EFFECTS OF WESTERN DIET ON ENDOCANNABINOID LEVELS IN MOUSE HEART

By

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APPROVED

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Abstract

Heart disease is a potent killer, claiming almost 650,000 lives annually in the US alone. Studies suggest that some classes of lipid-derived intercellular signaling messengers can potentially lower blood pressure and offer cardiac protection. There is evidence that both cannabinoid receptor subtypes 1 and 2 (CB1/CB2) activate the AMPK-eNOS pathway, which contributes to lower blood pressure and may offer cardiac protection [3]. Comparing the levels of the lipid-derived messengers, 2-arachidonoyl-sn-glycerol (2-AG), anandamide (AEA), oleoylethanolamide (OEA), in the left ventricle of male mice that have either been food deprived or free feeding 24 hours prior to organ collection showed no significant difference. However, the same experiment showed a significant increase in OEA levels in females. Interestingly, there was a significant increase of AEA and OEA but not 2-AG levels in the left ventricle of male mice that had been maintained on a western style diet high in fat and carbohydrate content for 60 days compared to mice on a standard chow diet. Females maintained on the western diet displayed significant increases in levels of AEA but not 2-AG ~~levels~~. In addition, we saw a significant increase in levels of OEA ~~levels~~ in free feeding females. This suggests that the levels of signaling messengers, under diet-induced stress of the heart, are dependent on gender. The physiological relevance for this biochemical event in the heart remains to be investigated.

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Acknowledgements

I would like to thank my faculty mentor Dr. Nicholas DiPatrizio, graduate students Donovan Argueta, Andrea Dillon, Pedro Anthony Perez and undergraduates Kevin Mortazavi and Doris Xie for all their guidance and support. This project could not happen without them. I would also like to express my gratitude to the University of California, Riverside for funding this project.

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Introduction

Heart disease is the leading cause of death, for both men and women, with more than half of the casualties being men [1]. The most common heart-related condition is coronary heart disease, which is caused by cholesterol-containing deposit build up (plaque) that narrows the coronary arteries [1][2]. Studies suggest that both cannabinoid receptor subtypes 1 and 2 (CB_1/CB_2) activate the AMPK-eNOS pathway, which eventually leads to vasodilation and possible cardiac protection [3]. There is also a suggested correlation between CB_1 and CB_2 activation and reduced hypertrophy in cardiomyocytes [3]. Endocannabinoids, such as 2-arachidonoyl-*sn*-glycerol (2-AG) and anandamide (AEA), are a class of lipid-derived intercellular signaling molecules that bind and activate CB_1 and CB_2 [4]. 2-AG is an endogenously produced potent CB_1/CB_2 agonist that has been shown to reduce blood pressure, though the exact mechanism is still unknown [3][4][5].

There is a strong correlation between obesity and heart disease [6]. The heart needs to accommodate damage caused by the excess fat and pump harder to supply blood to a larger body, which causes increased blood pressure [7]. Previous studies have shown changes in endocannabinoid levels in the gut of mice, based on food deprivation and free feeding conditions [8]. Oleoylethanolamide (OEA), an endocannabinoid-like molecule with a structure similar to AEA but does not bind CB_1/CB_2, has been shown to increase in feeding and decrease in fasting in the mouse gut [9]. ~~However, T~~the effects of feeding conditions and obesity-induced stress on endocannabinoids and endocannabinoid-like molecules in the heart of mice are still unknown. Understanding this possible cardiac

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~~protection~~ protective mechanism will provide the basis for ~~further cardiac drug developments.~~development of therapeutics to treat cardiovascular diseases.

Methodology

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Animals and Housing

Male C57BL/6 mice (Taconic Biosciences, La Verne, CA, USA) were housed in groups of 4 with 12-hour light and dark cycles (lights off at 1800 h). Food and water were provided ad libitum except 24 hours prior to organ harvest for noted conditions. For the 60 days duration of the experiments, the mice were either maintained on a western-style diet [(WD) Research Diets D12709B, New Brunswick, NJ, USA; 40% kcal as fat, 43% kcal from carbohydrates, mostly sucrose] or a standard lab rodent chow diet [(SD) Lab Diet 5001, St. Louis, MO, USA; 13.4% kcal as fat, 56% kcal from carbohydrates, mostly starch]. All procedures met the U.S. National Institute of Health guidelines for care and use of laboratory animals, and were approved by the Institutional Animal Care and Use Committee of the University of California, Riverside.

Lipid Extraction

An average of 2020~~.29~~ mg of heart tissue from the left ventricle of mice were used for extraction of lipids in 1.0 mL of methanol containing the following internal standards:

[$^2H_5$]-2-AG (Cayman Chemical, Ann Arbor, MI, USA). Lipids were extracted with chloroform (2.0 mL) and washed with 0.9% saline (1.0~~0.9~~ mL). Organic phases were collected and fractionated by open-bed silica gel column chromatography as previously described (DiPatrizio et al., 2011, *PNAS* and Argueta and DiPatrizio, 2017, Physiology and Behavior). Eluted fractions were dried under N_2 and reconstituted in 1.0 mL of methanol:chloroform (9:1) for liquid chromatography/mass spectrometry (LC/MS) analyses.

Lipid Analysis

Lipids were analyzed using a *Waters Acquity I-Class Ultra Performance Liquid Chromatography system coupled to a Waters TQS-micro Triple Quadrupole Mass Spectrometer*. Lipids were separated using an Acquity UPLC BEH C_{18} column (50 x 2.1 mm; i.d. 1.7 μm), eluted by a gradient of methanol (0.25% acetic acid, 5mM ammonium acetate) in water (0.25% acetic acid, 5mM ammonium acetate) (from 80 to 100% methanol in 2.5 min, 100% 2.5-3.0 min, 100-80% 3.0-3.1 min) at a flow rate of 0.4 mL/min. Column temperature was kept at 40˚ C and samples were maintained in the sample manager at 10˚ C. Argon was used as collision gas. 2-AG and [$^2H_5$] 2-AG were identified in the positive ionization mode, based on their retention times and MS^2 properties, using authentic standards (Cayman Chemical) as references. Multiple reaction monitoring was used to acquire full-scan tandem MS spectra of selected 2-AG ions. Lipids were quantified as ammonium adducts [M + NH4]. Extracted ion chromatograms were used to quantify 2-arachidonoyl-*sn*-glycerol (*m/z* = 379.3>287.3) and [$^2H_5$] 2-AG (*m/z* = 384.3 > 93.4), which were used as an internal standards.

Results

The levels of anandamide (AEA), oleoylethanolamide (OEA), and 2-arachidonoyl-*sn*-glycerol (2-AG) in the heart of male mice that were either free feeding, or food deprived 24 hours prior to tissue harvest showed no significant difference (Figure 1). T~~However, t~~he same experiment, however, resulted in a significant increase in OEA levels in female mice (Figure 2). When compared to male mice that were fed the standard chow diet, obese male mice that had chronic access (60 days) to a western style and food deprived 24 hours prior to tissue harvest exhibited increased levels of AEA and OEA but not 2-AG in the heart (Figure 3). While there was a significant increase in ~~AEA levels of~~levels of AEA in female mice that were maintained on the western diet for 60 days and food deprived compared to female mice on the standard chow diet, the levels of the other two endocannabinoids did not change (Figure 4). Interestingly, when the female mice were free feeding 24 hours prior to organ harvest, there was also a significant increase in levels of OEA (Figure 5). This suggests that both AEA and OEA play a role in the possible ~~cardioprotective~~cardio protective mechanisms and levels are affected by gender. ~~While there was no significant difference seen in 2-AG levels for all the experiment, it cannot be determined if there was not an increased rate of degradation as well. It may have been possible that although there were increased amounts of 2-AG being produced, it was also being degraded or utilized at a faster rate as well. Further experiments that~~

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~~measure the amounts of endocannabinoid precursors and synthesis and degradation enzymes are needed.~~

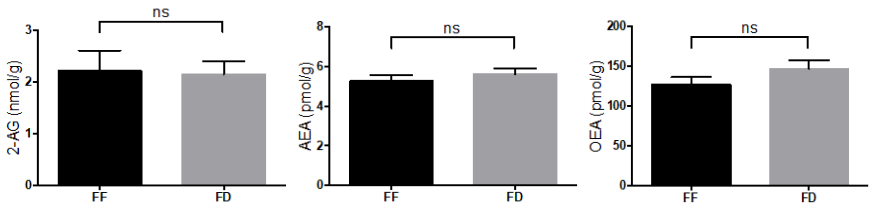

Figure 1. Levels of 2-AG, AEA, and OEA in the left ventricle of hearts of Male Mice after 0-days of Western Diet administration. Subjects were either free feeding or food deprived 24 hours prior to organ collection. Data points analyzed using student, two-tailed t-test. n = 8, ns = p≥0.05

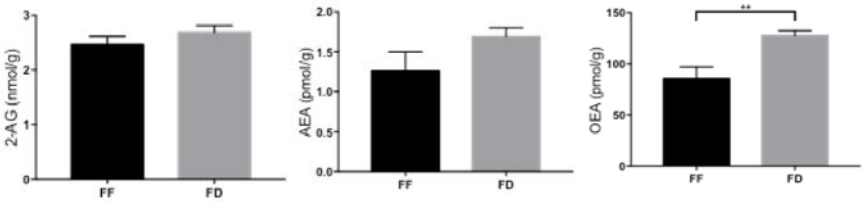

Figure 2. Levels of 2-AG, AEA, and OEA in the left ventricle of hearts of Female Mice after 0-days of Western Diet administration. Subjects were either free feeding or food deprived 24 hours prior to organ collection. Data points analyzed using student, two-tailed t-test. n = 8, ns = p≥0.05, **=p<0.01

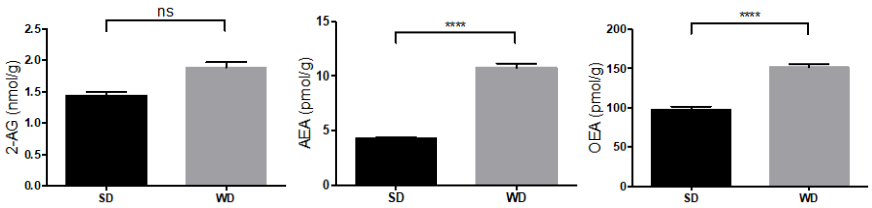

Figure 3. Levels of 2-AG, AEA, and OEA in the left ventricle of hearts of Male Mice after 60-days on either Standard Chow or Western Diet. All subjects were food deprived 24 hours prior to organ collection. Data set analyzed using student, two-tailed t-test. n=7-8, ns=p≥0.05, ****=p<0.0001

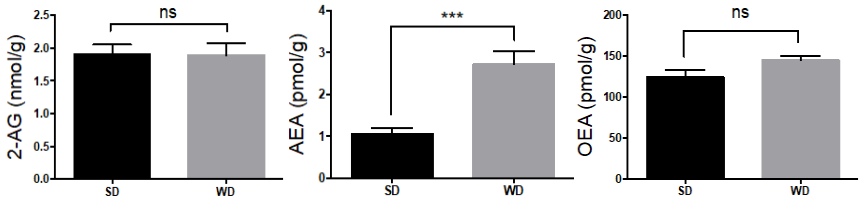

Figure 4. Levels of 2-AG, AEA, and OEA in the left ventricle of hearts of Female Mice after 60-days on either Standard Chow or Western Diet. All subjects were food deprived 24 hours prior to organ collection. Data set analyzed using student, two-tailed t-test. n=8, ns=p≥0.05, ***=p<0.001

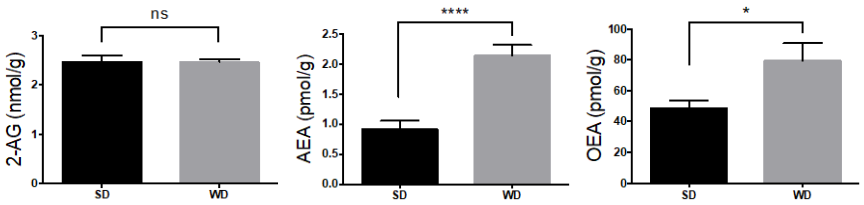

Figure 5. Levels of 2-AG, AEA, and OEA in the left ventricle of hearts of Female Mice after 60-days on either Standard Chow or Western Diet. All subjects were free feeding 24 hours prior to organ collection. Data set analyzed using student, two-tailed t-test. n=8, ns=p≥0.05, ****=p<0.0001, *=p<0.05

Discussion

There was significant increase in levels of AEA and OEA in the heart of both male and female mice that had been maintained on a Western Diet rather than Standard Chow Diet, but not 2-AG. Although AEA and OEA have different production and degradation pathways, they have very similar structures in comparison with 2-AG [13, 14]. This may suggest that the ethanolamide structure is significant downstream in the possible cardio protective pathway. Further studies, however, are still needed to establish this relationship.

There were different responses to the feeding conditions between the two sexes, which suggests males and females may regulate the endocannabinoid system differently.

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Females had a greater response to immediate changes in feeding conditions, while the changes in males were more significant over time. This difference may influence the different prevalence of heart disease in the two sexes over age [15].

While there was no significant difference seen in 2-AG levels for all the experiment, it cannot be determined if there was not an increased rate of degradation as well. It may have been possible that although there were increased amounts of 2-AG being produced, it was also being degraded or utilized at a faster rate as well. Further experiments that measure the amounts of endocannabinoid precursors and synthesis and degradation enzymes, such as FAAH and MAGL, are needed [16].

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Conclusion

Coronary heart disease alone costs the US almost $110 billion each year from lost productivity and medical costs [1]. The endocannabinoid system has already shown great potential for having cardiac protective properties [3], but the knowledge on this topic is still very limited. Further studies into the synthesis of this intercellular signaling system would allow researchers to gain a better understanding of the pathways and mechanisms involved in this system and could provide a clue for endocannabinoid synthesis pathways in other parts of the body, for instance the kidney or pancreas. Additionally, pharmaceutical drugs could be developed that target this signaling system and may have the potential to treat heart disease conditions, such as high blood pressure and high cholesterol.

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Bibliography

[1] CDC. "Heart Disease Fact Sheet." *Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention.

[2] NIH. "What Is Coronary Heart Disease?" US Department of Health & Human Services.

[3] Lu, Yan, Bolanle C. Akinwumi, Zongjun Shao, and Hope D. Anderson. "Ligand Activation of Cannabinoid Receptors Attenuates Hypertrophy of Neonatal Rat Cardiomyocytes." *Journal of Cardiovascular Pharmacology* 64.5 (2014): 420-30.

[4] Dipatrizio, Nicholas V., and Daniele Piomelli. "The Thrifty Lipids: Endocannabinoids and the Neural Control of Energy Conservation." *Trends in Neurosciences* 35.7 (2012): 403-11.

[5] Mechoulam, Raphael, Ester Fride, Shimon Ben-Shabat, Uri Meiri, and Michal Horowitz. "Carbachol, an Acetylcholine Receptor Agonist, Enhances Production in Rat Aorta of 2-arachidonoyl Glycerol, a Hypotensive Endocannabinoid." *European Journal of Pharmacology* 362.1 (1998): n. pag.

[6] Ecke, Robert H.. **"**Obesity and Heart Disease.**"** *Circulation* 96.9 (1997): 3248-3250

[7] "Health Risks of Being Overweight." National Institutes of Health. U.S. Department of Health and Human Services, n.d.

[8] Dipatrizio, N. V., A. Joslin, K.-M. Jung, and D. Piomelli. "Endocannabinoid Signaling in the Gut Mediates Preference for Dietary Unsaturated Fats." *The FASEB Journal* 27.6 (2013): 2513-520.

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[9] Gaetani, Silvana, Fariba Oveisi, and Daniele Piomelli. "Modulation of Meal Pattern in the Rat by the Anorexic Lipid Mediator Oleoylethanolamide." *Neuropsychopharmacology* 28.7 (2003): 1311-316.

[10] Argueta, Donovan A., and Nicholas V. Dipatrizio. "Peripheral Endocannabinoid Signaling Controls Hyperphagia in Western Diet-induced Obesity." *Physiology & Behavior* 171 (2017): 32-39.

[11] Ohno-Shosaku, Takako, Minoru Matsui, Yuko Fukudome, Jumpei Shosaku, Hiroshi Tsubokawa, Makoto M. Taketo, Toshiya Manabe, and Masanobu Kano. "Postsynaptic M1 and M3 Receptors Are Responsible for the Muscarinic Enhancement of Retrograde Endocannabinoid Signalling in the Hippocampus." *European Journal of Neuroscience Eur J Neurosci* 18.1 (2003): 109-16.

[12] DiPatrizio, Nicholas V., Miki Igarashi, Vidya Narayanaswami, Conor Murray, Joseph Gancayco, Amy Russell, Kwang-Mook Jung, and Daniele Piomelli. "Fasting Stimulates 2-AG Biosynthesis in the Small Intestine: Role of Cholinergic Pathways." *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology Am J Physiol Regul Integr Comp Physiol* 309.8 (2015): n. pag.

[13] Verme, J. Lo, S. Gaetani, J. Fu, F. Oveisi, K. Burton, and D. Piomelli. "Regulation of Food Intake by Oleoylethanolamide." *Cellular and Molecular Life Sciences* 62.6 (2005): 708-16.

[14] Mechoulam, Raphael, Lumir O. Hanus, Roger Pertwee, and Allyn C. Howlett. "Early Phytocannabinoid Chemistry to Endocannabinoids and beyond." *Nature Reviews Neuroscience* 15.11 (2014): 757-64.

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[15] Mosca, L., E. Barrett-Connor, and N. Kass Wenger. "Sex/Gender Differences in Cardiovascular Disease Prevention: What a Difference a Decade Makes." *Circulation* 124.19 (2011): 2145-154.

[16] Mazier, Wilfrid, Nicolas Saucisse, Blandine Gatta-Cherifi, and Daniela Cota. "The Endocannabinoid System: Pivotal Orchestrator of Obesity and Metabolic Disease." *Trends in Endocrinology & Metabolism* 26.10 (2015): 524-37.

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