Association of polymorphisms in the endocannabinoid system genes with myocardial infarction and plasma cholesterol levels

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Aims. The aim of this study was to investigate the relationship between selected symptoms of chronic heart failure (myocardial infarction, plasma cholesterol level) and single nucleotide polymorphisms (SNPs) in the *FAAH* and *CNR1* genes.

Methods. A case – control study involving 155 patients with chronic heart failure and 169 age- and sex-matched healthy subjects. We detected SNPs 385 C/A (rs324420) in the *FAAH* and 1359 G/A (rs1049353) in the *CNR1* genes using the polymerase chain reaction and restriction analysis. Genotype and allele frequencies were compared between patients and controls as well as between patients with and without myocardial infarction.

Results. No significant differences in genotype or allelic frequencies between patients and controls were found ($P > 0.05$). Carriers of the *FAAH* A allele had a 2.37-fold increase in the risk of myocardial infarction (odds ratio 2.37, 95% confidence interval 1.36-6.93, $P = 0.01$). Homozygous carriers of genotype AA of *CNR1* SNP 1359 had significantly higher plasma cholesterol levels than carriers of GG and GA genotypes in patients ($P = 0.04$).

Conclusions. The study results suggest a role for allele A of the *FAAH* 385 variant as a risk factor for myocardial infarction. Genotype AA of *CNR1* 1359 variant probably affects plasma cholesterol levels. Pharmacological intervention in this system could modify the therapeutic approach to certain cardiovascular disorders.

Key words: chronic heart failure, endocannabinoid system, fatty acid amide hydrolase, cannabinoid receptor, myocardial infarction, cholesterol

Received: January 3, 2014; Accepted: July 11, 2014; Available online: August 18, 2014
<http://dx.doi.org/10.5507/bp.2014.043>

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INTRODUCTION

The endocannabinoid system is an endogenous signaling system with numerous physiological functions, including cardiovascular system regulation¹. Endocannabinoids (ECS) are endogenous lipid mediators with a wide range of biological effects similar to those of tetrahydrocannabinol, the principal active component of *Cannabis sativa*. ECS are produced by virtually all cell types, however the most important sources of ECS are vascular smooth muscle cells, endothelial cells, nerve cells within the blood vessel walls and circulating blood cells^{2,3}. Two most widely studied ECS are anandamide (AEA) and 2-arachidonoylglycerol (2-AG) (ref.²). The effects that ECS have on the cardiovascular system are complex, including direct action on myocardium, vasculature and autonomic nerve system at peripheral and central levels⁴.

Their effects are mediated predominantly by G-protein coupled type 1 and 2 cannabinoid receptors (CB1 and CB2, respectively) (ref.⁵). CB1 receptors are highly expressed in the central nervous system and in tissues involved in the regulation of metabolic homeostasis. CB1 are also expressed in the myocardium and vascular endothelial and smooth muscle cells, suggesting the involvement of ECS in the modulation of endothelial function and a potential role in vascular remodelling^{1,6,7}. CB2 is

expressed predominantly in immune system cells with an established role in the modulation of immune function⁸.

The CB1 receptor is encoded by the gene *CNR1* located on chromosome 6q14-q15. Several nucleotide polymorphisms (SNPs) have been identified in the *CNR1*. Guanine to adenine substitution in the position 1359 in exon 4 (rs1049353) results in silent exchange in the codon 435 (threonine). The gene encoding CB2 lies in the region 1p36, in the close vicinity of the gene for fatty acid amide hydrolase, an enzyme linked to ECS metabolism¹.

Mechanisms underlying the *in vivo* cardiovascular effects of ECS and their synthetic analogues are multifaceted, involving modulation of autonomic out-flow through sites of action at presynaptic autonomic nerve terminals and in the central nervous system, as well as direct effects on myocardium and the vasculature⁵. AEA can cause relaxation of blood vessels by several mechanisms: (i) as a result of direct action on the vascular smooth muscle cells, (ii) indirectly through the release of calcitonin gene-related peptide from sensory nerve cells as a result of vanilloid receptor 1 activation and (iii) indirectly through endothelium dependent breakdown of AEA to arachidonic acid which is subsequently converted to vasoactive agents such as prostanoids or epoxides³. A functional relationship between ECS and nitric oxide has been identified which is thought to be dependent on ei-

ther CB1 or CB2 receptor stimulation⁹. ECS were found to stimulate AMP-activated protein kinase (AMPK) activity in the hypothalamus and in the heart, while inhibiting AMPK in the liver and adipose tissue. These effects of ECS provide a possible explanation for a number of their known actions, such as the reduction of infarct size in the myocardium, an increase in adipose tissue mass, and the stimulation of appetite¹⁰.

Fatty acid amide hydrolase (FAAH), an enzyme that degrades anandamide and other amides derived from polyunsaturated fatty acids is considered as a possible target of modulation of ECS signalling with potential use in therapy of pro-inflammatory states (such as colitis, arthritis, neurodegenerative disorders and atherosclerosis). Blockade of AEA degradation by FAAH inhibition increases myocardial levels of AEA and results in decrease of blood pressure and heart rate especially in hypertensive states due to reduction of contractility and vascular resistance¹¹. While chronic elevation of plasma AEA seems to have a negative effect on vasculature, acute elevations may be athero-protective^{12,13}.

Common C to A substitution in the position 385 (rs324420) in the *FAAH* gene (chromosome 1p35-p34) results in proline to threonine substitution (P129T) with reduced expression and stability of mutant enzyme form¹⁴.

This study aimed to investigate the relationship between certain symptoms of chronic heart failure (myocardial infarction, plasma cholesterol level) and SNPs in the *FAAH* and *CNR1* genes.

MATERIAL AND METHODS

Subjects

The study included 155 patients with chronic heart failure (functional class New York Heart Association [NYHA] II-IV, ejection fraction median 25%, cardiothoracic index > 50%) from the Department of Cardioangiology of St. Anne's University Hospital in Brno (116 men and 39 women, median age 56.0 years, 51–56 years interquartile range). All patients had chronic heart failure (due to ischemic heart disease or dilated cardiomyopathy) of at least 3 months duration and were stable on unchanged medication for at least 1 month. 44 % of patient had hypertension, 33.5% had type 2 diabetes mellitus and 27 % had angina pectoris. 169 age- and gender-matched control subjects (126 men and 43 women, median age 56.0 years, 51–61 years interquartile range) without any history of coronary artery disease, diabetes mellitus and obesity were recruited in a mass media campaign addressing the population of the south Moravia region of the Czech Republic.

The study was performed in accordance with principles of the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Medicine, Masaryk University Brno. Participants provided their written informed consent with involvement in the study.

Analysed material

Genomic DNA was extracted from peripheral blood leukocytes according to the standard protocol (phenol/chloroform method) and stored at -20 °C until analyses.

Detection of polymorphisms

The coding region containing *CNR1* SNP 1359 G/A was amplified by polymerase chain reaction (PCR) using a mismatch primer as described elsewhere¹⁵ to create *MspI* recognition sites. PCR run in a final volume of 20 µL under conditions as follows: initial denaturation (95 °C for 5 min), 37 cycles of 95 °C for 30 s (denaturation), annealing (56 °C for 50 s), extension (72 °C for 30 s) and final extension (72 °C for 7 min). The PCR product (111 bp) was digested by *MspI* in a final volume of 15 µL of reaction mixture (10 µL PCR products and 5 µL of reaction mixture contained 0.5 unit of *MspI*). Restriction analysis was performed in 37 °C for 4 h. Digested products (fragments 92 and 19 bp) were resolved on a 3% agarose gel containing ethidium bromide.

The coding region containing *FAAH* SNP 385 C/A was amplified by PCR using primer set according to Morita et al.¹⁴ in a final volume of 15 µL of reaction mixture. PCR conditions were as follows: initial denaturation (95 °C for 5 min), denaturation (95 °C for 35 s), annealing (63 °C for 30 s), extension (72 °C for 25 s) and final extension (72 °C for 7 min). PCR products (235 bp) were analysed using restriction analysis with *EcoO109I* with conditions as follows: 10 µL of PCR product and 7 µL of reaction mixture contained 0.5 unit of restriction enzyme (37 °C for 4 h). Digested products (two fragments 199 and 36 bp) were loaded on a 3% MetaPhor gel containing ethidium bromide.

All subjects included in the study were successfully genotyped. Repeated genotyping of 10% randomly selected samples yielded identical results.

Statistical analysis

Genotype distributions and allelic frequencies were compared using χ^2 and Fisher exact test. Hardy-Weinberg equilibrium was tested using the χ^2 test. Odds ratio (OR) and 95% confidence interval were calculated to estimate the risks related to respective polymorphism. To calculate the significance of OR, the Fisher exact test was used. Logic regression was used to test the effect of both studied SNPs. Comparison of cholesterol levels between genotypes was performed using a Kruskal-Wallis ANOVA. The software Statistica v. 8.0 (Statsoft Inc., Tulsa, OK) was used for all statistical analyses.

RESULTS

Characteristics of patients with chronic heart failure are shown in Table 1. Genotype and allele frequencies of both SNPs did not differ between subjects with and without chronic heart failure ($P > 0.05$). Comparisons were also made for men and women separately and again no difference was found ($P > 0.05$). Genotype distributions

and allele frequencies are shown in Table 2. Genotype distributions of both SNPs fulfilled the Hardy-Weinberg equilibrium criteria in each group ($P > 0.05$, χ^2 test).

For the purpose of further analysis, patients were divided into two groups: those (i) with and (ii) without history of myocardial infarction (MI). We found a significantly higher frequency of *FAAH* genotype CC in patients without any history of MI (73.3% vs. 53.8%, $P = 0.03$, χ^2 test). Allele frequencies of *FAAH* SNP also differed between the groups. The frequency of allele A was 14% in patients without MI and 26.3% in those with an MI history ($P = 0.007$, Fisher-exact test). Carriage of allele A with reported reduced *FAAH* activity (genotypes AA and CA) was associated with increased risk of MI in patients with chronic heart failure (OR = 2.37, 95% CI: 1.21 – 4.65, $P = 0.01$). Neither genotype nor allele frequencies of *CNR1* SNP differed between the groups ($P > 0.05$, χ^2 and Fisher-exact test). Logic regression confirmed that *FAAH* but not *CNR1* SNP is a risk factor for IM when both SNPs were considered ($P = 0.01$).

Finally, we tested whether plasma cholesterol levels differed between carriers of *CNR1* and *FAAH* genotypes. We found a significant differences in plasma cholesterol between *CNR1* genotypes carriers with levels highest in carriers of the AA genotype ($P = 0.04$, Kruskal-Wallis

Table 1. Basic characteristics of patients with heart failure.

Parameter	Median	Lower quartile	Upper quartile
Age (years)	56.0	51.0	61.0
Ejection fraction (%)	25.0	20.0	28.0
Systolic pressure (mm of Hg)	120.0	105.0	130.0
Diastolic pressure (mm of Hg)	80.0	70.0	80.0
Heart rate (beats/min)	79.0	70.0	84.0
Total cholesterol (mmol/L)	4.9	4.3	5.8
HDL cholesterol (mmol/L)	1.1	0.9	1.3
LDL cholesterol (mmol/L)	3.0	2.4	3.7
Triglycerides (mmol/L)	1.8	1.3	2.3

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein

Table 2. Genotype and allele frequencies of *CNR1* and *FAAH*.

	Genotypes			Alleles			
CNR1							
	GG	GA	AA	P	G	A	P
Patients	54.2	38.1	7.7	NS	73.2	26.8	NS
Controls	56.2	39.1	4.7		75.7	24.3	
FAAH							
	CC	CA	AA	P	C	A	P
Patients	63.2	32.9	3.9	NS	79.7	20.3	NS
Controls	56.8	40.8	2.4		77.2	22.8	

Data expressed as percentages.

Abbreviations: *CNR1*, cannabinoid receptor type 1; *FAAH*, fatty acid amide hydrolase

ANOVA). The results are shown in Fig. 1. Cholesterol levels did not differ between carriers of different *FAAH* genotypes ($P > 0.05$, Kruskal-Wallis ANOVA).

DISCUSSION

In the present study we assessed the relationship between genetic variation in the *CB1* and *FAAH* genes and phenotypes related to heart failure, namely myocardial infarction and plasma cholesterol levels.

The endocannabinoid system seems to play a limited role in cardiovascular regulation under physiological conditions. However, in various pathologic states (e.g. shock, cardiomyopathy or heart failure), it may become over-activated, thereby contributing to hypotension through cardiovascular CB1 receptors. Paradoxically, the ECS may also be activated as a compensatory mechanism in various

Fig. 1. Cholesterol levels according to the *CNR1* genotypes.

forms of hypertension to limit pathologically increased blood pressure and myocardial contractility⁵.

FAAH is one of the enzymes involved in the regulation of the endocannabinoid system and genetic variants affecting enzyme activity are therefore clear candidates for genetic susceptibility to MI in patients with heart failure. FAAH is a primary catabolizer of ECS and serves as a regulator of endogenous cannabinoid signalling in the brain^{16,17}. SNP 385C/A in the *FAAH* causes amino acid exchange in the position 129 (proline to threonine) with established functional effect. The threonine variant expresses increased sensitivity to proteolytic degradation and enzyme cellular expression and activity is decreased^{17,18}. Decreased anandamide degradation by an inhibitor of FAAH was shown to lower blood pressure and heart rate in hypertensive rodents through reductions of both cardiac contractility and vascular resistance⁵.

Numerous studies have suggested that the endocannabinoid system may modulate ischemic-reperfusion injury, the principal cause of tissue damage occurring in myocardial infarction¹⁰. ECS have been implicated in the protective effects of ischemic preconditioning through cannabinoid receptor dependent and independent mechanisms. However they may also contribute to the cardiovascular collapse associated with myocardial infarction and circulatory shock¹⁹.

Lower FAAH activity due to SNP results in decreased degradation of AEA with subsequent increase in plasma AEA levels which may have negative effects on vasculature and may represent a risk factor of atherosclerosis. These findings correspond with study results which indicate that AC and AA genotypes of FAAH SNP C385A were associated with increased risk of MI in individuals with personal history of chronic heart failure. This finding is depicted in Fig. 2. To our knowledge, no study has addressed the role of FAAH SNP in heart failure. Several studies have shown the association between FAAH 385 C/A SNP and lipid metabolism, insulin sensitivity and obesity in patients with metabolic syndrome compared to healthy subjects²⁰⁻²². Aberle et al showed that carriers of the A-allele had a significantly greater decrease in triglycerides and total cholesterol levels compared to wild type when following a low fat diet, without any relation to glucose metabolism²³. Mutant genotype of the FAAH gene in subjects with the metabolic syndrome had more

elevated TG levels and lower HDL-c concentrations than wild genotype. The 385 C/A polymorphism of the FAAH gene was found to be associated with increased serum TG and reduced levels of HDL-c and higher plasma insulin level, indicating the possible involvement of the FAAH in the development of insulin resistance²⁰.

There is increasing experimental evidence for modulation of endocannabinoid levels, receptors and related enzymes of the biosynthesis and degradation in different inflammatory conditions²⁴. Recent studies suggest that up-regulation of anandamide levels may be feasible pharmacological strategies to limit inflammatory organ injury. Anandamide dose dependently attenuates expression of certain pro-inflammatory cytokines such as TNF- α in human coronary artery endothelial cells that play an important role in the cardiovascular aging process and mediate their proatherogenic effects. Disruption of activation of this pathway with various cannabinoids may exert significant protective effects in numerous cardiovascular disorders associated with increased inflammatory response, such as atherogenesis, MI and cardiovascular aging²⁵.

Cardiovascular suppressive effects of cannabinoids are mediated by peripherally localized CB1 receptors that function as a target site of anandamide action. Considering the lack of blood pressure lowering action of anandamide in mice lacking CB1 receptors, polymorphism in the human CB1 receptor gene also might be expected to shift tonic levels of these compounds. The present study reports no case-control differences either in genotype distributions or allelic frequencies of the CB1 polymorphism in association to heart failure²⁶.

There is growing evidence that genetic variation in CNR1 directly influences metabolic traits in humans by altering the activity of CB1 receptor dependent signalling in peripheral tissues. Several studies have shown the association between SNPs in the cannabinoid receptor gene and lipid metabolism. A common variant of CNR1 SNP G1359A has frequently been studied in relation to obesity. The results of our case-control study indicate that there is an association between AA and AG genotypes of SNP G1359A and total cholesterol.

The endocannabinoid system plays an effective role in lipogenesis modulation. CB1 receptor blocking has resulted in increased expression of adiponectin, which has a crucial role in reducing the expression of enzymes involved in lipogenesis, with a potential role in atherogenic dyslipidaemia and dysglycaemia²⁷. Over-activity of the endocannabinoid system is recognized as contributing to the typical features of the metabolic syndrome, such as visceral obesity, dyslipidaemia, glucose intolerance and hypertension⁶. Chronic blockade of CB1 receptors in obese animal and humans reduces the symptoms of the metabolic syndrome (accumulation of fat, especially intra-abdominal fat, hypertriglyceridemia, low high density lipoprotein cholesterol, high arterial pressure, and high fasting glycaemia) (ref.¹⁶).

Fig. 2. Summary of the results of the study. Single nucleotide polymorphism C385A in the FAAH gene causes amino acid exchange (proline to threonine) with subsequent decrease in enzyme activity. This results in decreased degradation of anandamide and its increased plasma levels with negative consequences on vasculature (higher risk of atherosclerosis and myocardial infarction).

CONCLUSION

In conclusion, we have shown preliminary evidence of several cardiovascular risk factors associated with the 129Thr variant (carriers of allele A) and *CNR1* 1359 A/A polymorphism. We found that the A/A genotype of *CNR1* gene was associated with increased total cholesterol level and that 129Thr variant was associated with increased risk of myocardial infarction in patients with a history of heart failure.

ABBREVIATIONS

AMPK, adenosine monophosphate-activated protein kinase; CB1, cannabinoid receptor type - 1; CB2, cannabinoid receptor type 2; *CNR1*, cannabinoid receptor type 1; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; HDL-c, high-density lipoprotein-cholesterol; MI, myocardial infarction; NYHA, New York Heart Association; OR, odds ratio; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; TNF- α , tumour necrosis factor α .

Acknowledgement: The authors wish to thank Filip Zlamal for help with the statistical analysis.

Author contributions: MCH: manuscript writing; LP, AV: data analysis; AV: study design; LS: clinical data collection; LP, AV: manuscript revision.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

REFERENCES

- Szmitko PE, Verma S. The endocannabinoid system and cardiometabolic risk. *Atherosclerosis* 2008;199:248-56.
- Pacher P, Steffens A. The emerging role of the endocannabinoid system in cardiovascular diseases. *Semin Immunopathol* 2009;31(1):63-77.
- Hiley CR, Ford WR. Cannabinoid pharmacology in the cardiovascular system: potential protective mechanisms through lipid signalling. *Biol Rev* 2004;79:187-205.
- Randall MD, Harris D, Kendall DA, Ralevic V. Cardiovascular effects of cannabinoids. *Pharmacology and Therapeutics* 2002;95:191-202.
- Mukhopadhyay P, Mohanraj R, Btkai S, Pacher P. CB1 cannabinoid receptor inhibition: Promising approach for heart failure? *Congest Heart Fail* 2008;14(6):330-4.
- Jesudason D, Wittert G. Endocannabinoid system in food intake and metabolic regulation. *Current Opinion in Lipidology* 2008;19:344-8.
- Pacher P, Mukhopadhyay P, Mohanraj R, Godlewski G, Btkai S, Kunos G. Modulation of endocannabinoid system in cardiovascular disease: therapeutic potential and limitations. *Hypertension* 2008;52:601-7.
- Silvestri C, Di Marzo V. The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. *Cell Metab* 2013;17(4):475-90.
- Lagneux C, Lamontagne D. Involvement of cannabinoids in the cardioprotection induced of lipopolysaccharide. *British Journal of Pharmacology* 2001;132:793-6.
- Kola B, Hubina E, Tucci SA, Kirkham TC, Garcia EA, Mitchell SE, Williams LM, Hawley SA, Hardie DG, Grossman AB, Korbonits M. Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *The journal of biological chemistry* 2005;280:25196-201.
- Mach F, Montecucco F, Steffens S. Cannabinoid receptors in acute and chronic complications of atherosclerosis. *British Journal of Pharmacology* 2008;153:290-8.
- Pacher P, Steffens S. The emerging role of the endocannabinoid system in cardiovascular disease. *Semin Immunopathol* 2009;31(1):63-77.
- Quericioli A, Montecucco F, Pataky Z, Thomas A, Ambrosio G, Staub C, di Marzo V, Ratib O, Mach F, Golay A, Schindler TH. Improvement in coronary circulation function in morbidly obese individuals after gastric bypass-induced weight loss: relation to alterations in endocannabinoids and adipocytokines. *Europea Heart Journal* 2013;34:2063-73.
- Chiang KP, Gerber AL, Sipe JC, Cravatt BF. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. *Hum Mol Genet* 2004;13(18):2113-9.
- Gadzicki D, Mller-Vahl K, Stuhmann M. A frequent polymorphism in the coding exon of the human cannabinoid receptor (*CNR1*) gene. *Molecular and cellular probes* 1999;13:321-3.
- Morita Y, Ujike H, Tanaka Y, Uchida N, Nomura A, Ohtani K, Kishimoto M, Morio A, Imamura T, Sakai A, Inada T, Harano M, Komiyama T, Yamada M, Sekine Y, Iwata N, Iyo M, Sora I, Ozaki N, Kuroda S. A nonsynonymous polymorphism in the human fatty acid amide hydrolase gene did not associate with either methamphetamine dependence or schizophrenia. *Neuroscience letters* 2005;376:182-7.
- Jensen DP, Adreassen CH, Andersen MK, Hansen L, Eiberg H, Borch-Johnsen K, Jrgensen T, Hansen T, Pedersen O. The functional Pro129Thr variant of the FAAH gene is not associated with various fat accumulation phenotypes in a population-based cohort of 5,801 whites. *Journal of molecular medicine* 2007;85:445-9.
- Cravatt BF, Lichtman AH. The enzymatic inactivation of the fatty acid amide class of signaling lipids. *Chemistry and Physics of Lipids* 2002;12:135-48.
- Pacher P, Hask G. Endocannabinoids and cannabinoid receptors in ischaemia-reperfusion injury and preconditioning. *British Journal of Pharmacology* 2008;153:252-62.
- Zeng J, Li J, Huang G. 385 C/A polymorphism of the fatty acid amide hydrolyse gene is associated with metabolit syndrome in the Chinese Han population. *Arch Med Sci* 2011;7(3):423-7.
- Luis DA, Aller R, Izaola O, Conde R, Sagrado MG, Primo D, Castro MJ. Relationship among metabolic syndrome, C385A polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase (FAAH) and insulin resistance. *Journal of Diabetes and its complications* 2012;26(4):328-32.
- Onaivi ES, Leonard CM, Ishiguro H, Zhang PW, Lin Z, Akinshola BE, Uhl GR. Endocannabinoids and cannabinoid receptor genetics. *Progress in Neurobiology* 2002;66:307-44.
- Aberle J, Fedderwitz I, Klages N, George E, Beil FU. Genetic variation in two proteins of the endocannabinoid system and their influence on body mass index and metabolism under low fat diet. *Hormone and Metabolic research* 2007;39:395-7.
- Mach F, Steffens S. The role of the endocannabinoid system in atherosclerosis. *Journal of Neuroendocrinology* 2008;20:53-7.
- Btkai S, Rajesh M, Mukhopadhyay P, Hask G, Liaudet L, Cravatt BF, Csiszr A, Ungvri Z, Pacher P. Decreased age related cardiac dysfunction, myocardial nitrate stress, inflammatory gene expression and apoptosis in mice lacking fatty acid amide hydrolase. *Am J Physiol Heart Circ Physiol* 2007;293:909-18.
- Pacher P, Btkai S, Kunos G. Blood pressure regulation by endocannabinoids and their receptors. *Neuropharmacology* 2005;48:1130-8.
- Francischetti EA, Abreu V. The endocannabinoid system: a new perspective for cardiometabolic risk control. *Arq Bras Cardiol* 2006;87:499-508.