

Speckle-tracking strain assessment of left ventricular dysfunction in synthetic cannabinoid and heroin users

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ABSTRACT

Objective: There is growing evidence regarding the numerous adverse effects of synthetic cannabinoids (SCBs) on the cardiovascular system; however, no studies have shown the cardiovascular effects of opioids using strain echocardiography. This study examines the cardiac structure and function using echocardiographic strain imaging in heroin and synthetic cannabinoid users.

Methods: This double-blind study included patients who were admitted or referred to a rehabilitation center for heroin (n=31) and synthetic cannabinoid users (n=30). Heroin users and synthetic cannabinoid users were compared with healthy volunteers (n=32) using two-dimensional (2D) speckle-tracking (ST) echocardiography.

Results: No differences were found in the baseline characteristics and 2D echocardiography values. The mean global longitudinal strain value was $-20.5\% \pm 2.4\%$ for SCB users, $-22.3\% \pm 2.4\%$ for opioid users, and $-22.5\% \pm 2.2\%$ for healthy volunteers ($p=0.024$). The mean apical 2-chamber (AP2C) L-strain values were $-20.1\% \pm 3.1\%$, $-22.4\% \pm 3.0\%$, and $-22.3\% \pm 2.8\%$ for SCB users, opioid users, and healthy volunteers, respectively ($p=0.032$). The mean apical 4-chamber (AP4C) L-strain values were $-20.7\% \pm 2.5\%$ for SCB users, $-23.2\% \pm 3.2\%$ for opioid users, and $-23.8\% \pm 3.1\%$ for healthy volunteers ($p<0.001$).

Conclusion: SCBs are potential causes of subclinical left ventricular dysfunction. (*Anatol J Cardiol* 2018; 19: 388-93)

Keywords: strain, left ventricular function, synthetic cannabinoid, heroin

Introduction

Heroin (diamorphine) and synthetic cannabinoids (SCBs) are public health concerns. Heroin has a detection history of approximately 30 years and can be detected using routine toxicological screening; however, there is little information about SCBs (1-4). There is growing evidences regarding the adverse effects of SCBs and heroin on the cardiovascular system. However, till date, no studies have shown the cardiovascular effects of these drugs on the left ventricle using strain echocardiography. In addition, clinical trials are minimal, and most of the studies have been performed on animal models (5).

SCBs vary in their potency, efficacy, affinity, selectivity, and metabolic and molecular activity. The majority of SCBs' metabolites have longer half-lives. Notable among them is JWH-018, which retains its metabolic activity in the cannabinoid type 1 (CB1) receptors, indicating the increased prevalence of adverse

events with the use of JWH-018 compared with that of natural cannabis. In addition, JWH-018 also has a four-fold affinity to the CB1 receptor and a ten-fold affinity to the CB2 receptor (6, 7). Recent evidence has indicated that both cannabinoid receptors [CB1 and CB2 (type 2)] are expressed in healthy human left ventricular (LV) myocardium in a balanced distribution, whereas downregulation of CB1 is noted in patients with chronic heart failure (CHF). CB1 is a G-protein-coupled receptor that is coupled to Gi heterotrimeric proteins and adenylyl cyclase (AC). CB1 activation has been reported to inhibit the release of neurohormonal factors, improve myocardial energy metabolism, and suppress vasopressin-induced vasoconstriction (8). CB1 receptor activation by synthetic agonists induces reactive oxygen species production, mitogen-activated protein kinase activation, and cell death in human coronary endothelial cells (9, 10). CB1 receptors play an important role in vascular smooth muscle proliferation because receptor blockade is able to inhibit vascular smooth muscle proliferation and migration, in response to

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platelet-derived growth factor stimulation by inhibiting Ras and extracellular signal-regulated kinase 1/2 activation (11). The application of a CB2 receptor agonist showed a similar efficacy in the attenuation of vascular smooth muscle proliferation, indicating an opposing role of the CB receptors in both endothelial cell activation and vascular smooth muscle proliferation (12). Similar to β 1 adrenoceptor blockers, the activation of CB1 inhibits catecholamine release, suppresses AC activity, and decreases cAMP production (13, 14).

There are three main subtypes of heroin receptors, namely delta (DOR), kappa (KOR), and mu (MOR) receptors. They are more commonly found in the central nervous system than in the periphery. Heroin interacts with these receptors, primarily by exerting presynaptic inhibition, which results in reduced release of excitatory transmitters. It is thought that MOR and DOR activation causes respiratory depression. Analgesia is primarily mediated via activation of MOR receptors at supraspinal sites and KOR receptors within the spinal cord (15). Cardiac problems associated with heroin use include QT interval prolongation. The active metabolite of dextropropoxyphene, norpropoxyphene causes prolongation of the QRS interval and ventricular dysrhythmias, including ventricular fibrillation. These effects of norpropoxyphene, combined with the respiratory depressant effects of dextropropoxyphene, increase the risk of death from overdose (16). "Krokodil" is the street name for an impure homemade drug mixture containing desomorphine as the main heroin. The chronic use of krokodil has been shown to cause diffuse myocardial interstitial inflammatory neutrophilic infiltrates and endocardial vegetations. In addition, biochemical studies have shown creatine kinase, creatine kinase-MB, and uric acid changes. Significant alteration in the levels of reduced and oxidized glutathione in the kidney and heart suggest that oxidative stress may be involved in krokodil-mediated toxicity (17, 18).

Considering these mechanisms of action and conclusions from previous publications, it may be assumed that heroin and SCBs cause LV dysfunction in humans. We tested the following hypothesis: SCBs and heroin users will have impaired LV functions compared with healthy individuals. We used echocardiographic assessment and strain measurement using 2D speckle-tracking echocardiography.

Methods

Study design and inclusion criteria

This prospective and double-blind study included patients who were admitted or referred to Treatment and Training Center for Alcohol and Substance Dependence (AMATEM) in Tekirdağ between June 2015 and December 2016. Diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition) by experienced psychiatrists who did not participate in the study (19). Until the end of statistical assessment, researchers were not aware of study protocol and patient's substance abuse, with the exception of the project ad-

ministrator; furthermore, the researcher in charge of biochemical analysis was unaware about which subjects used heroin or SCBs. The biochemical analyst was also unaware about the nature of the study.

The inclusion criteria were as follows: heroin or SCBs users, males aged 18–60 years, sufficient mental ability to understand the aim of the study and provide voluntary, signed informed consent. The exclusion criteria were as follows: patients with prior diagnosis of any cardiovascular disease, physical or psychiatric disorders that could affect the cardiovascular system, taking any drugs that could alter cardiac functions, having any neurological diseases, having comorbid substance abuse (except smoking), alcoholism, unwillingness to participate to this study, and aged >60 or <18 years.

The study included 92 substance users. Patients with emotional fluctuations (n=14), depression (n=6), and suicidal inclinations (n=11) were excluded. Finally, 31 patients using SCBs and 30 using heroin were included (n=61). Additionally, 32 age- and cigarette smoking-matched males were included as healthy volunteers. Sociodemographic and clinical data obtained from the subjects were recorded. All patients had sufficient mental capacity to understand the aim of the study, and provide informed consent. Additionally, World Health Organization Alcohol, Smoking and Substance Involvement Screening Test version 3.0 was used (19).

Biochemical analysis

The Healgen® commercial kit (Healgen Scientific LLC, TX, USA) was used for routine toxicological urinalysis. Its methodology is based on a one-step multidrug screen lateral flow chromatographic immunoassay founded by Zhejiang Orient Gene Biotech in Shanghai.

The cutoff value was noted as 300 ng/mL for opiates and 50 ng/mL for SCBs. The SCB screening was used for JWH-073 butanoic acid, JWH-018 4-hydroxybutyl, JWH-018 5-hydroxybutyl, and JWH-073 4-hydroxybutyl. Sensitivity and specificity were reported as 99% for both urine toxicological SCB screening tests.

Echocardiography

Echocardiographic study was performed using a Philips® EPIQ 7 Cardiology Ultrasound Machine with an S5-1 transducer (Philips N.V., Amsterdam, Netherlands) within 24 h of opiate or SCB abuse. Parasternal, apical, and subcostal views were recorded. All data were stored on a workstation for offline analysis (EPIQ QLAB Automated Cardiac Motion Quantification software) by a cardiologist blinded to the clinical data. The conventional analysis of the echocardiogram preceded the two-dimensional strain analysis. For each measurement, at least two cardiac cycles were averaged. LV end-diastolic diameter and maximal end-diastolic LV wall thickness were measured in parasternal views. LV end-diastolic and end-systolic volumes and ejection fraction were measured using the biplane method of disks. Peak E-wave and A-wave velocities of the mitral inflow were measured using

pulsed-wave Doppler. Tissue Doppler imaging was recorded at the level of septal and lateral mitral annulus to obtain the average peak velocities during systole (s') and early diastole (e'). The E/e' ratio was calculated to assess LV filling pressure.

Longitudinal myocardial deformations were evaluated from standard two-dimensional images (frame rate, 60–90 frames/s), on the basis of the ST approach. Global longitudinal strain (GLS) was the average of the 16 segmental strain values from the apical four-, three-, and two-chamber views. The time to maximal myocardial shortening, including post systolic shortening if present, was measured from the electrocardiographic onset Q/ onset R wave in the 16 LV segments.

LV strain was measured from three parasternal short-axis views at the level of the mitral valve, papillary muscle, and apex. We analyzed circumferential strain in each of the 16 segments of LV. global circumferential strain (GCS) was calculated by averaging values of all 16 segmental strains.

Statistical analysis

All calculations were conducted using IBM SPSS Statistics 22.0 for Windows software (SPSS Inc., Chicago, IL, USA). Numerical data was analyzed using the Shapiro–Wilk test for

assessing the parametric qualities of data. The differences between numeric parameters were assessed using a series of one-way ANOVAs. The significances in ANOVA test were presented as F (dfbetween, dfwithin)= F value and p value. If p value is less than <0.05 and beside this, F value is greater than 1; the statistical significance can be demonstrated more clearly. In our study, we considered to use both F and p values for more clear interpretation of results. Differences between groups were evaluated with multiple pairwise comparison tests [Tukey honest significant difference (HSD)]. Tukey's HSD was applied for post-hoc analysis of multiple comparisons of the SCB users, opioid users and healthy volunteers. All numerical data was expressed as mean±standard deviation. The difference in categorical variables was assessed using the χ^2 test. Data was assessed by a confidence interval of 95%, and a 2-tailed p<0.05 was accepted as statistically significant.

Results

A total of 93 participants were included: 31 SCB users, 30 heroin users, and 32 healthy volunteers. There was no difference between groups in terms of cardiovascular risk factors (Table 1). The mean value of ejection fraction (biplane LVEF)

Table 1. Baseline characteristics and 2D echocardiographic values

	Healthy volunteers (n=32)	Synthetic cannabinoid users (n=30)	Heroin users (n=31)	
Men (%)	100	100	100	
Age (years)	25±8	24±6	24±4	F (2.88)=0.29, P=0.742
Diabetes mellitus	0	0	0	
Hypertension	0	0	0	
Systolic blood pressure (mm Hg)	128±13	130±13	130±11	F (2.88)=0.81, P=0.624
Diastolic blood pressure (mm Hg)	58±5	60±7	57±5	F (2.88)=0.73, P=0.425
Smoker (%)	100%	100%	100%	
Height (cm)	170±11	173±9	172±10	F (2.88)=0.55, P=0.342
Weight (kg)	83.4±6.2	80.6±5.1	81.1±5.6	F (2.88)=0.6, P=0.452
Heart rate (bpm)	77±7	76±8	76±6	F (2.88)=0.56, P=0.632
Biplane LVEF (%)	61.4±3.8	59.4±4.7	61.1±4.4	F (2.88)=1.00, P=0.374
Biplane LVEDV (mL)	96.3±19.3	102.8±19.5	101.6±19.0	F (2.88)=1.12, P=0.370
Biplane LVESV (mL)	37.5±9.6	40.9±11.0	40.6±11.1	F (2.88)=0.97, P=0.383
LVEDD (mm)	50.3±2.2	49.7±2.4	50.1±2.1	F (2.88)=0.87, P=0.212
LVESD (mm)	29.2±1.2	29.4±1.3	29.2±1.1	F (2.88)=0.75, P=0.320
PW (mm)	9±1	8±1	8±1	F (2.88)=0.86, P=0.423
IVS (mm)	10±1	10±1	10±1	F (2.88)=0.74, P=0.530
E/A	1.6	1.6	1.5	F (2.88)=0.51, P=0.201
LA (mm)	36±1	36±1	37±1	F (2.88)=0.67, P=0.612

NS - not significant, cm - centimeter, kg - kilogram, min- minute, LVEF - left ventricular ejection fraction, LVEDV - left ventricular end-diastolic volume, ml - milliliter, LVESV - left ventricular end-systolic volume, LVEDD - left ventricular end-diastolic diameter, mm - millimeter, LVESD - left ventricular end-systolic diameter, PW - posterior wall, IVS - interventricular septum, LA - left atrium

Table 2. Echocardiographic strain values

	Healthy volunteers (n=32)	Synthetic cannabinoid users (n=30)	Heroin users (n=31)	
Global longitudinal strain (%)	-22.5±2.2	-20.5±2.4	-22.3±2.4	F (2.88)=6.70, P=0.024
AP4C longitudinal strain (%)	-23.8±3.1	-20.7±2.5	-23.2±3.2	F (2.88)=9.04, P<0.001
AP2C longitudinal -strain (%)	-22.3±2.8	-20.1±3.1	-22.4±3.0	F (2.88)=3.50, P=0.032
Global circumferential strain (%)	-25.3±4.3	-23.1±5.4	-25.4±4.4	F (2.88)=1.09, P=0.393
SAX-B circumferential strain (%)	-21.0±5.1	-19.7±4.5	-20.8±5.0	F (2.88)=0.65, P=0.524
SAX-M circumferential strain (%)	-26.9±7.0	-23.3±5.9	-25.6±6.0	F (2.88)=0.81, P=0.120
SAX-A circumferential strain (%)	-38.0±14.0	-35.5±15.3	-35.7±11.8	F (2.88)=0.35, P=0.724

AP4C - apical 4-chamber, AP2C - apical 2-chambers, SAX-B - short-axis basal, SAX-M - short-axis medial, SAX-A - short-axis apical

was 59.4%±4.7% in SCB users, 61.1%±4.4% in heroin users, and 61.4%±3.8% in healthy volunteers [F (2.88)=1.00, p=0.374]. The mean values of end-diastolic volume (biplane) were 102.8±19.5 mL, 101.6±19.0 mL, and 96.3±19.3 mL in SCB users, heroin users, and healthy volunteers, respectively [F (2.88)=1.12, p=0.370]. The mean values of end-systolic volume (biplane) were 40.9±11.0 mL, 40.6±11.1 mL, and 37.5±9.6 mL in SCB users, heroin users, and healthy volunteers, respectively [F (2.88)=0.97, p=0.383].

The mean GLS values were -20.5%±2.4%, -22.3%±2.4%, and -22.5%±2.2% in SCB users, heroin users, and healthy volunteers, respectively [F (2.88)=6.70, p=0.024]. The mean GLS was lower in SCB users than in heroin users and healthy volunteers (p=0.012 and p=0.003). The heroin users and healthy volunteers had similar GLS values (p=0.931).

The mean apical-4 chamber (AP4) L-strain values were -20.7%±2.5% in SCB users, -23.2%±3.2% in heroin users, and -23.8%±3.1% in healthy volunteers [F (2.88)=9.04, p<0.001]. The mean AP4 L-strain value was lower in SCB users than in heroin users and healthy volunteers (p=0.05 and p<0.001). The heroin users and healthy volunteers were found to have similar AP4 L-strain values (p=0.723). The mean apical-2 chamber (AP2) L-strain values were -20.1%±3.1%, -22.4%±3.0%, and -22.3%±2.8% in SCB users, heroin users, and healthy volunteers, respectively [F (2.88)=3.50, p=0.032]. The mean AP2 L-strain value was lower in SCB users than in heroin users and healthy volunteers (p=0.042 and p=0.045 respectively). Other pairwise comparisons of mean AP2 L-strain values did not show any difference (p>0.05).

The mean values of GCS were -23.1%±5.4% in SCB users, -25.4%±4.4% in heroin users, and -25.3%±4.3% in healthy volunteers [F (2.88)=1.09, p=0.393]. The mean values of short-axis basal (SAX-B) circumferential strain were -19.7%±4.5%, -20.8%±5.0%, and -21.0%±5.1% in SCB users, heroin users, and healthy volunteers [F (2.88)=0.65, p=0.524]. The mean values of the apical short-axis (SAX-A) circumferential strain were -35.5%±15.3% in SCB users, -35.7%±11.8% in heroin users, and -38.0%±14.0% in healthy volunteers [F (2.88)=0.35, p=0.724]. The mean short-axis medial (SAX-M) circumferential strain values

were -23.30%±5.90% in SCB users, -25.64%±6.08% in heroin users, and -26.89%±7.0% in healthy volunteers [F (2.88)=0.81, p=0.120] (Table 2).

Discussion

Although all strain values were in the normal range, L-strain values in SCB users were significantly decreased compared with those in heroin users and healthy volunteers. Additionally, both traditional echocardiographic assessments and strain values were similar in heroin users and healthy volunteers. Longitudinal strain analysis helps in identifying the pathophysiology of myocardial longitudinal fibers. On a clinical level, the results of Stokke et al. (20) revealed interactions among different forms of strain and how they compensate for early LV dysfunction. Longitudinal fibers are typically oriented in the subendocardium and thus are more vulnerable to wall stress and fibrosis in contrast to the midwall circumferential fibers, which are not as greatly affected. Given its relatively greater contribution to LVEF, increased circumferential strain can therefore maintain stroke volume, despite significant loss of subendocardial strain. Stokke et al. (20) rightfully concluded that strain imaging probably better reflects systolic function in patients with a preserved estimated LVEF (20-24). Long-axis function might be a potential indicator of subclinical LV dysfunction in numerous diseases (24-26).

We suspect that the possible causes of LV dysfunction caused by SCBs include CB receptor activation, which plays an important role in cardiac remodeling. Inappropriate and excessive activation of CB1 receptors in cardiac and endothelial tissues leads to cell death. CB1 receptor deficiency promotes cardiac remodeling induced by pressure overload in mice. Liao et al. (10) found that CB1 receptor deficiency contributed to the exacerbation of chronic cardiac remodeling mediated by the AC-PKA-EGFR signaling pathway *in vivo* and *in vitro*, revealing that CB1 plays a role in the pathophysiology of CHF.

Although heroin has been shown to cause oxidative stress-induced myocarditis, heroin did not affect the LV function in this

study (16-18). Therefore, if there is no other risk factor to disrupt cardiac functioning (e.g., alcohol use), the use of heroin alone may not result in impairment of the LV function.

Study limitations

There are some limitations to the present study. This was a small study (n=61), and all participants were male. Most of the addicts used multiple drugs (e.g., SCBs, heroin, and cocaine) and alcohol. It was difficult to find individuals who used only SCBs, so the numbers of participants was low. As only few women were admitted or referred to the rehabilitation center for treatment, only male addicts were included. As majority of users did not accept the control and/or follow-up, echocardiographic examination was performed only once; therefore, a follow-up was not possible. Urine opiate test was positive in all heroin users. However, only four SCB users had a positive urine test. Spectroscopic urine analysis is another option to confirm the recent use of some SCBs. However, this technology is not yet commercially available, and the diagnosis remains primarily clinical (27, 28).

Conclusion

In conclusion, heroin does not affect the LV function, but SCBs are the potential cause of LV dysfunction. Further studies in larger groups of both genders and racial background are necessary to support our findings.

Ethics and permission: This research project was conducted with the permission of the Namık Kemal University School of Medicine Local Ethical Council. Permission was also granted by the state hospital to use participants' data.

Conflict of interest: None declared.

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References

1. Schep LJ, Slaughter RJ, Hudson S, Place R, Watts M. Delayed seizure-like activity following analytically confirmed use of previously unreported synthetic cannabinoid analogues. *Hum Exp Toxicol* 2015; 34: 557-60.
2. Badal S, Smith KN, Rajnarayanan R. Analysis of natural product regulation of cannabinoid receptors in the treatment of human disease. *Pharmacol Ther* 2017; 180: 24-48.
3. Le Boisselier R, Alexandre J, Lelong-Boulouard V, Debruyne D. Focus on cannabinoids and synthetic cannabinoids. *Clin Pharmacol Ther* 2017; 101: 220-9.
4. Reece AS, Hulse GK. Impact of lifetime opioid exposure on arterial stiffness and vascular age: cross-sectional and longitudinal studies in men and women. *BMJ Open*. 2014; 4: e004521.
5. Marcus DJ, Zee ML, Davis BJ, Haskins CP, Andrews MJ, Amin R, et al. Mice Expressing a "Hyper-Sensitive" Form of the Cannabinoid Receptor 1 (CB1) Are Neither Obese Nor Diabetic. *PLoS One* 2016; 11: e0160462.
6. Almada M, Costa L, Fonseca BM, Amaral C, Teixeira N, Correia-da-Silva G. The synthetic cannabinoid WIN-55,212 induced-apoptosis in cytotrophoblasts cells by a mechanism dependent on CB1 receptor. *Toxicology* 2017; 385: 67-73.
7. Alon MH, Saint-Fleur MO. Synthetic cannabinoid induced acute respiratory depression: Case series and literature review. *Respir Med Case Rep* 2017; 22: 137-41.
8. Steffens S, Pacher P. Targeting cannabinoid receptor CB (2) in cardiovascular disorders: promises and controversies. *Br J Pharmacol* 2012; 167: 313-23.
9. Zubrzycki M, Liebold A, Janecka A, Zubrzycka M. A new face of endocannabinoids in pharmacotherapy: part I: protective role of endocannabinoids in hypertension and myocardial infarction. *J Physiol Pharmacol* 2014; 65: 171-81.
10. Liao Y, Bin J, Luo T, Zhao H, Ledent C, Asakura M, et al. CB1 cannabinoid receptor deficiency promotes cardiac remodeling induced by pressure overload in mice. *Int J Cardiol* 2013; 167: 1936-44.
11. Hamilton RJ, Keyes V, Banka SS. Synthetic cannabinoid abuse resulting in ST-segment elevation myocardial infarction requiring percutaneous coronary intervention. *J Emerg Med* 2017; 52: 496-8.
12. Ibrahim S, Al-Saffar F, Wannenburg T. A unique case of cardiac arrest following K2 abuse. *Case Rep Cardiol* 2014; 2014: 120607.
13. Mukhopadhyay P, Horváth B, Rajesh M, Matsumoto S, Saito K, Bátkai S, et al. Fatty acid amide hydrolase is a key regulator of endocannabinoid-induced myocardial tissue injury. *Free Radic Biol Med* 2011; 50: 179-95.
14. Mckeever RG, Vearrier D, Jacobs D, Lasala G, Okaneku J, Greenberg MI. K2--not the spice of life; synthetic cannabinoids and ST elevation myocardial infarction: a case report. *J Med Toxicol* 2015; 11: 129-31.
15. Jordan B, Devi LA. Molecular mechanisms of opioid receptor signal transduction. *Br J Anaesth* 1998; 81: 12-9.
16. Dettmeyer R, Friedrich K, Schmidt P, Madea B. Heroin-associated myocardial damages--conventional and immunohistochemical investigations. *Forensic Sci Int* 2009; 187: 42-6.
17. Sorrentino A, Trotta S, Colucci AP, Aventaggiato L, Marzullo A, Solarino B. Lethal endomyocarditis caused by chronic "Krokodil" intoxication. *Forensic Sci Med Pathol* 2018; doi: 10.1007/s12024-018-9967-9. [Epub ahead of print]
18. Alves EA, Brandão P, Neves JF, Cravo SM, Soares JX, Grund JC, et al. Repeated subcutaneous administrations of krokodil causes skin necrosis and internal organs toxicity in Wistar rats: putative human implications. *Hum Psychopharmacol* 2017; 32. doi: 10.1002/hup.2572.
19. WHO. Management of substance abuse ASSIST Questionnaire 2015. http://www.who.int/substance_abuse/activities/assist_v3_english. 01 June 2015.
20. Stokke TM, Hasselberg NE, Smedsrud MK, Sarvari SI, Haugaa KH, Smiseth OA, et al. Geometry as a confounder when assessing ventricular systolic function: comparison between ejection fraction and strain. *J Am Coll Cardiol* 2017; 70: 942-54.
21. Amundsen BH, Helle-Valle T, Edvardsen T, Torp H, Crosby J, Lyseggen E, et al. Noninvasive myocardial strain measurement by speck-

- le tracking echocardiography: Validation against sonomicrometry and tagged magnetic resonance imaging. *J Am Coll Cardiol* 2006; 47: 789-93.
22. Serri K, Reant P, Lafitte M, Berhouet M, Le Bouffos V, Roudaut R, et al. Global and regional myocardial function quantification by two-dimensional strain: application in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2006; 47: 1175-81.
 23. Januzzi JL Jr, Chandrashekar Y. Strain Echocardiography: The New Gold Standard for Imaging Ventricular Function?. *J Am Coll Cardiol* 2017; 70: 955-7.
 24. Reant P, Lafitte S, Bougteb H, Sacher F, Mignot A, Douard H, et al. Effect of catheter ablation for isolated paroxysmal atrial fibrillation on longitudinal and circumferential left ventricular systolic function. *Am J Cardiol* 2009; 103: 232-7.
 25. Fang ZY, Leano R, Marwick TH. Relationship between longitudinal and radial contractility in subclinical diabetic heart disease. *Clin Sci (Lond)* 2004; 106: 53-60.
 26. Heinemann JC, Duerr GD, Keppel K, Breitbach M, Fleischmann BK, Zimmer A, et al. CB2 receptor-mediated effects of pro-inflammatory macrophages influence survival of cardiomyocytes. *Life Sci* 2015; 138: 18-28.
 27. Coulter C, Garnier M, Moore C. Synthetic cannabinoids in oral fluid. *J Anal Toxicol* 2011; 35: 424-30.
 28. Chang CE, Ai R, Gutierrez M, Marsella MJ. Homology modeling of cannabinoid receptors: discovery of cannabinoid analogues for therapeutic use. *Methods Mol Biol* 2012; 819: 595-613.