improvement in endothelial vasodilation response, increase in nitric oxide bioavailability, and reduction in endothelin levels. These effects are believed to be related to their benefit in populations with high cardiovascular risk, in which their use is associated with reduced risk of cardiovascular events.

One of the pleiotropic effects of the statins is the reduction in blood pressure (BP). A recent meta-analysis that included more than 20 thousand patients showed a reduction of 2.6 mmHg in systolic BP with statins. In addition, this effect was greater in patients undergoing pharmacological treatment for hypertension, suggesting a potential synergistic mechanism between the antihypertensive drugs and the statins.

The aim of this study was to analyze whether the use of statin would influence the effect of renin-angiotensin-aldosterone system (RAAS) inhibitors on BP, endothelial function, and vascular stiffness in a population of patients with hypertension and type 2 diabetes mellitus (T2DM).

Methods

Patients Selection

Patients selected for this project had hypertension and T2DM and were followed up as outpatients in the internal medicine service at Universidade do Estado do Rio de Janeiro (UERJ). The inclusion criteria were a previous diagnosis of hypertension and T2DM, age between 40 and 70 years, and systolic BP ≥ 130 mmHg and/or diastolic BP ≥ 80 mmHg. The exclusion criteria included resistant hypertension, insulin use, stages 4 and 5 chronic kidney disease, previous history of myocardial infarction and/or stroke, stages C and D heart failure, atrial fibrillation, symptomatic peripheral arterial disease, retinopathy with reduced visual acuity, nephrotic syndrome, and symptomatic diabetic neuropathy.
The study was approved by the Research Ethics Committee of Hospital Universitário Pedro Ernesto (UERJ) under the number 01539612.6.0000.5259. All participants read and signed a free and informed consent form. The study is registered at ClinicalTrials.gov under the number NCT01603940.

Study Design
The study was an open randomized clinical trial, with two active treatment groups and without use of placebo. At visit 1, patients underwent a clinical evaluation and had their antihypertensive medications replaced by amlodipine 5 mg/day. Visit 2 was conducted 6 weeks later and included clinical and laboratory evaluation, ambulatory BP monitoring (ABPM), and assessment of endothelial function and vascular stiffness. During this visit, the patients were randomized to benazepril 10 mg/day or losartan 50 mg/day while continuing on amlodipine. Two reevaluations (visits 3 and 4) were performed at intervals of 4 weeks for adjustment of BP, which was aimed at values < 130 x 80 mmHg. The antihypertensive medications in the study could have their doses doubled (benazepril 20 mg and losartan 100 mg), and the addition of hydrochlorothiazide 25 mg/day was allowed on visits 3 and 4. The dose of amlodipine was maintained at 5 mg/day. At the end of 12 weeks of treatment with RAAS inhibitors, the final visit (visit 5) was conducted, and the assessments performed on visit 2 were repeated. The dose of the T2DM medication was maintained constant.

All tests were conducted after a 12-hour fast for biochemical tests. When triglyceride levels were < 400 mg/dl, we calculated the LDL-cholesterol fraction with the Friedewald formula. Glomerular filtration rate (eGFR) was estimated with the MDRD formula: eGFR = 186*Creatinine^{-1.154}*Age^{-0.203}(0.742 if female). C-reactive protein was determined by nephelometry (Siemens AG Inc., Munich, Germany). To measure microalbuminuria, we used the albuminuria/creatinuria ratio determined in a sample of first-morning urine.

Vascular Tests

Brachial Artery Flow-Mediated Dilation (FMD)
The test was conducted according to the latest guidelines on the method. The examiner was blinded to the patients’ treatment. We used the ultrasound equipment Vivid 3 (GE Healthcare, Milwaukee, WI, USA) with a 10 MHz high-resolution linear transducer. The patient was placed in the supine position, with the right arm abducted. After locating the brachial artery, the transducer was placed on the anteromedial surface of the right arm, perpendicular to the axis of the arm, 2 to 5 cm above the antecubital fossa, on the topography of the brachial artery. The measurements were obtained at the end of the diastole (R wave on the electrocardiogram). Ischemia was induced by inflation of the cuff 50 mmHg above the systolic BP during 5 minutes. The largest diameter of the artery was recorded 30, 60, and 90 seconds after deflation of the cuff. FMD was calculated as the variation of the largest diameter of the artery in relation to its baseline measurement.

Pulse Wave Velocity (PWV)
Pulse waves were obtained with the equipment Complior SP (Alam Medical, Paris, France). The transducers were positioned on the right carotid, right radial (crPWV), and right femoral (cfPWV) arteries. The distances between
the pulses were obtained directly with a tape measure. The carotid-femoral distance was multiplied by 0.8, and this value was used by the equipment for the calculation. Two PWV measurements were carried out, and when the difference between them was greater than 0.5 m/s, a third measurement was obtained. The average of these measurements was used for the analysis.

**Determination of Central Aortic Pressures**

Pulse waves at the right radial artery were obtained with a tonometer (SPC-301 - Millar Instruments, Texas, USA) calibrated according to the pressure in the brachial artery. Analysis of this arterial wave by applanation tonometry was performed to derive central aortic pressures and other hemodynamic parameters using the system SphygmoCor (Atcor Medical, Sydney, Australia). This software calculated the central systolic BP, central pulse pressure (PP), augmentation pressure (AP), and augmentation index (AIx). Two measurements were obtained, and when the difference between them was greater than 10%, a third measurement was performed. The average of these measurements was used for the analysis.

**Statistical Analysis**

The data are presented as median (interquartile range) for numeric variables and distribution of frequencies and proportions for categorical variables. The Mann-Whitney test and Fisher's exact test were used to compare numerical and categorical variables, respectively. The Wilcoxon test was used for paired comparison of variables. Based on a maximum type I error of 0.05 and a standard deviation of 3.5%, a sample size of 14 patients in each group would have 80% power to find a difference ≥ 4% in FMD. The calculation of the sample size used the expected FMD difference between the benazepril and losartan groups according to the design of the original study. The statistical analysis was performed using a significance level of 5% and the software Statistica 12 (Statsoft Inc, Tulsa, OK, USA).

**Results**

According to the inclusion criteria, 47 patients were selected, and 30 were randomized to one of the treatment groups (Figure 1). On visit 1, there were 13 patients in the SU group and 17 patients in the NSU group. All patients showed good treatment adherence. Baseline demographic, clinical, and laboratory characteristics of these patients are shown in Table 1. After treatment with the angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB), there was no statistically significant difference in laboratory parameters between the groups (data not shown).

**Blood Pressure**

In the analysis of the office BP in the SU group, there was a non-significant decrease in diastolic (8.0 mmHg or 9% reduction) and systolic BP (11.6 mmHg or 8% reduction), although no statistical significance was observed (Figure 2). In the ABPM analysis, the SU group exhibited a greater reduction in mean 24-hour systolic BP (11.5 mmHg or 9% reduction) (Figure 3 and Table 2). A similar effect was observed in the mean 24-hour diastolic BP (4.0 mmHg or 5% reduction) and PP (7.0 mmHg or 13% reduction) (Table 2).

There was a similar percentage of patients using ACEI (54 vs 41%) and ARB (46 vs 59%) in the SU and SNU groups, and a similar proportion of patients who required dosage adjustment of these medications (61% vs 47%, p = 0.430) and/or association of hydrochlorothiazide for BP control (15% vs 17%, p = 0.865).

**Endothelial Function and Vascular Stiffness**

The SU group presented a greater FMD response and a higher reduction in systolic aortic BP when compared with the NSU group (Figure 4). There was no statistically significant difference in Alx, ciPWV, and crPWV responses (Table 3).

**Discussion**

The most recent guidelines recommend that patients with T2DM should use RAAS inhibitors (ACEI or ARB) and statins. The present study showed that simvastatin improves endothelial function and increases the antihypertensive effect of ACEI or ARB in association with amlodipine in patients with hypertension and diabetes.

The antihypertensive mechanisms of the statins are probably due to an increase in nitric oxide bioavailability with an increase in endothelium-dependent vasodilation response, and a reduction in endothelin-1 concentration and free radicals formation. In addition, a synergism is also speculated between statins and RAAS inhibitors through reduced expression of type 1 angiotensin receptors and blockade of intracellular pathways associated with angiotensin II action, both caused by statins. Many of these benefitting mechanisms of the statins are independent of the reduction in LDL-cholesterol. In the present study, the reduction in BP and improvement in endothelial function occurred despite statistically similar LDL-cholesterol values in the SU and NSU groups.

In a recent meta-analysis, Briasoulis et al. observed a mean systolic BP reduction of 2.6 mmHg in patients on statins, even in those without a diagnosis of hypertension. In hypertensive patients, the systolic BP may reduce up to 5.8 mmHg. In diabetics, the study estimated a reduction of 6.5 mmHg in the systolic BP and 4.0 mmHg in the diastolic BP. Another meta-analysis by Strazzullo et al. also examined the antihypertensive effect of statins and observed an average reduction of 4.0 mmHg in the systolic and 1.2 mmHg in the diastolic BP. Strazzullo et al. observed in a subgroup of diabetic patients a reduction of 3.7 mmHg in the systolic and 0.8 mmHg in the diastolic BP. None of these studies investigated the association of statins with ACEI or ARB.

Spósito et al. studied the interaction between ACEI and statins and observed that the group receiving a combination of both showed greater BP reduction when compared with the group receiving ACEI alone (21 vs. 14 mmHg, p < 0.05). However, Mancia et al. observed no reduction...
in BP with the association of pravastatin and fosinopril. Koh et al.\textsuperscript{19} evaluated the combination of losartan and ramipril with simvastatin and did not observe greater BP reduction with this association\textsuperscript{19,20}. In the present study, the association of benazepril or losartan in patients already receiving simvastatin and amlodipine promoted greater reduction in systolic and diastolic BP, both in casual measurements in the office and in 24-hour measurements.

Zhang et al.\textsuperscript{21} recently published a meta-analysis on the effects of statins in brachial artery FMD in patients with T2DM. The final result showed an improvement of 0.94% (95% CI 0.38-1.50%, p < 0.001) in absolute FMD values. Koh et al.\textsuperscript{19} conducted a study in which patients were randomized to three groups: ramipril, simvastatin, or a combination of both. Their cohort, comprised of 76% of hypertensive patients, included individuals with a mean age of 60 years, T2DM, and LDL-cholesterol > 100 mg/dL. The authors observed that the combined use of statins and ACEI promoted a greater increase in FMD than each medication alone. In contrast, no improvement in FMD was observed by Van Venrooij et al.\textsuperscript{22} with atorvastatin, and Beishizen et al. with cerivastatin and simvastatin. Tan et al.\textsuperscript{24}, in a population with T2DM and dyslipidemia, observed an improvement in FMD with atorvastatin when compared with placebo. In the present study, which assessed a population of diabetic patients with hypertension, only those already using simvastatin had a statistically significant improvement in brachial artery FMD when benazepril or losartan were associated.

Kanaki et al.\textsuperscript{25} evaluated the use of simvastatin in patients with hypertension and dyslipidemia and observed improvements in PWV, aortic BP, and AIx. However, no improvement in aortic pressure was observed with atorvastatin by William et al.\textsuperscript{26} in hypertensive patients and Fasset et al.\textsuperscript{27} in patients with chronic renal failure. Raisone et al.\textsuperscript{28} were one of the few to observe worsening in PWV with atorvastatin. In contrast, Pirro et al.\textsuperscript{29} and Maki-Petaja et al.\textsuperscript{30} observed improvement in PWV with statins. So far, there are no studies on the effects of statins on aortic pressure and PWV in diabetic patients with
hypertension. The present study showed a reduction in central aortic pressure, in parallel with reductions in office BP and ABPM in simvastatin users, although no statistically significant changes were observed in AIx and PWV.

The study has some limitations. Since this is a post hoc analysis, the groups were not randomized according to statin use. In addition, approximately half of the study sample had no prior use of statin. Although it was not possible to evaluate the reasons, the group without prior use of simvastatin could have pre-selected patients with poor adherence to antihypertensive drugs before recruitment for the study. These patients could have BP levels above the recommended target, higher prevalence of vascular lesions, and as a result, inadequate response to antihypertensive drugs or lower improvement in endothelial function with the treatment when compared with the group of statin users. There was also a trend towards more male patients in the group of simvastatin users. We believe that this fact had little relevance, because despite having a higher cardiovascular risk, this group had the greatest benefit in BP reduction. The open use of the medication is also a limiting factor. However, the results were reinforced by the BP reduction in the ABPM and in central aortic measurements, since these methods are known to be minimally influenced by the placebo effect.

Conclusions

Prior use of statin increases the antihypertensive effect and improves the endothelial function in hypertensive patients with T2DM treated with amlodipine associated with an RAAS inhibitor. Randomized studies with larger samples and 2x2 design are needed to evaluate the interaction between statins and RAAS inhibitors.

Author contributions

Conception and design of the research and Analysis and interpretation of the data: Gismondi RA, Bedirian R, Pozzobon CR, Ladeira MC, Oigman W, Neves MF; Acquisition of data: Gismondi RA, Bedirian R, Pozzobon CR, Ladeira MC; Statistical analysis and Critical revision of the manuscript for intellectual content: Gismondi RA, Oigman W, Neves MF; Writing of the manuscript: Gismondi RA, Bedirian R, Oigman W, Neves MF.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by Faperj and CNPq.

Study Association

This article is part of the thesis of Doctoral submitted by Ronaldo Altenburg Gismondi, from Universidade do Estado do Rio de Janeiro.
Figure 2 – Comparison of office systolic and diastolic blood pressure values between the SU and NSU groups before and after association of antihypertensive treatment; SU: Statin users group; NSU: Non-statin users group; *p = 0.100, patients in the SU group before and after treatment; †p = 0.016, patients in the SU group before and after treatment.
Figure 3 – Comparison of mean 24-hour systolic and diastolic blood pressures in the SU and NSU groups before and after association of antihypertensive treatment; SU: Statin users group; NSU: Non-statin users group; *p = 0.007, patients in the SU group before and after treatment.

Table 2 – Effect of statin use on ABPM

| Variables          | SU         | NSU         | p value     |
|--------------------|------------|-------------|-------------|
|                    | Before     | After       | Before      | After       | p1 | p2 | p3 |
| SBP 24-h ABPM      | 134 (120-146) | 122 (114-135) | 137 (122-149) | 128 (122-140) | 0.007 | 0.362 | 0.333 |
| DBP 24-h ABPM      | 79 (73-86) | 75 (67-84) | 78 (76-89) | 78 (63-90) | 0.007 | 0.209 | 0.976 |
| PP 24-h ABPM       | 56 (49-61) | 49 (41-55) | 51 (45-62) | 56 (44-61) | 0.032 | 0.813 | 0.177 |
| Awake SBP          | 139 (121-149) | 125 (115-136) | 139 (125-156) | 130 (123-147) | 0.015 | 0.167 | 0.333 |
| Awake DBP          | 81 (74-89) | 77 (72-83) | 81 (78-92) | 80 (66-90) | 0.058 | 0.099 | 0.930 |
| Nocturnal SBP      | 125 (112-139) | 113 (109-119) | 134 (116-137) | 121 (109-132) | 0.035 | 0.396 | 0.278 |
| Nocturnal DBP      | 72 (67-76) | 67 (58-73) | 73 (66-83) | 67 (62-80) | 0.035 | 0.615 | 0.769 |
| Nocturnal Descent (%) | 5.0 (2.2-11.7) | 7.4 (2.9-11.5) | 10.2 (4.3-13.3) | 6.4 (4.6-8.2) | 0.374 | 0.432 | 0.578 |

Values are expressed as median (interquartile range), except where specified otherwise; unit: mmHg; p1: Patients in the SU group before and after treatment; p2: Patients in the NSU group before and after treatment; p3: Comparison between the SU and NSU groups at the end of the treatment; SU: Statin users group; NSU: Non-statin users group; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; PP: Pulse pressure; ABPM: Ambulatory blood pressure monitoring.
Figure 4 – Comparison of the values of brachial artery flow-mediated dilation between the SU and NSU groups before and after association of antihypertensive treatment; SU: Statin users group; FMD: Brachial artery flow-mediated dilation; NSU: Non-statin users group; *p = 0.003, patients in the SU group before and after treatment.

Table 3 – Effect of statin use vascular tests

| Variables                 | SU Before | SU After | NSU Before | NSU After | p1   | p2   | p3   |
|---------------------------|-----------|----------|------------|-----------|------|------|------|
| Brachial FMD (%)          | 6.50 (5.10-7.10) | 10.90 (7.30-12.20) | 7.50 (6.00-10.20) | 8.30 (7.50-9.95) | 0.003 | 0.820 | 0.211|
| cPWV (m/s)                | 9.95 (9.35-10.55) | 9.90 (8.50-11.05) | 10.65 (9.60-12.35) | 11.05 (10.20-11.70) | 0.650 | 0.586 | 0.111|
| crPWV (m/s)               | 9.85 (9.05-10.40) | 10.00 (9.05-10.55) | 9.70 (9.35-10.90) | 9.65 (9.35-10.20) | 0.972 | 0.570 | 0.490|
| Aortic systolic BP (mmHg) | 139 (128-143)   | 125 (117-128)    | 127 (121-140)    | 126 (119-139)    | 0.046 | 0.623 | 0.477|
| Aortic PP (mmHg)          | 86 (84-92)     | 82 (68-84)       | 82 (79-87)       | 79 (74-88)       | 0.018 | 0.508 | 0.706|
| AIx (%)                   | 30 (28-43)     | 33 (25-40)       | 31 (23-33)       | 33 (27-37)       | 0.263 | 0.308 | 0.916|
| AP (mmHg)                 | 15 (13-24)     | 17 (10-21)       | 13 (9-16)        | 16 (10-20)       | 0.278 | 0.320 | 0.966|

Values are expressed as median (interquartile range), except where specified otherwise; p1: Patients in the SU group before and after treatment; p2: Patients in the NSU group before and after treatment; p3: Comparison between the SU and NSU groups at the end of the treatment; SU: Statin users group; NSU: Non-statin users group; Brachial FMD: Brachial artery flow-mediated dilation; cPWV: Carotid-femoral pulse wave velocity; crPWV: Carotid-radial pulse wave velocity; BP: Blood pressure; PP: Pulse pressure; AIx: Augmentation index; AP: Augmentation pressure.
References

1. Sarkar K, Sinha AK, Mehta JL. The role of statins in endothelial dysfunction in hypertension. Curr Opin Cardiol. 2006;21(4):316–21.

2. De Sotomayor MA, Pérez-Guerrero C, Herrera MD, Jimenez L, Marín R, Marhuenda E, et al. Improvement of age-related endothelial dysfunction by simvastatin: effect on NO and COX pathways. Br J Pharmacol. 2005;146(8):1130–8.

3. Ribeiro RA, Duncan BB, Ziegelmann FK, Stella SF, Vieira J, da C, Restelatto LMF, et al. Cost-effectiveness of high, moderate and low-dose statins in the prevention of vascular events in the Brazilian public health system. Arq Bras Cardiol. 2014;104(1):32–44.

4. De Vera MA, Bhole V, Burns LC, Lacaille D. Impact of statin adherence on cardiovascular disease and mortality outcomes: a systematic review. Br J Clin Pharmacol. 2014;78(4):684–98.

5. Briasoulis A, Agarwal V, Valachis A, Messerli FH. Antihypertensive effects of statins: a meta-analysis of prospective controlled studies. J Clin Hypertens (Greenwich). 2013;15(5):310–20.

6. Coretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilatation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257–65.

7. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588–605.

8. Pausch AL, O’Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. Hypertension. 2001;38(4):932–7.

9. Gismondi RA, Oigman W, Bedirian R, Pozzobon CR, Ladeira MB, Neves MF. Comparison of benazepril and losartan on endothelial function and vascular stiffness in patients with Type 2 diabetes mellitus and hypertension: A randomized controlled trial. J Renin Angiotensin Aldosterone Syst. 2015 Mar 17[epub ahead of print]

10. Xavier HT, Izar MC, Faria Neto JR, Assad MH, Rocha VZ, Sposito AC et al. V Brazilian Guidelines on Dyslipidemias and Prevention of Atherosclerosis. Arq Bras Cardiol. 2013;101(Suppl 1):1–20.

11. Mancia G, Parati G, Revera M, Bilo G, Giuliano A, Veglia F, et al. Statins, the renin-angiotensin-aldosterone system of hypertension. Hypertension. 2002;39(2):257–65.

12. Drapala A, Sikora M, Ufnal M. Statins, the renin-angiotensin-aldosterone system and the role of statins in myocardial infarction. J Renin Angiotensin Aldosterone Syst. 2014;15(3):250–8.

13. Beishuizen ED, Tamsma JT, Jukema JW, van de Ree MA, van der Vijver JCM, Banga PE, et al. Impact of statin therapy on central aortic pressures and hemodynamics: principal results of the Conduit Artery Function Evaluation-Lipid-Lowering Arm (CAFE-LLA) Study. Circulation. 2009;119(1):53–61.

14. Strazzullo P, Kenny SM, Baribato A, Versiero M, D’Elia L, Cappuccio FP. Do statins reduce blood pressure? A meta-analysis of randomized, controlled trials. Hypertension. 2007;49(4):792–8.

15. Spósito AC, Mansur AP, Coelho OR, Nikolaic IC, Ramieja A. Additional reduction in blood pressure after cholesterol-lowering treatment by statins (lovastatin or pravastatin) in hypercholesterolemic patients using angiotensin-converting enzyme inhibitors (enalapril or lisinopril). Am J Cardiol. 1999;83(10):1497–9.

16. Mancia G, Parati G, Revara M, Bilo G, Giuliano A, Veglia F, et al. Statins, antihypertensive treatment, and blood pressure control in clinic and over 24 hours: evidence from PHYLLIS randomised double blind trial. BMJ. 2010;340:c1197.

17. Koh KK, Son JW, Ahn JY, Kim DS, Jin DK, Kim HS, et al. Simvastatin combined with ramipril treatment in hypercholesterolemic patients. Hypertension. 2004;44(2):180–5.

18. Koh KK, Quon MJ, Lee Y, Han SH, Ahn JY, Chung WJ, et al. Additive beneficial cardiovascular and metabolic effects of combination therapy with ramipril and candesartan in hypertensive patients. Eur Heart J. 2007;28(12):1440–7.

19. Zhang L, Gong D, Li S, Zhou X. Meta-analysis of the effects of statin therapy on endothelial function in patients with diabetes mellitus. Atherosclerosis. 2012;223(1):78–85.

20. Van Venrooij FV, van de Ree MA, Bots ML, Stolp RK, Huisman MV, Banga PE. Aggressive lipid lowering does not improve endothelial function in type 2 diabetes: the Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial. Diabetes Care. 2002;25(7):1211–6.

21. Beishuizen ED, Tamsma JT, Jukema JW, van de Ree MA, van der Vierjer ECM, Meinders AE et al. The effect of statin therapy on endothelial function in type 2 diabetes without manifest cardiovascular disease. Diabetes Care. 2005;28(7):1668–74.

22. Tan KCB, Chow WS, Lam SC, Ai VHG, Lam CHL, Lam KSL. Atorvastatin lowers C-reactive protein and improves endothelium-dependent vasodilation in type 2 diabetes mellitus. J Clin Endocrinol Metab. 2002;87(2):563–8.

23. Kanaki AI, Sarafidis PA, Georgiannos PI, Kanavos K, Zebekakis PE, et al. Effects of low-dose atorvastatin on arterial stiffness and central aortic pressure augmentation in patients with hypertension and hypercholesterolemia. Am J Hypertens. 2013;26(5):608–16.

24. Williams B, Lacy PS, Cruickshank JK, Collier D, Hughes AD, Stanton A, et al. Impact of statin therapy on central aortic pressures and hemodynamics: principal results of the Conduit Artery Function Evaluation-Lipid-Lowering Arm (CAFE-LLA) Study. Circulation. 2009;119(1):53–61.

25. Fassett RG, Robertson IK, Ball MJ, Geraghty DP, Sharman JE, Coombes JS. Effects of atorvastatin on arterial stiffness in chronic kidney disease: a randomised controlled trial. J Atheroscler Thromb. 2010;17(3):235–41.

26. Raison J, Rudnichi A, Safar ME. Effects of atorvastatin on aortic pulse wave velocity in patients with hypertension and hypercholesterolaemia: a preliminary study. J Hum Hypertens. 2002;16(10):705–10.

27. Pirro M, Schillaci G, Mannarino MR, Savarese G, Vaudo G, Siepi D, et al. Effects of rosuvastatin on 3-nitrotyrosine and aortic stiffness in patients with hypertension and hypercholesterolemia. Nutr Metab Cardiovasc Dis. 2007;17(6):436–41.

28. Mäki-Petäjä KM, Booth AD, Hall FC, Wallace SML, Brown J, McEniery CM, et al. Ezetimibe and simvastatin reduce inflammation, disease activity, and aortic stiffness and improve endothelial function in rheumatoid arthritis. J Am Coll Cardiol. 2007;50(9):852–8.