IMPACT OF A WESTERN DIET ON LIPID SIGNALING MOLECULES IN THE LEFT VENTRICLE OF DIET-INDUCED OBESE MICE

By

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ABSTRACT

Seventy-one percent of men and sixty-six percent of women in the United States are considered overweight and over a third suffer from obesity which has been causally linked to cardiovascular disease resulting in 31% of all deaths worldwide. We require a better understanding of the underlying biochemical processes that affect heart health in diet-induced obese individuals to help develop effective therapeutic treatments.

Endocannabinoids are a class of lipid signaling molecules that regulate many physiological processes, including cardiovascular function and energy balance. However, the effects of western-diet containing high levels of carbohydrates and fats on the endocannabinoid system and resulting cardiovascular function remains largely unknown. This project investigates changes in production of endocannabinoids, 2-AG, AEA and OEA, in the left ventricle of mice. These lipids were extracted from the left ventricle of the heart of both males and females and subsequently analyzed using Ultra-Performance Liquid Chromatography/Tandem Mass Spectrometry. We found significant increases in AEA and OEA levels in the left ventricle in diet-induced obese mice, which suggests that there is a diet-dependent endocannabinoid response in the left ventricle of mice. Further exploration is needed to better understand the biochemical processes involved in this endocannabinoid response and its physiological relevance to cardiovascular disease. Better understanding of the role of endocannabinoids in diet-induced obesity can help identify sites for drug intervention to treat obesity and cardiovascular pathology.

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INTRODUCTION

The discovery of endocannabinoid receptors in the late 1980s opened the floodgates to the study of an entirely new biochemical pathway that affects nearly every cell in the body. Endocannabinoids are a class of lipid signaling molecules involved in energy homeostasis as well as cardiovascular function. The endocannabinoid system was originally classified by studying Δ^9-tetrahydrocannabinol, the psychoactive component in cannabis. Δ^9-tetrahydrocannabinol was identified as an agonist of Cannabinoid receptor type 1 (CB_1), a G-protein-coupled receptor. The endogenously produced cannabinoids, 2-arachidonoyl glycerol (2-AG) and anandamide (AEA), are known to have a high binding affinity for CB_1, which has been implicated in homeostasis regulation in multiple tissues [1].

The western world is currently plagued by obesity with 71% of men and 66% of women being reported as overweight or obese which has been credited to diets comprised of high fat, high carbs, and high sugar [2]. The recent discoveries implicating the endocannabinoid system in energy regulation suggests this system, if successfully inhibited, can reduce food intake and decrease obesity [3,4]. There is a strong association between obesity and cardiovascular disease indicating that obesity increases the chances of developing cardiovascular disease [3,5]. Therefore, it is worthwhile to explore the possibility of using the endocannabinoid system to target not only obesity, but cardiovascular pathology as well.

Although the endocannabinoid mechanism in glutamatergic neurons is well described, the mechanisms in which endocannabinoids function in cardiomyocytes remains unknown. *In-vitro* studies suggest the involvement of endocannabinoids in the

regulation of cardiovascular function occurs via nitric oxide production due to adenosine monophosphate activated protein kinase mediated activation of Endothelial Nitric Oxide Synthase [6]. *In-vivo* studies have indicated that endocannabinoids regulate blood pressure through CB_1 in spontaneously hypertensive rats [7]. Similar to the cardioprotective properties of CB_1 by regulating hypertension, lipid signaling molecules have also been implicated in cardioprotective properties during cardiovascular infarctions [8]. These studies suggest that under stressful conditions (i.e. hypertension and infarctions) endocannabinoids act to induce cardioprotective effects.

The DiPatrizio lab has found that endocannabinoids have been linked to vagal signaling that regulates food intake in fasting rats; specifically, 2-AG has been shown to increase food intake when levels rise in the jejunum of rats [9]. In contrast to AEA and 2-AG, oleoylethanolamide (OEA), an endocannabinoid-like lipid signaling molecule, has been linked to the feeling of satiety, which reduces feeding when active in the jejunum [10]. Due to the endocannabinoids' tissue-specific properties, more research is needed to explore how the stress of diet-induced obesity affects endocannabinoid levels in cardiac tissue. Literature suggests that endocannabinoids in cardiac tissue are linked to cardioprotective properties during hypertension and other cardiac stress [11]. The cardioprotective properties of endocannabinoids make this a promising approach for the treatment of cardiovascular pathology.

The role of endocannabinoids under diet-induced obesity in the heart, however, remains largely unknown. Determining the role of these lipid signaling molecules in respect to cardiovascular health in diet-induced obese conditions will lead to a better understanding of cardiovascular function. This study explores the changes in lipid

signaling molecule concentrations in the left ventricle of hearts of western diet-induced obese mice. This research will aid in the development of novel treatment of pathologic cardiovascular function associated with metabolic syndrome. We hypothesize Western-diet-induced obesity is sufficient to dysregulate the endocannabinoid system increasing concentrations in the left ventricle.

MATERIALS AND METHODS

All of the following methods have been previously described by Argueta and DiPatrizio, 2017 [12].

Animals

Eight-week old mice C57BL/6 mice (Taconic, Oxnard, CA, USA) were group-housed with access to water and food *ad libitum*, unless otherwise noted for food deprivation studies, and maintained on a 12 h light/dark cycle (lights off at 1800 h). Test diets consisted of standard lab rodent chow [(SD) Lab Diet 5001, St. Louis, MO, USA; 13.4% kcal as fat, 56% kcal from carbohydrates, mostly starch], or Western-style diet (WD) Research Diets D12709B, New Brunswick, NJ, USA; 40% kcal as fat, 43% kcal from carbohydrates, mostly sucrose]. Five days prior to tissue harvest, animals were single-housed in cages with raised wire mesh inserts to prevent coprophagia during 24 h food deprivation experiments. 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.

Tissue Harvest

Isofluorane was used to anesthetize animals at time of tissue harvest (0900 to 1100 h), following 24-hour food deprivation or *ad libitum* feeding. The hearts were rapidly collected, washed with phosphate-buffered saline (PBS) on ice, then snap-frozen in liquid nitrogen. All samples were stored at -80°C until processing.

Lipid Extraction

Frozen tissues were weighed and subsequently homogenized in 1.0 mL of methanol solution containing the internal standards, [$^2H_5$] 2-AG, [$^2H_4$] AEA, and [$^2H_4$] OEA (Cayman Chemical, Ann Arbor, MI, USA). All samples were extracted with a negative control to ensure there was no cross contamination between tissues. Lipids were extracted with chloroform (2 mL) and washed with water (1 mL). Organic phases were collected and separated by open-bed silica gel column chromatography as previously described [13]. Eluate was gently dried under N2 stream (99.998% pure) and resuspended in 0.2 mL of methanol:chloroform (9:1) for Ultra-Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC/MS/MS) analysis.

Statistical Analysis

Data was analyzed using Graphpad Prism7 software. Results are expressed as the mean ± S.E.M. Significant differences among groups were assessed using Student's two-tailed t-test. Differences were considered significant if $p<0.05$.

RESULTS

Impact of Western Diet with Males

Only free feeding male mice did not experience significant changes in 2-AG ($p=0.3049$). In male mice maintained on either a Standard Diet or Western Diet *ad libitum*, AEA increased from an average of 1.333 ± 0.2508 pmol/g in SD to 5.075 ± 0.2307 pmol/g in WD ($p<0.0001$). Under *ad libitum* conditions, OEA increased from an average of 95.97 ± 6.525 pmol/g in SD to 117.6 ± 5.09 pmol/g ($p=0.0202$).

Under 24-hour food deprivation conditions male mice experienced an increase in 2-AG by 0.4424 ± 0.1108 nmol/g ($p=0.0015$). Similarly, AEA in male mice increased from 4.26 ± 0.1426 pmol/g in SD to 10.74 ± 0.3961 pmol/g in WD under 24-hour food deprivation conditions ($p<0.0001$). OEA of male mice also increased under food deprivation by 53.97 ± 5.518 pmol/g ($p<0.0001$) [Figure 1].

Impact of Western Diet with Females

Female mice did not experience significant changes in 2-AG regardless of free feeding or food deprivation conditions. In free feeding female mice maintained on either a Standard Diet or Western Diet *ad libitum* AEA increased from an average of 0.9166 ± 0.1391 pmol/g in SD to 2.134 ± 0.1783 pmol/g in WD ($p<0.0001$). An increase in OEA also occurred from an average of 48.58 ± 4.868 pmol/g in SD to 79.55 ± 11.28 pmol/g in WD under *ad libitum* conditions ($p=0.0245$).

Under 24-hour food deprivation conditions female mice experienced an increase in AEA by 1.657 ± 0.3456 ($p=0.0003$). OEA in female mice increased from 123.7 ± 8.789 pmol/g in SD to 144.4 ± 5.444 pmol/g in WD under 24-hour food deprivation conditions ($p<0.0648$) [Figure 2].

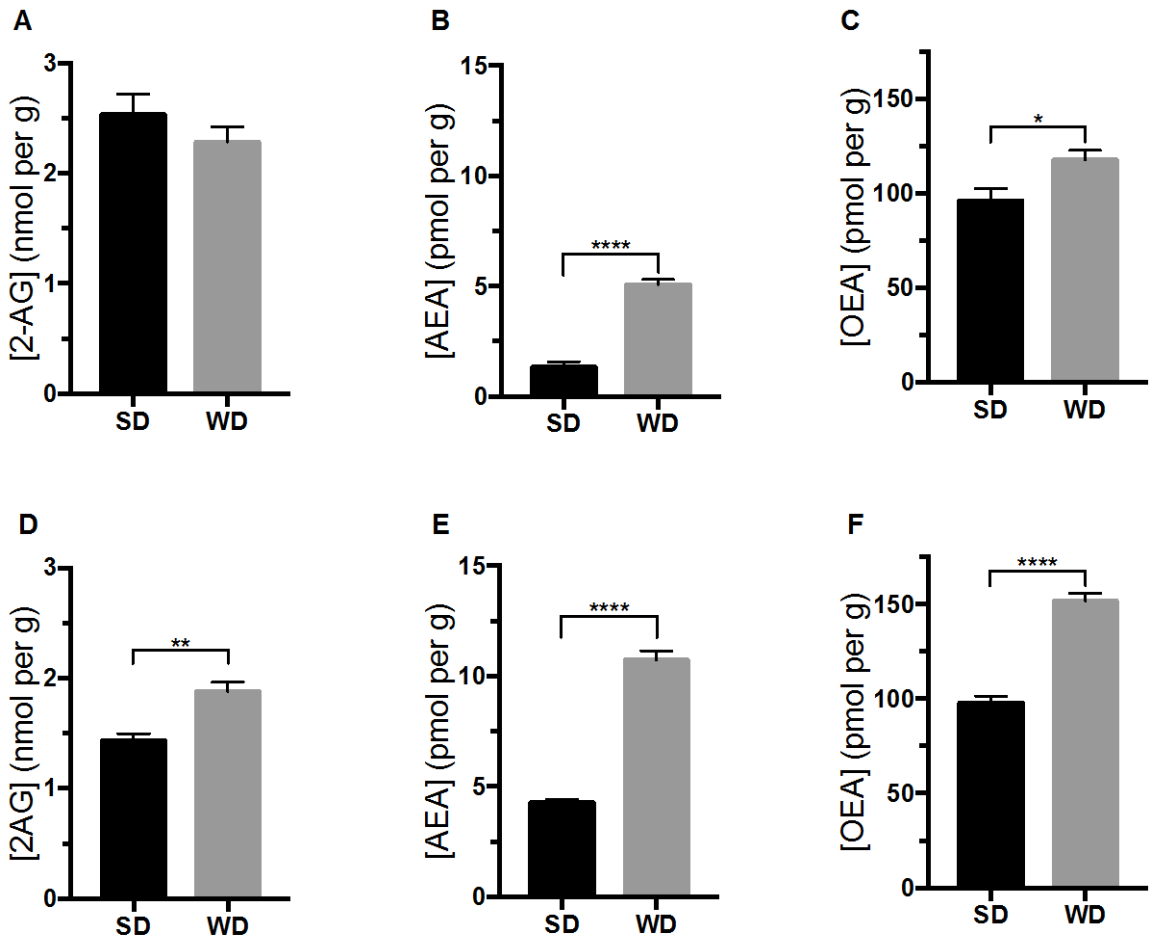

Figure 1. Increase in lipid signaling molecules. Samples collected from male mice after 60-days of Western diet or standard chow administration. Subjects were either free feeding (A-C) or food deprived 24 hours prior to organ collection (D-F). Data points analyzed using student, two-tailed t-test. n = 7-8, ns = $p \geq 0.05$, *=$p < 0.05$, **=$p < 0.01$, ****=$p < 0.0001$

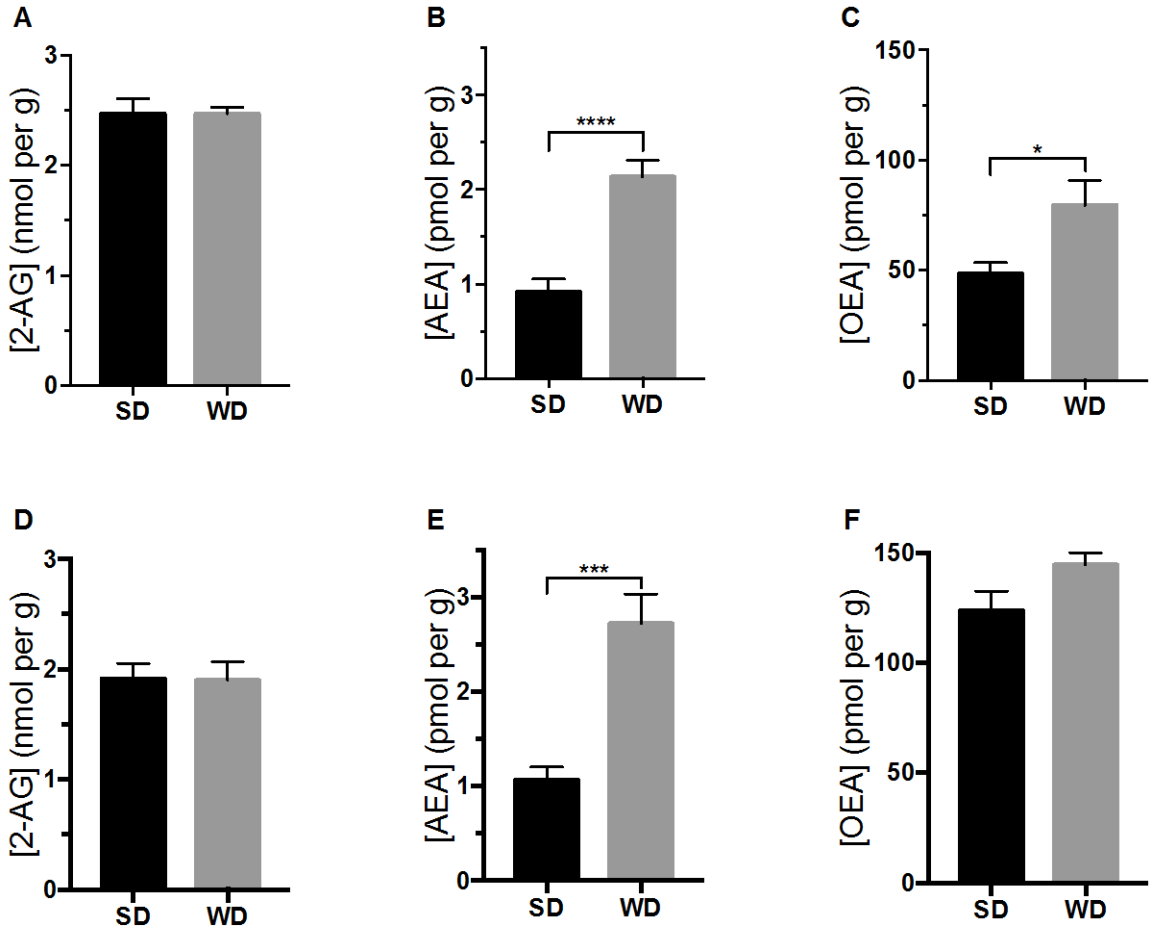

Figure 2. Increase in AEA after 60 Days of Western Diet. Samples collected from female after 60-days of Western diet or standard chow administration. Subjects were either free feeding (A-C) or food deprived 24 hours prior to organ collection (D-F). Data points analyzed using student, two-tailed t-test. n = 8, ns = $p \geq 0.05$, *=$p < 0.05$, ***=$p < 0.001$, ****=$p < 0.0001$

DISCUSSION

The results of this experiment suggest that diet-induced obesity alters endocannabinoid levels in cardiovascular tissue. The increase in AEA for all conditions suggest that, regardless of the sex of the mouse, western diet-induced obesity is sufficient to elicit an endocannabinoid response. This response is likely due to hypertensive stress on cardiovascular system as a result of the western diet-induced obesity [14]. This is consistent with literature that suggests AEA activation of CB_1 results in vasodilation and nitric oxide production [6]. The results of this study suggest that diet-induced obesity may have been sufficient to cause changes in AEA and OEA which have been linked to hypertension and heart failure respectively [7,13].

Like humans, male and female mice physiologically differ on a biochemical level as well as on an anatomical level. A similar increase in AEA suggest that females, like males, may be responding to hypertension as a result of their exposure to WD for 60 days. In response to WD, however, females did not experience significant changes under food deprived conditions compared to their male counterparts who exhibited a significant change. Although the role of OEA is not clearly understood in the heart, some findings suggest it may be involved during heart failure and in preventing apoptosis of cardiomyocytes [14,15]. The less dramatic changes in OEA in females [Figure 2] observed in this study may be indicative of physiological differences in response to cardiac stress in a sex-specific manner. Further research, however, is required to assess the role of OEA in diet-induced obesity and cardiovascular stress.

Although this study successfully investigated the influence of diet, sex, and food deprivation, it is difficult to identify if these changes are occurring in the cardiac tissue itself or the blood it pumps. Therefore, it remains a possibility that levels of lipid signaling molecules are increasing in other organs are then being transported to the heart via blood. To help account for this possibility, blood plasma was also collected from the mice and underwent LCMS analysis [12]. In comparison to blood plasma, cardiac endocannabinoids were nearly tenfold greater for most analytes. Therefore, although levels of lipid signaling molecules in the blood did contribute to the quantified data, our findings represent changes in the cardiac tissue itself. The presented data suggest that the cardiac tissue is altering either the rate of endocannabinoid production or degradation. Future studies should perform cardiac perfusions with PBS during tissue collection to minimize blood contamination of tissues and provide more accurate findings.

Despite the sex of the mice, the increased levels of lipid signaling molecules, which have been linked to cardioprotective effects, suggest that western diet-induced obesity is sufficient to elicit cardioprotective responses in mice. Unfortunately, this study was unable to explore markers for cardiovascular stress or measure signs of pathology such as heart rate, blood pressure, and ejection fraction. Therefore, future research should test for cardiovascular health in diet-induced obesity in addition to changes in endocannabinoid levels in cardiac tissue. Further exploring endocannabinoids during cardiovascular pathology can lead to the development of new drugs or better use of current therapeutics, such as marijuana.

This investigation concludes that diet-induced obesity is sufficient to induce changes in endocannabinoid levels in cardiac tissue. We propose that in response to this added stress, the cardiomyocytes produce excess endocannabinoids and OEA to restore cardiovascular homeostasis. This response is suggestive of the cardioprotective properties of the endocannabinoid system in cardiovascular tissue.

REFERENCES

1. N. V. DiPatrizio *et al.* (2012). The thrifty lipids: endocannabinoids and the neural control of energy conservation. Trends in Neurosciences. 35:403-411.

2. A. Ruopeng. (2013). Prevalence and Trends of Adult Obesity in the US, 1999-2012. Hindawi. 2014: Article ID 185132

3. (2017). New Initiative Launched to Tackle Cardiovascular Disease, the World's Number One Killer. World Health Organization. http://www.who.int/cardiovascular_diseases/global-hearts/Global_hearts_initiative/en/

4. J.-P. Després *et al.* (2008). Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. Arteriosclerosis, Thrombosis, and Vascular Biology. 28:1039-1049.

5. G. K. Andrikopoulos *et al.* (2008). Modification of cardiometabolic risk through cannabinoid type-1 receptor antagonism. Angiology. 59:44S-48S.

6. Y. Lu *et al.* (2014). Ligand activation of cannabinoid receptors attenuates hypertrophy of neonatal rat cardiomyocytes. Journal of Cardiovascular Pharmacology. 64:420-430.

7. N. V. DiPatrizio *et al.* (2011). Endocannabinoid signal in the gut controls dietary fat intake. Proceedings of the National Academy of Sciences of the United States of America. 108:12904-12908.

8. S. Bátkai *et al.* (2004). Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. Circulation. 110:1996-2002.

9. M. Joyeux *et al.* (2002). Endocannabinoids are implicated in the infarct size-reducing effect conferred by heat stress preconditioning in isolated rat hearts. Cardiovascular Research. 55:619-625.

10. G. J. Schwartz *et al.* (2008). The Lipid Messenger OEA Links Dietary Fat Intake to Satiety. Cell Metabolism. 8:281-288.

11. A. J. Wheal *et al.* (2009). Cardiovascular effects of cannabinoids in conscious spontaneously hypertensive rats: Cannabinoids in spontaneously hypertensive rats. British Journal of Pharmacology. 152:717-724.

12. D. A. Argueta, *et al.* (2017). Peripheral endocannabinoid signaling controls hyperphagia in western diet-induced obesity. Physiology & Behavior. 171:32-39.

13. N. Cluny *et al.* (2010). A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents: Peripheral CB antagonist reduces food intake. British Journal of Pharmacology. 161:629-624.

14. H.-F. Su *et al.* (2006). Oleylethanolamide activates Ras-Erk pathway and improves myocardial function in doxorubicin-induced heart failure. Endocrinology. 147:827-834.

15. N. Azad *et al.* (2011). Gender differences in the etiology of heart failure: A systematic review. Journal of Geriatric Cardiology. 8:15-23.