ORIGINAL ARTICLE

Sequence of cardiovascular autonomic alterations after fingolimod initiation

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Background: Homeostasis between heart rate and blood pressure is based on several interacting regulatory reflexes, which become influenced by fingolimod initiation. The aim of this study was to determine the sequence of changes in cardiovascular autonomic regulation after fingolimod initiation.

Methods: Twenty-seven patients with relapsing–remitting multiple sclerosis underwent continuous electrocardiogram recording during the first 6 hr after the first dose of fingolimod. In addition to the time interval between two consecutive R-peaks (RR interval), blood pressure and heart rate variability (HRV) were measured on hourly basis. Cardiac parasympathetic and sympathetic regulation were assessed by the different components of HRV.

Results: HRV demonstrated an enhancement in cardiac parasympathetic regulation starting 1 hr after the first dose of fingolimod. Blood pressure started to decrease 2 hr and sympathetic cardiac regulation 3 hr after fingolimod initiation. Recovery in RR interval, systolic and diastolic blood pressure, as well as in cardiac autonomic regulation started after 5 hr postdose, whereas pulse pressure (difference between systolic and diastolic blood pressure) continued to increase at the time of hospital discharge.

Conclusions: RR interval, blood pressure, as well as the parasympathetic and sympathetic components of cardiac autonomic regulation alter sequentially in different temporal pattern after fingolimod initiation. These findings enhance the understanding of the effects of fingolimod initiation on cardiovascular autonomic regulation in real life.

KEYWORDS

cardiac autonomic regulation, fingolimod, heart rate variability

1 | INTRODUCTION

Fingolimod is a sphingosine-1-phosphate (S1P) receptor modulator and has disease modifying effect on relapsing–remitting multiple sclerosis (RRMS) (Calabresi et al., 2014; Cohen et al., 2010; Kappos et al., 2006, 2010). In addition to lymphatic and neural tissue (Brinkmann, 2007; Brinkmann et al., 2010; Mandala et al., 2002; Matloubian et al., 2004), S1P receptors are found in heart and blood vessels (Camm et al., 2014; Vanoli, Pentimalli, & Botto, 2014).

Initial S1P receptor agonism of fingolimod causes heart rate decrease by activation of G protein-coupled inwardly rectifying potassium (GIRK) channels and vasodilatation via endothelial nitric oxide synthase (eNOS) pathway (Camm et al., 2014). Fingolimod-related S1P receptor agonism activates Rho-kinase (ROCK) and results in vasoconstriction as well (Camm et al., 2014). Physiological homeostasis between heart rate and blood pressure is based on several interacting regulatory reflexes, which are altered in part after fingolimod initiation. However, the sequence of changes in cardiovascular

autonomic regulation after fingolimod initiation has not been completely revealed.

We studied the cardiovascular autonomic function through heart rate variability (HRV). HRV is a noninvasive method to investigate cardiac autonomic regulation. The components of HRV reflect the modulation of cardiac autonomic regulation and other regulatory systems (Camm et al., 1996). In addition to systolic and diastolic blood pressure, the difference between these measures, called pulse pressure, mirrors the features of cardiovascular autonomic regulation, too.

In this study, we investigated the first-dose effects of fingolimod on the sequence of changes in cardiovascular autonomic regulation in RRMS patients by measuring HRV and blood pressure during the first 6 hr after fingolimod initiation.

2 | METHODS

2.1 | Population

Twenty-seven patients with RRMS including 16 (59%) women and 11 (41%) men were studied. The characteristics of patients are expressed in Table 1. The fingolimod treatment was prescribed on clinical basis strictly by an established drug label without randomization. All patients had fingolimod as a second-line treatment due to side effects or lack of efficacy during a first-line treatment. Preceding immunomodulative treatment for RRMS was discontinued at least a day before fingolimod initiation if changed from interferon-1b or glatiramer acetate and at least 2 months before if shifted from natalizumab. The first dose of fingolimod was given at hospital before 10:00 a.m. and all the patients were followed subsequently 6 hr according to the safety instructions.

2.2 | Study setup

All patients underwent 24-hr ambulatory ECG recording at the day of fingolimod initiation. To assess cardiovascular autonomic regulation, heart rate was recorded as RR intervals (duration between two consecutive R-peaks in ECG), which was further generated to different HRV parameters in time and frequency domain. Blood pressure was measured in sitting position just before the first dose of fingolimod (0 hr) and subsequently six times hourly postdose (1, 2, 3, 4, 5, and

6 hr). Expanded disability status scale (EDSS) was used to assess neurological disability related to RRMS.

2.3 | Ethics approval

Before participating in the study, each patient gave written informed consent after full explanation of the aim and risk of procedures used. The study was approved by the local ethics committee and was conducted according to the Declaration of Helsinki (2013) of the World Medical Association (World Medical Association 2013). The study was registered at ClinicalTrials.gov (NCT01704183).

2.4 | Acquisition of ECG signal

Twenty-four-hour ambulatory electrocardiogram (ECG) recordings were performed using Schiller Medilog AR12plus recorders (Schiller Medilog; Schiller AG, Switzerland) with a sampling frequency of 250 Hz. Three bipolar ECG leads were modified chest lead V5, modified chest lead V1, and modified aVF. Digital ECG recordings were read to Darwin Holter analysis system (Schiller Medilog; Schiller AG) and they were exported in MIT-format for the further analyses. Ambulatory ECG recordings were started 1 hr before fingolimod initiation. During the six postdose hours, the patients were observed under sedentary conditions at hospital, although physical activity was not restricted anyway.

2.5 | Analysis of heart rate variability

The mean of RR interval was calculated for each postdose hour. The values for different HRV measures were calculated according to the recommendations (Camm et al., 1996) using the WinCPRS software (Absolute Aliens Oy, Turku, Finland) on hourly basis. For the assessment of HRV in time domain, the standard deviation of all RR intervals (SDNN), the percentage of normal RR interval with duration more than 50 ms different from the previous normal RR interval (pNN50) and the root mean square of successive differences in RR interval (rMSSD) were measured. Frequency domain analysis of HRV was performed with a nonparametric fast Fourier transformation. The power spectrum of RR intervals was divided into low frequency (LF) (0.04–0.15 Hz) and high frequency (HF) (0.15–0.40 Hz) bands. The integrals under the power spectral density functions were measured and expressed as normalized units by dividing the power of LF (LFnu) and HF (HFnu) components by the total power, from which the <0.04 Hz spectral component had been subtracted and multiplied by 100. In addition, the ratio of the LF power band and HF power band (LF/HF ratio) was calculated.

2.6 | Physiological background of heart rate variability

Heart rate has an inverse relation to the most HRV measures, and thus, lower heart rate (equal to longer RR interval) tends to associate with higher HRV (Monfredi et al., 2014). Typically, parasympathetic stimulation results in increased HRV, whereas sympathetic stimulation is associated with a decrease in HRV (Camm et al., 1996). In time

TABLE 1 Clinical characteristics

Age (years)	43 ± 11
Time from MS diagnosis (years)	10 ± 7
EDSS score	3.4 ± 1.8
Comorbidities	
Asthma bronchiale	1 (4%)
Diabetes mellitus	2 (7%)
Hypertension	1 (4%)
Hypothyroidism	3 (11%)

EDSS, Expanded Disability Status Scale; MS, multiple sclerosis. Values are mean ± SD or n (%).

domain, parasympathetic activation is typically reflected by increase in pNN50 and rMSSD parameters. In frequency domain, LFnu component is considered mainly to reflect sympathetic and HFnu component to be a marker of parasympathetic cardiac autonomic regulation (Akselrod et al., 1981; Pomeranz et al., 1985). Correspondingly, LF/HF ratio indicates sympatho-vagal balance of cardiac autonomic regulation (Camm et al., 1996). In addition, interactions between vasomotor tone, peripheral vascular resistance, thermoregulation and renin-angiotensin system, for example, has an effect on HRV (Akselrod et al., 1981; Reid, 1992; Virtanen et al., 2003).

2.7 | Statistical analyses

Kolmogorov–Smirnov test was applied to verify the normal distribution of variables. In case of nonnormal distribution, logarithmic (ln) transformation was applied for further statistical testing. Results are expressed as mean $\pm$ SD. The repeated measures ANOVA was applied to study the overall differences between the measurements at different time points. Thereafter, the significances of the differences between two specific time points were tested by paired sample *t* test. All analyses were conducted at the 2-tailed level and a *p*-value $< .05$ was considered statistically significant. All statistical analyses were performed using IBM SPSS statistics (version 19; 1989–2010 SPSS Inc, Chicago, IL, USA).

3 | RESULTS

The mean of RR interval before fingolimod initiation (0 hr) was 741 ± 94 ms. It prolonged significantly already at 1 hr (803 ± 101 ms; $p < .01$ vs. 0 hr), while the peak value was demonstrated at 5 hr (974 ± 153 ms; $p < .05$ vs. 0 hr) (Figure 1).

The mean of systolic blood pressure was 133 ± 11 mmHg at 0 hr. As compared to 0 hr, it started to decrease at 2 hr (128 ± 12 mmHg; $p < .05$), reached its nadir at 4 hr (124 ± 12 mmHg; $p < .001$) and showed a complete recovery by discharge at 6 hr (135 ± 11 mmHg) (Figure 1).

The mean of diastolic blood pressure was 83 ± 11 mmHg at 0 hr. It decreased similarly to systolic blood pressure at 2 hr (78 ± 12 mmHg; $p < .01$), reached its nadir at 4 hr (74 ± 13 mmHg; $p < .001$) as well but remained significantly lower at 6 hr (78 ± 10 mmHg; $p < .001$) as compared to 0 hr (Figure 1).

Pulse pressure was 50 ± 10 mmHg at 0 hr and remained stable during the first five postdose hours. However, significantly higher pulse pressure was demonstrated at 6 hr (57 ± 12 mmHg; $p < .01$) as compared to 0 hr (Figure 1).

3.1 | Cardiac autonomic regulation after fingolimod initiation

Overall HRV assessed by the SDNN was 91 ± 31 ms at 0 hr. It increased at 2 hr (104 ± 40 ms; $p < .05$) and reached the highest value at 3 hr (115 ± 46 ms; $p < .01$) (Figure 2).

Cardiac parasympathetic regulation was enhanced already at 1 hr. In the time domain, this was reflected by higher percentage of

FIGURE 1 Changes in RR interval (time between two consecutive R-peaks) and blood pressure during the 6 hr after the first dose of fingolimod. dBP: diastolic blood pressure; sBP: systolic blood pressure. Values are expressed as mean $\pm$ SEM. Significances: ***p* $< .01$ and ****p* $< .001$ vs. baseline (0 hr)

normal RR interval with duration more than 50 ms different from the previous normal RR interval (pNN50) ($12 \pm 11\%$; $p = .01$) and higher root mean square of successive differences in RR interval (rMSSD) (34 ± 19 ; $p < .01$) as compared to 0 hr ($8 \pm 7\%$ and 28 ± 15 , respectively) (Figure 2). The highest value of pNN50 was recorded at 5 hr ($23 \pm 17\%$; $p < .01$), whereas rMSSD reached its highest value an hour earlier at 4 hr (55 ± 34 ; $p < .001$), as compared to 0 hr (Figure 2).

In frequency domain, low frequency in normalized units (LFnu) reflecting sympathetic cardiac regulation was lower (71 ± 11 ; $p < .05$) and high frequency in normalized units (HFnu) reflecting parasympathetic cardiac regulation was higher (28 ± 10 ; $p < .05$) at 3 hr as compared to 0 hr (77 ± 11 and 21 ± 11 , respectively) (Figure 2). Correspondingly, LF/HF ratio was significantly lower at 3 hr (3.17 ± 2.12 ; $p < .01$) as compared to 0 hr (5.15 ± 3.71) (Figure 2). The peak of HFnu (33 ± 12) as well as the nadir of LFnu (65 ± 14) and LF/HF ratio (2.94 ± 3.15) were recorded at the fifth postdose hour (Figure 2).

3.2 | Recovery of cardiac autonomic regulation

The recovery of cardiac autonomic regulation started 5 hr postdose but was incomplete at the time of discharge (6 hr). RR interval was significantly longer at 6 hr (919 ± 111 ms) than at 0 hr (741 ± 94 ms; $p < .001$). In addition, pNN50 ($18 \pm 14\%$ vs. $9.2 \pm 7.3\%$; $p < .001$), rMSSD (39 ± 19 vs. 28 ± 15 ; $p < .01$), and HFnu (28 ± 10 vs. 21 ± 11 ; $p < .01$), all reflecting parasympathetic cardiac regulation, were significantly higher at 6 hr as compared to 0 hr, respectively (Figure 2). LFnu (71 ± 11 vs. 77 ± 11 ; $p < .01$) reflecting sympathetic cardiac modulation and LF/HF ratio (3.40 ± 2.78 vs. 5.15 ± 3.72 ; $p < .01$), an index of sympatho-vagal balance, both remained significantly lower at 6 hr as compared to 0 hr, respectively (Figure 2).

FIGURE 2 Changes in heart rate variability during the 6 hr after the first dose of fingolimod. Each time point is compared to predose values at 0 hr. HF, high frequency; LF, low frequency; nu: normalized units; pNN50, the percentage of normal RR interval with duration more than 50 ms different from the previous normal RR interval; rMSSD, the root mean square of successive differences in RR interval SDNN, the standard deviation of all RR intervals. Values are expressed as mean $\pm$ SEM. Significances: * $p < .05$, ** $p < .01$, and *** $p < .001$ vs. baseline (0 hr)

4 | DISCUSSION

In this study, we demonstrated that several components of cardiovascular autonomic regulation were influenced by fingolimod initiation. Along with a decrease in heart rate, an enhancement in cardiac parasympathetic regulation was found already 1 hr after the first dose of fingolimod. Systolic and diastolic blood pressure started to show lower values 2 hr and sympathetic cardiac regulation 3 hr after fingolimod initiation. Recovery in heart rate, systolic and diastolic blood pressure, as well as in the parameters of cardiac autonomic regulation started to recover toward baseline values started at 5 hr but did not resume completely at the time of discharge. In addition, pulse pressure continued to increase at the time of discharge.

Fingolimod initiation results in heart rate decrease equal to RR interval prolongation (Camm et al., 2014; Vanoli et al., 2014). This was demonstrated to occur already at the first postdose hour in our study. An enhancement in the parasympathetic components of HRV was logically demonstrated at the first postdose hour, too. As known, both these phenomena relate to the initial vagomimetic effects of S1P receptor agonism. A close temporal association between RR interval and the parasympathetic components of HRV was demonstrated during the whole entire observation period.

The components of HRV reflecting mainly sympathetic cardiac regulation and sympatho-vagal balance, started to decrease later, 3 hr after fingolimod initiation. Indeed, this is 2 hr later than an enhancement in the parasympathetic components of HRV was demonstrated. This finding supports the hypothesis that more than one cardiovascular regulatory mechanism becomes adjusted after fingolimod initiation. According to our present findings, the different components of cardiovascular autonomic regulation become altered sequentially in real-life RRMS patients.

Systolic and diastolic blood pressure decreased parallel from the second to the fifth postdose hour. However, pulse pressure continued to increase at the time of discharge as systolic blood pressure recovered but diastolic did not. Previously, in large studies, systolic blood pressure has been reported to increase slightly more than diastolic blood pressure after fingolimod initiation (Calabresi et al., 2014; Gold et al., 2014; Kappos et al., 2010), whereas some studies (Cohen

et al., 2010; Kappos et al., 2006) report only the values of mean arterial pressure. To the best of our knowledge, the effect of fingolimod on pulse pressure has not been previously reported. In general, an elevated pulse pressure has been connected with an impaired blood pressure regulation and an increased cardiac stroke volume (Alfie et al., 1999; Virtanen et al., 2003). An elevated pulse pressure predicts cardiovascular events and even mortality in unselected populations (Benetos et al., 1997; Franklin et al., 1999). Unfortunately, we do not have any blood pressure data beyond the hospital discharge.

The recovery (shortening) of RR interval started 5 hr after fingolimod initiation, which is consistent with previously described time window (Cohen et al., 2010; Gold et al., 2014; Hilz et al., 2015; Kappos et al., 2010; Rossi et al., 2015). In our study, recovery of RR interval was demonstrated to occur along with a withdrawal of parasympathetic cardiac enhancement and increase in sympathetic cardiac regulation. However, none of the studied parameters reflecting cardiovascular autonomic regulation demonstrated full recovery by the time of discharge. Indeed, we have previously reported that heart rate and HRV do not recover to pretreatment values even 3 months after fingolimod treatment (Simula et al., 2015, 2016).

The affinity of fingolimod is the highest for S1P1 receptors expressed abundantly on atrial myocytes as well as on endothelial cells, and somewhat lower for S1P3 receptors being more numerous on vascular smooth muscle cells (Camm et al., 2014; Koyrakh et al., 2005). Initial S1P agonism by fingolimod is known to activate GIRK channels resulting in heart rate decrease and also activate eNOS promoting vasodilatation. On the contrary, activation of ROCK by fingolimod results in vasoconstriction. Eventually, fingolimod causes down-regulation of S1P1 receptors and consequently shifts endogenous S1P signaling toward S1P2–3 receptors. These well-described pharmacological effects of fingolimod on different regulatory pathways at myocardium and vascular wall obviously occur in different temporal pattern, and thus, may explain the sequential changes demonstrated in cardiovascular autonomic regulation after fingolimod initiation. In addition, adjustment of physiological cardiovascular autonomic regulation in relation to altered S1P receptor profile may rebalance cardiovascular homeostasis.

5 | CONCLUSION

Cardiovascular autonomic regulation becomes influenced shortly after fingolimod initiation. Heart rate, blood pressure, as well as the parasympathetic and sympathetic components of cardiac autonomic regulation alter sequentially in different temporal pattern. These real-life findings enhance the understanding of the effects of fingolimod initiation and S1P receptor modulation on cardiovascular autonomic regulation in RRMS patients. The possible effect of fingolimod on pulse pressure and its clinical significance needs further evaluation.

CONFLICT OF INTEREST

SS has been the congress representative of Mikkeli Central Hospital sponsored by industry (BiogenIdec, Boehringer Ingelheim, Genzyme, GlaxoSmithKline, Novartis, OrionPharma, Sanofi, TEVA) and has been a speaker sponsored by industry (BiogenIdec, Genzyme, Merck, Novartis, TEVA). TPL has received a research grant from the Finnish Foundation for Cardiovascular Research. TML: None. PH has been the congress representative of Kuopio University Hospital sponsored by industry (BiogenIdec, Genzyme, TEVA). JEKH has received research grants from the Finnish Foundation for Cardiovascular Research and the European Union Seventh Framework Programme and has been a speaker sponsored by industry (Amgen, AstraZeneca, Biotronic, Cardiome, MSD).

REFERENCES

- Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A. C., & Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: A quantitative probe of beat to beat cardiovascular control. *Science*, 213, 220–222.
- Alfie, J., Waisman, G. D., Galarza, C. R., & Cámera, M. I. (1999). Contribution of stroke volume to the change in pulse pressure pattern with age. *Hypertension*, 34, 808–812.
- Benetos, A., Safar, M., Rudnicki, A., Smulyan, H., Richard, J. L., Ducimetière, P., & Guize, L. (1997). Pulse pressure: A predictor of long-term cardiovascular mortality in a French male population. *Hypertension*, 30, 1410–1415.
- Brinkmann, V. (2007). Sphingosine 1-phosphate receptors in health and disease: Mechanistic insights from gene deletion studies and reverse pharmacology. *Pharmacology & Therapeutics*, 115, 84–105.
- Brinkmann, V., Billich, A., Baumruker, T., Heining, P., Schmouder, R., Francis, G., ... Burtin, P. (2010). Fingolimod (FTY720): Discovery and development of an oral drug to treat multiple sclerosis. *Nature Reviews Drug Discovery*, 9, 883–897.
- Calabresi, P. A., Radue, E. W., Goodin, D., Jeffery, D., Rammohan, K. W., Reder, A. T., ... Lublin, F. D. (2014). Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): A double-blind, randomized, placebo-controlled, phase 3 trial. *Lancet Neurology*, 13, 545–556.
- Camm, J., Bigger, J. T. Jr, Breithardt, G., Cerutti, S., Cohen, R. J., Coumel, P., ... Singer, D. H. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*, 93, 1043–1065.
- Camm, J., Hla, T., Bakshi, R., & Brinkmann, V. (2014). Cardiac and vascular effects of fingolimod: Mechanistic basis and clinical implications. *American Heart Journal*, 168, 632–634.
- Cohen, J. A., Barkhof, F., Comi, G., Hartung, H. P., Khatri, B. O., Montalban, X., ... Kappos, L. (2010). Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *New England Journal of Medicine*, 362, 402–415.
- Franklin, S. S., Khan, S. A., Wong, N. D., Larson, M. G., & Levy, D. (1999). Is pulse pressure useful in predicting risk for coronary heart disease? The Framingham Heart Study. *Circulation*, 100, 354–360.
- Gold, R., Comi, G., Palace, J., Siever, A., Gottschalk, R., Bijarnia, M., ... Kappos, L. (2014). Assessment of cardiac safety during fingolimod treatment initiation in a real-world relapsing multiple sclerosis population: A phase 3b, open-label study. *Journal of Neurology*, 261, 267–276.
- Hilz, M. J., Intravooth, T., Moeller, S., Wang, R., Lee, D. H., Koehn, J., & Linker, R. A. (2015). Central autonomic dysfunction delays recovery of fingolimod induced heart rate slowing. *PLoS One*, 10, e0132139.
- Kappos, L., Antel, J., Comi, G., Montalban, X., O'Connor, P., Polman, C. H., ... Radue, E. W. (2006). Oral fingolimod (FTY720) for relapsing multiple sclerosis. *New England Journal of Medicine*, 355, 1124–1140.
- Kappos, L., Radue, E. W., O'Connor, P., Polman, C., Hohlfeld, R., Calabresi, P., ... Burtin, P. (2010). A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *New England Journal of Medicine*, 362, 387–401.
- Koyrakh, L., Roman, M. I., Brinkmann, V., & Wickman, K. (2005). The heart rate decrease caused by acute FTY720 administration is mediated by the G protein-gated potassium channel I. *American Journal of Transplantation*, 5, 529–536.
- Mandala, S., Hajdu, R., Bergstrom, J., Quackenbush, E., Xie, J., Milligan, J., ... Rosen, H. (2002). Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science*, 296, 346–349.
- Matloubian, M., Lo, C. G., Cinamon, G., Lesneski, M. J., Xu, Y., Brinkmann, V., ... Cyster, J. G. (2004). Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature*, 427, 355–360.
- Monfredi, O., Lyashkov, A. E., Johnsen, A. B., Inada, S., Schneider, H., Wang, R., ... Boyett, M. R. (2014). Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. *Hypertension*, 64, 1334–1343.
- Pomeranz, B., Macaulay, R. J., Caudill, M. A., Kutz, I., Adam, D., Gordon, D., ... Cohen, R. J. (1985). Assessment of autonomic function in humans by heart rate spectral analysis. *American Journal of Physiology*, 248, H151–H153.
- Reid, I. A. (1992). Interactions between ANG II, sympathetic nervous system and baroreceptor reflexes in regulation of blood pressure. *American Journal of Physiology*, 262, E763–E778.
- Rossi, S., Rocchi, C., Studer, V., Motta, C., Lauretti, B., Germani, G., ... Centonze, D. (2015). The autonomic balance predicts cardiac responses after the first dose of fingolimod. *Multiple Sclerosis*, 21, 206–216.
- Simula, S., Laitinen, T., Laitinen, T. M., Tarkiainen, T., Hartikainen, J. E., & Hartikainen, P. (2015). Effects of three months fingolimod therapy on heart rate. *Journal of Neuroimmune Pharmacology*, 10, 651–654.
- Simula, S., Laitinen, T., Laitinen, T. M., Tarkiainen, T., Hartikainen, P., & Hartikainen, J. E. (2016). Effect of fingolimod on cardiac autonomic regulation in patients with multiple sclerosis. *Multiple Sclerosis*, 22, 1080–1085.
- Vanoli, E., Pentimalli, F., & Botto, G. (2014). Vagomimetic effects of fingolimod: Physiology and clinical implications. *CNS Neuroscience & Therapeutics*, 20, 496–502.
- Virtanen, R., Jula, A., Kuusela, T., Helenius, H., & Vopio-Pulkki, L. M. (2003). Reduced heart rate variability in hypertension: Associations with lifestyle factors and plasma renin activity. *Journal of Human Hypertension*, 17, 171–179.
- World Medical Association (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA*, 310, 2191–2194.

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