Verapamil Eliminates the Hierarchical Nature of Activation Frequencies from the Pulmonary Veins to the Atria during Paroxysmal Atrial Fibrillation

Yasunori Kushiyama, MD[1,2], Toshiyuki Osaka, MD, PhD[1], Eriko Yokoyama, MD[1]

Hideyuki Hasebe, MD[1], Yusuke Kuroda, MD[1], Kaichiro Kamiya, MD, PhD[2]

Itsuo Kodama, MD, PhD[2]

[1]Division of Arrhythmia and Electrophysiology, Shizuoka Saiseikai General Hospital, Shizuoka, Japan

[2]Department of Cardiovascular Research, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

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Address correspondence to Dr. Toshiyuki Osaka at

Division of Arrhythmia and Electrophysiology

Shizuoka Saiseikai General Hospital

Oshika1-1-1, Suruga-ku

Shizuoka 422-8527, Japan

E-mail: t130915@siz.saiseikai.or.jp

Fax: 81-54-285-5197

ABSTRACT

Background: There is evidence that verapamil promotes the persistence of paroxysmal atrial fibrillation (AF). Little is known about the underlying mechanisms.

Objective: Our aim was to determine the effect of verapamil on dominant frequencies (DFs) in the pulmonary veins (PVs) and atria during paroxysmal AF with reference to its potential arrhythmogenicity.

Methods: Forty-three patients with paroxysmal AF were studied. Bipolar electrograms were recorded simultaneously during AF from the right atrial free wall (RAFW), coronary sinus (CS) and three PVs or two PVs and the left atrial appendage (LAA). The DFs were obtained by fast Fourier transform analysis before and after infusion of verapamil (0.1 mg/kg, i.v.).

Results: At baseline, the maximum DF among the PVs (6.9 ± 0.9 Hz) was significantly higher than the DF in the RAFW (6.2 ± 0.7 Hz), CS (5.7 ± 0.5 Hz), or LAA (5.9 ± 0.7 Hz) ($P < 0.01$); there was a substantial PV-to-atrial DF gradient (RAFW 0.7 ± 0.9, CS 1.1 ± 0.7, LAA 0.7 ± 0.9 Hz). Verapamil increased the atrial DF to 6.9 ± 0.8, 6.6 ± 0.7, and 7.2 ± 1.0 Hz in the RAFW, CS, and LAA, respectively ($P < 0.0001$), but did not affect the maximum PV DF (7.1 ± 0.7 Hz). The PV-to-atrial DF gradient was eliminated after verapamil (RAFW 0.2 ± 0.8, CS 0.5 ± 0.6, LAA -0.4 ± 0.8 Hz; $P < 0.01$ vs baseline).

Conclusion: Verapamil increases the activation frequency in the atria but not in the PVs, eliminating the PV-to-atrial DF gradient during paroxysmal AF.

Key Words: paroxysmal atrial fibrillation, verapamil, dominant frequency, pulmonary vein, atrium

Abbreviations

AF=atrial fibrillation

APD=action potential duration

BP=blood pressure

CL=cycle length

CS=coronary sinus

DF=dominant frequency

ERP=effective refractory period

$I_{Ca,\ L}$=L-type Ca^{2+}-current

ISDN=isosorbide dinitrate

LA/LAA=left atrium/left atrial appendage

PV=pulmonary vein

RA/RAFW=right atrium/right atrial free wall

SR=sinus rhythm

INTRODUCTION

Verapamil, a blocker of L-type Ca^{2+}-current ($I_{Ca, L}$), is commonly used to control the ventricular response during atrial fibrillation (AF). Despite its usefulness for controlling the ventricular rate, there is evidence that verapamil shortens the AF cycle lengths (CLs) and promotes the maintenance of AF.[1-3] It was demonstrated in a goat model of AF that verapamil converted paroxysmal AF into sustained AF in association with a shortening of the atrial refractory period and an enhancement of reentrant activities.[3] As to the profibrillatory effects of verapamil in humans, however, the available information is still limited and much remains to be clarified.

High-frequency sources of excitation in the pulmonary veins (PVs) play a crucial role in the genesis of paroxysmal AF, and the presence of a PV-to-atiral frequency gradient is a sign of high-frequency activity arising from the PVs.[4-7] The absence of the PV-to-atrial frequency gradient, on the other hand, suggests the important role of focal reentrant activities within the atria in maintaining AF.[4-7]

We investigated the effects of verapamil on the activation frequencies of the PVs and atria during paroxysmal AF in order to obtain an insight into the mechanisms underlying the profibrillatory action of this drug in humans.

METHODS

The present study included 43 patients with paroxysmal AF (57±11 years, 33 males). All patients were admitted to Shizuoka Saiseikai General Hospital (Shizuoka, Japan) for radiofrequency catheter ablation therapy. None had structural heart disease except for controlled hypertension in 12. Paroxysmal AF was defined as AF episodes terminating spontaneously within 48 hours. The study was approved by the Institutional Ethics

Committee, and all the patients gave their written informed consent.

Study protocol

All antiarrhythmic drugs including Ca^{2+}-channel blocking agents, beta-blocking agents, and digoxin were withheld for periods of at least five times their half-lives before the study. An electrophysiological study was performed under conscious sedation with propofol (3-5 mg/kg/h). A 5-bipolar catheter (5-mm edge-to-edge spacing, St. Jude Medical, Minnetonka, MN, USA) was placed in the right atrium (RA) on the free wall lateral to the crista terminalis (RAFW) via the femoral vein. An internal cardioversion catheter was inserted into the distal coronary sinus (CS) via the right internal jugular vein (7 electrodes with 3-mm spacing in the CS, Irvine Biomedical, Irvine, CA, USA). Three 8F sheaths (St Jude Medical) were advanced to the left atrium (LA) via a transseptal approach. Three 10-bipolar circular catheters (6-mm center-to-center spacing, Biosense Webster, Diamond Bar, CA, USA) were placed through the sheaths, and positioned in the proximal portion of the right superior, left superior, and left inferior PVs in 17 patients. In the remaining 26 patients, one circular catheter was placed in the left atrial appendage (LAA) and two were placed in the left superior and right inferior PVs (n=13) or in the left superior and right superior PVs (n=13). The PV catheters were positioned carefully ~1 cm inside the ostium to avoid recording far-field atrial potentials.

Bipolar signals from the 5 recording sites were acquired simultaneously during AF for 64 seconds, filtered between 30 and 500 Hz, and stored digitally on an EP lab system (Bard, Billerica, MA, USA). In 4 patients presenting with AF to the laboratory, recordings were made 10 minutes after catheter placement. In 39 patients presenting with sinus rhythm (SR), induction of AF was attempted by decremental pacing from the CS; the pacing CL was decreased progressively in 20 ms-steps from 400 to 300 ms, and in 10

ms-steps from 300 to 150 ms or until the failure of 1:1 capture. The pulses used for pacing were 1 ms in duration and five times the diastolic threshold in intensity. Attempts were made at least 5 times until sustained AF (lasting>10 minutes) was induced. When sustained AF was not induced by the CS pacing, the decremental pacing was applied from the RAFW. Baseline recordings were made after a 10-minute waiting period for stabilization of the AFCL. After the baseline recordings, verapamil was administered intravenously as a bolus of 0.1 mg/kg over 5 minutes in 26 patients (4 presenting with AF, and 22 presenting with SR); the verapamil data were acquired 4 minutes after the completion of the drug infusion. In 5 patients (presenting with SR), we administered isosorbide dinitorate (ISDN), a vasodilator, as a bolus of 1.0 mg, and added 0.5 mg every 20 seconds, if necessary, to achieve a ~20% decrease in the systolic blood pressure (BP) from baseline in order to examine the influence of a reflex increase in sympathetic tone in response to BP reduction. In 6 patients (presenting with SR), only saline was administered. Six patients were excluded from spectral analysis because sustained AF was not induced in the pacing protocol. In the 6 patients, verapamil was administered during sinus rhythm, and AF induction was attempted again using the same pacing protocol.

Spectral analysis

The details of spectral analysis have been described previously.[7] In brief, bipolar signals were high-pass filtered at 1 Hz, rectified, and then low-pass filtered with a 20-Hz cutoff. A 4096-point fast Fourier transform was performed for each successive 4.096-second segment of the respective bipolar signals with 50% overlap to obtain the power spectrum. The frequency with the largest amplitude was assigned as the dominant frequency (DF). The DF at each bipole was expressed as an averaged value for the 30 segments. To ensure the reliability of the DF detection, only points demonstrating a

regularity index (RI) >0.2 were included.[5-7] The DF for each PV was determined as the averaged value for the 10 bipoles. We defined the highest or higher DF among the 3 PVs or 2 PVs, respectively, as the maximum PV DF. The DFs for the LAA, RAFW, and CS were determined as the averaged values for the 10, 5, and 3 bipoles, respectively. Bipoles showing large ventricular components were discarded. One set of data from the CS was excluded from the analysis because of a poor signal quality.

Statistical Analysis

Data are presented as mean ± SD unless otherwise specified. Analysis of variance (ANOVA) followed by Bonferroni's test was used for multiple comparisons. Student's unpaired *t*-test or Mann-Whitney U test was used, as appropriate, to compare data between the different patient groups. Comparisons of data in the same patient group were made by Student's paired *t*-test. Statistical significance was established at $P < 0.05$.

RESULTS

The left atrial dimension and left ventricular ejection fraction estimated by echocardiography in the 43 patients prior to the electrophysiological study were 38±5 mm and 71±7 %, respectively. Four patients were in sustained AF (lasting 18.3±7.1 hours) at the time of the examination. The remaining 39 patients were in SR; AF episode had not been documented at least for 48 hours before the examination. Among the 39 patients, sustained AF emerged during catheter manipulation in 5, and was induced by burst atrial pacing in 28. All the AFs (4 presenting and 33 emerged or induced) employed for the DF analysis lasted >20 minutes, and required DC cardioversion (n=24) or ablation (n=13) for their termination.

Spatiotemporal Stability of DF

Figure 1 shows temporal changes in the DF in the RAFW, CS and left superior PV after AF induction in 6 patients with saline administration. The DFs obtained at 1, 5, 10, 15 and 20 minutes after the AF induction are plotted. The DF at each recording site tended to increase during the first 5 minutes after the induction. Then, the DF at each region remained constant until the end of the recording, indicating spatiotemporal stability of the activation frequencies. There were no significant differences in the DFs at 10 and 20 minutes after the AF induction in the respective recording sites. The effects of verapamil and ISDN were examined during the 10-minute stable period.

Effect of verapamil on DF

Figure 2 demonstrates bipolar electrograms obtained from 5 recording sites and the corresponding power spectra (a 4.096-second segment) in a patient before and after the administration of verapamil. At baseline, the highest DF among the 5 recording sites was recognized at the left superior PV (7.4 Hz). The highest DF among the 3 atrial sites was recognized at the RAFW (6.1 Hz), giving rise to a PV-to-atrial DF gradient of 1.3 Hz. Verapamil increased the DF at each recording site; however, the increase was much greater in the atria compared with the PVs. The highest DF among the 5 recording sites shifted to the LAA (7.6 Hz) after verapamil; the increase in the DF at the left superior PV was minimal (7.5 Hz), resulting in a reversal of the PV-to-atrial DF gradient to -0.1 Hz.

The effects of verapamil on the atrial and PV DFs were examined in 26 patients. The results are summarized in Table 1. At baseline, the maximum PV DF was significantly higher than the DF in the RAFW ($P < 0.01$), CS ($P < 0.0001$), or LAA ($P < 0.0005$). No significant difference was recognized among the DFs in the 3 atrial regions (RAFW, CS and LAA). Verapamil significantly decreased the systolic BP and ventricular rate from 122±16 to 102±14 mmHg ($P < 0.0001$) and from 93±18 to 67±11 bpm ($P < 0.0001$),

respectively. Verapamil significantly increased the DFs in the RAFW, CS, and LAA (P <0.0001). Verapamil also significantly increased the DFs in the right inferior PV (P=0.001) and left inferior PV (P=0.036), but not right superior PV (P=0.13) and left superior PV (P=0.061). The maximum PV DF was unaffected by verapamil (P=0.26). No significant difference was recognized after verapamil between the maximum PV DF and DF in the RAFW, CS or LAA.

Figures 3 and 4 plot the changes in the DF before and after verapamil administration for the individual patients. The DFs in the RAFW, CS, and LAA increased after verapamil in all the patients examined but 2 in whom the RAFW DF remained unchanged (Figure 3). In contrast, there were considerable variations in the effect of verapamil on the PV DF; the DF decreased or was unchanged after verapamil in 2/13 right superior PVs, 2/13 right inferior PVs, 7/26 left superior PVs, and 2/11 left inferior PVs. As for the maximum PV DF, the value decreased in 9/26 patients (Figure 4). In Figure 5, the verapamil-induced changes in the DF (ΔDF) in all the PVs examined are plotted against the respective baseline DFs. There was a significant negative correlation between the ΔDF and baseline DF (R=-0.57, P <0.0001, n=63); the higher the baseline PV DF, the smaller the DF increase after verapamil.

Figure 6 illustrates the verapamil-induced change in the DF in each region examined. The percent increases in the DF in the RAFW (10.2±5.7%), CS (14.7±8.4%), and LAA (21.5±6.8%) were significantly higher than that in the maximum PV DF (3.3±10.2%, P <0.05 vs RAFW, P <0.0001 vs CS, P <0.0001 vs LAA). The verapamil-induced increase in the DF in the LAA was comparable with that in the CS, but was significantly higher than that in the RAFW (P <0.001); no significant difference was recognized in the increase in the DF between the CS and RAFW. There was no significant correlation

between the percent increase in the DF and percent decrease in the systolic BP caused by verapamil at any atrial region examined (R=-0.0003, P=0.99 for RAFW, R=-0.08, P=0.70 for CS, R=-0.69, P=0.74 for LAA).

The PV-to-atrial DF gradient at baseline was 0.7±0.9, 1.1±0.7, and 0.7±0.9 Hz in the RAFW, CS, and LAA, respectively. After verapamil, the PV-to-atrial DF gradient decreased significantly to a level of virtually no gradient at 0.2±0.8 (P=0.005), 0.5±0.6 (P=0.001) and -0.4±0.8 Hz (P=0.0001) in the RAFW, CS, and LAA, respectively.

Influence of AF burden

To evaluate the influence of preexisting AF (AF burden) on the effects of verapamil,[3] we compared the data obtained from 4 patients presenting with sustained AF to the laboratory and those from 22 patients presenting with SR (Supplemental Table). There were no significant differences between the 2 patient groups in the baseline DFs in the RAFW, CS and LAA, and in the baseline maximum PV DF. The effects of verapamil on the DFs in the 2 groups were essentially similar. The atrial DFs (RAFW, CS and LAA) were increased significantly by 8.8-20.6% in the group presenting with SR, and 13.6-23.9% in the group presenting with AF. The maximum PV DF was unaffected by verapamil in the both groups. The verapamil-induced change in the DF in the RAFW was larger in the group presenting with AF than with SR, but there were no significant differences in other regions. These observations suggest that AF burden, by and large, may not affect the verapamil action to modify the hierarchical organization of activation frequencies in the PVs and atria.

Effects of verapamil on AF inducibility

No sustained AF was induced by burst pacing from both the CS and RAFW in 6 patients at baseline; the shortest pacing CL applied was 160±11 ms, and the maximum

duration of the induced AF was 17.6±11.7 seconds. In all of the 6 patients, sustained AF was easily induced after verapamil by burst pacing from the CS; the pacing CL (193±28 ms) was significantly longer than that at baseline (P=0.025). DC shock (n=3) or ablation (n=3) was required to terminate the induced AF.

Effect of ISDN on DF

The effects of ISDN were examined in 5 patients. The mean dose applied was 2.4±2.2 mg. ISDN significantly decreased the systolic BP from 135±34 to 103±27 mmHg (P=0.006) and significantly increased the ventricular rate from 103±30 to 111±32 bpm (P=0.041). ISDN significantly increased the DFs in the CS (5.2±0.3 Hz at baseline vs 5.5±0.2 Hz after ISDN, P=0.013) and LAA (5.3±0.7 vs 5.6±0.7 Hz, P=0.007), whereas no significant changes were recognized in the RAFW DF (5.6±0.7 vs 5.9±0.8 Hz, P=0.16) and maximum PV DF (6.9±1.8 vs 7.0±1.7 Hz, P=0.49). Despite a comparable decrease in the systolic BP by ISDN and verapamil (23.1±8.1 vs 20.4±8.1%, P=0.40), the DF increases in the CS and LAA by ISDN were significantly lower than those by verapamil (5.3 ±2.9 vs 14.7±8.4%, P=0.021 for CS; 4.8±2.1 vs 21.5±6.8%, P<0.0001 for LAA). The PV-to-atrial DF gradient did not decrease significantly after ISDN in both the CS (1.7±1.7 vs 1.5±1.7 Hz, P=0.58) and LAA (1.6±2.1 vs 1.4±2.0 Hz, P=0.25).

DISCUSSION

The key observations in the present study are as follows. First, a PV-to-atrial DF gradient was present during AF in the majority of patients with paroxysmal AF. Second, verapamil caused a consistent increase in the atrial DF, whereas variable changes were produced for the PV DF. The PV-to-atrial DF gradient was eliminated by verapamil. Third, verapamil facilitated the induction of sustained AF. Fourth, ISDN caused a minimal

increase in the DF in the atria despite the induction of a similar decrease in the systolic BP compared with verapamil.

Role of PVs in the maintenance of paroxysmal AF

Emerging evidence suggests that AF is maintained by high-frequency sources of excitation (drivers) causing a hierarchical organization in the rate of activation in different regions of the atria.[4-7] In the present study, the averaged value of the maximum PV DF was significantly higher compared with the averaged DFs in the RAFW, CS and LAA, giving rise to a substantial PV-to-atrial DF gradient. These observations are concordant with the previous reports suggesting the importance of the PVs as a driver of paroxysmal AF in humans.[4-7] Clinical and experimental studies have shown that the action potential duration (APD) and effective refractory period (ERP) in the PV are shorter than those in the LA in favor of the faster activation in the PV.[8, 9] Both reentry and focal discharges may contribute to the rapid activity in the PV, and each mechanism can lead to one another.[10-12] Anisotropic fiber orientation and increased interstitial fibrosis as well as pronounced heterogeneity of the repolarization in the PV muscular sleeves can provide substrates for reentry.[10-12] The PV muscular sleeves have also be shown to exhibit phase 3 early after depolarizations (EADs) or delayed after depolarizations (DADs) in association with intracellular calcium overload.[10-14]

Effects of verapamil on DFs in the PVs

To the best of our knowledge, this is the first report showing the effect of verapamil on the PV DF during paroxysmal AF in humans. We have revealed that there are considerable variations in the effect of verapamil; the maximum PV DF increased in 65% of the patients, but decreased in the remaining 35%. With regard to individual PVs, there was a negative correlation between the baseline DF level and verapamil-induced change in the

DF; the higher the baseline DF, the smaller the increase in the DF (the verapamil-induced decrease in the PV DF was generally observed in the PVs exhibiting the DFs >7.0 Hz at baseline). The increase in the PV DF by verapamil can be attributable to a shortening of the APD and ERP resulting from a rate-dependent $I_{Ca,\,L}$ block.[3, 15, 16] In a simulation study of remodeled human atria, Pandit et al.[17] demonstrated that $I_{Ca,\,L}$ reduction and consequent APD shortening accelerates and stabilizes functional reentry in association with an increase in the DFs. The decrease in the PV DF by verapamil could be the result of suppression of $I_{Ca,L}$-dependent focal discharges such as phase 3 EADs or DADs.[13, 14] Yamazaki et al.[18] reported in a sheep model of AF that reentry and focal discharges can work synergistically in increasing the DF. They showed that suppression of focal discharges resulted in a substantial decrease in the DF in such conditions. A similar mechanism may work in the human PVs exhibiting extremely high DFs.

Effects of verapamil on DFs in the atria

In contrast to the PVs, verapamil consistently increased the DFs in the atria, and eliminated the PV-to-atrial DF gradient. In isolated canine myocytes, Cha et al.[9] demonstrated a longer APD and higher $I_{Ca,\,L}$ density in the LA compared with the PVs at baseline. The regional differences were shown to be minimized after tachycardia-induced electrical remodeling. The present results can be explained by a similar mechanism; atrial muscle is considered to be more susceptible to the verapamil action to shorten the APD through $I_{Ca,\,L}$ reduction compared with the PV myocardial sleeves. The consequently greater APD shortening in the atria would allow for a more prominent acceleration of the atrial frequency. The verapamil-induced increase in the atrial DF was significantly higher in the LAA than in the RAFW. This may be attributable, in part, to regional heterogeneities of action potential morphology and ionic currents responsible for the

repolarization in the artia.[19]

The atrial-selective increase in DF by verapamil was not prevented by preexisting sustained AF (AF burden). However, we cannot rule out some difference in the effects of verapamil in the absence and presence of AF burden.[3] Further studies will be required to elucidate the point.

Sympathetic reflex

Previous experimental studies reported that verapamil exaggerated AF through a reflex increase in sympathetic tone in response to a reduction in BP.[20, 21] The verapamil-induced increase in the atrial DF observed in the present study may not be attributable to such a reflex increase in sympathetic tone because of the following reasons. First, there was no significant correlation between the percent increase in the atrial DF and percent decrease in the systolic BP after verapamil. Second, the ISND-induced increase in the atrial DF was much lower than the verapamil-induced increase in the atrial DF despite a comparable decrease in the systolic BP. In line with this, Lee et al.[22] demonstrated in dog that verapamil increased the duration of induced AF in the presence of total autonomic blockade.

Clinical implications

The present results suggest that verapamil facilitates paroxysmal AF by creating new high-frequency sources in the atria as observed in patients with persistent AF.[4-6] In accordance with this, sustained AF was more easily induced after verapamil. The choice of drugs for rate control of paroxysmal AF should be determined not only by the efficacy of regulating the ventricular rate, but also by their effects on AF itself. The usefulness of verapamil in the ventricular rate control is limited by its profibrillatory action on the atrial excitation.

Study limitations

First, the DF was obtained from limited regions of the PVs and atria because of the limitation of the simultaneous DF mapping technique. The spatiotemporal resolution was insufficient to reliably track the preferential driver sites. Second, DF analysis of regional excitation properties is known to be perturbed by far-field potentials.[23,24] We did not subtract ventricular components from the CS signals. This might have led to an underestimation of the DF. As for the PV potential recordings, we placed the catheter ~1 cm inside the ostium to avoid the potential risk for double counting of PV spikes and atrial signals. We compared the DF in the anterior segment of the LSPV, the region most susceptible to the influence of far-field atrial potentials because of the proximity to the LAA, with the DF in the posterior segment in 26 patients; the DF in each segment was determined as an averaged value for the 3 bipoles. There were no differences in the DF between the anterior and posterior segments both at baseline (6.6±0.9 vs 6.7±1.1 Hz, P=0.53) and after verapamil (6.9±0.7 vs 7.1±0.8 Hz, P=0.53). This may support that our catheter positioning was appropriate for the PV DF analysis. Third, potential influence of sympathetic reflex on the DF in response to BP reduction by verapamil cannot be completely excluded because the present study was not carried out in the presence of sympathetic blockade with an intolerable bradycardia in mind. Finally, we examined only the effects of verapamil, so whether the present findings can be extrapolated to other $I_{Ca,\,L}$ blockers, like diltiazem, is an issue for further investigation.

Conclusion

Verapamil increases the activation frequency in the atria but not in the PVs, eliminating the PV-to-atrial DF gradient during paroxysmal AF.

REFERENCES

1. Shenasa M, Kus T, Fromer M, LeBlanc RA, Dubuc M, Nadeau R. Effect of intravenous and oral calcium antagonists (diltiazem and verapamil) on sustenance of atrial fibrillation. Am J Cardiol. 1988;62:403-407.
2. Suttorp MJ, Kingma JH, Lie-A-Huen L, Mast EG. Intravenous flecainide versus verapamil for acute conversion of paroxysmal atrial fibrillation or flutter to sinus rhythm. Am J Cardiol. 1989;63:693-696
3. Duytschaever MF, Garratt CJ, Allessie MA. Profibrillatory effects of verapamil but not of digoxin in the goat model of atrial fibrillation. J Cardiovasc Electrophysiol. 2000;11:1375-1385.
4. Lazar S, Dixit S, Marchlinski FE, Callans DJ, Gerstenfeld EP. Presence of left-to-right atrial frequency gradient in paroxysmal but not persistent atrial fibrillation in humans. Circulation. 2004;110:3181-3186.
5. Sanders P, Berenfeld O, Hocini M, et al. Spectral analysis identifies sites of high-frequency activity maintaining atrial fibrillation in humans. Circulation. 2005;112:789-797.
6. Atienza F, Almendral J, Moreno J, et al. Activation of inward rectifier potassium channels accelerates atrial fibrillation in humans: evidence for a reentrant mechanism. Circulation. 2006;114:2434-2442.
7. Yokoyama E, Osaka T, Takemoto Y, et al. Paroxysmal atrial fibrillation maintained by nonpulmonary vein sources can be predicted by dominant frequency analysis of atriopulmonary electrograms. J Cariovasc Electrophysiol 2009;20:630-636
8. Jaïs P, Hocini M, Macle L, et al. Distinctive electrophysiological properties of pulmonary veins in patients with atrial fibrillation. Circulation. 2002;106:2479-2485.

9. Cha TJ, Ehrlich JR, Zhang L, Chartier D, Leung TK, Nattel S. Atrial tachycardia remodeling of pulmonary vein cardiomyocytes: comparison with left atrium and potential relation to arrhythmogenesis. Circulation. 2005;111:728-735.
10. Arora R, Verheule S, Scott L, et al. Arrhythmogenic substrate of the pulmonary veins assessed by high-resolution optical mapping. Circulation. 2003;107:1816-1821.
11. Chou CC, Nihei M, Zhou S, et al. Intracellular calcium dynamics and anisotropic reentry in isolated canine pulmonary veins and left atrium. Circulation. 2005;111:2889-2897.
12. Miyauchi Y, Hayashi H, Miyauchi M, et al. Heterogeneous pulmonary vein myocardial cell repolarization implications for reentry and triggered activity. Heart Rhythm. 2005;2:1339-1345.
13. Chen YJ, Chen SA, Chen YC, Yeh HI, Chang MS, Lin CI. Electrophysiology of single cardiomyocytes isolated from rabbit pulmonary veins: implication in initiation of focal atrial fibrillation. Basic Res Cardiol. 2002;97:26-34.
14. Hirose M, Laurita KR. Calcium-mediated triggered activity is an underlying cellular mechanism of ectopy originating from the pulmonary vein in dogs. Am J Physiol Heart Circ Physiol. 2007;292:H1861-H1867.
15. Hassan SA, Oral H, Scharf C, et al. Rate-dependent effect of verapamil on atrial refractoriness. J Am Coll Cardiol. 2003;41:446-451.
16. Li GR, Nattel S. Properties of human atrial I_{Ca} at physiological temperatures and relevance to action potential. Am J Physiol. 1997;272:H227-H235.
17. Pandit SV, Berenfeld O, Anumonwo JM, et al. Ionic determinants of functional reentry in a 2-D model of human atrial cells during simulated chronic atrial fibrillation. Biophys J. 2005;88:3806-3821.

18. Yamazaki M, Vaquero LM, Hou L, et al. Mechanisms of stretch-induced atrial fibrillation in the presence and the absence of adrenocholinergic stimulation: interplay between rotors and focal discharges. Heart Rhythm. 2009;6:1009-1017.
19. Li D, Zhang L, Kneller J, Nattel S. Potential ionic mechanism for repolarization differences between canine right and left atrium. Circ Res. 2001;88:1168-1175.
20. Friedman HS, Rodney E, Sinha B, et al. Verapamil prolongs atrial fibrillation by evoking an intense sympathetic neurohumoral effect. J Investig Med. 1999;47:293-303.
21. Bénardeau A, Fareh S, Nattel S. Effects of verapamil on atrial fibrillation and its electrophysiological determinants in dogs. Cardiovasc Res. 2001;50:85-96.
22. Lee SH, Yu WC, Cheng JJ, et al. Effect of verapamil on long-term tachycardia-induced atrial electrical remodeling. Circulation. 2000;101:200-206.
23. Ng J, Kadish AH, Goldberger JJ. Effect of electrogram characteristics on the relationship of dominant frequency to atrial activation rate in atrial fibrillation. Heart Rhythm. 2006;3:1295-1305.
24. Grzeda KR, Noujaim SF, Berenfeld O, Jalife J. Complex fractionated atrial electrograms: properties of time-domain versus frequency-domain methods. Heart Rhythm. 2009;6:1475-1482.

FIGURE LEGENDS

Figure 1: Temporal change in the dominant frequencies (DFs) after the induction of AF. The DFs were obtained from the right atrial free wall (RAFW), coronary sinus (CS), and left superior pulmonary vein (LSPV) at 1, 5, 10, 15 and 20 minutes after the AF induction in 6 patients with saline administration. Data are mean ± SD. Error bars are the standard error of the mean (SEM).

Figure 2: Bipolar electrograms and corresponding power spectra in a patient before (baseline) and after the administration of verapamil. The data were obtained simultaneously from 5 sites: the right atrial free wall (RAFW), coronary sinus (CS), left atrial appendage (LAA), left superior pulmonary vein (LSPV) and right inferior pulmonary vein (RIPV). The highest dominant frequency (DF) among the 5 recording sites (star mark) shifted from the LSPV (7.4 Hz) to LAA (7.6 Hz).

Figure 3: Effects of verapamil on the dominant frequencies in the atria. The data were obtained from 26 patients before (baseline) and after verapamil. The individual values and means ± SD in the RAFW, CS and LAA are presented. Base: baseline, Ver: after verapamil.

Figure 4: Effects of verapamil on the dominant frequencies in the pulmonary veins. The data were obtained 26 patients before (baseline) and after verapamil. The individual values and means ± SD in the right superior pulmonary vein (RSPV), right inferior pulmonary vein (RIPV), left superior pulmonary vein (LSPV), and left inferior pulmonary vein (LIPV) are presented. Max PV: maximum DF among 3 or 2 PVs, Base: baseline, Ver: after

verapamil.

Figure 5: Relationship between the dominant frequency (DF) in the pulmonary vein (PV) at baseline and change in the DF after verapamil. Verapamil-induced changes in the DF in all the PVs examined are plotted against the respective baseline DFs (n=63). There was a significant negative correlation between the two parameters (R=-0.57, P <0.0001).

Figure 6: Site-dependent changes in the dominant frequency (DF) caused by verapamil in 26 patients. Percent changes in the DF after verapamil from baseline in the RAFW (n=26), CS (n=25), LAA (n=15), and in the maximum DF among the pulmonary veins (Max PV, n=26) are shown. Data are mean ± SD. * P <0.05 vs RAFW, $^{\#}$ P <0.05 vs RAFW, CS, or LAA.

Table 1

Effects of Verapamil on Dominant Frequencies (Hz)

	RAFW (n=26)	CS (n=25)	LAA (n=15)	RSPV (n=13)	RIPV (n=13)	LSPV (n=26)	LIPV (n=11)	Max PV (n=26)
Baseline	6.2±0.7	5.7±0.5	5.9±0.7	6.4±0.7	6.1±0.4	6.7±0.9	6.7±0.5	6.9±0.9[#]
Verapamil	6.9±0.8*	6.6±0.7*	7.2±1.0*	6.6±0.6	6.5±0.5*	7.0±0.7	7.1±0.6*	7.1±0.7

Values are mean ± SD. *P <0.05 vs baseline, [#]P <0.05 vs RAFW, CS, or LAA

RAFW: right atrial free wall, CS: coronary sinus, LAA: left atrial appendage, RSPV: right superior pulmonary vein, RIPV: right inferior pulmonary vein, LSPV: left superior pulmonary vein, LIPV: left inferior pulmonary vein, Max PV: maximum DF among PVs.

Supplemental Table

Effects of AF burden on Dominant Frequency

	DFs in Patients Presenting with SR				DFs in Patients Presenting with AF			
	n	**Baseline (Hz)**	**Verapamil (Hz)**	**Change (%)**	**n**	**Baseline (Hz)**	**Verapamil (Hz)**	**Change (%)**
RAFW	22	6.2±0.7	6.7±0.7*	8.8±5.0	4	6.6±0.6	7.8±0.8*,#	18.1±2.4#
CS	21	5.7±0.5	6.6±0.8*	14.9±8.8	4	5.7±0.2	6.4±0.6*	13.6±6.9
LAA	11	5.8±0.8	7.0±1.0*	20.6±6.4	4	6.2±0.5	7.7±0.9*	23.9±8.0
Max PV	22	6.9±0.9	7.1±0.7	3.1±10.6	4	6.8±1.0	7.0±0.8	3.9±0.5

Values are mean ± SD. *P <0.05 vs baseline, #P <0.05 vs patients with sinus rhythm (SR), DFs: dominant frequencies, RAFW: right atrial free wall, CS: coronary sinus, LAA: left atrial appendage, Max PV: maximum DF among PVs.

Figure
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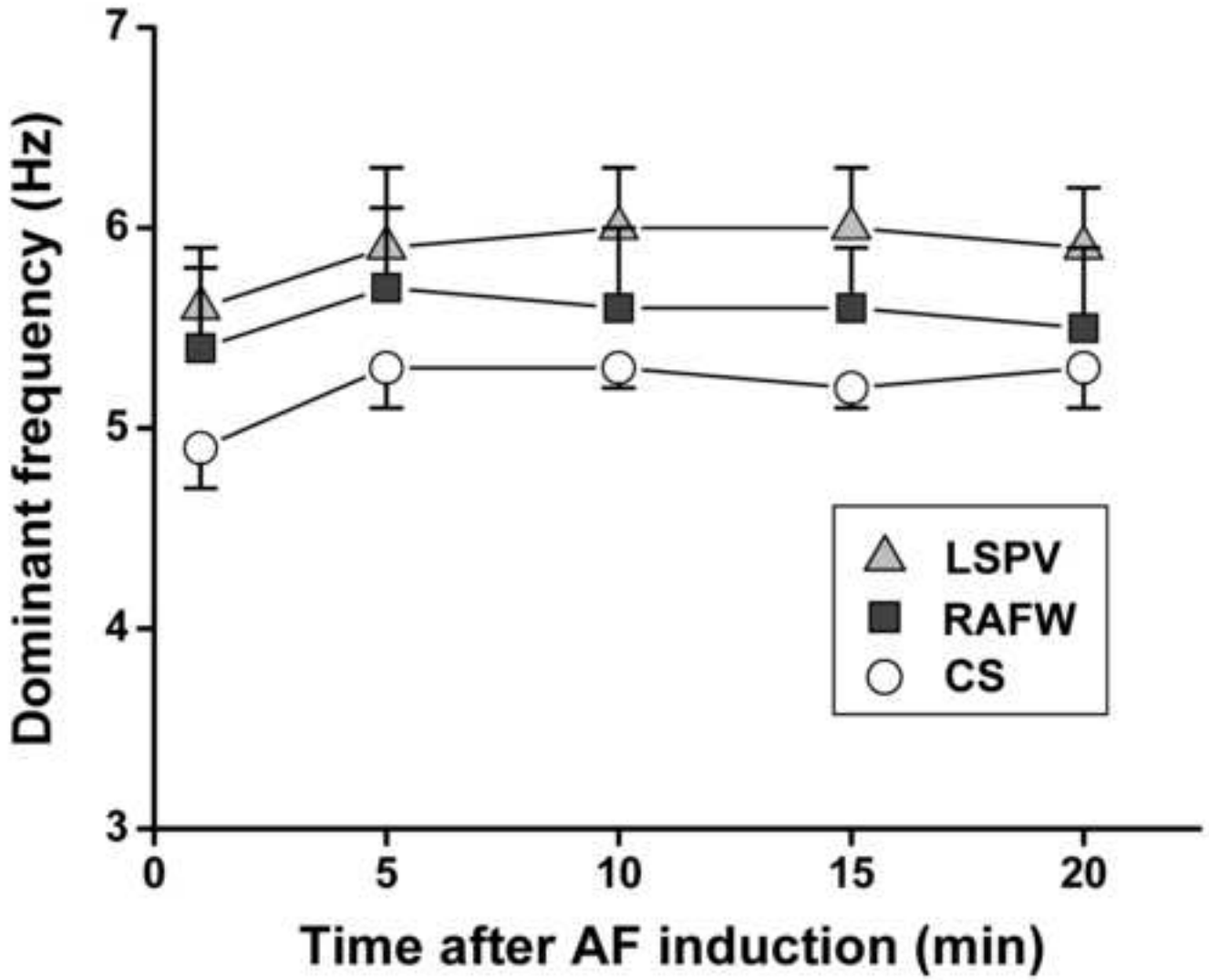

Fig 1

Figure

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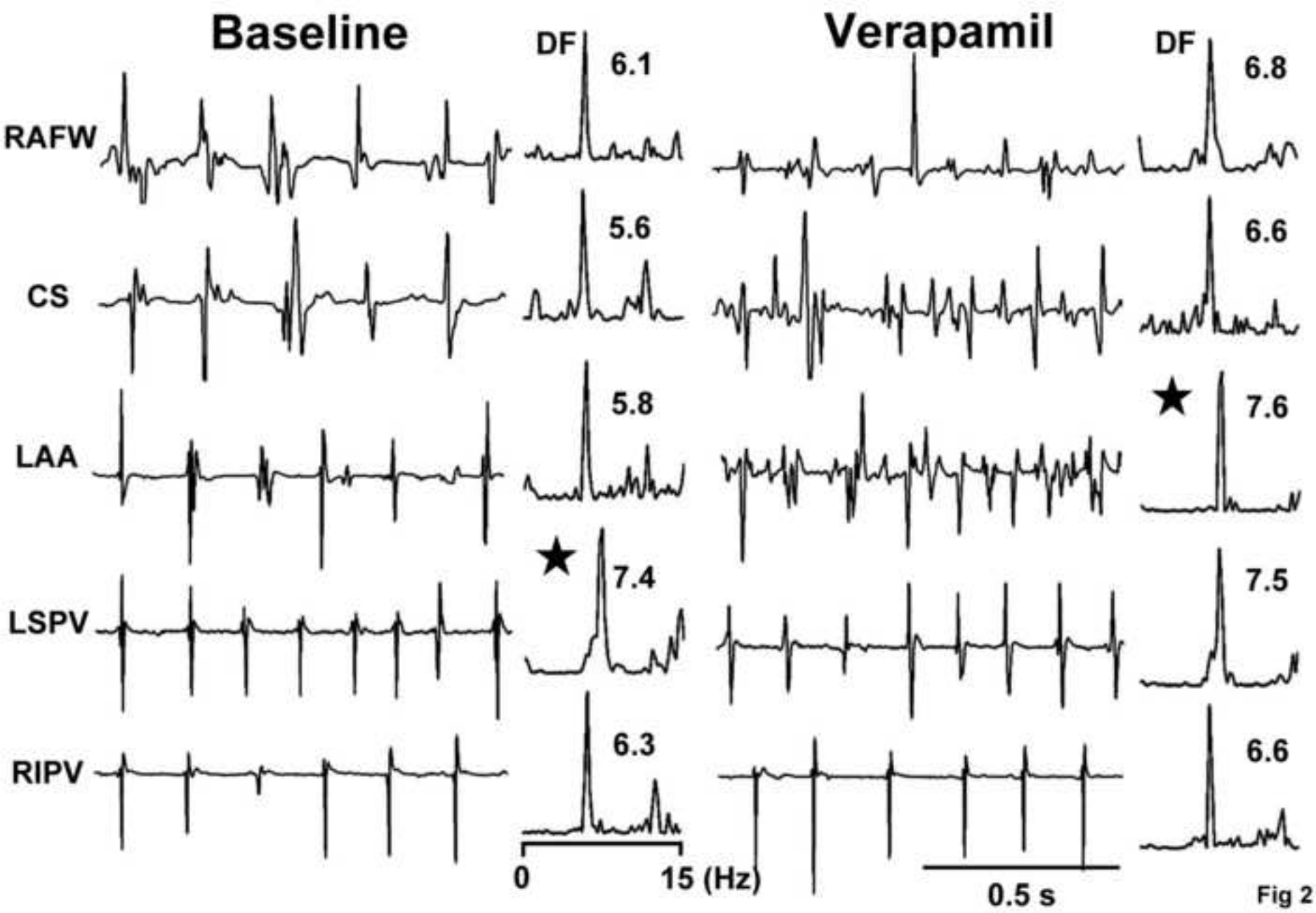

Fig 2

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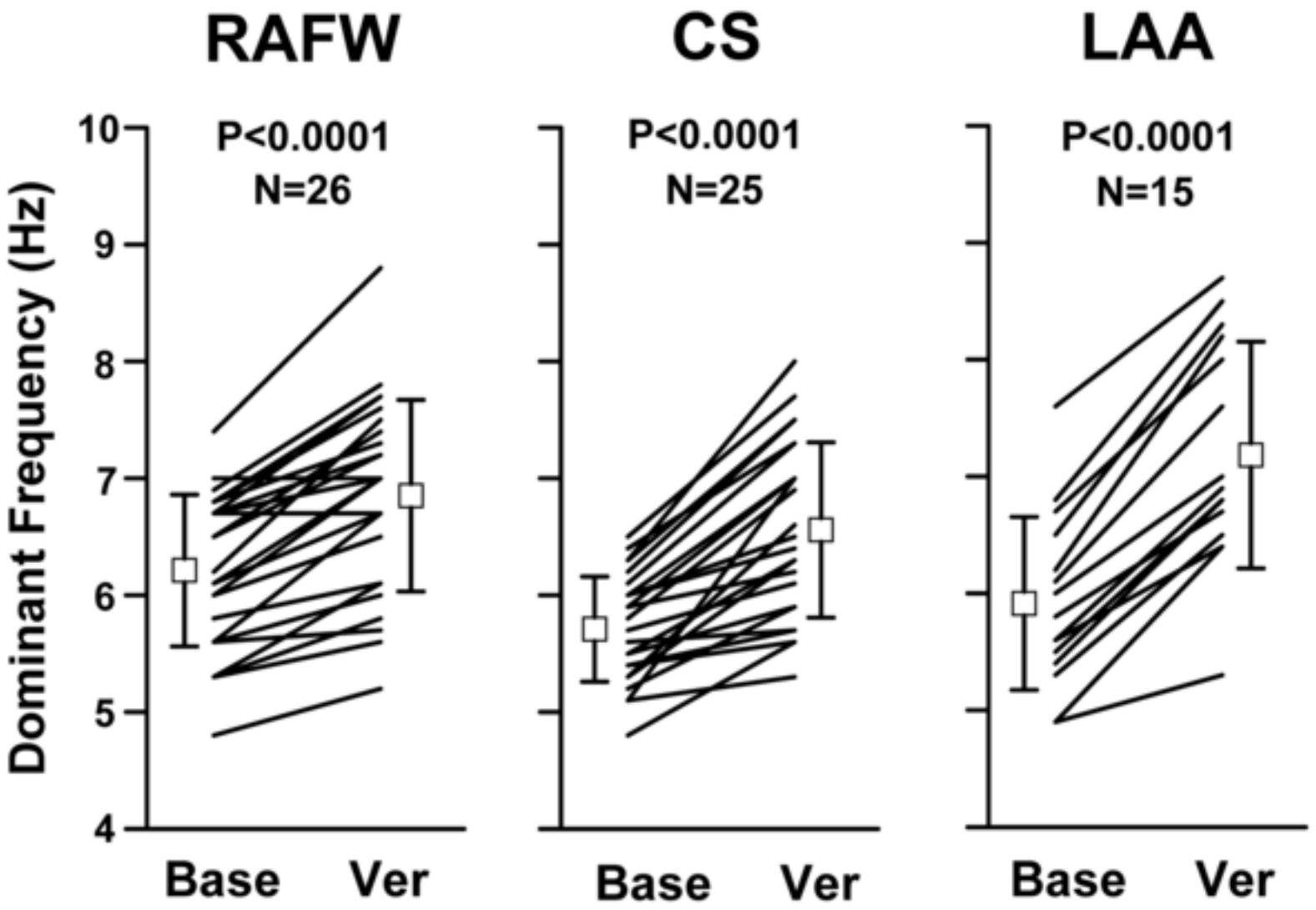

Fig 3

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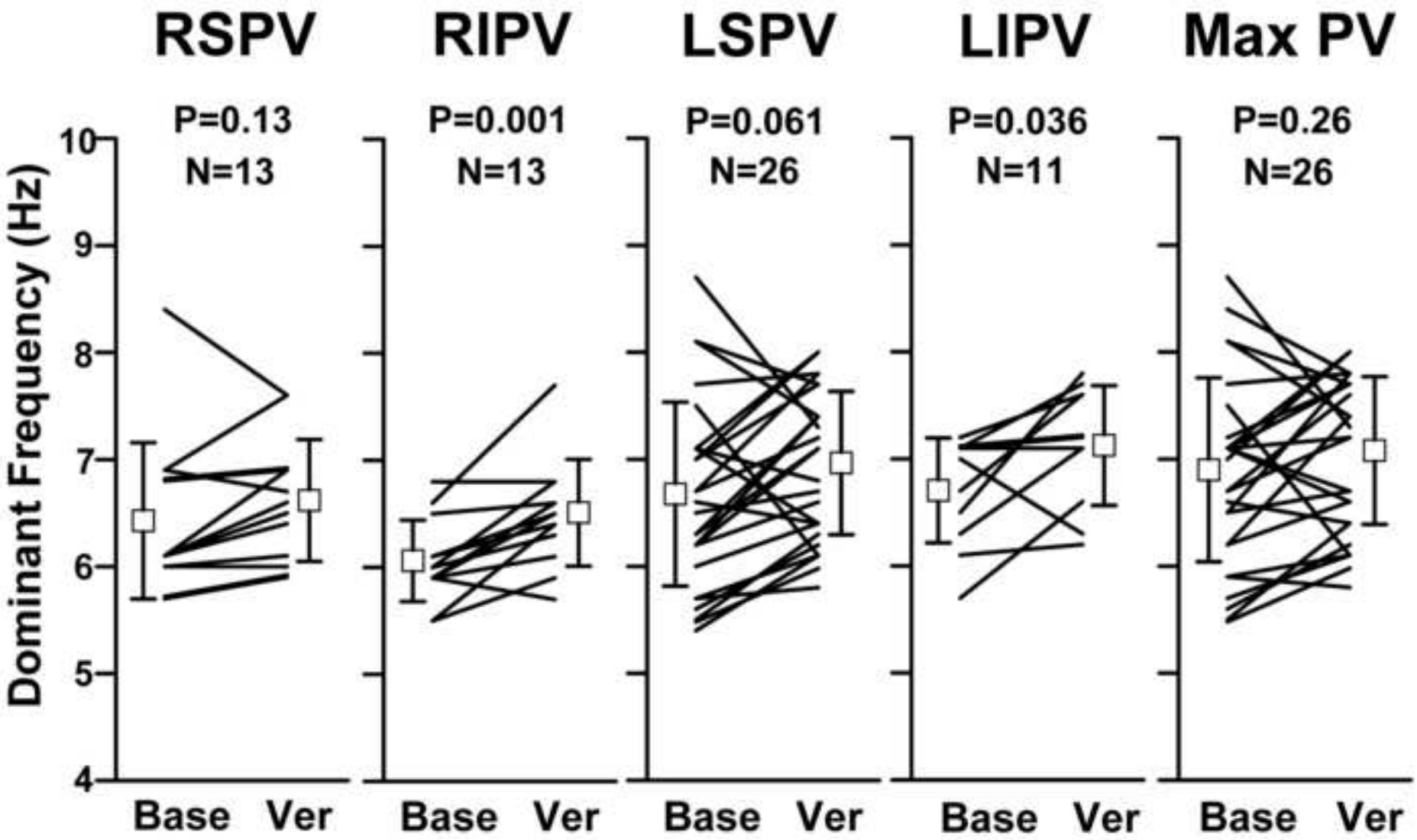

Fig 4

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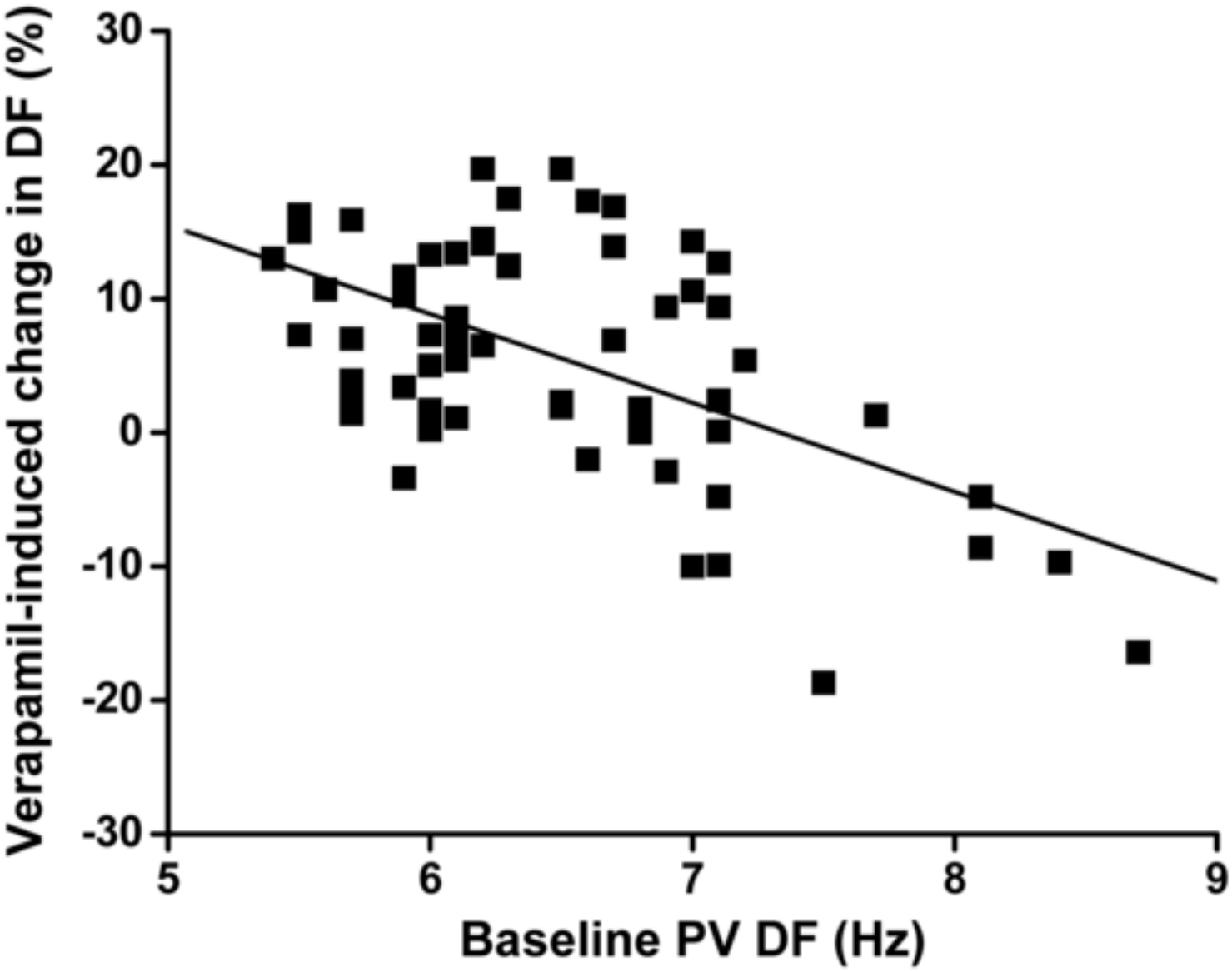

Fig 5

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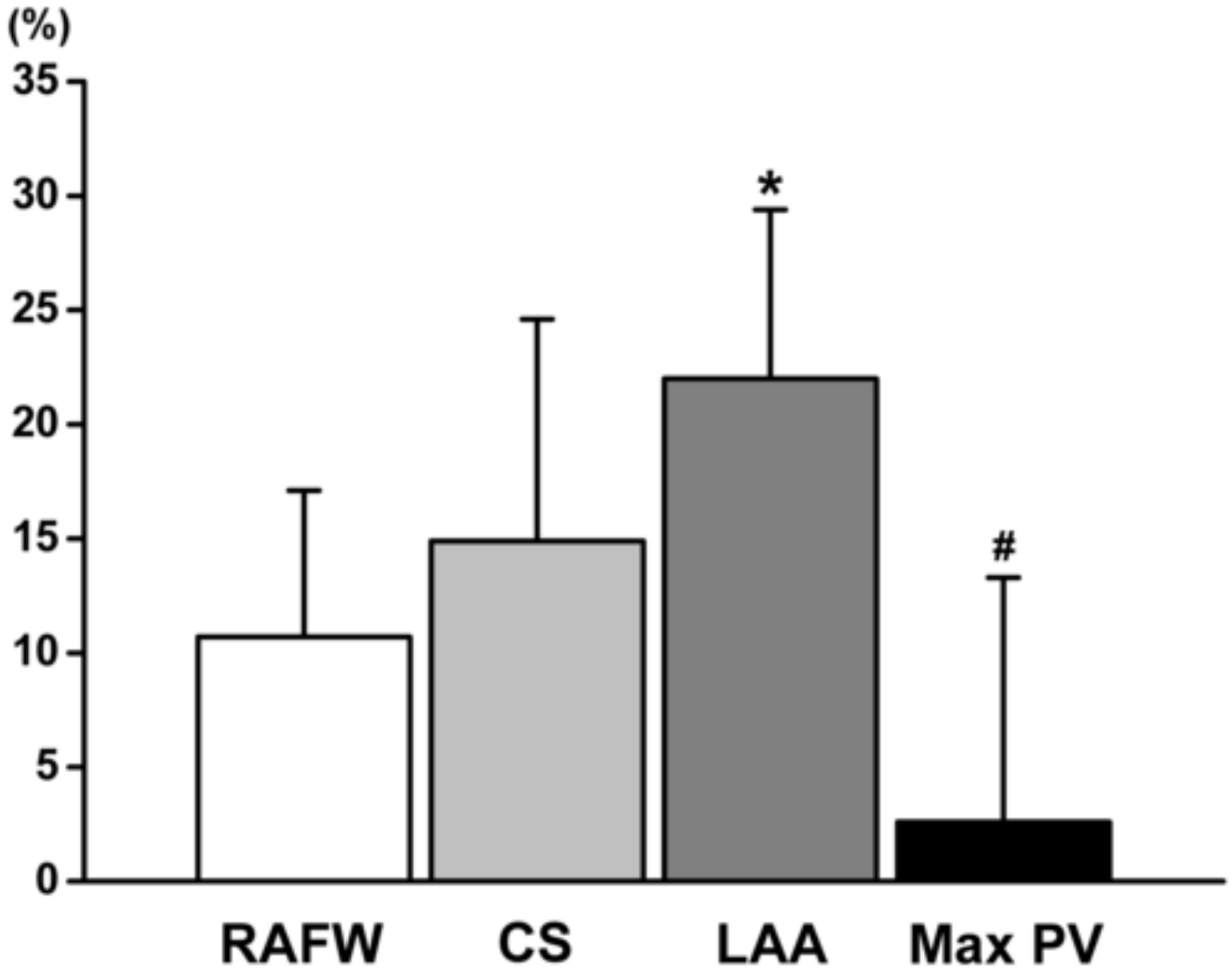

Fig 6