UNIVERSITY OF CALIFORNIA
RIVERSIDE

Endogenous and Exogenous Cannabinoids Regulate Energy Homeostasis During Obesity

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Biomedical Sciences

by

Bryant Avalos Leyva

September 2023

Dissertation Committee:
Dr. Nicholas V. DiPatrizio, Chairperson
Dr. Christian Y. Lytle
Dr. Declan McCole

Copyright by
Bryant Avalos Leyva
2023

The Dissertation of Bryant Avalos Leyva is approved:

____

____

____

Committee Chairperson

University of California, Riverside

Acknowledgements

This body of work was made possible with the collective support of multiple individuals over several years. First and foremost, I would like to acknowledge the mentorship and support provided by my primary faculty mentor Dr. Nicholas V. DiPatrizio who provided sustained guidance that perpetually pushed the boundaries of my knowledge. Additional support was provided by members of my qualifying and dissertation committee, including Dr. Christian Y. Lytle and Dr. Declan F. McCole, which ensured that I received the proper exposure to different expert scientific visions that is necessary for growth as a scientist. To these three individuals, I am forever grateful for their time, attention, and patience throughout my graduate studies.

Some of the most important advice and general support that I received as a graduate student came from previous members of the DiPatrizio lab. Drs. Donovan A. Argueta, Pedro A. Perez, and Mark B. Wiley were instrumental in my early success as a PhD student. I would also like to acknowledge Dr. Courtney Wood for jointly navigating scientific and life challenges throughout our concurrent time in the DiPatrizio lab. Although these individuals were initially labmates, they all became life-long friends that I truly appreciate unconditionally.

Acknowledgements of previous publications must be made for the text of this dissertation, including reprint of material that appears in “Cannabinoid CB_1 receptors in the intestinal epithelium are required for acute western-diet preferences in mice” Nutrients, 2020 (Chapter 1). Dr. Nicholas DiPatrizio directed and supervised this published work as well as the remaining research that forms the basis of this dissertation. Additional co-authors include 1) Donovan Argueta, 2) Pedro Antonio Perez, and 3) Mark Wiley:

1. Assisted with conceptualization, methodology for investigations, data analysis, and writing (review and editing)

2. Assisted with methodology for investigations, data analysis and curation, and writing (review and editing)

3. Assisted with methodology for investigations, data analysis

Copyright ©

The author maintains copyright for the use and distribution of material used in this dissertation.

Funding

These studies were funded by the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases grants DK119498 and DK114978, the Tobacco-Related Disease Research Program (TRDRP) from the University of California Office of the President grant T29KT0232 to NVD, and the Dean’s Distinguished Fellowship and departmental BMSC quarterly fellowships presented to BA. Additionally, we thank the Ford Foundation Dissertation Fellowship to DA.

Dedication

I dedicate this body of work to:

1. The countless first-generation students who struggle with self-doubt

2. The city of Palmdale, CA

3. The late great Kobe Bryant

ABSTRACT OF THE DISSERTATION

Endogenous and Exogenous Cannabinoids
Regulate Energy Homeostasis During Obesity

by

Bryant Avalos Leyva

Doctor of Philosophy, Graduate Program in Biomedical Sciences
University of California, Riverside, September 2023
Dr. Nicholas V. DiPatrizio, Chairperson

The peripheral endocannabinoid (eCB) system is a key determinant of energy homeostasis in various biological tissues. Studies indicate eCB dysregulation in multiple peripheral organs, including the small intestine and adipose tissue, is strongly associated with diet-induced obesity (DIO). Moreover, elevated levels of peripheral endogenous cannabinoids can perpetuate the obesity phenotype by promoting appetite. To assess the impact of intestinal eCB activity on food intake, these studies first examined the role of cannabinoid type-1 receptor (CB_1R) in modulating dietary preferences. CB_1R, a key component of the ECS, has been extensively studied for its involvement in the modulation of appetite, adipogenesis, and adipokine secretion. Cannabinoid-mediated influence on appetite is well-established with certain exogenous cannabinoids found in *Cannabis sativa*, such as Δ9-tetrahydrocannabinol (THC), which stimulates appetite by activating the eCB system. Paradoxically, however, recent evidence from retrospective human observational studies reports lower prevalence of

obesity and decreased incidence of diabetes in cannabis users compared to non-users. It remains unclear if cannabinoids like THC are responsible for any metabolic improvements associated with long-term cannabis use. Thus, these studies also assessed the impact of chronic cannabinoid exposure on the development of DIO. By utilizing ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC/MS/MS), we compare the metabolic outcomes of chronic exposure to pure THC versus whole cannabis extract with dose-matched THC content. Furthermore, we examined the role of adipocyte CB_1R in regulating energy homeostasis within adipose tissue in response to chronic cannabinoid exposure *in vivo* and *in vitro*. Collectively, these studies use a combination of genetic and pharmacological tools to understand the role of peripheral CB_1R in facilitating shifts in energy homeostasis. Findings from these studies indicate a substantial role for intestinal CB_1R in modulating food intake and dietary preference. This body of work also provides novel insight into the role of adipocyte CB_1R in mitigating the differential metabolic improvements following chronic cannabinoid exposure.

Table of Contents

Acknowledgements……………………………………………………………….iv

Dedication……………………………………………………………………….vi

Abstract………………………………………………………………………….vii

List of Figures…………………………………………………………………...xi

Introduction……………………………………………………………………...1

References…………………………………………………………………8

Chapter 1………………………………………………………………………..14

Abstract……………………………………………………………………15

Introduction………………………………………………………………..15

Materials & Methods……………………………………………………..17

Results……………………………………………………………………22

Discussion………………………………………………………………...26

References………………………………………………………………..32

Tables & Figures…………………………………………………………41

Chapter 2………………………………………………………………………..46

Abstract……………………………………………………………………46

Introduction………………………………………………………………..47

Materials & Methods……………………………………………………..50

Results……………………………………………………………………58

Discussion………………………………………………………………...65

References………………………………………………………………..70

Tables & Figures……………………………………………………….……81

Conclusion…………………………………………………………………………...89

References…………………………………….…………………………..92

List of Figures

Chapter 1

Figure 1.1. Cannabinoid CB_1Rs control acute preferences for Western Diet…….41

Figure 1.2. CB_1R immunoreactivity is absent in the upper small-intestinal epithelium of conditional intestinal epithelium-specific CB_1R-deficient mice……42

Figure 1.3. Expression of endocannabinoid system genes in conditional intestinal epithelium-specific CB_1R-deficient mice and controls……………………………..43

Figure 1.4. Conditional intestinal epithelium CB_1R-deficient mice display no changes in baseline feeding parameters, motor activity, or glucose clearance….44

Figure 1.5. Acute preferences for western diet are absent in conditional intestinal epithelium-specific CB_1R-deficient mice……………………………………………45

Chapter 2

Figure 2.1. Changes in body weight, motor activity, and food intake in mice chronically treated with THC or extract…………………………………………...81

Figure 2.2. Amelioration of glucose dyshomeostasis is associated with reduced adiposity in cannabinoid-treated mice……………………………………………….82

Figure 2.3. Visceral fat gene expression is differentially impacted by 30d exposure to THC and extract……………………………………………………….83

Figure 2.4. Endocannabinoid tone associated with DIO becomes normalized following chronic THC and extract exposure……………………………………….84

Figure 2.5. Adipocyte CB_1R deletion impacts glucose homeostasis but does not affect body weight reductions caused by chronic cannabinoid exposure……….85

Figure 2.6. Chronic cannabinoid exposure in 3T3-L1 cultured adipocytes results in reduced lipid accumulation……………………………………………………...86

Figure 2.7. Chronic THC exposure produces dose-dependent changes in cellular bioenergetic profile of 3T3-L1 adipocytes…………………………………………..88

Introduction

Obesity

Obesity is defined as an abnormal or excessive accumulation of adipose tissue, which is the primary factor dictating the rising trend of body mass index (BMI) measurements throughout the world. Chronic consumption of high-fat, high-sugar foods (i.e. the westernized diet (WD)) contributes immeasurably to the development of diet-induced obesity (DIO) in the United States[1-3]. Current projections estimate that 51% of America adults will suffer from obesity by the year 2030[4], further increasing the current $147-342 billion spent in healthcare costs related to being severely overweight[5,6]. In 2013, the American Medical Association first classified obesity as a disease as accumulating evidence showed that the disease can significantly increase the risk of developing other major health concerns such as diabetes. Obesity and type 2 diabetes mellitus (T2DM) are intricately linked through the common denominator of insulin resistance[7]. While obesity often precedes and exacerbates T2DM, diabetes can contribute to weight gain and complications. Understanding the multifaceted mechanisms connecting these conditions is crucial for developing effective prevention and treatment strategies.

The Peripheral Endocannabinoid System

The peripheral endocannabinoid (eCB) system, a key determinant of energy homeostasis that becomes dysregulated in obesity, has recently become a target for therapeutic treatment of DIO. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the endogenous cannabinoids that activate the cannabinoid type-1 (CB_1) and type-2 (CB2) receptors, contribute pivotal roles to the development of obesity. In DIO, elevated levels of AEA and 2-AG cause overactivation of peripheral CB_1 which drives increased food intake[8]. Conversely, pharmacological inactivation of CB_1 reduces food intake, adipose tissue mass, and insulin hypersecretion[9,10]. Targeting the eCB system has been considered as a pharmacological approach to tackling the obesity epidemic. In 2006, Rimonabant became the first approved drug in Europe to be used as an anti-obesity medication behaving as an inverse agonist of the CB_1 receptor. However, the restoration of adipoinsular function linked with eCB system inhibition is heavily outweighed by severe psychiatric side effects resulting from non-specific CB_1R antagonism within the central nervous system[11-14]. The drug was subsequently removed from the market after its serious adverse effects and was never approved in the United States.

Antagonizing the ECS has been pursued with an array of cannabinoid receptor antagonists. The peripherally restricted CB_1R antagonist AM6545 has been shown to ameliorate the dysregulation of triglycerides, cholesterol, and fat phospholipids, in monosodium glutamate-induced obese mice[15,16]. In mice that

become diet-induced obese from a high fat/high sugar diet, AM6545 blocked hyperphagia associated with the calorically dense and innately palatable diet[17]. This compound, which is unable to cross the blood brain barrier, maintains its receptor-blocking activity in the periphery however this effect has only been studied in animal models and has yet to display clinical efficacy in humans. Other peripherally restricted compounds, such as URB447 have also led to lower food intake and reduced weight gain in mice closely mimicking the effects of Rimonabant[18,19]. Conversely, agonists of the ECS such as WIN 55,212-2 have been shown to increase food intake by up to 80% in mice[20]. This effect appears to be altered in rats, in which exposure to THC during adolescence caused an increase propensity for the rats to self-administer WIN 55,212-2 during adulthood[21-23]. Furthermore, studies have shown that co-administration of WIN 55,212-2 with a CB_1R antagonist, such as AM251, results in a reduction of hyperphagic behavior illustrating a CB_1R-dependent hunger signaling event occurring within the central nervous system or periphery[24,25]. These findings encapsulate the homeostatic nature of a system that is capable of being pharmacologically manipulated to produce opposing physiological effects depending on the compound, while also depicting the developmental dependency the system holds that ultimately affects a healthy metabolism.

The Adipoinsular Axis

Adipose tissue is no longer considered solely a passive energy storage depot; it is now recognized as an active endocrine organ capable of secreting numerous bioactive molecules, collectively termed adipokines[26]. These adipokines have emerged as key regulators of energy metabolism, inflammation, and insulin sensitivity[27]. The interaction between adipokines and cannabinoids contributes to the intricate regulatory network governing obesity and related metabolic disorders. Leptin and adiponectin are crucial adipokines that modulate energy balance. Leptin, known as the "satiety hormone," suppresses appetite and stimulates energy expenditure[28]. In obesity, leptin resistance often develops, contributing to increased food intake. Adiponectin levels are inversely correlated with adiposity; as obesity progresses, adiponectin levels tend to decrease[26]. Reduced adiponectin availability contributes to insulin resistance, as this hormone is known to enhance insulin sensitivity by stimulating fatty acid oxidation and glucose uptake in skeletal muscles and liver[29]. Dysregulated adipsin levels have been associated with obesity and metabolic dysfunction, making it a potential link between CB_1 receptors, adipose tissue, and obesity-related complications[30].

Shifts in metabolic activity within adipose tissue depots are not exclusively pathological. Unlike white adipose tissue (WAT), brown adipose tissue (BAT) serves a specialized role in thermogenesis, utilizing its dense mitochondrial reserve high in uncoupling protein 1 (UCP1) activity to oxidize fat and release energy in the form of heat[31,32]. White and brown adipocytes share characteristics

with a subset of fat cells referred to as "beige" or "brite" adipocytes[33]. Certain WAT depots like inguinal fat maintain the ability to undergo "browning" and produce an intermediate beige phenotype CB_1R signaling. WAT depots capable of browning maintain a different thermogenic gene profile compared to BAT until stimulated to express high levels of the UCP1, a mitochondrial membranous protein committed to adaptive thermogenesis[34-36]. This transdifferentiation potential can shift the energy balance in adipocytes by reducing the storage of triglyercides, increasing uptake of glucose, and decreasing the release of appetite-suppressing hormones[37,38]. Given that CB_1R blockade can induce this phenotypic change, the therapeutic relevance of targeting the ECS is further signified.

Cannabis & Metabolic Health

Archaeological artifacts containing residues of the *Cannabis sativa* plant indicate its historical use originated in the continent of Asia, more specifically the steppes of Central Asia[39,40]. Having served a medicinal, spiritual, and recreational use throughout its history, cannabis' complex molecular makeup has been shown to provide more than just a psychoactive effect to its users. The natural compounds found in cannabis, known as phytocannabinoids, are abundant in nature and up until the end of the 19th century, were unidentified. Purification and extraction of oil via fractional distillation of cannabis marked the first step in elucidating the physiological and biochemical relevance of the plant[41]. Soon after, isolation of the first two inactive natural cannabinoids, cannabinol (CBN) and cannabidiol (CBD),

paved way to uncovering the constituents of cannabis that promote psychoactivity[42]. Successful isolation and structural characterization of the primary psychoactive constituent of the plant, Δ9-tetrahydrocannabinol (THC), was reported in 1964 and further prompted investigation into the stereochemistry of the two previously discovered cannabinoids[43,44].

Also known as marijuana, the psychoactive herb and its pharmacodynamics remained unearthed for several decades following the discovery of the early cannabinoids. Towards the end of the century, the discoveries of the receptor binding sites marked the start of the elucidation of cannabis' mechanism of action within the mammalian body. Cannabinoid-mediated influence on appetite is well-established with certain phytocannabinoids found in Cannabis sativa, like Δ9-tetrahydrocannabinol (THC), which stimulates appetite via CB_1R activation[41,43,45]. Dronabinol (Marinol), a synthetic analog of THC, was first approved by the Food and Drug Administration in 1985 as an antiemetic agent prescribed to combat nausea and stimulate appetite during chemotherapy or in acquired immune deficiency syndrome (AIDS) patients. Paradoxically, however, several prospective studies have shown positive metabolic benefits associated with cannabis use among adults. The National Epidemiological Survey on Alcohol and Related Conditions (NESARC) reports an inverse association between cannabis and body mass index (BMI) in a study consisting of adult cannabis users, age 18+, within the United States when compared to never-users[46,47]. This attenuated BMI response in recapitulated in the data from the National Health and Nutrition

Examination Survey (NHANES) III, where current cannabis users reported slightly lower BMI values compared to non-current cannabis users despite higher caloric intake[48-50]. The endocannabinoid system's role in regulating glucose and insulin homeostasis is further restated in the findings illustrating cannabis users maintain a lower HOMA-IR index, a Homeostasis Model Assessment of Insulin Resistance, when compared to now users[51-53].

Together, these studies provide accumulating evidence elucidating cannabis' potential in altering the phenotype associated with obesity and marijuana consumption. Additionally, habitual use of cannabis has been associated with a down regulation of CB_1 receptors serving as a plausible explanation for effects seen in both the central and peripheral ECS. It is important to note that data from the mentioned prospective studies are dependent on self-reporting which may cause a response bias. Regardless, the endocannabinoid system's ability to be manipulated by cannabis which correlates with several beneficial metabolic effects should be explored further to avoid heavily relying on correlational information concerning acute and chronic cannabis use. Understanding the complex interplay between cannabinoids and adipokines is essential for developing targeted interventions that address the global health burden of obesity.

References

1. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will All Americans Become Overweight or Obese? Estimating the Progression and Cost of the US Obesity Epidemic. Obesity 2008;16(10):2323-2330. DOI: 10.1038/oby.2008.351.

2. Cordain L, Eaton SB, Sebastian A, et al. Origins and evolution of the Western diet: health implications for the 21st century. The American Journal of Clinical Nutrition 2005;81(2):341-354. DOI: 10.1093/ajcn.81.2.341.

3. Kopp W. How Western Diet And Lifestyle Drive The Pandemic Of Obesity And Civilization Diseases. Diabetes Metab Syndr Obes 2019;12:2221-2236. DOI: 10.2147/DMSO.S216791.

4. Hales CM CM, Fryar CD, Ogden CL. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. National Center for Health Statistics.

5. Cawley J, Meyerhoefer C. The medical care costs of obesity: an instrumental variables approach. J Health Econ 2012;31(1):219-30. (In eng). DOI: 10.1016/j.jhealeco.2011.10.003.

6. Biener A, Cawley J, Meyerhoefer C. The High and Rising Costs of Obesity to the US Health Care System. J Gen Intern Med 2017;32(Suppl 1):6-8. (In eng). DOI: 10.1007/s11606-016-3968-8.

7. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L. Overfeeding Rapidly Induces Leptin and Insulin Resistance. Diabetes 2001;50(12):2786. DOI: 10.2337/diabetes.50.12.2786.

8. Argueta DA, DiPatrizio NV. Peripheral endocannabinoid signaling controls hyperphagia in western diet-induced obesity. Physiol Behav 2017;171:32-39. (In eng). DOI: 10.1016/j.physbeh.2016.12.044.

9. Jbilo O, Ravinet-Trillou C, Arnone M, et al. The CB_1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. The FASEB Journal 2005;19(11):1567-1569. (https://doi.org/10.1096/fj.04-3177fje).

10. Bensaid M, Gary-Bobo M, Esclangon A, et al. The cannabinoid CB_1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. Mol Pharmacol 2003;63(4):908-14. (In eng). DOI: 10.1124/mol.63.4.908.

11. Sam AH, Salem V, Ghatei MA. Rimonabant: From RIO to Ban. J Obes 2011;2011:432607. (In eng). DOI: 10.1155/2011/432607.

12. Arnone M, Maruani J, Chaperon F, et al. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB_1) receptors. Psychopharmacology (Berl) 1997;132(1):104-6. (In eng). DOI: 10.1007/s002130050326.

13. Rinaldi-Carmona M, Barth F, Héaulme M, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 1994;350(2-3):240-4. (In eng). DOI: 10.1016/0014-5793(94)00773-x.

14. Randall PA, Vemuri Vk Fau - Segovia KN, Segovia Kn Fau - Torres EF, et al. The novel cannabinoid CB_1 antagonist AM6545 suppresses food intake and food-reinforced behavior. (1873-5177 (Electronic)) (In eng).

15. Cluny NL, Vemuri VK, Chambers AP, et al. A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. British journal of pharmacology 2010;161(3):629-642. (In eng).

16. Ma H, Zhang G, Mou C, Fu X, Chen Y. Peripheral CB_1 Receptor Neutral Antagonist, AM6545, Ameliorates Hypometabolic Obesity and Improves Adipokine Secretion in Monosodium Glutamate Induced Obese Mice. Front Pharmacol 2018;9:156. (In eng). DOI: 10.3389/fphar.2018.00156.

17. Argueta DA, Perez PA, Makriyannis A, DiPatrizio NV. Cannabinoid CB(1) Receptors Inhibit Gut-Brain Satiation Signaling in Diet-Induced Obesity. Front Physiol 2019;10:704. (In eng). DOI: 10.3389/fphys.2019.00704.

18. LoVerme J, Duranti A, Tontini A, et al. Synthesis and characterization of a peripherally restricted CB_1 cannabinoid antagonist, URB447, that reduces feeding and body-weight gain in mice. Bioorg Med Chem Lett 2009;19(3):639-43. (In eng). DOI: 10.1016/j.bmcl.2008.12.059.

19. Foloppe N, Allen NH, Bentley CH, et al. Discovery of a novel class of selective human CB_1 inverse agonists. Bioorg Med Chem Lett 2008;18(3):1199-206. (In eng). DOI: 10.1016/j.bmcl.2007.11.133.

20. Pontieri FE, Conti G, Zocchi A, Fieschi C, Orzi F. Metabolic mapping of the effects of WIN 55212-2 intravenous administration in the rat. Neuropsychopharmacology 1999;21(6):773-6. (In eng). DOI: 10.1016/S0893-133X(99)00064-0.

21. Scherma M, Dessì C, Muntoni AL, et al. Adolescent Δ(9)-Tetrahydrocannabinol Exposure Alters WIN55,212-2 Self-Administration in Adult Rats. Neuropsychopharmacology 2016;41(5):1416-26. (In eng). DOI: 10.1038/npp.2015.295.

22. Fattore L, Cossu G, Martellotta CM, Fratta W. Intravenous self-administration of the cannabinoid CB_1 receptor agonist WIN 55,212-2 in rats. Psychopharmacology (Berl) 2001;156(4):410-6. (In eng). DOI: 10.1007/s002130100734.

23. Martellotta MC, Cossu G, Fattore L, Gessa GL, Fratta W. Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. Neuroscience 1998;85(2):327-30. (In eng). DOI: 10.1016/s0306-4522(98)00052-9.

24. Baur R, Gertsch J, Sigel E. The cannabinoid CB_1 receptor antagonists rimonabant (SR141716) and AM251 directly potentiate GABA(A) receptors. British journal of pharmacology 2012;165(8):2479-2484. (In eng). DOI: 10.1111/j.1476-5381.2011.01405.x.

25. Spiller KJ, Bi GH, He Y, Galaj E, Gardner EL, Xi ZX. Cannabinoid CB_1 and CB2 receptor mechanisms underlie cannabis reward and aversion in rats. Br J Pharmacol 2019;176(9):1268-1281. (In eng). DOI: 10.1111/bph.14625.

26. Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends in Endocrinology & Metabolism 2002;13(2):84-89. DOI: 10.1016/s1043-2760(01)00524-0.

27. Mäuser W, Perwitz N, Meier B, Fasshauer M, Klein J. Direct adipotropic actions of atorvastatin: differentiation state-dependent induction of apoptosis, modulation of endocrine function, and inhibition of glucose uptake. Eur J Pharmacol 2007;564(1-3):37-46. (In eng). DOI: 10.1016/j.ejphar.2007.02.024.

28. Jo Y-H, Chen Y-JJ, Chua SC, Jr., Talmage DA, Role LW. Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit. Neuron 2005;48(6):1055-1066. (In eng). DOI: 10.1016/j.neuron.2005.10.021.

29. Stern JH, Rutkowski JM, Scherer PE. Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. Cell Metab 2016;23(5):770-84. (In eng). DOI: 10.1016/j.cmet.2016.04.011.

30. Sarmento-Cabral A, Peinado JR, Halliday LC, et al. Adipokines (Leptin, Adiponectin, Resistin) Differentially Regulate All Hormonal Cell Types in Primary Anterior Pituitary Cell Cultures from Two Primate Species. Scientific Reports 2017;7(1):43537. DOI: 10.1038/srep43537.

31. Wu J, Boström P, Sparks LM, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 2012;150(2):366-376. (In eng). DOI: 10.1016/j.cell.2012.05.016.

32. Gómez-Hernández A, Beneit N, Díaz-Castroverde S, Escribano Ó. Differential Role of Adipose Tissues in Obesity and Related Metabolic and Vascular Complications. International journal of endocrinology 2016;2016:1216783-1216783. (In eng). DOI: 10.1155/2016/1216783.

33. Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 2010;285(10):7153-7164. (In eng). DOI: 10.1074/jbc.M109.053942.

34. Fayyad AM, Khan AA, Abdallah SH, Alomran SS, Bajou K, Khattak MNK. Rosiglitazone Enhances Browning Adipocytes in Association with MAPK and PI3-K Pathways During the Differentiation of Telomerase-Transformed Mesenchymal Stromal Cells into Adipocytes. Int J Mol Sci 2019;20(7):1618. (In eng). DOI: 10.3390/ijms20071618.

35. Dickson LM, Gandhi S, Layden BT, Cohen RN, Wicksteed B. Protein kinase A induces UCP1 expression in specific adipose depots to increase energy expenditure and improve metabolic health. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 2016;311(1):R79-R88. DOI: 10.1152/ajpregu.00114.2016.

36. Ricquier D. Uncoupling Protein 1 of Brown Adipocytes, the Only Uncoupler: A Historical Perspective. Front Endocrinol (Lausanne) 2011;2(85) (Mini Review) (In English). DOI: 10.3389/fendo.2011.00085.

37. Bartelt A, Bruns OT, Reimer R, et al. Brown adipose tissue activity controls triglyceride clearance. Nature Medicine 2011;17(2):200-205. DOI: 10.1038/nm.2297.

38. Cohen P, Levy JD, Zhang Y, et al. Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. Cell 2014;156(1-2):304-316. (In eng). DOI: 10.1016/j.cell.2013.12.021.

39. Kalant OJ. Report of the Indian Hemp Drugs Commission, 1893-94: a critical review. Int J Addict 1972;7(1):77-96. (In eng). DOI: 10.3109/10826087209026763.

40. Agurell S, Leander K. Stability, transfer and absorption of cannabinoid constituents of cannabis (hashish) during smoking. Acta Pharm Suec 1971;8(4):391-402. (In eng).

41. Pertwee RG, Thomas A, Stevenson LA, et al. The psychoactive plant cannabinoid, Delta9-tetrahydrocannabinol, is antagonized by Delta8- and Delta9-tetrahydrocannabivarin in mice in vivo. British journal of pharmacology 2007;150(5):586-594. (In eng). DOI: 10.1038/sj.bjp.0707124.

42. Huff HC, Vasan A, Roy P, Kaul A, Tajkhorshid E, Das A. Differential Interactions of Selected Phytocannabinoids with Human CYP2D6 Polymorphisms. Biochemistry 2021;60(37):2749-2760. (In eng). DOI: 10.1021/acs.biochem.1c00158.

43. Foltin RW, Brady JV, Fischman MW. Behavioral analysis of marijuana effects on food intake in humans. Pharmacol Biochem Behav 1986;25(3):577-82. (In eng). DOI: 10.1016/0091-3057(86)90144-9.

44. Rawitch AB, Rohrer R, Vardaris RM. Delta-9-tetrahydrocannabinol uptake by adipose tissue: Preferential accumulation in gonadal fat organs. General Pharmacology: The Vascular System 1979;10(6):525-529. DOI: https://doi.org/10.1016/0306-3623(79)90019-3.

45. Järbe TU, DiPatrizio NV. Delta9-THC induced hyperphagia and tolerance assessment: interactions between the CB_1 receptor agonist delta9-THC and the CB_1 receptor antagonist SR-141716 (rimonabant) in rats. Behav Pharmacol 2005;16(5-6):373-80. (In eng). DOI: 10.1097/00008877-200509000-00009.

46. Alshaarawy O, Anthony JC. Are cannabis users less likely to gain weight? Results from a national 3-year prospective study. Int J Epidemiol 2019;48(5):1695-1700. (In eng). DOI: 10.1093/ije/dyz044.

47. Le Strat Y, Le Foll B. Obesity and cannabis use: results from 2 representative national surveys. Am J Epidemiol 2011;174(8):929-33. (In eng). DOI: 10.1093/aje/kwr200.

48. Kim D, Kim W, Kwak MS, Chung GE, Yim JY, Ahmed A. Inverse association of marijuana use with nonalcoholic fatty liver disease among adults in the United States. PLoS One 2017;12(10):e0186702. (In eng). DOI: 10.1371/journal.pone.0186702.

49. Vidot DC, Prado G, Hlaing WM, Florez HJ, Arheart KL, Messiah SE. Metabolic Syndrome Among Marijuana Users in the United States: An Analysis of National Health and Nutrition Examination Survey Data. The American Journal of Medicine 2016;129(2):173-179.

50. Rajavashisth TB, Shaheen M, Norris KC, et al. Decreased prevalence of diabetes in marijuana users: cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) III. BMJ Open 2012;2:e000494. (In eng). DOI: 10.1136/bmjopen-2011-000494.

51. Clark TM, Jones JM, Hall AG, Tabner SA, Kmiec RL. Theoretical Explanation for Reduced Body Mass Index and Obesity Rates in Cannabis Users. Cannabis and cannabinoid research 2018;3(1):259-271. (In eng). DOI: 10.1089/can.2018.0045.

52. Ravi D, Ghasemiesfe M, Korenstein D, Cascino T, Keyhani S. Associations Between Marijuana Use and Cardiovascular Risk Factors and Outcomes: A Systematic Review. Ann Intern Med 2018;168(3):187-194. (In eng). DOI: 10.7326/M17-1548.

53. Penner EA, Buettner H, Mittleman MA. The impact of marijuana use on glucose, insulin, and insulin resistance among US adults. Am J Med 2013;126(7):583-9. (In eng). DOI: 10.1016/j.amjmed.2013.03.002.

54. Abioye A, Ayodele O, Marinkovic A, Patidar R, Akinwekomi A, Sanyaolu A. Δ9-Tetrahydrocannabivarin (THCV): a commentary on potential therapeutic benefit for the management of obesity and diabetes. Journal of Cannabis Research 2020;2(1). DOI: 10.1186/s42238-020-0016-7.

55. Wargent ET, Zaibi MS, Silvestri C, et al. The cannabinoid Δ(9)-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in two mouse models of obesity. Nutr Diabetes 2013;3(5):e68-e68. (In eng). DOI: 10.1038/nutd.2013.9.

Chapter 1: Cannabinoid CB_1 receptors in the intestinal epithelium are required for acute western-diet preferences in mice

Authors: Avalos B[1], Argueta DA[1], Perez PA[1], Wiley MB[1], Wood CP[1], DiPatrizio NV[1]

[1] Division of Biomedical Sciences, School of Medicine, University of California, Riverside, Riverside, CA 92521

Abstract

The endocannabinoid system plays an important role in controlling intake of palatable food. Endocannabinoid signaling in the upper small-intestinal epithelium is increased (i) in rats after tasting dietary fats, which controls intake of fats, and (ii) in a mouse model of diet-induced obesity, which promotes overeating via impaired nutrient-induced gut-brain satiation signaling. We now utilized a combination of genetic, pharmacological, and behavioral approaches to identify roles for cannabinoid CB_1Rs in upper small-intestinal epithelium in preferences for a western-style diet (WD, high-fat/sucrose) versus a standard rodent diet (SD, low-fat/no sucrose). Mice were maintained on SD in automated feeding chambers. During testing, mice were given simultaneous access to SD and WD, and intakes were recorded. Mice displayed large preferences for WD, which were inhibited by systemic pretreatment with the cannabinoid CB_1R antagonist/inverse agonist, AM251, for up to 12 h. We next used our novel intestinal epithelium-specific conditional cannabinoid CB_1R-deficient mice (IntCB_1-/-) to investigate the necessity of intestinal CB_1Rs in preferences for WD. Similar to AM251 treatment, preferences for WD were largely absent in IntCB_1-/- mice when compared to control mice for up to 12 h. Together, these data suggest that CB_1Rs in the murine intestinal epithelium are required for acute WD preferences.

Introduction

Humans and other mammals, when given a choice, generally prefer food that contains fats, sugars, or a combination of both [1]. Homeostatic and hedonic

feeding are controlled by diverse, albeit overlapping, neural and molecular signaling pathways throughout the brain, including those regulated by the endocannabinoid (eCB) system [see for review 2-5]. Recent studies, however, suggest important roles for the peripheral eCB system in energy homeostasis and intake of palatable food [6-29]. For example, we reported that tasting dietary lipids was sufficient to increase levels of eCBs in the rat upper small-intestinal epithelium, which required an intact vagus nerve, and pharmacological inhibition of cannabinoid subtype-1 receptors (CB_1Rs) in the periphery blocked consumption of lipids [14,15]. Moreover, levels of eCBs in the upper small-intestinal epithelium were increased in mice maintained for eight weeks on a western-style diet high in fat and sugar (WD) when compared to mice fed a standard diet low in fat and sugar, and pharmacological inhibition of CB_1Rs in the periphery blocked overeating associated with WD-induced obesity [17].

Nutrients are sensed by gustatory mechanisms in the oral cavity and enteroendocrine cells in the intestinal epithelium. In response, these cells release several satiation- and satiety-related molecules that communicate with the brain via the afferent vagus nerve [30-38]. We recently reported that eCB signaling in the gut controls nutrient-induced release of satiation peptides [16]. Gene transcripts for CB_1Rs were enriched in a subpopulation of enteroendocrine cells in the upper small-intestinal epithelium that secrete the satiation peptide, cholecystokinin [16,39]. Notably, the ability for nutrients to stimulate an increase in levels of circulating cholecystokinin was impaired in mice fed WD for eight weeks

when compared to lean control mice, and pharmacological inhibition of overactive eCB signaling at peripheral CB_1Rs in mice fed WD restored the ability for nutrients to induce release of cholecystokinin [16]. Furthermore, the appetite-suppressing effects of peripheral CB_1R inhibition in mice maintained on WD were attenuated by co-treatment with an antagonist for cholecystokinin-A receptors [16], which are expressed by sensory vagal neurons and other organs [40]. Collectively, these studies suggest that eCB signaling in upper small-intestinal epithelium is dysregulated in WD-induced obese mice and promotes overeating by a mechanism that includes blocking nutrient-induced gut-brain satiation signaling.

In the current study, we used a novel conditional intestinal epithelium-specific CB_1R-deficient mouse model to investigate the necessity for intestinal CB_1Rs in preferences for WD.

Materials & Methods

Animals

C57BL/6 male mice (Taconic, Oxnard, CA, USA) or transgenic mice (described below in Transgenic Mouse Generation) 8-10 weeks of age were group-housed with ad-libitum access to standard rodent laboratory diet (SD; Teklad 2020x, Envigo, Huntingdon, UK; 16% kcal from fat, 24% kcal from protein, 60% kcal from carbohydrates) and water throughout all experiments. Mice were maintained on a12-h dark/light cycle beginning at 1800 h. 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 (IACUC) of the University of California, Riverside.

Transgenic Mouse Generation

Conditional intestinal epithelium-specific CB_1R-deficient mice (Cnr1tm1.1 mrl/vil-cre ERT2) were generated by crossing Cnr1-floxed mice (Cnr1tm1.1 mrl; Taconic, Oxnard, CA, USA; Model # 7599) with Vil-CRE ERT2 mice donated by Dr. Randy Seeley (University of Michigan, Ann Arbor, MI, USA) with permission from Dr. Sylvie Robin (Curie Institute, Paris, France). Cre recombinase expression in the intestinal epithelium is driven by the villin promotor, which allows for conditional tamoxifen-dependent Cre recombinase action to remove the Cnr1 gene from these cells, as described by el Marjou et al., [41]. Cnr1tm1.1 mrl/vil-cre ERT2 mice used in these experiments are referred to as $IntCB_1$-/-, and Cnr1tm1.1 mrl control mice (lacking Cre recombinase) are referred to as $IntCB_1$+/+. Tail snips were collected from pups at weaning and DNA was extracted and analyzed by conventional PCR using the following primers (5'-3'): GCAGGGATTATGTCCCTAGC (CNR1-ALT), CTGTTACCAGGAGTCTTAGC (1415-35), GGCTCAAGGAATACACTTATACC (1415-37), GAACCTGATGGACATGTTCAGG (vilcre, AA), AGTGCGTTCGAACGCTAGAGCCTGT (vilcre, SS), TTACGTCCATCGTGG-ACAGC (vilcre, MYO F), TGGGCTGGGTGTTAGCCTTA (vilcre, MYO R).

Western Diet Preference Test

Mice were single-housed in two-hopper feeding chambers (TSE Systems, Chesterfield, MO, USA) for five days to acclimate, and received ad-libitum access to SD and water throughout behavioral testing. At the time of testing, mice were given access for the first time to the hopper containing Western Diet (WD; Research Diets D12709B, New Brunswick, NJ, USA; 40% kcal from fat, 17% kcal from protein, 43% kcal from carbohydrates as mostly sucrose). Preferences for WD versus SD (% total kcals from WD), total caloric intake of each diet (kcals), and average meal size of each diet (kcals) were calculated every minute across the testing period, beginning one hour before dark cycle (1700 h).

Chemical Preparation and Administration

$IntCB_1$-/- and $IntCB_1$+/+ mice were administered tamoxifen (IP, 40 mg per kg) every 24 h for five consecutive days. Tamoxifen (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in corn oil at a concentration of 10 mg per mL then stored at $37^{o}C$ protected from light until administration. Tamoxifen in corn oil was placed in a bath sonicator for 10 minutes prior to administration. Mice were group housed in disposable cages throughout the injection window and for a 3-day post-injection period. The CB_1R antagonist/inverse agonist, AM251 (Tocris, Minneapolis, MN, USA), was administered (IP 3 mg per kg per 2 mL) 30 minutes prior to testing. Vehicle consisted of 7.5% dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA), 7.5% Tween 80 (Chem Implex Intl Inc., Wood Dale, IL, USA), and 85% sterile saline.

Immunohistochemistry

Proximal small intestinal tissue was collected from $IntCB_1$-/- and $IntCB_1$+/+ control mice 7 days after the completion of tamoxifen schedule. Tissue was flushed with ice-cold 4% paraformaldehyde/PBS, then fixed for 4 hours at 4^oC. Cross sections of the upper small intestine were cut and frozen in OCT (Fisher Healthcare, Chino, CA, USA) on dry ice. Approximately 16 μm sections were obtained using a cryostat (Leica, Wetzlar, Germany) then mounted onto charged glass slides. Sections were permeabilized with 0.5% Tween20/PBS and then blocked with 0.1% Tween20 in casein solution (Thermo Fisher, Waltham, MA, USA). Primary antibodies for CB_1Rs (kindly provided by Dr. Ken Mackie, Indiana University, USA) raised in rabbit were diluted 1:500 in blocking buffer, slides were incubated for 1 h at room temperature. Sections were washed three times with 0.1% Tween20/PBS solution then incubated for 1 h at room temperature with goat anti-rabbit secondary antibodies conjugated with alexafluor 647. Following repeated washes, coverslips were mounted with Prolong Gold Antifade reagent with DAPI (Thermo Fisher) for nuclear counterstaining. Images were obtained at room temperature using an Axio Observer Z1 Inverted Microscope (Zeiss, Oberkochen, Germany) as previously described [16].

Gene Expression

Total RNA from intestinal epithelium tissue was extracted using RNeasy kit (Qiagen, Valencia, CA, USA) and first-strand cDNA was generated using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Areas used for tissue

collection and processing were sanitized with 70% ethanol solution then treated with RNAse inhibitor (RNAse out, G-Biosciences, St. Louis, MO, USA). Reverse transcription of total RNA was performed as previously described [16]. Quantitative RT-PCR was performed using PrimePCR Assays (Biorad, Irvine, CA, USA) with primers for CB_1R (Cnr1), CB2R (Cnr2), g-protein coupled receptor 55 (Gpr55), diacylglycerol lipase alpha (Dagla), diacylglycerol lipase beta (Daglb), monoacylglycerol lipase (Mgll), alpha beta hydrolase domain containing 6 (Abhd6), N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (Napepld), and fatty acid amide hydrolase (Faah) gene transcripts under preconfigured SYBR Green assays (Biorad, Irvine, CA, USA). Hprt was used as a housekeeping gene for stomach, duodenum intestinal epithelium, jejunum intestinal epithelium, ileum intestinal epithelium, small-intestinal submucosa/muscle/serosal layer, large intestinal epithelium, and liver; □-actin (Actb) as housekeeping gene for pancreas; and □2-microglobulin (B2m) as housekeeping gene for epididymal fat. Reactions were run in triplicates and values are expressed as relative mRNA expression.

<u>Statistical Analysis</u>

Data were analyzed by GraphPad Prism 8 software using unpaired Student's t-tests (two-tailed) or two-way ANOVA with Holm-Sidak's multiple comparisons post-hoc test when appropriate. Results are expressed as means ± S.E.M. and significance was determined at $p<0.05$.

Results

Systemic Pharmacological Blockade of CB_1Rs Reduces Acute Preferences for Western Diet in Mice

We investigated roles for cannabinoid CB_1 receptors in preferences for Western Diet (WD). Naïve mice maintained on ad-libitum standard laboratory chow diet (SD) were administered vehicle or the cannabinoid CB_1R antagonist/inverse agonist, AM251, and subjected to a 24 h preference test for WD versus SD. Vehicle-treated mice displayed robust preferences for WD when compared to SD, an effect inhibited by AM251 by 3 h (Fig 1a, from 84.7 ± 7.1% total kcals from WD in vehicle-treated mice to 58.7 ± 8.3% in AM251-treated mice; $p = 0.042$), 6 h (Fig 1b, from 89.1 ± 3.7% total kcals from WD in vehicle-treated mice to 64.0 ± 7.4% in AM251-treated mice; $p = 0.012$), and 12 h (Fig 1c from 92.4 ± 2.8% total kcals from WD in vehicle-treated mice to 72.2 ± 7.2% in AM251-treated mice; $p = 0.028$) after initiation of the preference test. AM251 had no significant effect on WD preferences by 24 h after administration (Fig 1d, from 86.9 ± 3.1% total kcals from WD in vehicle-treated mice to 75.0 ± 6.7% in AM251-treated mice; $p = 0.176$).

Consistent with these data, vehicle-treated mice ate significantly more kcals from WD than from SD by 3 h (Fig 1e, $p = 0.046$), 6 h (Fig 1f, $p = 0.014$), 12 h (Fig 1g, $p = 0.002$), and 24 h (Fig 1h, $p<0.001$) after initiation of the preference test. These effects were absent in mice treated with AM251 by 3 h (Fig 1e, $p = 0.951$), 6 h (Fig 1f, $p = 0.679$), and 12 h (Fig 1g, $p = 0.244$); however, mice consumed

more WD than SD by 24 h (Fig 1g, $p = 0.021$), regardless of treatment. Moreover, vehicle-treated mice displayed larger meal sizes of WD versus SD by 3 h (Fig 1i, $p = 0.028$), 6 h (Fig 1j, $p = 0.018$), 12 h (Fig 1k, $p = 0.009$), and 24 h after initiation of the preference test (Fig 1l, $p = 0.006$). These effects were absent in mice treated with AM251 by 3 h (Fig 1i, $p = 0.816$), 6 h (Fig 1j, $p = 0.827$), 12 h (Fig 1k, $p = 0.803$), and 24 h (Fig 1l, $p = 0.939$). In addition, there were no significant differences in total cumulative caloric intake (i.e., total kcals from WD+SD) between treatment groups by 3 h (Fig 1m, $p = 0.501$), 6 h (Fig 1n, $p = 0.444$), 12 h (Fig 1o, $p = 0.734$), and 24 h after initiation of the preference test (Fig 1p, $p = 0.151$). Collectively, these results suggest that cannabinoid CB_1Rs control acute preferences for WD in mice.

Acute Preferences for Western Diet are Absent in Mice with CB_1R Deletion in the Intestinal Epithelium

Endocannabinoid signaling in the rodent upper small-intestinal epithelium is important for consumption of dietary fats based on their taste properties [14,15], re-feeding after a fast [10], and hyperphagia in a mouse model of WD-induced obesity via a mechanism that includes blocking nutrient-induced gut-brain satiation signaling [16,17]. We used our novel intestinal epithelium-specific conditional CB_1R-deficient mice (IntCB_1-/-) to probe the necessity for CB_1Rs in the intestinal epithelium in preferences for WD. Moreover, AM251 is reported to have some off-target effects [42,43]; therefore, this mouse model allows for direct evaluation of roles for CB_1Rs in the intestinal epithelium in these processes. CB_1R deficiency in

the intestinal epithelium of IntCB$_1$-/- mice was verified by immunohistochemistry (Figure 2a-d).

CB$_1$R deficiency in the intestinal epithelium of IntCB$_1$-/- mice was further confirmed by qRT-PCR (Fig 3a, b). IntCB$_1$-/- mice, when compared to IntCB$_1$+/+ controls, were deficient in expression of mRNA for CB$_1$Rs (Cnr1) in the jejunum epithelium (Fig 3a, $p = 0.031$). Expression of mRNA for other components of the endocannabinoid system in the jejunal epithelium were unaffected, including cannabinoid CB2Rs (Cnr2; Fig 3a, $p = 0.892$), G-protein coupled receptor 55 (Gpr55; Fig 3a, $p = 0.736$), diacylglycerol lipase alpha (Dagla; Fig 3a, $p = 0.825$), diacylglycerol lipase beta (Daglb; Fig 3a, $p = 0.798$), monoacylglycerol lipase (Mgll; Fig 3a, $p = 0.872$), alpha beta hydrolase domain containing 6 (Abhd6; Fig 3a, $p = 0.314$), N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (Napepld; Fig 3a, $p = 0.217$), and fatty acid amide hydrolase (Faah; Fig 3a, $p = 0.986$). In addition to the jejunum epithelium, IntCB$_1$-/- mice were deficient in expression of mRNA for CB$_1$Rs (Cnr1) in the duodenum epithelium (Fig 3b, $p = 0.009$), ileum epithelium (Fig 3b, $p = 0.038$), large intestine epithelium (Fig 3b, $p = 0.039$), but not in the small-intestinal submucosa/muscle/serosal layers (Fig 3b, $p = 0.633$), stomach (Fig 3b, $p = 0.602$), liver (Fig 3b, $p = 0.593$) , pancreas (Fig 3b, $p = 0.9$), and epididymal fat (Fig 3b, $p = 0.14$).

IntCB$_1$-/- and IntCB$_1$+/+ control mice displayed similar body weights (Fig 4a, $p = 0.404$), and baseline 24-h caloric intake (Fig 4b, $p = 0.52$), 24-h water intake (Fig 4c, $p = 0.487$), average meal size (Fig 4d, $p = 0.653$), ambulation (Fig 4e, p =

0.741), and glucose clearance during an oral glucose tolerance test (Fig 4f-g; 15, 30, 60, 120 min, ns; total area under curve, $p = 0.847$). Control IntCB$_1$+/+ mice displayed robust preferences for WD when compared to SD, an effect largely absent in IntCB$_1$-/- mice by 3 h (Fig 5a, from 92.8 ± 2.8% total kcals from WD in IntCB$_1$+/+ mice to 46.5 ± 12.5% in IntCB$_1$-/- mice; $p = 0.029$), 6 h (Fig 5b, from 95.0 ± 2.0% total kcals from WD in IntCB$_1$+/+ mice to 49.9 ± 13.8% in IntCB$_1$-/- mice; $p = 0.048$), and 12 h (Fig 5c, from 94.3 ± 0.2% total kcals from WD in IntCB$_1$+/+ mice to 72.2 ± 6.8% in IntCB$_1$-/- mice; $p = 0.049$) after initiation of the preference test. Preferences for WD in IntCB$_1$-/- mice were not significantly different from controls by 24 h (Fig 5d, from 94.9 ± 1.1% total kcals from WD in IntCB$_1$+/+ mice to 76.3 ± 6.3% in IntCB$_1$-/- mice; $p = 0.069$). Congruent with these data, control IntCB$_1$+/+ mice ate significantly more kcals from WD when compared to SD by 3 h (Fig 5e, $p = 0.02$), 6 h (Fig 5f, $p = 0.002$), 12 h (Fig 5g, $p<0.001$), and 24 h (Fig 5h, $p<0.001$) after initiation of the preference test. These effects were absent in IntCB$_1$-/- mice by 3 h (Fig 5e, $p = 0.732$, ns), 6 h (Fig 5f, $p = 0.92$), and 12 h (Fig 5g, $p = 0.06$); however, IntCB$_1$-/- mice consumed more WD than SD by 24 h (Fig 5g, $p = 0.003$). Moreover, IntCB$_1$+/+ mice consumed larger meal sizes of WD versus SD by 3 h (Fig 5i, $p = 0.031$), 6 h (Fig 5j, $p = 0.009$), 12 h (Fig 5k, $p<0.001$), and 24 h after initiation of the preference test (Fig 5l, $p<0.001$). These effects were absent in IntCB$_1$-/- mice by 3 h (Fig 5i, $p = 0.404$), 6 h (Fig 5j, $p = 0.387$), 12 h (Fig 5k, $p = 0.076$), and 24 h (Fig 5l, $p = 0.149$). In addition, there were no significant changes in total cumulative caloric intake (i.e., total kcals from WD+SD) for IntCB$_1$-/- mice

when compared to $IntCB_1$+/+ controls by 3 h (Fig 5m, p = 0.196), 6 h (Fig 5n, p = 0.105), 12 h (Fig 5o, p = 0.111), and 24 h after initiation of the preference test (Fig 5p, p = 0.051).

Discussion

We report that acute preferences for WD (i) were inhibited by global pharmacological blockade of CB_1Rs, and (ii) were largely absent in mice conditionally deficient in CB_1Rs selectively in the intestinal epithelium. These results suggest that CB_1Rs in the intestinal epithelium are required for acute WD preferences in mice. Moreover, these studies expand our understanding of critical pathways for gut-brain communication in the control of preferences for palatable foods.

Dietary components are detected by receptors located throughout the oral cavity [36] and intestinal epithelium [44], which provide feedback associated with the nutritional content of food and contribute to determination of food preferences. For example, we reported that tasting dietary unsaturated lipids – but not sugar or protein – triggered production of endocannabinoids in the rat upper small-intestinal epithelium, and pharmacological inhibition of endocannabinoid signaling at CB_1Rs in the periphery blocked intake and preferences for fats in a sham-feeding model [14,15]. These studies suggest that endocannabinoid signaling in the gut contributes to the positive feedback control of fat intake based on its unique taste properties. Despite localized increases of endocannabinoids selectively in the upper small-intestinal epithelium and blockade of intake following pharmacological

treatment with a peripherally-restricted neutral CB_1R antagonist, these studies were limited in ability to identify necessity for CB_1Rs in the intestinal epithelium in food intake and dietary preferences. To overcome these challenges and examine whether CB_1Rs in the small-intestinal epithelium were required for WD preferences, we generated a novel conditional intestinal epithelium-specific CB_1R-deficient mouse. Preferences for WD compared to a low-fat, no-sucrose, chow were largely absent in $IntCB_1$-/- mice when compared to $IntCB_1$+/+ control mice during the first 12 h of preference testing. Moreover, these effects were mimicked by systemic treatment with the globally acting CB_1R antagonist/inverse agonist, AM251, in wild-type mice. Collectively, these results provide evidence of a critical role for CB_1Rs in the rodent intestinal epithelium in acute preferences for food containing high levels of fats and sugars.

The specific mechanism(s) underlying intestinal epithelium CB_1R-mediated preferences for WD are unknown, but may include CB_1R control of gut-brain signaling. We reported that hyperphagia and increased meal size associated with WD-induced obesity in mice are dependent on (i) elevated levels of endocannabinoids in the upper small-intestinal epithelium; and (ii) CB_1R-mediated inhibition of nutrient-induced signaling of the satiation peptide, cholecystokinin [16]. Cholecystokinin is secreted from enteroendocrine cells in the upper small-intestinal epithelium when nutrients arrive in the lumen, and transmits satiation signals to the brain by interacting with cholecystokinin A receptors on the afferent vagus nerve [30,40,45-48] and possibly the brain [49,50]. Bohorquez and

colleagues recently characterized enteroendocrine cells (i.e., neuropods) in the mouse intestinal epithelium that form functional synapses with afferent vagal fibers [33]. Neuropods sense nutrients in the lumen and in response, release glutamate and cholecystokinin, which activate afferent vagal neurons in a coordinated manner [51]. Moreover, afferent vagal neurons participate in reward-related behaviors – including flavor and place preferences – and control dopamine outflow in the mouse striatum [48]. Notably, however, studies suggest that the afferent vagus nerve is required for nutrient-induced negative feedback from the gut associated with satiation and satiety, but is dispensable for positive feedback [i.e., appetition [52]] associated with nutrient reinforcement and flavor-nutrient preference conditioning [53]. Nonetheless, it is possible that CB_1Rs in the intestinal epithelium participate in preferences for WD by a mechanism that includes control of nutrient-induced, neuropod-mediated, afferent vagal activity and recruitment of brain reward circuits. A direct test of this hypothesis and evaluation of distinct roles for intestinal CB_1Rs in satiation versus appetition remain for future studies.

We propose that CB_1Rs indirectly regulate afferent vagal activity by controlling nutrient sensing and release of satiation peptides from enteroendocrine cells in the small-intestinal epithelium that directly interact with the afferent vagus nerve [16,39]. Recent studies also suggest that CB_1Rs in the mouse stomach participate in alcohol intake by controlling formation of the appetite-stimulating hormone, ghrelin, which interacts with ghrelin receptors on afferent vagal fibers [54]. In addition to these indirect mechanisms, CB_1Rs may also directly control

afferent vagal neurotransmission and food intake [55]. For example, Burdyga and colleagues reported that fasting was associated with increased expression of CB_1Rs in the rat vagal afferent neurons [56]. Refeeding or administration of cholecystokinin rapidly reversed fasting-induced expression of CB_1Rs [56], which was also blunted in rats maintained on a high-fat diet [57]. In addition, administration of ghrelin blocked the effects of refeeding on CB_1R expression [58]. Moreover, Christie and colleagues reported that low and high concentrations of methanandamide – a stable analog of anandamide – differentially modified mechanosensitivity of mouse gastric vagal afferents in vitro via a mechanism that included CB_1Rs, TRPV1, and ghrelin receptors [59], and these effects were dysregulated in mice fed a high-fat diet for 12 weeks [60]. These studies suggest that CB_1Rs on the afferent vagus nerve may participate in gut-brain signaling important for food intake and energy balance. Interestingly, mice with genetic deletion of CB_1Rs on afferent vagal neurons displayed no changes in body weight or food intake, irrespective of test diet (i.e., standard versus high-fat), which suggests that vagal CB_1Rs may not be necessary for long-term maintenance of body weight and feeding [61]. Further investigations are necessary to expand our understanding of physiological roles for the endocannabinoid system in vagal afferent neurons.

It is noteworthy that attenuation of preferences for WD were limited to the first 12 h in, both, AM251-treated wild-type mice and $IntCB_1$-/- mice when compared to vehicle and $IntCB_1$+/+ mice, respectively. It is plausible that restricted

temporal effects of AM251 reflect the pharmacokinetic properties of this compound, which displays a half-life of 22 h in rats [62]. IntCB_1-/- mice, however, displayed a similar restriction of preferences for WD to the first 12 h of the test. The mechanism(s) in this restricted response remains unknown. Post-prandial cues at later time-points, however, may provide compensatory feedback and reinforcement, and restore preferences for WD in the absence of CB_1Rs in the intestinal epithelium. One candidate in this proposed mechanism is the satiety factor oleoylethanolamide, which is synthesized in the intestinal epithelium from dietary fats and controls food intake and possibly reward through a mechanism that requires peroxisome proliferator-activated receptor (PPARα) and the afferent vagus [63-65]. Studies examining interactions between orexigenic endocannabinoid and anorexic oleoylethanolamide signaling pathways in acute and long-term dietary preferences remain for future inquiry.

Conclusion

In summary, these studies extend our understanding beyond central roles for the endocannabinoid system in intake and reward value of palatable food [66-82], and provide evidence that CB_1Rs in the intestinal epithelium are an integral component of a gut-brain axis that controls dietary preferences. Future studies will be important to elucidate (i) specific mechanism(s) of intestinal CB_1R-mediated preferences for palatable food, (ii) roles for CB_1Rs in the intestinal epithelium in recruitment of brain reward circuits and the “wanting” or “liking” of palatable food [83], (iii) roles for intestinal CB_1Rs in satiation versus appetition, (iv) interactions

between CB_1R and PPARα signaling pathways in preferences for palatable food, (v) roles CB_1Rs in the intestinal epithelium in development and maintenance of diet-induced obesity, and (vi) physiological roles for CB_1Rs on vagal neurons.

Author Disclosure Statement: The authors declare no conflicts of interest.

References

1. Levine, A.S.; Kotz, C.M.; Gosnell, B.A. Sugars and fats: the neurobiology of preference. J Nutr 2003, 133, 831S-834S.

2. Lau, B.K.; Cota, D.; Cristino, L.; Borgland, S.L. Endocannabinoid modulation of homeostatic and non-homeostatic feeding circuits. Neuropharmacology 2017, 124, 38-51.

3. Rossi, M.A.; Stuber, G.D. Overlapping Brain Circuits for Homeostatic and Hedonic Feeding. Cell Metab 2018, 27, 42-56.

4. DiPatrizio, N.V.; Piomelli, D. The thrifty lipids: endocannabinoids and the neural control of energy conservation. Trends Neurosci 2012, 35, 403-411.

5. Di Marzo, V.; Ligresti, A.; Cristino, L. The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. Int J Obes (Lond) 2009, 33 Suppl 2, S18-24.

6. Gomez, R.; Navarro, M.; Ferrer, B.; Trigo, J.M.; Bilbao, A.; Del Arco, I.; Cippitelli, A.; Nava, F.; Piomelli, D.; Rodriguez de Fonseca, F. A peripheral mechanism for CB_1 cannabinoid receptor-dependent modulation of feeding. J Neurosci 2002, 22, 9612-9617.

7. Izzo, A.A.; Sharkey, K.A. Cannabinoids and the gut: New developments and emerging concepts. Pharmacology & therapeutics 2010, 126, 21-38.

8. Randall, P.A.; Vemuri, V.K.; Segovia, K.N.; Torres, E.F.; Hosmer, S.; Nunes, E.J.; Santerre, J.L.; Makriyannis, A.; Salamone, J.D. The novel cannabinoid CB_1 antagonist AM6545 suppresses food intake and food-reinforced behavior. Pharmacol Biochem Behav 2010, 97, 179-184.

9. Cluny, N.L.; Vemuri, V.K.; Chambers, A.P.; Limebeer, C.L.; Bedard, H.; Wood, J.T.; Lutz, B.; Zimmer, A.; Parker, L.A.; Makriyannis, A., et al. A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. Br J Pharmacol 2011, 161, 629-642.

10. DiPatrizio, N.V.; Igarashi, M.; Narayanaswami, V.; Murray, C.; Gancayco, J.; Russell, A.; Jung, K.M.; Piomelli, D. Fasting stimulates 2-AG biosynthesis in the small intestine: role of cholinergic pathways. Am J Physiol Regul Integr Comp Physiol 2015, 309, R805-813.

11. Tam, J.; Vemuri, V.K.; Liu, J.; Batkai, S.; Mukhopadhyay, B.; Godlewski, G.; Osei-Hyiaman, D.; Ohnuma, S.; Ambudkar, S.V.; Pickel, J., et al. Peripheral CB_1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. The Journal of clinical investigation 2010, 120, 2953-2966.

12. Tam, J.; Szanda, G.; Drori, A.; Liu, Z.; Cinar, R.; Kashiwaya, Y.; Reitman, M.L.; Kunos, G. Peripheral cannabinoid-1 receptor blockade restores hypothalamic leptin signaling. Molecular metabolism 2017, 6, 1113-1125.

13. Bellocchio, L.; Soria-Gomez, E.; Quarta, C.; Metna-Laurent, M.; Cardinal, P.; Binder, E.; Cannich, A.; Delamarre, A.; Haring, M.; Martin-Fontecha, M., et al. Activation of the sympathetic nervous system mediates hypophagic and anxiety-like effects of CB_1 receptor blockade. Proceedings of the National Academy of Sciences of the United States of America 2013, 110, 4786-4791.

14. DiPatrizio, N.V.; Astarita, G.; Schwartz, G.; Li, X.; Piomelli, D. Endocannabinoid signal in the gut controls dietary fat intake. Proceedings of the National Academy of Sciences of the United States of America 2011, 108, 12904-12908.

15. DiPatrizio, N.V.; Joslin, A.; Jung, K.M.; Piomelli, D. Endocannabinoid signaling in the gut mediates preference for dietary unsaturated fats. Faseb J 2013, 27, 2513-2520.

16. Argueta, D.A.; Perez, P.A.; Makriyannis, A.; DiPatrizio, N.V. Cannabinoid CB_1 Receptors Inhibit Gut-Brain Satiation Signaling in Diet-Induced Obesity. Front Physiol 2019, 10, 704.

17. Argueta, D.A.; DiPatrizio, N.V. Peripheral endocannabinoid signaling controls hyperphagia in western diet-induced obesity. Physiol Behav 2017, 171, 32-39.

18. Niki, M.; Jyotaki, M.; Yoshida, R.; Yasumatsu, K.; Shigemura, N.; DiPatrizio, N.V.; Piomelli, D.; Ninomiya, Y. Modulation of sweet taste sensitivities by endogenous leptin and endocannabinoids in mice. The Journal of physiology 2015, 593, 2527-2545.

19. Ruiz de Azua, I.; Mancini, G.; Srivastava, R.K.; Rey, A.A.; Cardinal, P.; Tedesco, L.; Zingaretti, C.M.; Sassmann, A.; Quarta, C.; Schwitter, C., et al. Adipocyte cannabinoid receptor CB_1 regulates energy homeostasis and alternatively activated macrophages. The Journal of clinical investigation 2017, 127, 4148-4162.

20. Simon, V.; Cota, D. MECHANISMS IN ENDOCRINOLOGY: Endocannabinoids and metabolism: past, present and future. Eur J Endocrinol 2017, 176, R309-R324.

21. Monteleone, P.; Piscitelli, F.; Scognamiglio, P.; Monteleone, A.M.; Canestrelli, B.; Di Marzo, V.; Maj, M. Hedonic eating is associated with increased peripheral levels of ghrelin and the endocannabinoid 2-arachidonoyl-glycerol in healthy humans: a pilot study. J Clin Endocrinol Metab 2012, 97, E917-924.

22. Price, C.A.; Argueta, D.A.; Medici, V.; Bremer, A.A.; Lee, V.; Nunez, M.V.; Chen, G.X.; Keim, N.L.; Havel, P.J.; Stanhope, K.L., et al. Plasma fatty acid ethanolamides are associated with postprandial triglycerides, ApoCIII, and ApoE in humans consuming a high-fructose corn syrup-sweetened beverage. Am J Physiol Endocrinol Metab 2018, 315, E141-E149.

23. Little, T.J.; Cvijanovic, N.; DiPatrizio, N.V.; Argueta, D.A.; Rayner, C.K.; Feinle-Bisset, C.; Young, R.L. Plasma endocannabinoid levels in lean, overweight, and obese humans: relationships to intestinal permeability markers, inflammation, and incretin secretion. Am J Physiol Endocrinol Metab 2018, 315, E489-E495.

24. Engeli, S.; Bohnke, J.; Feldpausch, M.; Gorzelniak, K.; Janke, J.; Batkai, S.; Pacher, P.; Harvey-White, J.; Luft, F.C.; Sharma, A.M., et al. Activation of the peripheral endocannabinoid system in human obesity. Diabetes 2005, 54, 2838-2843.

25. Bluher, M.; Engeli, S.; Kloting, N.; Berndt, J.; Fasshauer, M.; Batkai, S.; Pacher, P.; Schon, M.R.; Jordan, J.; Stumvoll, M. Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. Diabetes 2006, 55, 3053-3060.

26. Cote, M.; Matias, I.; Lemieux, I.; Petrosino, S.; Almeras, N.; Despres, J.P.; Di Marzo, V. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. Int J Obes (Lond) 2007, 31, 692-699.

27. Di Marzo, V.; Cote, M.; Matias, I.; Lemieux, I.; Arsenault, B.J.; Cartier, A.; Piscitelli, F.; Petrosino, S.; Almeras, N.; Despres, J.P. Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with changes in metabolic risk factors. Diabetologia 2009, 52, 213-217.

28. Matias, I.; Gatta-Cherifi, B.; Tabarin, A.; Clark, S.; Leste-Lasserre, T.; Marsicano, G.; Piazza, P.V.; Cota, D. Endocannabinoids measurement in human saliva as potential biomarker of obesity. PLoS One 2012, 7, e42399.

29. Hillard, C.J. Circulating Endocannabinoids: From Whence Do They Come and Where are They Going? Neuropsychopharmacology 2017, 43, 155-72.

30. Steinert, R.E.; Feinle-Bisset, C.; Asarian, L.; Horowitz, M.; Beglinger, C.; Geary, N. Ghrelin, CCK, GLP-1, and PYY(3-36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. Physiol Rev 2017, 97, 411-463.

31. Schwartz, G.J. Roles for gut vagal sensory signals in determining energy availability and energy expenditure. Brain Res 2018, 1693, 151-153.

32. DiPatrizio, N.V.; Piomelli, D. Intestinal lipid-derived signals that sense dietary fat. The Journal of clinical investigation 2015, 125, 891-898.

33. Kaelberer, M.M.; Rupprecht, L.E.; Liu, W.W.; Weng, P.; Bohorquez, D.V. Neuropod Cells: The Emerging Biology of Gut-Brain Sensory Transduction. Annual review of neuroscience 2020, 43, 337-353.

34. Berthoud, H.R. The vagus nerve, food intake and obesity. Regul Pept 2008, 149, 15-25.

35. Dockray, G.J. Enteroendocrine cell signalling via the vagus nerve. Curr Opin Pharmacol 2013, 13, 954-958.

36. Roper, S.D. Taste buds as peripheral chemosensory processors. Seminars in cell & developmental biology 2013, 24, 71-79.

37. Sclafani, A. From appetite setpoint to appetition: 50years of ingestive behavior research. Physiol Behav 2018, 192, 210-217.

38. Raybould, H.E. Gut chemosensing: interactions between gut endocrine cells and visceral afferents. Auton Neurosci 2010, 153, 41-46.

39. Sykaras, A.G.; Demenis, C.; Case, R.M.; McLaughlin, J.T.; Smith, C.P. Duodenal enteroendocrine I-cells contain mRNA transcripts encoding key endocannabinoid and fatty acid receptors. PLoS ONE 2012, 7, e42373.

40. Raybould, H.E. Mechanisms of CCK signaling from gut to brain. Curr Opin Pharmacol 2007, 7, 570-574.

41. El Marjou, F.; Janssen, K.P.; Chang, B.H.; Li, M.; Hindie, V.; Chan, L.; Louvard, D.; Chambon, P.; Metzger, D.; Robine, S. Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. Genesis 2004, 39, 186-193.

42. Henstridge, C.M. Off-target cannabinoid effects mediated by GPR55. Pharmacology 2012, 89, 179-187.

43. Seely, K.A.; Brents, L.K.; Franks, L.N.; Rajasekaran, M.; Zimmerman, S.M.; Fantegrossi, W.E.; Prather, P.L. AM-251 and rimonabant act as direct antagonists at mu-opioid receptors: implications for opioid/cannabinoid interaction studies. Neuropharmacology 2012, 63, 905-915.

44. Gribble, F.M.; Reimann, F. Enteroendocrine Cells: Chemosensors in the Intestinal Epithelium. Annu Rev Physiol 2016, 78, 277-299.

45. Smith, G.P.; Jerome, C.; Cushin, B.J.; Eterno, R.; Simansky, K.J. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science 1981, 213, 1036-1037.

46. Smith, G.P.; Jerome, C.; Norgren, R. Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. Am J Physiol 1985, 249, R638-641.

47. Schwartz, G.J.; Moran, T.H. CCK elicits and modulates vagal afferent activity arising from gastric and duodenal sites. Ann N Y Acad Sci 1994, 713, 121-128.

48. Han, W.; Tellez, L.A.; Perkins, M.H.; Perez, I.O.; Qu, T.; Ferreira, J.; Ferreira, T.L.; Quinn, D.; Liu, Z.W.; Gao, X.B., et al. A Neural Circuit for Gut-Induced Reward. Cell 2018, 175, 665-678.

49. Reidelberger, R.D.; Hernandez, J.; Fritzsch, B.; Hulce, M. Abdominal vagal mediation of the satiety effects of CCK in rats. Am J Physiol Regul Integr Comp Physiol 2004, 286, R1005-1012.

50. Ripken, D.; van der Wielen, N.; van der Meulen, J.; Schuurman, T.; Witkamp, R.F.; Hendriks, H.F.; Koopmans, S.J. Cholecystokinin regulates satiation independently of the abdominal vagal nerve in a pig model of total subdiaphragmatic vagotomy. Physiol Behav 2015, 139, 167-176.

51. Kaelberer, M.M.; Buchanan, K.L.; Klein, M.E.; Barth, B.B.; Montoya, M.M.; Shen, X.; Bohorquez, D.V. A gut-brain neural circuit for nutrient sensory transduction. Science 2018, 361.

52. Sclafani, A. Gut-brain nutrient signaling. Appetition vs. satiation. Appetite 2013, 71, 454-458.

53. Shechter, A.; Schwartz, G.J. Gut-brain nutrient sensing in food reward. Appetite 2018, 122, 32-35.

54. Godlewski, G.; Cinar, R.; Coffey, N.J.; Liu, J.; Jourdan, T.; Mukhopadhyay, B.; Chedester, L.; Liu, Z.; Osei-Hyiaman, D.; Iyer, M.R., et al. Targeting Peripheral CB_1 Receptors Reduces Ethanol Intake via a Gut-Brain Axis. Cell Metab 2019, 29, 1320-1333.

55. Burdyga, G.; Varro, A.; Dimaline, R.; Thompson, D.G.; Dockray, G.J. Expression of cannabinoid CB_1 receptors by vagal afferent neurons: kinetics and role in influencing neurochemical phenotype. Am J Physiol Gastrointest Liver Physiol 2010, 299, G63-69.

56. Burdyga, G.; Lal, S.; Varro, A.; Dimaline, R.; Thompson, D.G.; Dockray, G.J. Expression of cannabinoid CB_1 receptors by vagal afferent neurons is inhibited by cholecystokinin. J Neurosci 2004, 24, 2708-2715.

57. Cluny, N.L.; Baraboi, E.D.; Mackie, K.; Burdyga, G.; Richard, D.; Dockray, G.J.; Sharkey, K.A. High fat diet and body weight have different effects on cannabinoid CB(1) receptor expression in rat nodose ganglia. Auton Neurosci 2013, 179, 122-130.

58. Burdyga, G.; Varro, A.; Dimaline, R.; Thompson, D.G.; Dockray, G.J. Ghrelin receptors in rat and human nodose ganglia: putative role in regulating CB-1 and MCH receptor abundance. Am J Physiol Gastrointest Liver Physiol 2006, 290, G1289-1297.

59. Christie, S.; O'Rielly, R.; Li, H.; Wittert, G.A.; Page, A.J. Biphasic effects of methanandamide on murine gastric vagal afferent mechanosensitivity. The Journal of physiology 2020, 598, 139-150.

60. Christie, S.; O'Rielly, R.; Li, H.; Nunez-Salces, M.; Wittert, G.A.; Page, A.J. Modulatory effect of methanandamide on gastric vagal afferent satiety signals depends on nutritional status. The Journal of physiology 2020, 598, 2169-2182.

61. Vianna, C.R.; Donato, J., Jr.; Rossi, J.; Scott, M.; Economides, K.; Gautron, L.; Pierpont, S.; Elias, C.F.; Elmquist, J.K. Cannabinoid receptor 1 in the vagus nerve is dispensable for body weight homeostasis but required for normal gastrointestinal motility. J Neurosci 2012, 32, 10331-10337.

62. McLaughlin, P.J.; Winston, K.; Swezey, L.; Wisniecki, A.; Aberman, J.; Tardif, D.J.; Betz, A.J.; Ishiwari, K.; Makriyannis, A.; Salamone, J.D. The cannabinoid CB_1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. Behav Pharmacol 2003, 14, 583-588.

63. Fu, J.; Gaetani, S.; Oveisi, F.; Lo Verme, J.; Serrano, A.; Rodriguez De Fonseca, F.; Rosengarth, A.; Luecke, H.; Di Giacomo, B.; Tarzia, G., et al. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature 2003, 425, 90-93.

64. Schwartz, G.J.; Fu, J.; Astarita, G.; Li, X.; Gaetani, S.; Campolongo, P.; Cuomo, V.; Piomelli, D. The lipid messenger OEA links dietary fat intake to satiety. Cell Metab 2008, 8, 281-288.

65. Tellez, L.A.; Medina, S.; Han, W.; Ferreira, J.G.; Licona-Limon, P.; Ren, X.; Lam, T.T.; Schwartz, G.J.; de Araujo, I.E. A gut lipid messenger links excess dietary fat to dopamine deficiency. Science 2013, 341, 800-802.

66. Higuchi, S.; Irie, K.; Yamaguchi, R.; Katsuki, M.; Araki, M.; Ohji, M.; Hayakawa, K.; Mishima, S.; Akitake, Y.; Matsuyama, K., et al. Hypothalamic 2-arachidonoylglycerol regulates multistage process of high-fat diet preferences. PLoS One 2012, 7, e38609.

67. Higuchi, S.; Ohji, M.; Araki, M.; Furuta, R.; Katsuki, M.; Yamaguchi, R.; Akitake, Y.; Matsuyama, K.; Irie, K.; Mishima, K., et al. Increment of hypothalamic 2-arachidonoylglycerol induces the preference for a high-fat diet via activation of cannabinoid 1 receptors. Behav Brain Res 2011, 216, 477-480.

68. Deshmukh, R.R.; Sharma, P.L. Stimulation of accumbens shell cannabinoid CB(1) receptors by noladin ether, a putative endocannabinoid, modulates food intake and dietary selection in rats. Pharmacol Res 2012, 66, 276-282.

69. DiPatrizio, N.V.; Simansky, K.J. Activating parabrachial cannabinoid CB_1 receptors selectively stimulates feeding of palatable foods in rats. J Neurosci 2008, 28, 9702-9709.

70. DiPatrizio, N.V.; Simansky, K.J. Inhibiting parabrachial fatty acid amide hydrolase activity selectively increases the intake of palatable food via cannabinoid CB_1 receptors. Am J Physiol Regul Integr Comp Physiol 2008, 295, R1409-1414.

71. Mahler, S.V.; Smith, K.S.; Berridge, K.C. Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. Neuropsychopharmacology 2007, 32, 2267-2278.

72. Wei, D.; Lee, D.; Li, D.; Daglian, J.; Jung, K.M.; Piomelli, D. A role for the endocannabinoid 2-arachidonoyl-sn-glycerol for social and high-fat food reward in male mice. Psychopharmacology (Berl) 2016, 233, 1911-1919.

73. Mendez-Diaz, M.; Rueda-Orozco, P.E.; Ruiz-Contreras, A.E.; Prospero-Garcia, O. The endocannabinoid system modulates the valence of the emotion associated to food ingestion. Addict Biol 2012, 17, 725-735.

74. De Luca, M.A.; Solinas, M.; Bimpisidis, Z.; Goldberg, S.R.; Di Chiara, G. Cannabinoid facilitation of behavioral and biochemical hedonic taste responses. Neuropharmacology 2012, 63, 161-168.

75. Jarrett, M.M.; Scantlebury, J.; Parker, L.A. Effect of delta9-tetrahydrocannabinol on quinine palatability and AM251 on sucrose and quinine palatability using the taste reactivity test. Physiol Behav 2007, 90, 425-430.

76. Melis, T.; Succu, S.; Sanna, F.; Boi, A.; Argiolas, A.; Melis, M.R. The cannabinoid antagonist SR 141716A (Rimonabant) reduces the increase of extra-cellular dopamine release in the rat nucleus accumbens induced by a novel high palatable food. Neuroscience letters 2007, 419, 231-235.

77. Droste, S.M.; Saland, S.K.; Schlitter, E.K.; Rodefer, J.S. AM 251 differentially effects food-maintained responding depending on food palatability. Pharmacol Biochem Behav 2010, 95, 443-448.

78. South, T.; Deng, C.; Huang, X.F. AM 251 and beta-Funaltrexamine reduce fat intake in a fat-preferring strain of mouse. Behav Brain Res 2007, 181, 153-157.

79. Thornton-Jones, Z.D.; Vickers, S.P.; Clifton, P.G. The cannabinoid CB_1 receptor antagonist SR141716A reduces appetitive and consummatory responses for food. Psychopharmacology (Berl) 2005, 179, 452-460.

80. Feja, M.; Leigh, M.P.K.; Baindur, A.N.; McGraw, J.J.; Wakabayashi, K.T.; Cravatt, B.F.; Bass, C.E. The novel MAGL inhibitor MJN110 enhances responding to reward-predictive incentive cues by activation of CB_1 receptors. Neuropharmacology 2020, 162, 107814.

81. Salamone, J.D.; McLaughlin, P.J.; Sink, K.; Makriyannis, A.; Parker, L.A. Cannabinoid CB_1 receptor inverse agonists and neutral antagonists: effects on food intake, food-reinforced behavior and food aversions. Physiol Behav 2007, 91, 383-388.

82. Williams, C.M.; Kirkham, T.C. Anandamide induces overeating: mediation by central cannabinoid (CB_1) receptors. Psychopharmacology (Berl) 1999, 143, 315-317.

83. Castro, D.C.; Berridge, K.C. Advances in the neurobiological bases for food 'liking' versus 'wanting'. Physiol Behav 2014, 136, 22-30.

Figures

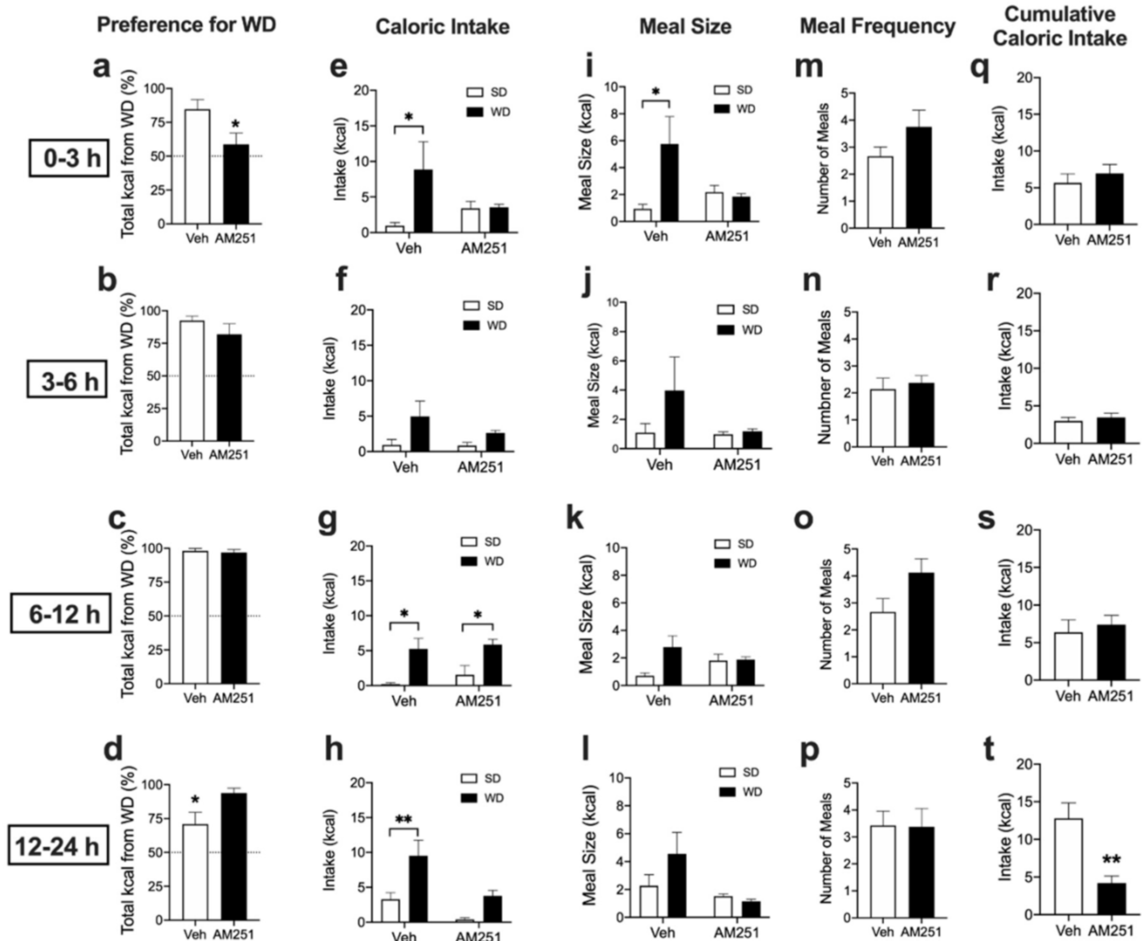


Figure 1.1. Cannabinoid CB_1Rs control acute preferences for Western Diet. When compared to vehicle treatment (Veh), preferences for western diet (WD) are reduced in AM251-treated animals for up to 12 hours (a-c), an effect absent after 24 hours (d). Similarly, cumulative intake of WD is increased versus SD in vehicle-treated mice, an effect absent in AM251-treated mice (e-g) that is regained in AM251-treated mice after 24 hours (h). Average meal size for WD, when compared to SD, is increased throughout the 24 hour preference test in Veh mice, an effect absent in AM251-treated mice (i-l). Total cumulative intakes were similar between treatment groups throughout all time points (m-p). Unpaired Student's t-test, two-tailed (a-d, m-p); Two-way ANOVA with Holm-Sidak's multiple comparison tests (e-l); * = p<0.05, ** = p<0.01, *** = p<0.001. Results are expressed as means ± S.E.M; n = 7-8 per condition.

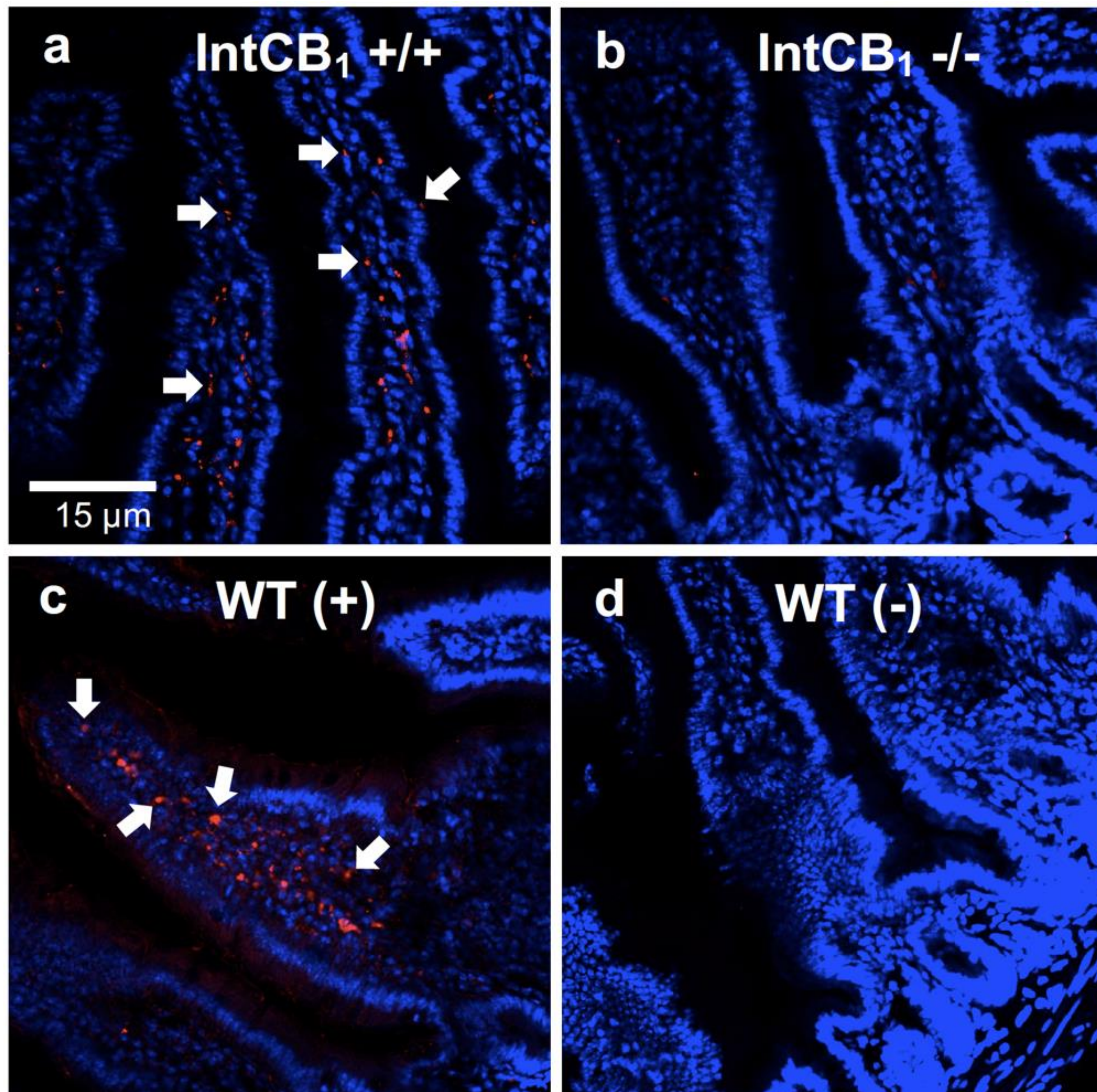


Figure 1.2. CB_1R immunoreactivity is absent in the upper small-intestinal epithelium of conditional intestinal epithelium-specific CB_1R-deficient mice.

When compared to control mice (a, IntCB +/+), conditional intestinal epithelium-specific CB_1R-null mice (b, $IntCB_1$ -/-) are deficient in immunoreactivity for CB_1Rs in the upper small-intestinal epithelium. Wild-type C57BL/6Tac mice display immunoreactivity for CB_1Rs in the upper small-intestinal epithelium [c, WT (+)], which is absent when the primary CB_1R antibody is not included [d, WT(-)]. White arrows point to representative red immunoreactivity for CB_1Rs. Red = CB_1R immunoreactivity; blue = DAPI. WT = wild-type mice. (+) = with CB_1R primary antibody; (-) = without CB_1R primary antibody.

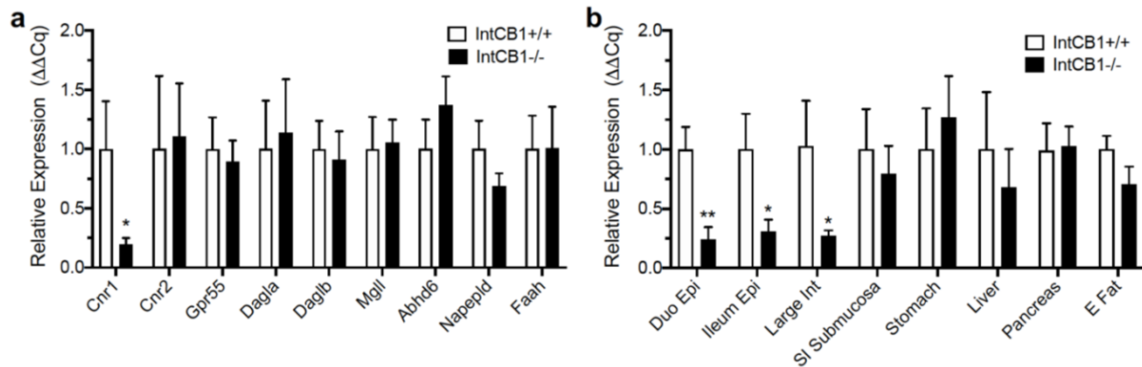
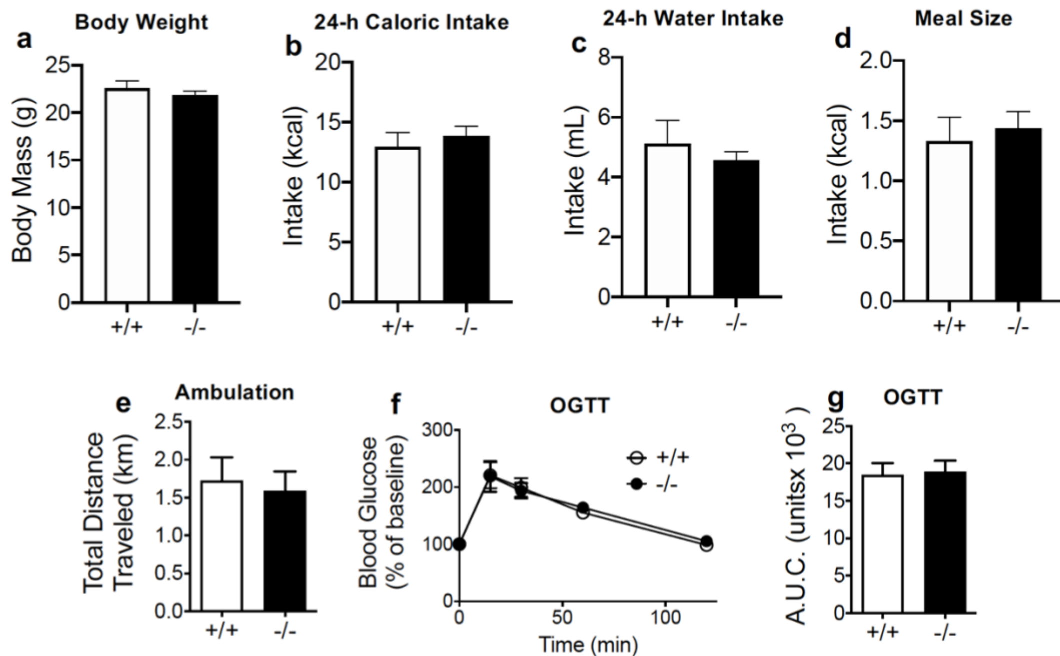


Figure 1.3. Expression of endocannabinoid system genes in conditional intestinal epithelium-specific CB_1R-deficient mice and controls.

Expression of cannabinoid CB_1Rs (Cnr1) was reduced in the jejunum epithelium of conditional intestinal epithelium-specific CB_1R deficient mice (IntCB_1-/-) when compared to control mice (IntCB_1+/+), and expression of mRNA for other components of the endocannabinoid system were unaffected, including cannabinoid CB2Rs (Cnr2), g-protein coupled receptor 55 (Gpr5), diacylglycerol lipase alpha (Dagla), diacylglycerol lipase beta (Daglb), monoacylglycerol lipase (Mgll), alpha beta hydrolase domain containing 6 (Abhd6), N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (Napepld), and fatty acid amide hydrolase (Faah)(a). IntCB_1-/- mice, when compared to IntCB_1+/+ controls, were deficient in expression of mRNA for CB_1Rs (Cnr1) in the duodenum epithelium (Duo Epi), ileum epithelium (Ileum Epi), large intestine (Large Int), but not in the small-intestinal submucosa/muscle/serosal layers (SI Submucosa), stomach, liver, pancreas, and epididymal fat (E Fat). Unpaired Student's t-tests, two-tailed; * = $p<0.05$, ** = $p<0.01$. Results are expressed as means ± S.E.M; n = 5-8 per condition.



<u>Figure 1.4. Conditional intestinal epithelium CB_1R-deficient mice display no changes in baseline feeding parameters, motor activity, or glucose clearance.</u> Unpaired Student's t-test, two-tailed (a-e, g; $p>0.05$); Two-way Repeated Measures ANOVA with Holm-Sidak's multiple comparison tests (f; not significant). Results are expressed as means ± S.E.M; n=7-8 per condition (a-e), n = 3-4 (f-g). +/+ = $IntCB_1$+/+ mice, -/- = $IntCB_1$-/- mice; OGTT = oral glucose tolerance test; AUC = area under the curve.

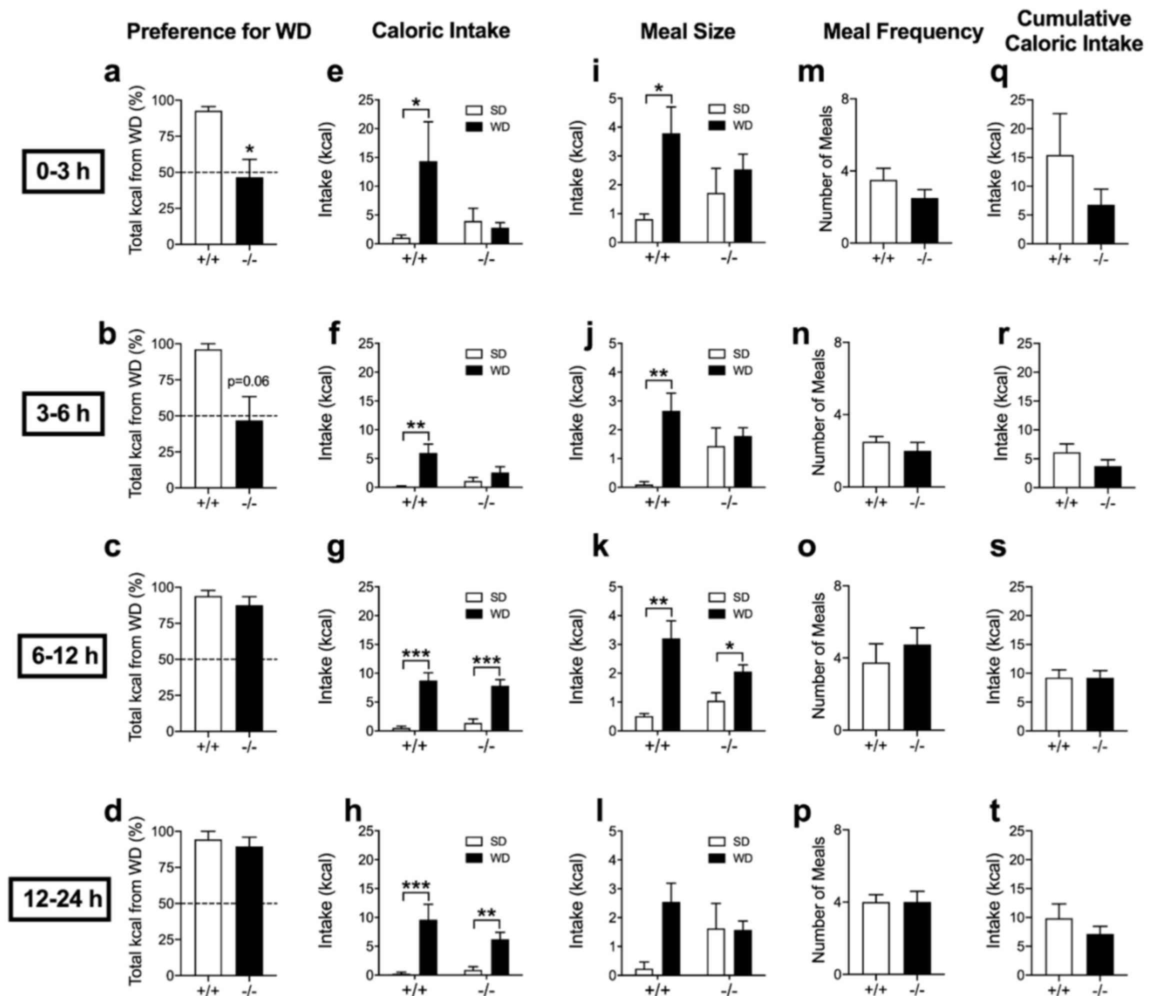


Figure 1.5. Acute preferences for western diet are absent in conditional intestinal epithelium-specific CB_1R-deficient mice.

When compared to IntCB_1+/+ control mice, preferences for western diet (WD) are reduced in IntCB-/- mice for up to 12 hours (a-c), an effect absent after 24 hours (d). Similarly, cumulative intake of WD is increased versus SD in IntCB_1+/+ control mice, an effect absent in IntCB-/- mice (e-g) that is regained after 24 hours (h). Average meal size for WD, when compared to SD, is increased throughout the 24 hour preference test in IntCB_1+/+ control mice, an effect absent in IntCB-/- mice (i-l). Total cumulative intakes were similar between groups throughout all time points (m-p). Unpaired Student's t-test, two-tailed (a-d, m-p); Two-way ANOVA with Holm-Sidak's multiple comparison tests (e-l); * = $p<0.05$, ** = $p<0.01$, *** =$p<0.001$. Results are expressed as means ± S.E.M; n = 4-8 per condition.

Chapter 2: Chronic Phytocannabinoid Exposure Reduces Adipoinsular Dysfunction During Diet-Induced Obesity

Authors: Avalos B[1], Wood CP[1], Udompholkul P[1], Cabrera H[2], Pellechia M[1], Garland T[2], DiPatrizio NV[1]

[1] Division of Biomedical Sciences, School of Medicine, University of California, Riverside, Riverside, CA 92521

[2] Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, Riverside, CA 92521

Abstract

Chronically elevated endocannabinoid (eCB) system activity is associated with caloric overconsumption during obesity. Emerging evidence reports inverse associations between cannabis use and prevalence of obesity, type 2 diabetes, and metabolic syndrome in humans. In this study, we investigated the impact of chronic cannabinoid exposure on the development of DIO and examined the potential mechanisms involved with the amelioration of metabolic dysfunction following cannabinoid exposure. Adipoinsular function was assessed following chronic exposure to pure THC or THC dose-match whole cannabis in an adult mouse model of obesity. Body weights and food intake were measured daily throughout the 30d exposure period before assessing glucose tolerance and insulin sensitivity. Changes in adipose tissue biology were assessed by histological

and genetic analysis. Additional in vivo and in vitro exposure studies in adipose-specific CB_1R knockout mice and 3T3-L1 adipocytes, respectively, further examined the role of the eCB system in mediating the pro-metabolic effects of cannabinoid exposure. Both THC and extract led to decreases in body weight and white adipose tissue mass in DIO mice, which was accompanied by transient reductions in food intake. Drug treatments also reversed DIO-associated changes in expression of genes that regulate the adipoinsular axis. Specifically, extract more potently restored levels of adipokine levels found in lean control mice fed a low-fat/no sucrose standard lab chow. Moreover, chronic exposure to extract, but not THC, was associated with improved glucose clearance in DIO mice. Similar outcomes were seen in adipose-specific CB_1R knockout mice whereas in vitro studies revealed contrasting effects in adipocyte biology. The relationship between cannabis, THC, and obesity is complex and multifaceted, involving adipose tissue dysfunction and altered adipokine secretion. Altogether, these data suggest chronic cannabinoid exposure influences production of fat-derived adipokines that control glucose homeostasis. Moreover, results highlight an ability for cannabis extract to affect the adipoinsular axis and ameliorate dysfunctional energy metabolism associated with obesity.

Introduction

Adipose tissue overabundance is a hallmark of diet-induced obesity (DIO) and a strong risk factor for development of type 2 diabetes mellitus (T2DM) [1, 2].

DIO is primarily driven by an excessive intake of high-calorie foods which are typically high in fat and sugar content. The caloric surplus from a Westernized Diet (WD) leads to hypertrophy and hyperplasia of white adipose tissue (WAT). As the primary storage units for excess energy, adipocytes composing WAT also secrete adipokines involved with various autocrine, paracrine, and endocrine processes. Adipokines influence insulin secretion and insulin reciprocally regulates adipokine production, thus forming a dynamic feedback loop termed the adipoinsular axis[3]. Adipoinsular dysfunction in DIO can be characterized by the dysregulation of prominent adipokines which modulate insulin sensitivity, lipid metabolism, and appetite. Adiponectin, adipsin (also known as complement factor D; Cfd), leptin, fibroblast growth factor 21 (FGF21), and secreted frizzled-related protein 5 (SFRP5) are among the dysregulated adipokines implicated in DIO-associated insulin resistance. Thus, factors influencing adiposity can directly impact the dysregulated production of adipokines involved with glucose homeostasis.

While dietary habits and sedentary lifestyles play pivotal roles in the development of obesity, emerging research suggests that the endocannabinoid (eCB) system, primarily influenced by compounds in cannabis, may contribute to its etiology. One of the well-known effects of THC is its impact on appetite stimulation, which is often referred to as the "munchies." This phenomenon is primarily mediated through CB_1 receptor activation as THC is a partial agonist of CB_1. Despite these appetite-inducing effects, epidemiological studies on the association between cannabis use and obesity have reported paradoxical findings.

Chronic cannabis use has been associated with lower rates of obesity and T2DM, which suggests a more complex interaction between cannabinoids, adipokines, and obesity. Moreover, peripheral eCB signaling is a key determinant of energy homeostasis that becomes dysregulated in DIO. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the endogenous cannabinoids that activate the cannabinoid type-1 (CB_1) and type-2 (CB2) receptors, contribute pivotal roles to the development of obesity by driving increased food intake [4]. In adipose tissue, CB_1 activation has been linked to significant alterations in the secretion of several adipokines including adiponectin, adipsin, and leptin. Additional research is required to understand cannabis' impact on adipokine and endocannabinoid dysregulation. These studies could offer novel insight for the development of therapeutic strategies to improve insulin sensitivity, manage inflammation, and mitigate the impact of adipose dysfunction on the development of T2DM.

In this study, we evaluated the metabolic impact of chronic THC exposure in a DIO mouse model. Specifically, we aimed to investigate the effects of chronic (30d) daily exposure to pure THC or whole cannabis extract matched for THC content quantified via Ultrahigh Performance Liquid Chromatography and Tandem Mass Spectrometry (UPLC/MS/MS). Utilization of UPLC/MS/MS technology also allowed for the detection of discrete differences in endocannabinoids (eCBs) levels within WAT and in circulation. Generation of conditional genetic knockout mice lacking adipose-specific CB_1R (AdiCB_1-/-) via CreLox recombination was used to further explore the role of adipocyte CB_1R in response to chronic THC exposure.

Additional in vitro experimentation in cultured 3T3-L1 adipocytes was also performed to assess ECS function and adipocyte biology during chronic (14d) cannabinoid exposure. Altogether, data from these investigations recapitulate many of the pro-metabolic improvements observed in cannabis users and overall demonstrates differences in energy metabolism that arise in response to pure THC versus whole cannabis extract.

Materials & Methods

<u>Mouse models & diets</u>

Male C57BL/6 mice (Taconic, Oxnard, CA, USA) were group-housed and maintained on a 12h:12h light:dark cycle with ad libitum access to food and water, unless indicated otherwise. Conditional adipose-specific CB_1R knockout mice (Ati-CB_1R-KO) were generated by breeding Cnr1loxP/loxP mice (Cnr1tm1.1 mrl; Taconic, Oxnard, CA; Model #7599) with AdipoqCreERT2 mice, both of C57BL/6 background. The resulting Cnr1fl/flAdipoqCreERT2 transgenic mice expressed excisable loxP genetic sequences flanking the CB_1 gene (Cnr1) with Cre-recombinase expression under the control of the adipose-specific adiponectin (adipoq) promoter. Gene deletion was induced in 4- to 6-week-old Cnr1fl/flAdipoqCreERT2 mice treated with tamoxifen (40 mg/kg per 4 mL corn oil), which was intraperitoneally (IP) administered daily for five consecutive days. Cre-negative Cnr1loxP/loxP mice served as wildtype controls following tamoxifen treatment. After 72h following the final tamoxifen injection, mice were transferred

back to home cages for a one week acclimation period. All mice were genotyped for transgenes via PCR using DNA extracted from tail clippings using the following primers (5'-3'): GCAGGGATTATGTCCCTAGC (CNR1-ALT), CTGTTACCAGGAGTCTTAGC (1415-35), GGCTCAAGGAATACACTTATACC (1415-37), GAGTCTGCCTTTCCCATGAC (22925), and TCCCTCACATCCTCAGGTTC (22926).

Adult mice, 7-8 weeks-of-age, were placed on a high-fat, high-sugar western-style diet (WD; Research Diets D12709B, New Brunswick, NJ, USA; 40% kcal from fat, 17% kcal from protein, 43% kcal from carbohydrates as mostly sucrose) or maintained on a standard rodent chow diet (SD; Teklad 2020x, Envigo, Huntingdon, UK; 16% kcal from fat, 24% kcal from protein, 60% kcal from carbohydrates) for a total of 60 days. Mice were age- and weight-matched to begin experiments with body weight and food intake measurements taken daily. Motor activity was measured in mice that were single-housed in two-hopper feeding chambers (TSE Systems, Chesterfield, MO, USA) after being given 3 days to acclimate before 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 (IACUC) of the University of California Riverside.

Cannabis extraction and LCMS quantitation

Whole cannabis flower was weighed and submerged in ice-cold 100% ethanol (KOPTEC, Decon Labs, PA, USA) before being placed on a shaker for 15

minutes at 125 rpm. The extract was filtered and dried under N2 stream 37C (ultrapure 99.9%) and then heated for 1h at 120ºC for complete decarboxylation of cannabis oil concentrate. Chemical analysis of phytocannabinoid content in extracts, including acidic precursors such as THCA and CBDA, was performed using Acquity Class I UPLC coupled with Xevo TQ-S Micro Mass Spectrometry (Waters Corporation, MA, USA). Chromatographic separation of cannabinoids using an Acquity UPLC BEH C18 column was performed using previously established methods for lipid quantification[5]. Multiple Reaction Monitoring (MRM) under positive ion mode (ES+) conditions for analyte detection used the following mass-to-charge ratios (m/z), cone voltages, and collision energy values, respectively: Δ9-THC (315.2>193.0) = 8v, 18v; CBD (315.2>122.9) = 8v, 30v; THCa (359.3>94.9) = 2v, 26v; CBDa (359.2>219.0) = 26v, 30v; Δ9-THC-D3 (318.3>196.1) and CBD-D3 (318.3>196.1) = 18v, 18v. Generation of standard calibration curves included deuterated standards used for cannabinoid quantification. Extracts were prepared in ethanol and stored at -20ºC until day-of-use.

Drug preparation and administration

After 30 days on respective diets, mice began the chronic exposure to pure THC (5 mg/kg) or whole cannabis oil extract matched for THC content. Vehicle consisted of 5% dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA), 5% Tween-80 (Chem Implex Intl Inc., Wood Dale, IL, USA), and 90% sterile saline (0.9% w/v; LabChem Inc., PA, USA). Compounds were prepared freshly each day

by adding Tween-80 to drug aliquots followed by heated-bath sonication for 30 minutes. Saline was added drop-wise before additional sonication for 10 minutes. Daily IP administration, using 25G needles, occurred 1h before the dark cycle (6pm). Injection sites alternated between the left and right peritoneal area to minimize tissue scarring.

Assessment of glucose homeostasis

Metabolic tests were performed in separate cohorts of mice after 3 weeks (21d) of daily cannabinoid exposure. Mice were fasted overnight (16h) or 6h while housed in wire-bottom cages to prevent copraphagia leading up to glucose tolerance tests (IP GTT) and insulin tolerance tests (IP ITT), respectively. Blood-glucose measurements were taken with an Accu-Chek Aviva Plus glucometer (Roche, IN, USA) after administering either glucose (2g/kg) or insulin (1 IU/kg) solution. Tail-snip blood samples were collected at baseline (0 minutes), 15, 30, 60, and 120 minutes post-injection for both tolerance tests. After metabolic tests, mice were returned to home cages with respective diets for the remaining duration of the daily exposure period.

Tissue processing and histology

Animals were euthanized the morning following the final injection and tissues were harvested, rinsed in phosphate-buffered saline (PBS; pH 7.4 at 1X, Alpha Teknova, CA, USA), flash-frozen in liquid nitrogen, and stored at -80ºC for future analysis. Epididymal fat (eFat) was weighed prior to being flash-frozen and subcutaneous, or inguinal fat (iFat), was collected from beneath the hind legs.

Blood samples collected in EDTA-coated tubes (BD and Co., NJ, USA) via cardiac puncture were centrifuged at 1,500g for 10 minutes at 4 ºC before storing plasma at -80ºC. Plasma collected from mice that completed the 30d exposure without any metabolic testing was used for enzyme-linked immunoassays (ELISA) measuring circulating levels of leptin, insulin (Invitrogen, Thermo Fisher Scientific, USA), adipsin (Boster Biological Technology, Pleasanton, CA, USA), and triglycerides (Cayman Chemical, Ann Arbor, MI, USA). For histological analyses, 1-2 gram(s) of eFat was fixed in 10 mL of 10% w/v buffered formalin (4% paraformaldehyde) for 24h at 4ºC before embedding in tissue freezing medium (Electron Microscopy Sciences, PA, USA). Using a cryostat set at -35°C (Leica CM1950, Leica Biosystems, IL, USA), samples were sectioned at 10 μM-thickness then mounted on charged glass slides (Fisher Scientific, PA, USA) before drying overnight. Sections were stained with hematoxylin (Abcam, MA, USA) and then rinsed with xylene (Sigma-Aldrich Co., MO, USA) before imaging using an Olympus EP50 bright-field microscope at 10x magnification. Image J software was used to quantify average adipocyte area.

Gene expression analyses

Areas used for RNA isolation and processing were sanitized with 70% ethanol solution and treated with RNAse inhibitor (RNAse out, G-Biosciences, St. Louis, MO, USA). Total RNA from eFat and iFat was extracted by method of RNeasy kit (Qiagen, Valencia, CA, USA) and first-strand cDNA was generated from normalized RNA using M-MLV reverse transcriptase (Invitrogen, Carlsbad,

CA, USA). Quantitative RT-PCR was performed with preconfigured SYBR Green assays (Biorad, Irvine, CA, USA) and PrimePCR gene-specific primers. Reactions were run in duplicates and expression was measured using 2-ΔΔCt. Normalized RNA from both fat depots was used for NanoString CodeSet (NanoString Technologies, Seattle, WA, USA) analysis designed with probe sequences of genes related to adipose tissue maintenance, metabolism, and differentiation. NanoString gene expression was normalized to lean controls and values are expressed as relative fold-change.

Endocannabinoid measurement

Lipid extractions and subsequent UPLC/MS/MS analyses were performed on collected tissues, as previously described [5]. Briefly, 10-20 mg of tissue (100 μl plasma) was homogenized in methanol containing internal standards for 2-AG and AEA (Caymen Chemicals, Ann Arbor, MI). Tissue lipids were extracted using chloroform and water washes prior to organic phase collection and separation via silica gel column chromatography. Collected eluates were dried under a gentle N2 stream at 37°C before immediate resuspension in 100 μl of 1:1 UPLC-grade chloroform:methanol, then analyzed via ultra-performance liquid chromatography couple to tandem mass spectrometry (UPLC/MS2). Multiple Reaction Monitoring (MRM) under positive ion mode (ES+) conditions for analyte detection used the following mass-to-charge ratios (m/z), cone voltages, and collision energy values, respectively: AEA (348.3>62.0) = 30v, 14v; 2-AG (379.3>287.3) = 30v, 12v; [2H4] AEA (352.3>66.1) = 26v, 16v and [2H5] 2-AG (384.3>93.4) = 25v, 44v. Generation

of standard calibration curves included deuterated standards used for cannabinoid quantification.

<u>3T3-L1 cell culture differentiation & exposure</u>

3T3-L1 pre-adipocytes (C-173, ATCC, USA) were cultured and maintained at 37°C in 5% CO2 (HeraCell Vios 160i CO2 incubator, Thermo Scientific). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2nM L-glutamine, 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA). Pre-adipocytes were passaged upon reaching 80% confluency in 25 or 75 cm2 polystyrene tissue culture flasks (CC7682-4825, CytoOne). Cultures were split by incubating in 0.25% trypsin-EDTA (Sigma-Aldrich Co., MO, USA) for 5 minutes at 37°C. Cells were seeded in 12- or 24-well polystyrene cell culture plates (Costar REF-3513, Corning Inc., USA) at a density of 4.0x105 cells per well. After 48-72h, confluent cells were washed twice with DPBS before inducing differentiation by adding differentiation media (DM), which was prepared day-of-use with supplemented DMEM containing 3-isobutyl-1-methylxanthine (IBMX; 0.5 mM; Sigma), dexamethasone (DEX; 1 µM; Sigma), and insulin (INS; 20 µg/mL; Sigma). After 48-72h, DM was replaced with post-differentiation maintenance media (MM) consisting of supplemented DMEM with insulin (20 µg/mL). MM was renewed every 48-72h throughout the 14-day differentiation period.

Fully differentiated adipocytes were washed twice with DPBS before beginning the cannabinoid exposure period. THC (1-10 µM) or THC-dose matched

whole cannabis extract was added to wells with MM and renewed with each media change over 14 days, with 100% ethanol serving as the vehicle control. Following exposure periods, media was collected and stored at -80°C for future ELISA analyses. Cells were washed twice with phosphate-buffered saline (DPBS 1x; Thermo Scientific) then incubated with trypsin for 5 minutes before adding Qiazol for RNA extraction (Qiagen, USA). Isolated RNA from cells was processed according to the manufacture's protocol and was used for gene expression analysis via RT-qPCR.

Oil Red O staining

Mature 3T3-L1 adipocytes were fixed with 4% paraformaldehyde (10% formalin) for 30 minutes at room temperature. Cells were then washed with 60% isopropanol and wells were allowed to completely dry before adding 500 µL of Oil Red O (ORO) solution for 10 minutes at room temperature. ORO was subsequently aspirated from wells before washing with 1 mL dH2O. Plates were then washed gently 4 times under running tap water and additional dH2O was added to wells until live imaging analysis (Incucyte S3, Sartorius) was completed. Plates were dried overnight before 500 µL of 100% isopropanol was added to each well with gentle shaking for 10 minutes at room temperature. ORO was eluted from cells and absorbance was measured at 490nm (BioRad iMark Plate Reader).

Seahorse analysis

Cellular bioenergetic profiling of 3T3-L1 adipocytes following chronic cannabinoid exposure was performed via Seahorse ATP XF Real-Time ATP Rate

Assay (Agilent Technologies, Santa Clara, CA). 3T3-L1 cells were seeded in Seahorse XFe96 plates at a density of 4.0x104 cells per well. After 48-72h, pre-adipocytes began the respective 14d differentiation and exposure periods, as detailed above. One day prior to the assay, the XFe96 cartridge was filled with Seahorse XF calibrant (200 µL per well) and incubated overnight in a CO2-free incubator at 37°C. On the day of assay, 3T3-L1 cells were primed with Seahorse XF DMEM (pH 7.4, 10mM glucose, 2nM L-glutamine, 1 mM sodium pyruvate) and were kept in a 37°C humidified incubator at 5% CO2 for one hour prior to beginning the Seahorse XF Real-Time ATP Rate assay.

Statistical analysis

Data were analyzed by GraphPad Prism 8 software using either unpaired Student's t-tests (two-tailed), ordinary one-way ANOVA, or two-way ANOVA with Holm-Sidak's multiple comparisons post-hoc test when appropriate. Results are expressed as means ± S.E.M. and significance was determined at $p<0.05$.

Results

Body weight reductions are concurrent with transient differences in food intake following chronic cannabinoid exposure

To assess the impact of chronic cannabinoid exposure on DIO, measurements for body weight, motor activity, food intake were recorded daily during the 30d drug exposure period (Figure 1A-C). Prior to the initial exposure, DIO animals (obese controls) weighed significantly more than lean control

counterparts fed a standard rodent chow (Figure 1D, $p < 0.001$). Overall change in body weight indicates significant reductions in both THC and extract cohorts, while vehicle controls gained weight throughout the exposure (Figure 1E, $p < 0.001$). Final body weights for THC- and extract-treated animals were significantly reduced compared to obese controls after the 30d exposure period (Figure 1F, $p < 0.05$, $p < 0.01$). Daily motor activity, represented as daily distance traveled, showed no significant differences across groups (Figure 1G). Despite maintaining significantly higher cumulative intake compared to lean controls (Figure 1H, $p < 0.001$), THC- and extract-treated mice initially displayed transient reductions in food intake that normalized by day 10 (Figure 1I). Collectively, these data suggest that the time-dependent gain in body weight seen in DIO mice fed WD is ameliorated following chronic exposure to THC or cannabis extract.

<u>Amelioration of glucose dyshomeostasis is associated with reduced adiposity in cannabinoid-treated mice.</u>

To determine if THC and extract exposure differentially affect glucose homeostasis, metabolic testing was performed during the exposure period. Glucose tolerance testing revealed impaired glucose clearance capacity in DIO mice that was significantly improved with extract treatment, an effect that was absent in THC-treated animals (Figure 2A, $p < 0.05$). To further investigate mechanisms of this response, an insulin tolerance test was performed in a separate cohort of mice. Although not statistically significant, extract treatment

improved insulin sensitivity (Figure 2B, $p < 0.054$). Furthermore, both THC and extract treatment resulted in significantly reduced fasting blood glucose levels comparable to lean controls (Figure 2C, $p < 0.05$, $p < 0.01$). Similarly, elevated levels of circulating insulin found in DIO mice were reversed following chronic exposure to THC or extract (Figure 2D, $p < 0.05$). To assess if reduced adiposity was associated with improvements in glucose homeostasis, we also investigated the impact of chronic cannabinoid exposure on circulating adipokines. Circulating levels of leptin (Figure 2E, $p < 0.05$, $p < 0.01$) and adipsin (Figure 2F, $p < 0.01$) were comparable to lean controls and overall more potently restored by extract treatment. Moreover, significant reductions in visceral epididymal fat mass were observed in both THC- and extract-treated mice (Figure 2G, $p < 0.01$). Histological analysis of epididymal fat tissue revealed differences in adipocyte morphology (Figure 2H), with significantly reduced average adipocyte area seen in THC and extract treatment groups.

Visceral WAT gene expression is differentially impacted by THC and extract

Gene Ontology (GO) and enrichment analyses revealed altered expression of genes involved with energy metabolism in visceral WAT of THC- and extract-treated mice (Figure 3A). When compared to vehicle, dysregulated adipokine gene expression is normalized following extract treatment and, to a lesser degree, in mice treated with THC (Figure 3B-F, $p < 0.05$). GLUT4 expression within adipose tissue of DIO mice remained significantly downregulated relative to lean controls,

regardless of treatment (Figure 3G, $p < 0.05$). Minimal changes occurred in gene expression related to fatty acid metabolism, as compared to DIO controls (Figure 3H-I, $p < 0.05$). Further normalization of dysregulated gene expression associated with DIO, but restored with THC or extract treatment, includes Insr (Figure 3J, $p < 0.05$), Pparα (Figure 3K), Pparγ (Figure 3L), and Sirt1 (Figure 3M, $p < 0.01$). Collectively, restoration of dysregulated adipokines levels is recapitulated by normalized gene expression in WAT of mice treated with THC or extract.

<u>Endocannabinoid tone associated with DIO becomes normalized following chronic THC and extract exposure</u>

Heat map depicting DIO-associated changes in epididymal fat depot endocannabinoid system gene expression following chronic cannabinoid treatment (Figure 4A). Predominant changes were seen in Cnr1 (Figure 4B, $p < 0.05$) and Faah (Figure 4E, $p < 0.05$), but no differences in Cnr2, Dagla, Mgll, or Nape-pld expression (Figure 4C, 4D, 4F, 4G, respectively). DIO-induced changes in expression of the ECS were reversed following chronic cannabinoid exposure. Specifically, levels of 2-AG (Figure 4H) and AEA (Figure 4I) in epididymal fat were lowered with THC or extract exposure. While circulating levels of endocannabinoids were also impacted by chronic cannabinoid exposure, extract treatment significantly restored plasma levels of 2-AG (Figure 4J, $p < 0.05$) and AEA (Figure 4K, $p < 0.05$). These alterations to the ECS suggest that the adipocyte

CB_1R may play an important role in restoring adipoinsular axis function following chronic cannabinoid exposure.

Adipocyte CB_1R deletion impacts glucose homeostasis but does not affect body weight reductions caused by chronic cannabinoid exposure

To assess a role of adipocyte CB_1R in adipoinsular function, transgenic mice with conditional deletion of CB_1R adipose-specific were generated. Overall 30d body weight trends in $AdiCB_1$+/+ (Figure 5A) and $AdiCB_1$-/- (Figure 5B) mice following chronic cannabinoid exposure show similar reductions in body weight (Figure 5C, 5E, $p < 0.001$). EchoMRI analyses revealed differences in body mass composition with no changes in lean mass (Figure 5D, 5F). Extract treated $AdiCB_1$+/+ animals displayed moderate improvement in glucose tolerance (Figure 5G-H, $p < 0.05$) whereas no differences in glucose clearance capacity were seen in $AdiCB_1$ -/- animals chronically treated with THC or extract (Figure 5I-J). These data suggest adipocyte CB_1R plays an important role in regulating glucose homeostasis, but is not necessary to observe the reduced adiposity phenotype following THC or extract exposure.

Chronic cannabinoid exposure in 3T3-L1 cultured adipocytes results in reduced lipid accumulation

To further investigate the impact of chronic cannabinoid exposure on adipocyte physiology, in vitro experimentation was performed in 3T3-L1

adipocytes. Cultured 3T3-L1 pre-adipocytes were differentiated for 14d before beginning a 14d exposure to THC or extract (Figure 6A). Oil Red O staining revealed reduced lipid accumulation in adipocytes exposed to 1-10 μM THC and THC-dose matched extract (Figure 6B, 6D). Corresponding optical density of eluted ORO measured at 490 nm revealed significant reductions at high THC concentrations, however, both extract concentrations produced similar reduced lipid accumulation (Figure 6C, 6E, $p < 0.01$). Extract treatment led to more potent downregulation of adipokine genes including Adipoq, Cfd, and Lep (Figure 6F, 6G, $P < 0.01$, $p < 0.001$). Gene expression in 3T3-L1 adipocytes treated with 10 μM THC shows a downregulation in the Cnr1 gene (Figure 6G, $p < 0.05$). In contrast, low concentrations of THC in extract caused downregulation of Insr (Figure 6F, $p < 0.01$) whereas high THC concentrations led to upregulation of Insr (Figure 6G, $p < 0.05$, $p < 0.01$). Minimal changes were seen in Pparg expression (Figure 6G, $p < 0.05$) and no differences in Ucp1 expression were observed (Figure 6F, 6G). Only high THC concentrations caused significant reductions in adipsin (Figure 6H, $p < 0.01$) and leptin (Figure 6I, $p < 0.001$) levels found in media from 3T3-L1 adipocytes. In the presence of the CB_1R antagonist AM251 or the CB2R antagonist AM630, extract was still capable of reducing lipid content in cultured adipocytes at low concentrations whereas reductions were more prominent at high THC concentrations (Figure 6J, $p < 0.05$). In the absence of THC or extract, cannabinoid receptor antagonists displayed reductions in lipid accumulation, with CB_1R antagonism causing greater reductions than CB2R antagonism; no synergistic

effects in dual-antagonist treatment were observed (Figure 6K, $p < 0.01$, $p < 0.001$). The dose-dependent differences observed here prompted further investigation into possible dose-dependent shift in energy homeostasis in 3T3-L1 adipocytes.

Chronic THC exposure produces dose-dependent changes in cellular bioenergetic profile of 3T3-L1 adipocytes

We next investigated the impact of THC and extract on cellular bioenergetics. A Seahorse assay was performed following 14d exposure in mature adipocytes. Extracellular acidification rate (ECAR), oxygen consumption rate (OCR), and proton efflux rate (PER) measurements in 3T3-L1 cells revealed dose dependent differences in basal rates following low and high THC and THC-dose matched extract treatment (Figure 7A-F, $p < 0.01$, $p < 0.001$). A shift in the bioenergetic profile depicts low THC and extract concentrations are associated with an energetic phenotype, whereas high THC and extract concentrations are associated with a quiescent phenotype (Figure 7G). Glycolytic and mitochondrial respiration ATP production rates are both reduced at high THC and extract concentrations (Figure 7H, $p < 0.05$, $p < 0.01$). As an overall percentage, only extract treatment at both low and high THC concentrations displayed a shift towards more ATP production from glycolysis than from oxidative phosphorylation (Figure 7I, $p < 0.01$, $p < 0.05$). Together, these results indicate dose-dependent

differences in adipocyte bioenergetics for 3T3-L1 cells treated with low and high THC and extract concentrations.

Discussion

DIO is a multifactorial disorder influenced by various molecular mechanisms, including the intricate interplay between adipokines and cannabinoids. CB_1R activation in adipose tissue contributes to insulin resistance through various mechanisms, including impaired insulin signaling, dysregulated adipokine secretion, and altered glucose uptake[54-57]. Despite the dysregulation of appetite and glucose homeostasis associated with eCB system hyperactivity, results from these studies suggest that chronic cannabinoid exposure improves adipoinsular dysfunction associated with DIO. Specifically, chronic exposure to THC and extract reduced adiposity following initial transient reductions in food intake during the first week daily exposure. Regarding glycemic improvements, we postulated that cannabis extract differs from THC in part by differentially impacting the production of multiple adipokines involved with glucose homeostasis. Fat cell enlargement is a major contributor to the development of insulin and leptin resistance[58], thus morphological analyses showing reductions in average adipocyte area served as a strong indicator of normalized adipokine production. Hyperleptinemia, a key indicator of leptin resistance commonly observed in obesity[7,59], was attenuated following chronic exposure to THC and especially cannabis extract. Extract exposure resulted in more potent restoration of additional

circulating adipokines like adipsin. These data align with the effects observed in adipokine gene expression within WAT, wherein THC and extract caused normalization of these and other adipokines.

Chronic cannabinoid exposure reversed many of the typical eCB characteristics associated with DIO. Levels of AEA and 2-AG in epididymal adipose tissue and in circulation more closely resembled levels of the lean phenotype following chronic THC and extract treatment. Downregulation of *Cnr1*, the gene encoding CB_1, within the WAT of cannabinoid-treated mice suggested an altered role for CB_1R in DIO during chronic cannabinoid exposure. By generating a transgenic mouse line with inducible genetic deletion of adipocyte-CB_1R, we observed similar changes in adiposity seen in control and wildtype mice. EchoMRI analyses of body mass composition revealed similar reductions in body fat mass with no changes in lean mass, further indicative of CB_1R-independent alterations to adipose tissue function. Despite its impact shown in glucose tolerance tests, adipocyte-CB_1R appears to be only partially responsible for THC-mediated reductions in adipose dysfunction during DIO. To further study the impact of CB_1R on adipose tissue, we utilized the 3T3-L1 pre-adipocyte cell line, a widely used cell culture model of adipogenesis[60,61]. These cells can differentiate into mature adipocytes and are responsive to cannabinoid ligands, providing novel insights into the molecular mechanisms underlying CB_1R-mediated effects on adipocyte differentiation, lipid metabolism, and adipokine secretion.[62,63] Activation of CB_1R in cultured 3T3-L1 adipocytes can mimic the energy-conserving effects observed in

vivo, including enhanced adipogenesis and lipid accumulation. Furthermore, treatment of differentiating adipocyte cells with the antidiabetic drug rosiglitazone, a PPARγ ligand, enhances thermogenesis in adipocytes as well as causes a reduced expression of CB_1R and increased expression of FAAH[64]. These data are in conjunction with the data on eCB system gene expression in WAT of DIO mice.

Interestingly, chronic exposure to THC and extract caused reductions in lipid content of 3T3-L1 adipocytes. Furthermore, we observed concentration-dependent effects present in cultured adipocytes treated with low (1uM) and high (10uM) concentrations of pure THC and THC content-matched extract. This range represents the theoretical concentration of THC in blood circulation following consumption of one cannabis cigarette, approximately 3-5uM. In addition to reduced lipid accumulation, low and high THC concentrations in cannabis extract resulted in changes in gene expression in many DIO-associated genes. *Lep* and *Cfd*, the genes encoding leptin and adipsin, respectively, were subject to downregulation most potently by extract. Measuring leptin in media following cannabinoid exposure indicates similar reductions in leptin seen in vivo. Adipsin, however, was also decreased in the media of adipocytes cultured in high THC conditions, unlike the increased expression and circulation of adipsin seen in vivo. Notably, THC activation of the adipocyte-CB_1R produces a concentration-dependent decrease in lipid accumulation, however, this effect was not attenuated by CB_1R and/or CB2R antagonism suggesting a possible receptor-independent mechanism[65,66]. Nevertheless, differences between THC and extract were further

apparent following a Seahorse assay of cellular bioenergetics, which showed concentration-dependent shifts in ECAR and OCR in fully differentiated 3T3-L1 cells. Only extract, however, caused an overall shift in ATP production which favored glycolysis over oxidative phosphorylation at low and high THC concentrations. We recognize the limitations of in vitro assessment of adipocyte biology in response to chronic cannabinoid exposure given the absence of metabolic challenges presented in DIO, such as chronic low-grade inflamatiion. Recent evidence suggests a role for alternatively activated macrophages in adipocyte-CB_1R mediated regulation of energy homeostasis[67]. Nevertheless, cannabis extract collectively showed a pronounced impact on adipokine production in vivo and in vitro.

Overall, these studies indicate that in addition to reducing adipose tissue accumulation, THC and extract exposure positively impacted adipoinsular function by ameliorating hyperglycemia, hyperinsulinemia, and dysadipokinemia associated with DIO. The modulation of energy homeostasis governed by dynamic cannabinoid and adipokine signaling evidently highlights the therapeutic potential targeting the eCB system for obesity treatment. Cannabis' therapeutic potential in the context of T2DM remains unclear; however, other phytocannabinoids may be affecting the hormonal feedback loop between adipose tissue and the pancreas. In addition to THC, cannabis contains several cannabinoids that influence energy metabolism including cannabidiol (CBD), tetrahydrocannabivarin (THCV), cannabinol (CBN), and cannabigerol (CBG). Lipophilic metabolites of THC, such

as 11-hydroxy-tetrahydracannabinol (11-OH-THC) and 11-nor-9-carboxy-tetrahydracannabol (THC-COOH), are prone to long-term storage in adipose tissue[68-70]. Other cannabis-derived compounds, such as limonene, also accumulate in adipose tissue and can modify the bioenergetic profile of adipocytes[71-73]. Understanding the impact of cannabis metabolites on energy homeostasis is crucial given the increasing prevalence of cannabis use, and further research is needed to elucidate the specific molecular mechanisms through which cannabis-derived metabolites stored in adipose tissue may affect glucose homeostasis. Future studies will aim to further elucidate specific mechanisms in the pro-metabolic effects of cannabis exposure and will focus on identifying differential effects on energy homeostasis for THC and other chemical constituents in cannabis.

References

1. Barlow, S.E., Expert Committee Recommendations Regarding the Prevention, Assessment, and Treatment of Child and Adolescent Overweight and Obesity: Summary Report. Pediatrics, 2007. 120(Supplement 4): p. S164.

2. Argueta, D.A., et al., Cannabinoid CB(1) Receptors Inhibit Gut-Brain Satiation Signaling in Diet-Induced Obesity. Front Physiol, 2019. 10: p. 704.

3. Avalos, B., et al., Cannabinoid CB(1) Receptors in the Intestinal Epithelium Are Required for Acute Western-Diet Preferences in Mice. Nutrients, 2020. 12(9).

4. Sticht, M.A., et al., Endocannabinoid regulation of homeostatic feeding and stress-induced alterations in food intake in male rats. Br J Pharmacol, 2019. 176(10): p. 1524-1540.

5. Monteleone, A., et al., Peripheral Endocannabinoid Responses to Hedonic Eating in Binge- Eating Disorder. Nutrients, 2017. 9(12): p. 1377.

6. DiPatrizio, N.V., et al., Fasting stimulates 2-AG biosynthesis in the small intestine: role of cholinergic pathways. (1522-1490 (Electronic)).

7. Tarragon, E. and J.J. Moreno, Role of Endocannabinoids on Sweet Taste Perception, Food Preference, and Obesity-related Disorders. Chemical Senses, 2017. 43(1): p. 3-16.

8. Pertwee, R.G., The diverse CB_1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. British journal of pharmacology, 2008.

9. Pertwee, R.G., et al., International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. Pharmacological reviews, 2010. 62(4): p. 588-631.

10. Russo, E.B., Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British journal of pharmacology, 2011. 163(7): p. 1344-1364.

11. Järbe, T.U. and N.V. DiPatrizio, Delta9-THC induced hyperphagia and tolerance assessment: interactions between the CB_1 receptor agonist delta9-THC and the CB_1 receptor antagonist SR-141716 (rimonabant) in rats. Behav Pharmacol, 2005. 16(5-6): p. 373-80.

12. Foltin, R.W., J.V. Brady, and M.W. Fischman, Behavioral analysis of marijuana effects on food intake in humans. Pharmacol Biochem Behav, 1986. 25(3): p. 577-82.

13. Weltens, N., et al., Effect of acute Δ9-tetrahydrocannabinol administration on subjective and metabolic hormone responses to food stimuli and food intake in healthy humans: a randomized, placebo-controlled study. Am J Clin Nutr, 2019. 109(4): p. 1051-1063.

14. Alshaarawy, O. and J.C. Anthony, Are cannabis users less likely to gain weight? Results from a national 3-year prospective study. Int J Epidemiol, 2019. 48(5): p. 1695-1700.

15. Rajavashisth, T.B., et al., Decreased prevalence of diabetes in marijuana users: cross- sectional data from the National Health and Nutrition Examination Survey (NHANES) III. BMJ Open, 2012. 2: p. e000494.

16. Kim, D., et al., Inverse association of marijuana use with nonalcoholic fatty liver disease among adults in the United States. PLoS One, 2017. 12(10): p. e0186702.

17. Vázquez-Bourgon, J., et al., Effect of cannabis on weight and metabolism in first-episode non- affective psychosis: Results from a three-year longitudinal study. J Psychopharmacol, 2019. 33(3): p. 284-294.

18. Meier, M.H., et al., Associations Between Cannabis Use and Cardiometabolic Risk Factors: A Longitudinal Study of Men. Psychosom Med, 2019. 81(3): p. 281-288.

19. Dibba, P., et al., Potential Mechanisms Influencing the Inverse Relationship Between Cannabis and Nonalcoholic Fatty Liver Disease: A Commentary. Nutr Metab Insights, 2019. 12: p. 1178638819847480.

20. Penner, E.A., H. Buettner, and M.A. Mittleman, The impact of marijuana use on glucose, insulin, and insulin resistance among US adults. Am J Med, 2013. 126(7): p. 583-9.

21. Le Foll, B., et al., Cannabis and Δ9-tetrahydrocannabinol (THC) for weight loss? Med Hypotheses, 2013. 80(5): p. 564-7.

22. Hayatbakhsh, M.R., et al., Cannabis use and obesity and young adults. Am J Drug Alcohol Abuse, 2010. 36(6): p. 350-6.

23. Vidot, D.C., et al., Metabolic Syndrome Among Marijuana Users in the United States: An Analysis of National Health and Nutrition Examination Survey Data. The American Journal of Medicine, 2016. 129(2): p. 173-179.

24. Le Strat, Y. and B. Le Foll, Obesity and cannabis use: results from 2 representative national surveys. Am J Epidemiol, 2011. 174(8): p. 929-33.

25. Clark, T.M., et al., Theoretical Explanation for Reduced Body Mass Index and Obesity Rates in Cannabis Users. Cannabis and cannabinoid research, 2018. 3(1): p. 259-271.

26. Bancks, M.P., et al., Self-reported marijuana use over 25 years and abdominal adiposity: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Addiction, 2018. 113(4): p. 689-698.

27. Lone, J. and J.W. Yun, Monoterpene limonene induces brown fat-like phenotype in 3T3-L1 white adipocytes. Life Sciences, 2016. 153: p. 198-206.

28. Bielawiec, P., E. Harasim-Symbor, and A. Chabowski, Phytocannabinoids: Useful Drugs for the Treatment of Obesity? Special Focus on Cannabidiol. Frontiers in Endocrinology, 2020. 11.

29. Fellous, T., et al., Phytocannabinoids promote viability and functional adipogenesis of bone marrow-derived mesenchymal stem cells through different molecular targets. Biochemical Pharmacology, 2020. 175: p. 113859.

30. Hales CM, C.M., Fryar CD, Ogden CL. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. NCHS Data Brief 2020 cited 2021 January 2021; 360:[Available from: https://www.cdc.gov/nchs/products/databriefs/db360.htm.

31. Wang, Y., et al., Will All Americans Become Overweight or Obese? Estimating the Progression and Cost of the US Obesity Epidemic. Obesity, 2008. 16(10): p. 2323-2330.

32. Cawley, J. and C. Meyerhoefer, The medical care costs of obesity: an instrumental variables approach. J Health Econ, 2012. 31(1): p. 219-30.

33. Cordain, L., et al., Origins and evolution of the Western diet: health implications for the 21st century. The American Journal of Clinical Nutrition, 2005. 81(2): p. 341-354.

34. Kopp, W., How Western Diet And Lifestyle Drive The Pandemic Of Obesity And Civilization Diseases. Diabetes Metab Syndr Obes, 2019. 12: p. 2221-2236.

35. Khaodhiar, L., K.C. McCowen, and G.L. Blackburn, Obesity and its comorbid conditions. Clin Cornerstone, 1999. 2(3): p. 17-31.

36. Bays, H.E., et al., Obesity, adiposity, and dyslipidemia: A consensus statement from the National Lipid Association. Journal of Clinical Lipidology, 2013. 7(4): p. 304-383.

37. Argueta, D.A., et al., Cannabinoid CB(1) Receptors Inhibit Gut-Brain Satiation Signaling in Diet-Induced Obesity. Frontiers in physiology, 2019. 10: p. 704-704.

38. Argueta, D.A. and N.V. DiPatrizio, Peripheral endocannabinoid signaling controls hyperphagia in western diet-induced obesity. Physiol Behav, 2017. 171: p. 32-39.

39. Mechoulam, R., et al., Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochemical pharmacology, 1995. 50(1): p. 83-90.

40. Krott, L.M., et al., Endocannabinoid regulation in white and brown adipose tissue following thermogenic activation. Journal of Lipid Research, 2016. 57(3): p. 464-473.

41. Motaghedi, R. and T.E. McGraw, The CB_1 Endocannabinoid System Modulates Adipocyte Insulin Sensitivity. Obesity, 2008. 16(8): p. 1727-1734.

42. Sidibeh, C.O., et al., Role of cannabinoid receptor 1 in human adipose tissue for lipolysis regulation and insulin resistance. Endocrine, 2017. 55(3): p. 839-852.

43. Tedesco, L., et al., Cannabinoid receptor stimulation impairs mitochondrial biogenesis in mouse white adipose tissue, muscle, and liver: the role of eNOS, p38 MAPK, and AMPK pathways. Diabetes, 2010. 59(11): p. 2826-2836.

44. Ruiz de Azua, I., et al., Adipocyte cannabinoid receptor CB_1 regulates energy homeostasis and alternatively activated macrophages. J Clin Invest, 2017. 127(11): p. 4148-4162.

45. Bensaid, M., et al., The cannabinoid CB_1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. Mol Pharmacol, 2003. 63(4): p. 908-14.

46. Cota, D., et al., The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest, 2003. 112(3): p. 423-31.

47. Vázquez-Vela, M.E.F., N. Torres, and A.R. Tovar, White Adipose Tissue as Endocrine Organ and Its Role in Obesity. Archives of Medical Research, 2008. 39(8): p. 715-728.

48. Longo, M., et al., Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. International journal of molecular sciences, 2019. 20(9): p. 2358.

49. Skurk, T., et al., Relationship between Adipocyte Size and Adipokine Expression and Secretion. The Journal of Clinical Endocrinology & Metabolism, 2007. 92(3): p. 1023-1033.

50. Weyer, C., et al., Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts Type II diabetes independent of insulin resistance. Diabetologia, 2000. 43(12): p. 1498-1506.

51. Juge-Aubry, C.E., et al., Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. Diabetes, 2003. 52(5): p. 1104-10.

52. Jo, J., et al., Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. PLoS computational biology, 2009. 5(3): p. e1000324-e1000324.

53. Di Marzo, V., A. Ligresti, and L. Cristino, The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. International Journal of Obesity, 2009. 33(S2): p. S18-S24.

54. Jo, Y.-H., et al., Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit. Neuron, 2005. 48(6): p. 1055-1066.

55. Sarmento-Cabral, A., et al., Adipokines (Leptin, Adiponectin, Resistin) Differentially Regulate All Hormonal Cell Types in Primary Anterior Pituitary Cell Cultures from Two Primate Species. Scientific Reports, 2017. 7(1): p. 43537.

56. Kershaw, E.E. and J.S. Flier, Adipose Tissue as an Endocrine Organ. The Journal of Clinical Endocrinology & Metabolism, 2004. 89(6): p. 2548-2556.

57. Wu, J., et al., Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell, 2012. 150(2): p. 366-376.

58. Gómez-Hernández, A., et al., Differential Role of Adipose Tissues in Obesity and Related Metabolic and Vascular Complications. International journal of endocrinology, 2016. 2016: p. 1216783-1216783.

59. Petrovic, N., et al., Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. The Journal of biological chemistry, 2010. 285(10): p. 7153-7164.

60. Petersen, R.K., et al., Cyclic AMP (cAMP)-Mediated Stimulation of Adipocyte Differentiation Requires the Synergistic Action of Epac- and cAMP-Dependent Protein Kinase-Dependent Processes. Molecular and Cellular Biology, 2008. 28(11): p. 3804-3816.

61. Matias, I., et al., Regulation, Function, and Dysregulation of Endocannabinoids in Models of Adipose and β-Pancreatic Cells and in Obesity and Hyperglycemia. The Journal of Clinical Endocrinology & Metabolism, 2006. 91(8): p. 3171-3180.

62. Howlett, A.C., Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. Life Sciences, 1984. 35(17): p. 1803-1810.

63. Armani, A., et al., Phosphodiesterase type 5 (PDE5) in the adipocyte: a novel player in fat metabolism? Trends in Endocrinology & Metabolism, 2011. 22(10): p. 404-411.

64. Lihn, A.S., et al., AICAR stimulates adiponectin and inhibits cytokines in adipose tissue. Biochem Biophys Res Commun, 2004. 316(3): p. 853-8.

65. Kola, B., et al., Cannabinoids and Ghrelin Have Both Central and Peripheral Metabolic and Cardiac Effects via AMP-activated Protein Kinase. Journal of Biological Chemistry, 2005. 280(26): p. 25196-25201.

66. Verty, A.N., et al., The Cannabinoid Receptor Agonist THC Attenuates Weight Loss in a Rodent Model of Activity-Based Anorexia. Neuropsychopharmacology, 2011. 36(7): p. 1349- 1358.

67. Towler, M.C. and D.G. Hardie, AMP-Activated Protein Kinase in Metabolic Control and Insulin Signaling. Circulation Research, 2007. 100(3): p. 328-341.

68. Cousin, B., et al., Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. Journal of Cell Science, 1992. 103(4): p. 931.

69. Ricquier, D., Uncoupling Protein 1 of Brown Adipocytes, the Only Uncoupler: A Historical Perspective. Frontiers in Endocrinology, 2011. 2(85).

70. Bartelt, A., et al., Brown adipose tissue activity controls triglyceride clearance. Nature Medicine, 2011. 17(2): p. 200-205.

71. Dodd, G.T., et al., Leptin and insulin act on POMC neurons to promote the browning of white fat. Cell, 2015. 160(1-2): p. 88-104.

72. Cohen, P., et al., Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. Cell, 2014. 156(1-2): p. 304-316.

73. Perwitz, N., et al., Cannabinoid type 1 receptor blockade induces transdifferentiation towards a brown fat phenotype in white adipocytes. Diabetes, Obesity and Metabolism, 2010. 12(2): p. 158-166.

74. Perwitz, N., M. Fasshauer, and J. Klein, Cannabinoid receptor signaling directly inhibits thermogenesis and alters expression of adiponectin and visfatin. Horm Metab Res, 2006. 38(5): p. 356-8.

75. Herling, A.W., et al., Increased Energy Expenditure Contributes More to the Body Weight- Reducing Effect of Rimonabant than Reduced Food Intake in Candy-Fed Wistar Rats. Endocrinology, 2008. 149(5): p. 2557-2566.

76. Verty, A.N.A., A.M. Allen, and B.J. Oldfield, The Effects of Rimonabant on Brown Adipose Tissue in Rat: Implications for Energy Expenditure. Obesity, 2009. 17(2): p. 254-261.

77. Jbilo, O., et al., The CB_1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. The FASEB Journal, 2005. 19(11): p. 1567-1569.

78. Teixeira, D., et al., Modulation of Adipocyte Biology by Δ9 - Tetrahydrocannabinol. Obesity, 2010. 18(11): p. 2077-2085.

79. Baur, R., J. Gertsch, and E. Sigel, The cannabinoid CB_1 receptor antagonists rimonabant (SR141716) and AM251 directly potentiate GABA(A) receptors. British journal of pharmacology, 2012. 165(8): p. 2479-2484.

80. Pagano, C., et al., The Endogenous Cannabinoid System Stimulates Glucose Uptake in Human Fat Cells via Phosphatidylinositol 3-Kinase and Calcium-Dependent Mechanisms. The Journal of Clinical Endocrinology & Metabolism, 2007. 92(12): p. 4810-4819.

81. Fayyad, A.M., et al., Rosiglitazone Enhances Browning Adipocytes in Association with MAPK and PI3-K Pathways During the Differentiation of Telomerase-Transformed Mesenchymal Stromal Cells into Adipocytes. International journal of molecular sciences, 2019. 20(7): p. 1618.

82. Lefterova, M.I., et al., PPARγ and the global map of adipogenesis and beyond. Trends in endocrinology and metabolism: TEM, 2014. 25(6): p. 293-302.

83. Uppenberg, J., et al., Crystal structure of the ligand binding domain of the human nuclear receptor PPARγ. Journal of Biological Chemistry, 1998. 273(47): p. 31108-31112.

84. Fu, J., et al., Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. Nature, 2003. 425(6953): p. 90-93.

85. Guzmán, M., et al., Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-alpha). J Biol Chem, 2004. 279(27): p. 27849-54.

86. Bouaboula, M., et al., Anandamide induced PPARγ transcriptional activation and 3T3-L1 preadipocyte differentiation. European Journal of Pharmacology, 2005. 517(3): p. 174-181.

87. Elmes, M.W., et al., Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ9- tetrahydrocannabinol (THC) and cannabidiol (CBD). The Journal of biological chemistry, 2015. 290(14): p. 8711-8721.

88. Collins, S., et al., Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol Behav, 2004. 81(2): p. 243-8.

89. Wang, C.-Y. and J.K. Liao, A mouse model of diet-induced obesity and insulin resistance. Methods in molecular biology (Clifton, N.J.), 2012. 821: p. 421-433.

90. Flurkey, K., J. Currer, and D. Harrison, The Mouse in Aging Research. The Mouse in Biomedical Research, 2007. 3.

91. Karastergiou, K., et al., Sex differences in human adipose tissues – the biology of pear shape. Biology of Sex Differences, 2012. 3(1): p. 13.

92. Cuttler, C., L.K. Mischley, and M. Sexton, Sex Differences in Cannabis Use and Effects: A Cross-Sectional Survey of Cannabis Users. Cannabis and cannabinoid research, 2016. 1(1): p. 166-175.

93. Bagchi, D.P. and O.A. MacDougald, Identification and Dissection of Diverse Mouse Adipose Depots. Journal of visualized experiments : JoVE, 2019(149): p. 10.3791/59499.

94. Han, W., et al., Influence of food texture on energy metabolism and adiposity in male rats. Experimental Physiology, 2018. 103(10): p. 1347-1356.

95. Banni, S., et al., Vagus Nerve Stimulation Reduces Body Weight and Fat Mass in Rats. PLOS ONE, 2012. 7(9): p. e44813.

96. Frontini, A. and S. Cinti, Distribution and Development of Brown Adipocytes in the Murine and Human Adipose Organ. Cell Metabolism, 2010.

97. Rawitch, A.B., R. Rohrer, and R.M. Vardaris, Delta-9-tetrahydrocannabinol uptake by adipose tissue: Preferential accumulation in gonadal fat organs. General Pharmacology: The Vascular System, 1979. 10(6): p. 525-529.

98. Gunasekaran, N., et al., Reintoxication: the release of fat-stored delta(9)-tetrahydrocannabinol (THC) into blood is enhanced by food deprivation or ACTH exposure. British journal of pharmacology, 2009. 158(5): p. 1330-1337.

99. Bentley-Hewitt, K.L., et al., Comparison of quantitative real-time polymerase chain reaction with NanoString® methodology using adipose and liver tissues from rats fed seaweed. New Biotechnology, 2016.

100. Cirera, S., Highly efficient method for isolation of total RNA from adipose tissue. BMC research notes, 2013. 6: p. 472-472.

101. Hu, E., P. Liang, and B.M. Spiegelman, AdipoQ Is a Novel Adipose-specific Gene Dysregulated in Obesity. Journal of Biological Chemistry, 1996. 271(18): p. 10697-10703.

102. Ruiz de Azua, I., et al., Adipocyte cannabinoid receptor CB_1 regulates energy homeostasis and alternatively activated macrophages. The Journal of clinical investigation, 2017. 127(11): p. 4148-4162.

103. Sassmann, A., S. Offermanns, and N. Wettschureck, Tamoxifen-inducible Cre-mediated recombination in adipocytes. Genesis, 2010. 48(10): p. 618-25.

104. Hansen, H.S. and V. Vana, Non-endocannabinoid N-acylethanolamines and 2- monoacylglycerols in the intestine. British Journal of Pharmacology, 2019. 176(10): p. 1443- 1454.

105. Wargent, E.T., et al., The cannabinoid Δ(9)-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in two mouse models of obesity. Nutrition & diabetes, 2013. 3(5): p. e68-e68.

106. Green, H. and O. Kehinde, Sublines of mouse 3T3 cells that accumulate lipid. Cell, 1974. 1(3): p. 113-116.

107. Kraus, N.A., et al., Quantitative assessment of adipocyte differentiation in cell culture. Adipocyte, 2016. 5(4): p. 351-358.

108. Ramirez-Zacarias, J., F. Castro-Munozledo, and W. Kuri-Harcuch, Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry, 1992. 97(6): p. 493-497.

109. Yang, Y., et al., Emodin with PPARgamma ligand-binding activity promotes adipocyte differentiation and increases glucose uptake in 3T3-LI cells. Biochem Biophys Res Commun, 2007. 353(2): p. 225-30.

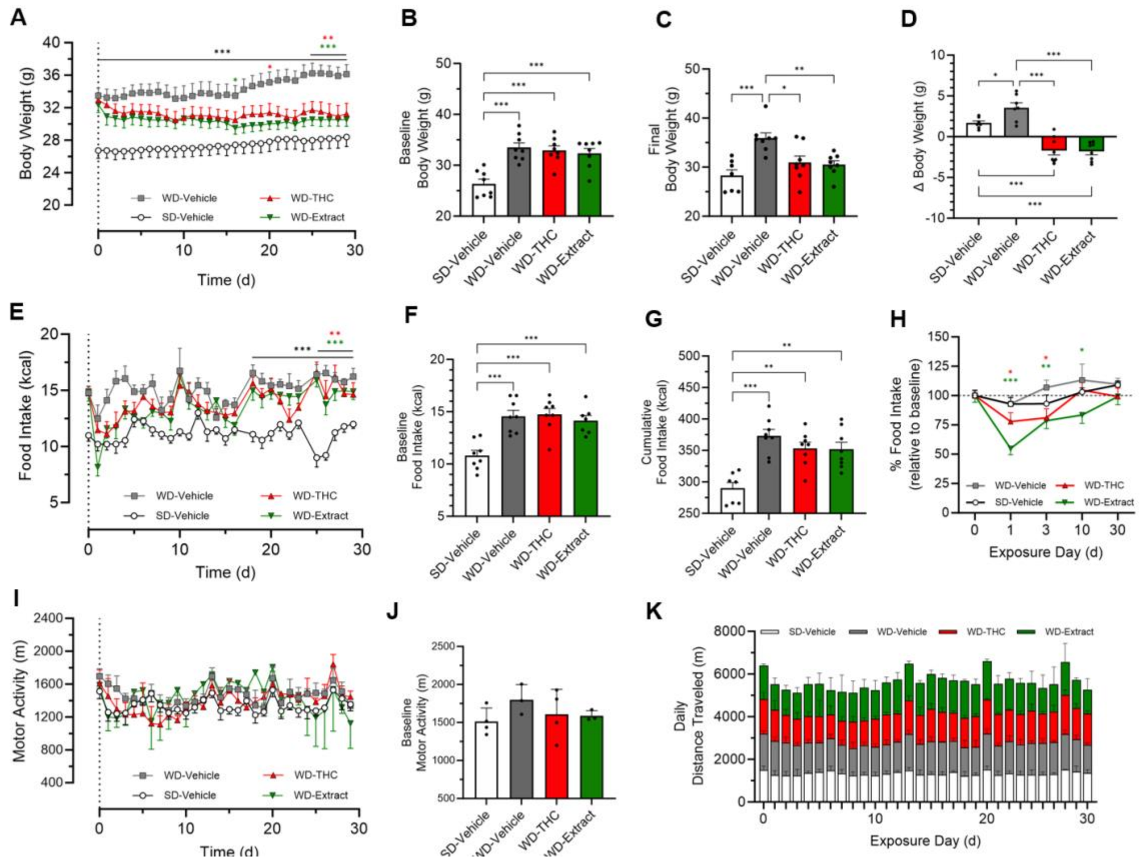
110. Mäuser, W., et al., Direct adipotropic actions of atorvastatin: differentiation state-dependent induction of apoptosis, modulation of endocrine function, and inhibition of glucose uptake. Eur J Pharmacol, 2007. 564(1-3): p. 37-46.

111. Rodbell, M., METABOLISM OF ISOLATED FAT CELLS. I. EFFECTS OF HORMONES ON GLUCOSE METABOLISM AND LIPOLYSIS. J Biol Chem, 1964. 239: p. 375-80.

112. Abreu-Vieira, G., et al., Cidea improves the metabolic profile through expansion of adipose tissue. Nature Communications, 2015. 6(1): p. 7433.

113. Esposito, G., et al., Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. PLoS One, 2011. 6(12): p. e28668.

Figures



<u>Figure 2.1. Changes in body weight, motor activity, and food intake in mice chronically treated with THC or extract.</u>

Complete 30d trends for (A) body weight, (B) motor activity, and (C) food intake. (D) Baseline body weights after 30d of respective diets. (E) Final body weights and (F) overall change in body weights after 30d of drug exposure. (G) Average daily distance traveled throughout 30d exposure. (H) Cumulative and (I) transient food intake measured during 30d exposure. Two-way ANOVA (A-C, I) and one-way ANOVA (D-H) with Holm-Sidak's multiple comparisons post-hoc tests; * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. Data are expressed as means ± S.E.M; n=6-8 per condition; n=4 for motor activity.

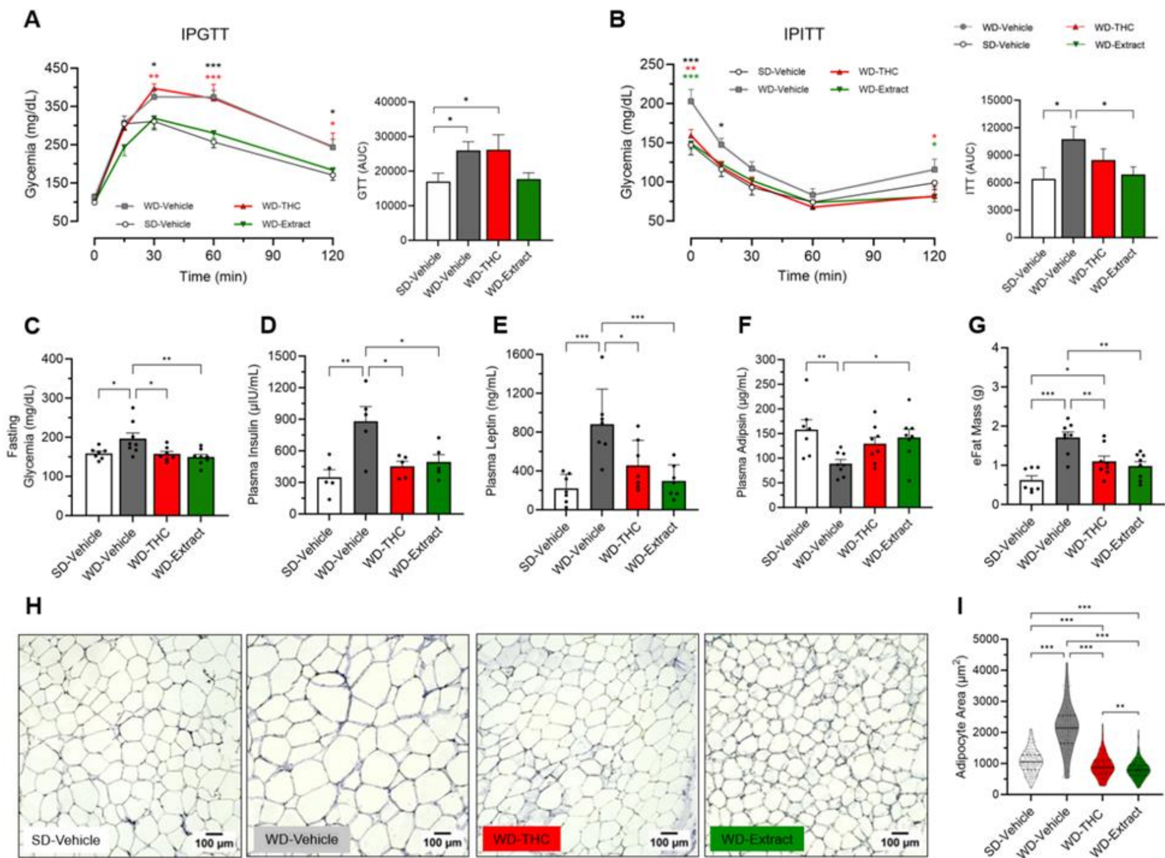


Figure 2.2. Amelioration of glucose dyshomeostasis is associated with reduced adiposity in cannabinoid-treated mice.

Glucose tolerance test curve with GTT AUC (A) following glucose administration (2 g/kg BW) in mice fasted overnight. Insulin tolerance test curve with ITT AUC (B) following insulin administration (1 IU/kg) in mice fasted for 6h. (C) Fasting blood-glucose levels and (D) non-fasted circulating insulin levels following 30d chronic exposure in mice. Circulating adipokine levels for (E) leptin and (F) adipsin measured via ELISA. (G) Overall eWAT mass after 30d exposure. (H) Representative histological sections of hematoxylin-stained eWAT; 10× magnification at 10 μm-thickness; 100 μm scale bar. (I) Average adipocyte area from eWAT of chronically treated mice. Two-way ANOVA (A, B) and one-way ANOVA (C-G, I) with Holm-Sidak's multiple comparisons post-hoc test; * = p<0.05, ** = p<0.01, *** = p<0.001. Data are expressed as means ± S.E.M; n=5-8 per condition; n=3 for histological sections.

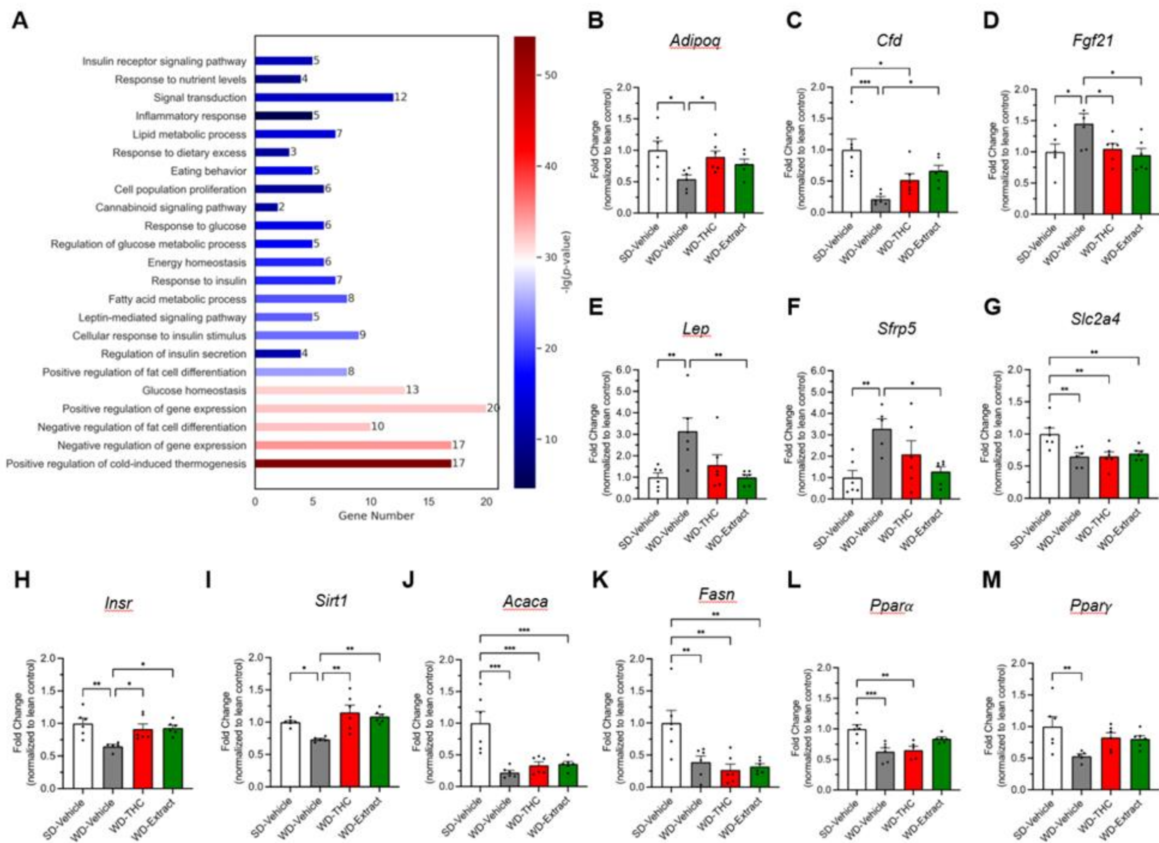


Figure 2.3. Visceral fat gene expression is differentially impacted by 30d exposure to THC and extract.

Gene Ontology (GO) with enrichment analyses (A) for adipose tissue genes impacted by chronic cannabinoid exposure. Dysregulated expression for genes related to the adipoinsular axis (B-I), fatty acid synthesis (J-K), and regulation of transcription and differentiation (L-M). Adipoq: adiponectin, Cfd: adipsin, Fgf21: fibroblast growth factor 21, Lep: leptin, Sfrp5: secreted frizzled related protein 5, Slc2a4: glucose transporter 4 (GLUT4), Insr: insulin receptor, Acaca: acetyl-CoA carboxylase, Fasn: fatty acid synthase, Pparα/γ: peroxisome proliferator activated receptor alpha/gamma. NanoString gene expression is normalized to lean controls. One-way ANOVA with Holm-Sidak's multiple comparisons post-hoc test (B, D). * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. Results are expressed as means ± S.E.M; n=5-8 per condition.

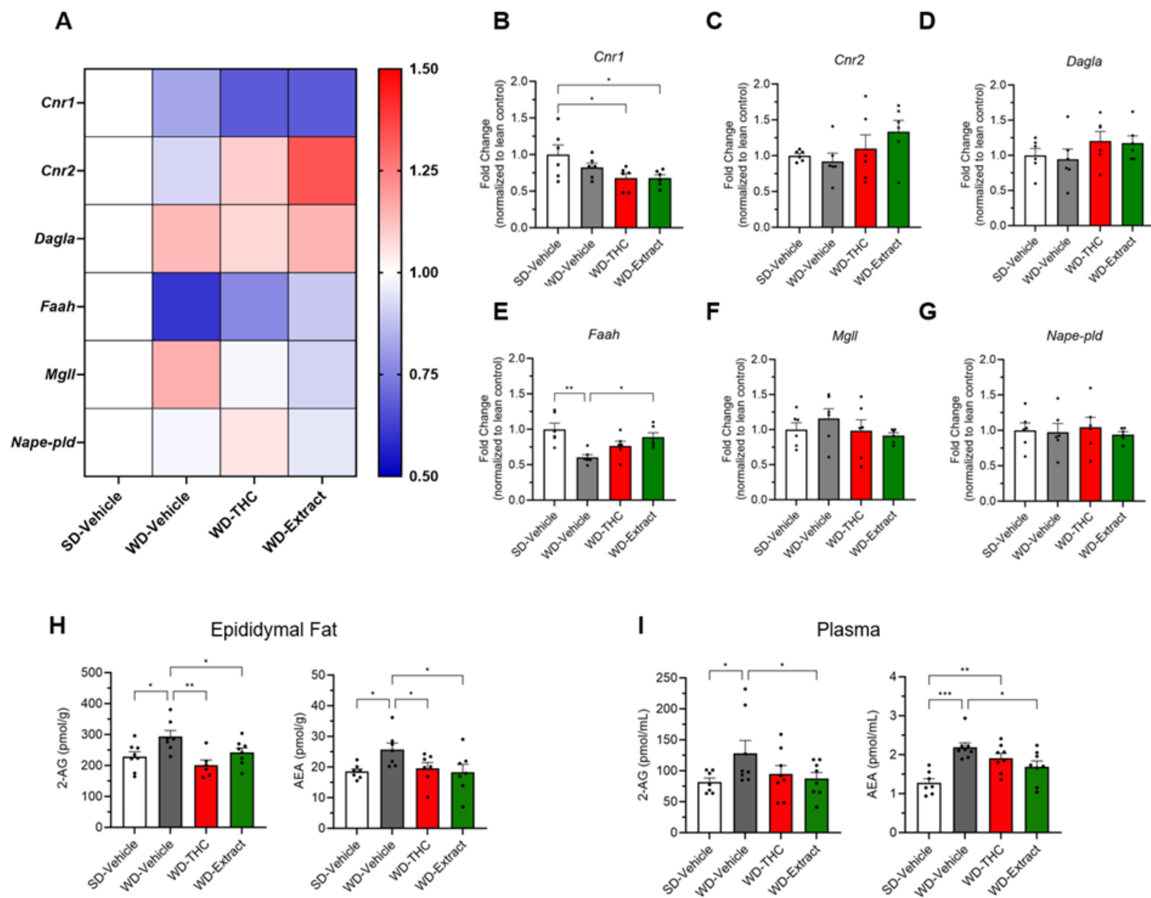


Figure 2.4. Endocannabinoid tone associated with DIO becomes normalized following chronic THC and extract exposure.

Heat map (A) depicting DIO-associated changes in epididymal fat depot endocannabinoid system gene expression following chronic cannabinoid treatment. (B-G) ECS gene expression relative to lean controls. Endocannabinoid levels in eWAT (H-I) and in plasma (J-K). One-way ANOVA with Holm-Sidak's multiple comparisons post-hoc test (B-K); * = p<0.05, ** = p<0.01, *** = p<0.001. Results are expressed as means ± S.E.M; n=5-8 per condition.

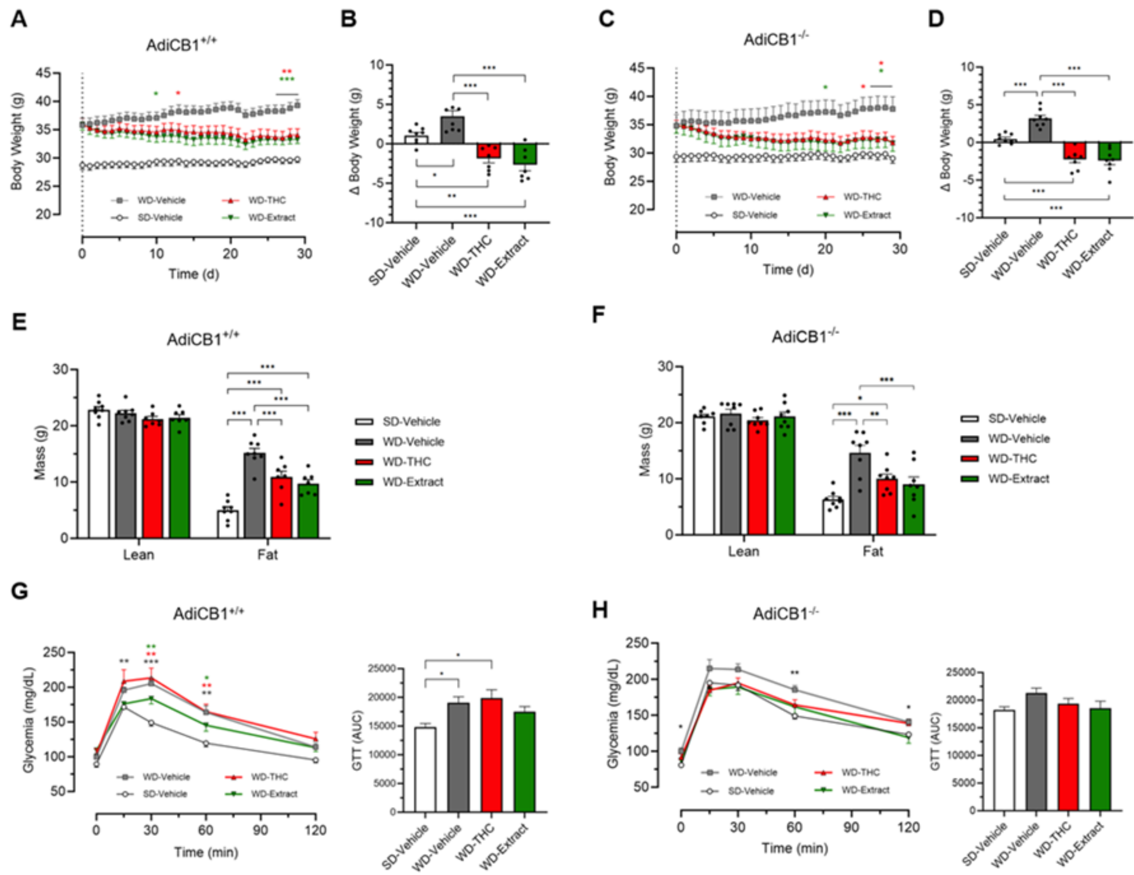
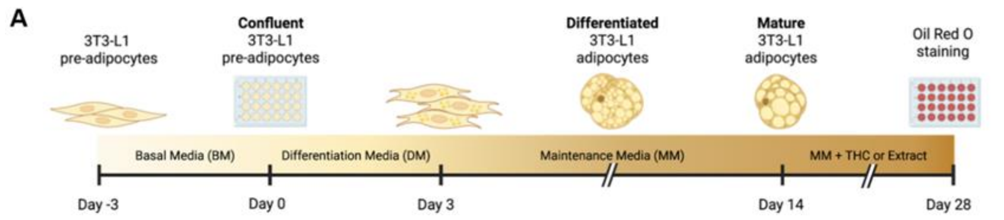
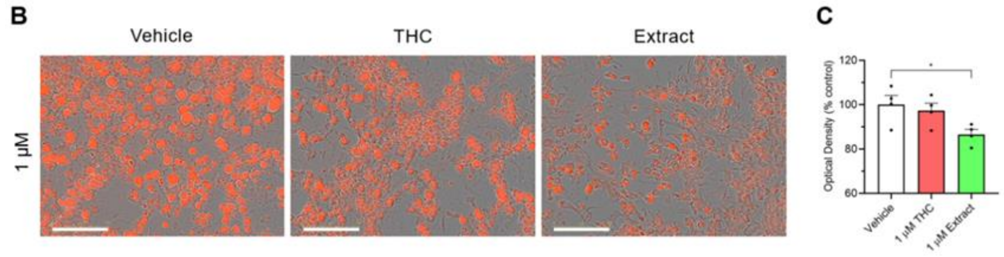
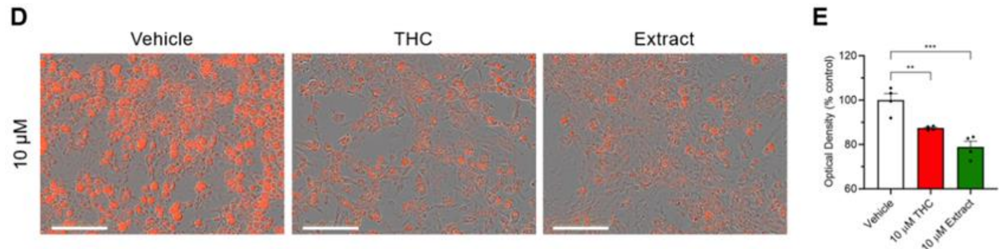
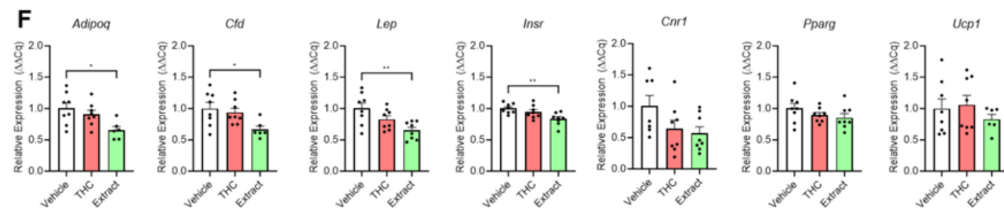


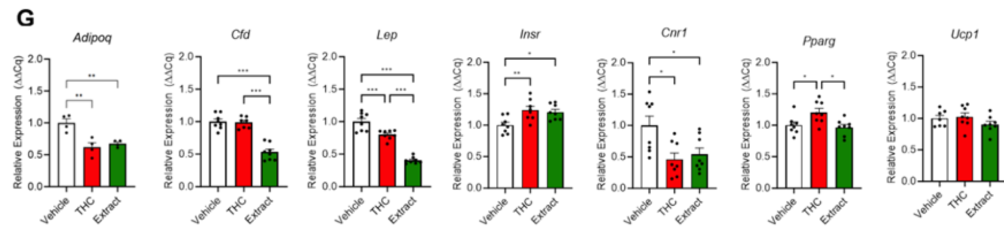
Figure 2.5. Adipocyte CB_1R deletion impacts glucose homeostasis but does not affect body weight reductions caused by chronic cannabinoid exposure.
Complete 30d body weight measurements for AdiCB$_1$+/+ (A) and AdiCB$_1$-/- (C) mice. Overall change in body weight (B, D) and body mass composition (E, F) in AdiCB$_1$+/+ and AdiCB$_1$-/- mice, respectively. Glucose tolerance test curve with GTT AUC (G) for AdiCB$_1$+/+ mice. Glucose tolerance test curve with GTT AUC (H) for AdiCB$_1$-/- mice. Two-way ANOVA (A, C, E, F, G, H) and one-way ANOVA (B, D; G, H AUC data) with Holm Sidak multiple comparisons post hoc test, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. Results are expressed as means ± S.E.M; n=5-8 per condition.


A
3T3-L1
pre-adipocytes
Confluent
3T3-L1
pre-adipocytes
Differentiated
3T3-L1
adipocytes
Mature
3T3-L1
adipocytes
Oil Red O
staining
Basal Media (BM)
Differentiation Media (DM)
Maintenance Media (MM)
MM + THC or Extract
Day -3
Day 0
Day 3
Day 14
Day 28


B
Vehicle
THC
Extract
1 µM
C
Optical Density (% control)
Vehicle
1 µM THC
1 µM Extract


D
Vehicle
THC
Extract
10 µM
E
Optical Density (% control)
Vehicle
10 µM THC
10 µM Extract


F
Adipoq
Cfd
Lep
Insr
Cnr1
Pparg
Ucp1
Relative Expression (ΔΔCq)
Vehicle
THC
Extract


G
Adipoq
Cfd
Lep
Insr
Cnr1
Pparg
Ucp1
Relative Expression (ΔΔCq)
Vehicle
THC
Extract

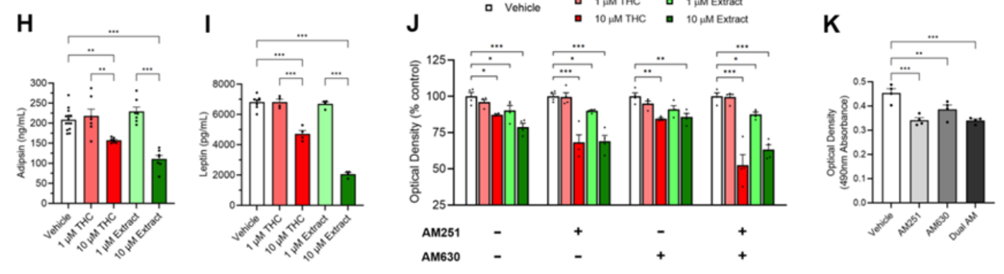
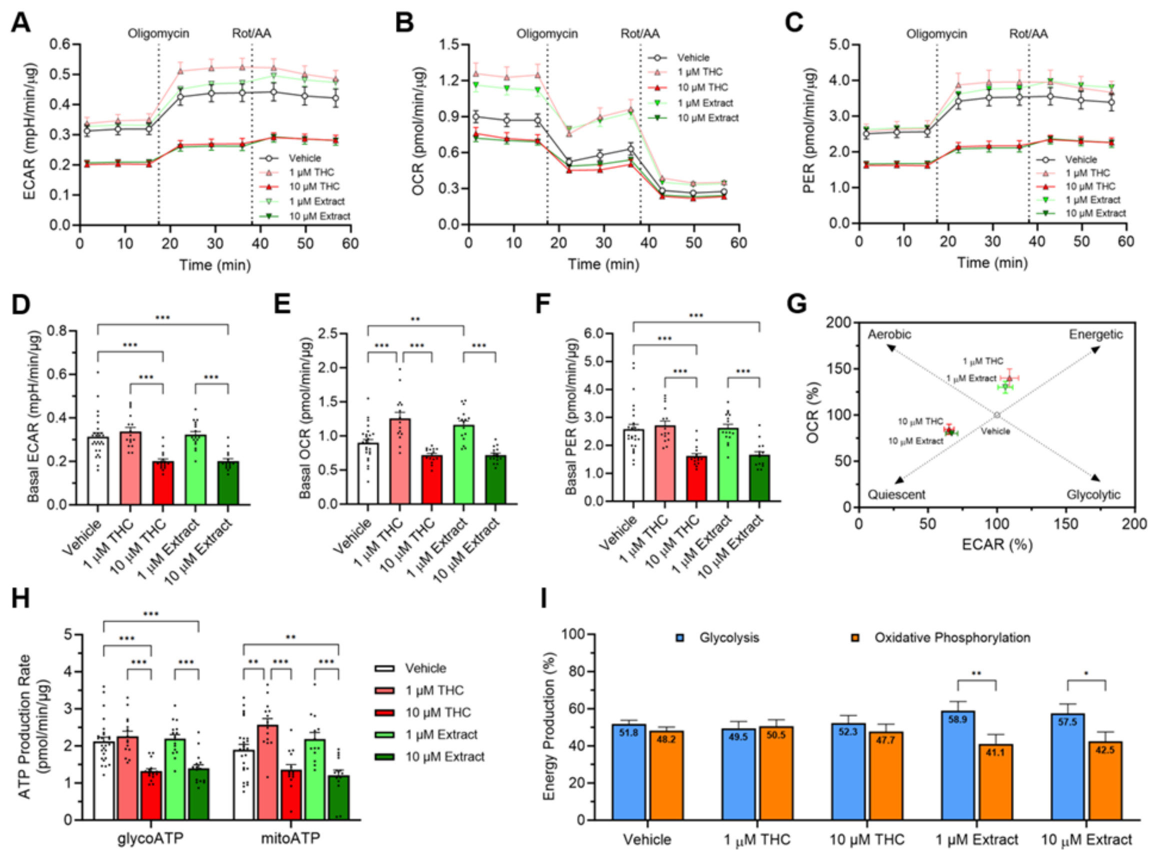

H
Adipsin (ng/mL)
I
Leptin (pg/mL)
Vehicle
1 µM THC
10 µM THC
1 µM Extract
10 µM Extract
J
Vehicle
1 µM THC
10 µM THC
1 µM Extract
10 µM Extract
Optical Density (% control)
AM251
AM630
K
Optical Density
(490nm Absorbance)
Vehicle
AM251
AM630
Dual AM

Figure 2.6. Chronic cannabinoid exposure in 3T3-L1 cultured adipocytes results in reduced lipid accumulation.

(A) Differentiation and exposure paradigm in 3T3-L1 cells. (B, D) Oil Red O stained adipocytes exposed to 1 or 10 µM THC and THC-dose matched extract with (C, E) corresponding optical density of eluted ORO measured at 490 nm. (F, G) Gene expression in 3T3-L1 adipocytes treated with 1 or 10 µM THC. (H) Adipsin and (I) ELISA measurements of adipokines in media of cannabinoid-treated adipocytes. (J) Normalized optical density of low and high THC treatment in the presence of CB_1R (AM251) and/or CB2R (AM630) antagonists. (K) Raw optical density values of control wells treated with only cannabinoid antagonists. One-way ANOVA with Holm-Sidak multiple comparisons post hoc test, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. Results are expressed as means ± S.E.M; n=4-8 per condition.



<u>Figure 2.7. Chronic THC exposure produces dose-dependent changes in cellular bioenergetic profile of 3T3-L1 adipocytes.</u>

(A) Extracellular acidification rate (ECAR), (B) oxygen consumption rate (OCR), and (C) proton efflux rate (PER) measuring real-time ATP production in 3T3-L1 cells treated with low (1 uM) and high (10uM) THC and THC-dose matched extract concentrations. (D-F) Basal rates for ECAR, OCR, and PER indicating differences in cellular phenotypes based on drug concentration. (G) Energy map of THC- and extract-treated adipocytes based on OCR and ECAR. (H) ATP production rates from glycolysis and mitochondrial respiration. (I) Overall ATP produced from glycolysis and oxidative phosphorylation represented as a percentage. Two-way ANOVA (A-C, H), one-way ANOVA (D-F), and multiple unpaired t-test (I) with Holm-Sidak multiple comparisons post hoc test, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. All data are expressed as means ± S.E.M; n=16 per condition.

Conclusion

The eCB system plays a multifaceted role in adipose tissue biology, influencing appetite regulation, adipogenesis, and the secretion of adipokines. The interaction between CB_1 receptors and cannabinoids, particularly THC, has profound effects on energy balance and metabolic function. Understanding the complex interplay between CB_1 receptors, adipose tissue, and adipokines is essential for developing targeted interventions that address the global health burden of obesity. DIO is primarily driven by an excessive intake of high-calorie, energy-dense foods coupled with sedentary lifestyles. This caloric surplus leads to the expansion of adipose tissue mass and the subsequent dysregulation of adipokines. As obesity progresses, adipose tissue undergoes changes in its cellular composition and function. Adipogenesis, the process of adipocyte differentiation, is a critical determinant of adipose tissue expansion. Studies using the 3T3-L1 adipocyte cell line have demonstrated that CB_1 receptor activation enhances adipogenesis by promoting preadipocyte differentiation into mature adipocytes[61,62,74,75]. This effect is mediated by the activation of intracellular signaling pathways involved in adipogenic gene expression[10]. Consequently, CB_1 receptor blockade has been proposed as a potential strategy to mitigate excessive adipocyte differentiation, thereby limiting adipose tissue expansion.

Adipokines are central players in the intricate web of interactions between adipose tissue, energy metabolism, glucose homeostasis, and insulin resistance. Their multifaceted roles underscore the importance of adipose tissue as a dynamic

endocrine organ with far-reaching systemic effects. By elucidating the mechanisms by which cannabinoids and adipokines influence metabolic health, researchers and clinicians can develop targeted strategies for preventing and treating obesity-related metabolic disorders, ultimately improving the quality of life for individuals affected by these conditions. The CB_1 receptor's presence in adipose tissue underscores its importance in adipocyte function, adipokine secretion, and metabolic regulation. Dysregulated CB_1 signaling contributes to adipose tissue dysfunction, altered adipokine profiles, appetite dysregulation, and insulin resistance, all of which are key components of obesity-related metabolic complications. A thorough understanding of CB_1 receptor-mediated effects on adipose tissue could pave the way for novel therapeutic strategies targeting this pathway to effectively manage obesity and its associated metabolic consequences. Thus, targeting peripheral cannabinoid receptors, particularly CB_1 in the small intestine and in adipose tissue, with selective antagonists or modulators could help mitigate obesity-related metabolic disturbances.

As a homeostatic system of endogenous lipid signaling molecules, the eCB system is also a target of exogenous cannabinoids, such as those found in cannabis. The primary psychoactive constituent of cannabis is Δ9-tetrahydrocannabinol (THC), which is among more than 100 phytocannabinoids found within the plant. These phytocannabinoids, especially THC, are capable of binding to the cannabinoid receptors found both in the central nervous system and the periphery ultimately altering appetite and energy homeostasis[43,76,77].

Furthermore, the consequences associated with the dysregulation of energy homeostasis caused by obesity extend to the small intestine and pancreas. Upon introduction of dietary nutrients to the small intestine, the peripheral endocannabinoid system found here affects gut-derived hormone release of GIP, GLP, and CCK, which collectively can control up to 70% of insulin release from the pancreas[78-80]. In this regard, dysfunction of the eCS may influence the unbalanced glucose homeostasis seen in type 2 diabetes, one of obesity's most common comorbidities. Currently, the consequences on glucose homeostasis and pancreatic function associated with cannabis exposure remain unclear.

Overall, this work highlights the pro-metabolic effects of chronic cannabinoid exposure in DIO mice, which promoted a metabolic profile comparable to lean control mice fed a low-fat/no sucrose standard diet. Together, these results highlight cannabis' therapeutic potential to target the molecular mechanism(s) linking obesity and T2D. Whole cannabis extract contains several non-psychoactive compounds such as tetrahydrocannabivarin (THCV) that can affect adipocyte biology, however the precise biological underpinnings remain unclear[81,82]. Moreover, the potential anti-inflammatory and metabolic-enhancing effects of CBD warrant further investigation[83-85]. With an increase in popularity and overall societal acceptance, it is imperative to further investigate the role of both acute and chronic cannabis use and how the endocannabinoid system may adapt to the increasingly potent marijuana consumed across the world.

References

1. DiPatrizio, N.V., Endocannabinoids in the Gut. Cannabis Cannabinoid Res, 2016. 1(1): p. 67-77.

2. Christie, S., et al., Biphasic effects of methanandamide on murine gastric vagal afferent mechanosensitivity. The Journal of Physiology, 2019. n/a(n/a).

3. Fenwick, A.J., et al., Direct Anandamide Activation of TRPV1 Produces Divergent Calcium and Current Responses. Frontiers in molecular neuroscience, 2017. 10: p. 200-200.

4. Biener, A., J. Cawley, and C. Meyerhoefer, The High and Rising Costs of Obesity to the US Health Care System. J Gen Intern Med, 2017. 32(Suppl 1): p. 6-8.

5. Chia, C.W., et al., Incretin secretion in humans is under the influence of cannabinoid receptors. Am J Physiol Endocrinol Metab, 2017. 313(3): p. E359-E366.

6. Cluny, N.L., et al., Prevention of Diet-Induced Obesity Effects on Body Weight and Gut Microbiota in Mice Treated Chronically with Δ9-Tetrahydrocannabinol. PLoS One, 2015. 10(12): p. e0144270.

7. D'Souza, D.C., et al., Rapid Changes in CB_1 Receptor Availability in Cannabis Dependent Males after Abstinence from Cannabis. Biol Psychiatry Cogn Neurosci Neuroimaging, 2016. 1(1): p. 60-67.

8. el Marjou, F., et al., Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. Genesis, 2004. 39(3): p. 186-93.

9. ElSohly, M.A., et al., Changes in Cannabis Potency Over the Last 2 Decades (1995-2014): Analysis of Current Data in the United States. Biol Psychiatry, 2016. 79(7): p. 613-9.

10. Foltin, R.W., J.V. Brady, and M.W. Fischman, Behavioral analysis of marijuana effects on food intake in humans. Pharmacol Biochem Behav, 1986. 25(3): p. 577-82.

11. Han, W., et al., A Neural Circuit for Gut-Induced Reward. Cell, 2018. 175(3): p. 887-888.

12. Jadoon, K.A., et al., Efficacy and Safety of Cannabidiol and Tetrahydrocannabivarin on Glycemic and Lipid Parameters in Patients With Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled, Parallel Group Pilot Study. Diabetes Care, 2016. 39(10): p. 1777-86.

13. Kirkham, T.C., Endocannabinoids in the regulation of appetite and body weight. Behav Pharmacol, 2005. 16(5-6): p. 297-313.

14. Kleckner, A.S., et al., Opportunities for cannabis in supportive care in cancer. Ther Adv Med Oncol, 2019. 11: p. 1758835919866362.

15. Sam, A.H., V. Salem, and M.A. Ghatei, Rimonabant: From RIO to Ban. J Obes, 2011. 2011: p. 432607.

16. Scheinker, D., A. Valencia, and F. Rodriguez, Identification of Factors Associated With Variation in US County-Level Obesity Prevalence Rates Using Epidemiologic vs Machine Learning Models. JAMA Netw Open, 2019. 2(4): p. e192884.

17. Seino, Y., M. Fukushima, and D. Yabe, GIP and GLP-1, the two incretin hormones: Similarities and differences. J Diabetes Investig, 2010. 1(1-2): p. 8-23.

18. Shire, D., et al., Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. Biochim Biophys Acta, 1996. 1307(2): p. 132-6.

19. Smitka, K., et al., The role of "mixed" orexigenic and anorexigenic signals and autoantibodies reacting with appetite-regulating neuropeptides and peptides of the adipose tissue-gut-brain axis: relevance to food intake and nutritional status in patients with anorexia nervosa and bulimia nervosa. Int J Endocrinol, 2013. 2013: p. 483145.

20. Steinert, R.E., et al., Ghrelin, CCK, GLP-1, and PYY(3-36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. Physiol Rev, 2017. 97(1): p. 411-463.

21. Turcotte, C., et al., The CB 2 receptor and its role as a regulator of inflammation. Cell Mol Life Sci, 2016. 73(23): p. 4449-4470.

22. Pacek, L.R., R.J. Malcolm, and S.S. Martins, Race/ethnicity differences between alcohol, marijuana, and co-occurring alcohol and marijuana use disorders and their association with public health and social problems using a national sample. Am J Addict, 2012. 21(5): p. 435-44.

23. Wu, L.T., H. Zhu, and M.S. Swartz, Trends in cannabis use disorders among racial/ethnic population groups in the United States. Drug Alcohol Depend, 2016. 165: p. 181-90.

24. Hales CM, C.M., Fryar CD, Ogden CL. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. NCHS Data Brief 2020 [cited 2021 January 2021]; 360:[Available from: https://www.cdc.gov/nchs/products/databriefs/db360.htm.

25. Arnone, M., et al., Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB_1) receptors. Psychopharmacology (Berl), 1997. 132(1): p. 104-6.

26. Avalos, B., et al., Cannabinoid CB(1) Receptors in the Intestinal Epithelium Are Required for Acute Western-Diet Preferences in Mice. Nutrients, 2020. 12(9).

27. Barlow, S.E., Expert Committee Recommendations Regarding the Prevention, Assessment, and Treatment of Child and Adolescent Overweight and Obesity: Summary Report. Pediatrics, 2007. 120(Supplement 4): p. S164.

28. Bensaid, M., et al., The Cannabinoid CB₁ Receptor Antagonist SR141716 Increases Acrp30 mRNA Expression in Adipose Tissue of Obese fa/fa Rats and in Cultured Adipocyte Cells. Molecular Pharmacology, 2003. 63(4): p. 908.

29. Bensaid, M., et al., The cannabinoid CB_1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. Mol Pharmacol, 2003. 63(4): p. 908-14.

30. Bouret, S., B.E. Levin, and S.E. Ozanne, Gene-environment interactions controlling energy and glucose homeostasis and the developmental origins of obesity. Physiological reviews, 2015. 95(1): p. 47-82.

31. Cawley, J. and C. Meyerhoefer, The medical care costs of obesity: an instrumental variables approach. J Health Econ, 2012. 31(1): p. 219-30.

32. Cota, D., et al., The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest, 2003. 112(3): p. 423-31.

33. Eid, B.G., et al., Effects of the CB_1 Receptor Antagonists AM6545 and AM4113 on Insulin Resistance in a High-Fructose High-Salt Rat Model of Metabolic Syndrome. Medicina (Kaunas), 2020. 56(11).

34. Fryar, C.D., et al., Fast Food Consumption Among Adults in the United States, 2013-2016. NCHS Data Brief, 2018(322): p. 1-8.

35. Hirsch, S. and J. Tam, Cannabis: From a Plant That Modulates Feeding Behaviors toward Developing Selective Inhibitors of the Peripheral Endocannabinoid System for the Treatment of Obesity and Metabolic Syndrome. Toxins, 2019. 11(5): p. 275.

36. Jo, Y.-H., et al., Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit. Neuron, 2005. 48(6): p. 1055-1066.

37. Johansson, E., et al., Determination of delta 1-tetrahydrocannabinol in human fat biopsies from marihuana users by gas chromatography-mass spectrometry. Biomed Chromatogr, 1989. 3(1): p. 35-8.

38. Kershaw, E.E. and J.S. Flier, Adipose Tissue as an Endocrine Organ. The Journal of Clinical Endocrinology & Metabolism, 2004. 89(6): p. 2548-2556.

39. Khaodhiar, L., K.C. McCowen, and G.L. Blackburn, Obesity and its comorbid conditions. Clin Cornerstone, 1999. 2(3): p. 17-31.

40. Kirkham, T.C., et al., Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. Br J Pharmacol, 2002. 136(4): p. 550-7.

41. Nguyen, T., B.F. Thomas, and Y. Zhang, Overcoming the Psychiatric Side Effects of the Cannabinoid CB_1 Receptor Antagonists: Current Approaches for Therapeutics Development. Current topics in medicinal chemistry, 2019. 19(16): p. 1418-1435.

42. Powell, L.M., B.T. Nguyen, and E. Han, Energy intake from restaurants: demographics and socioeconomics, 2003-2008. American journal of preventive medicine, 2012. 43(5): p. 498-504.

43. Randall, P.A., et al., The novel cannabinoid CB_1 antagonist AM6545 suppresses food intake and food-reinforced behavior. Pharmacol Biochem Behav, 2010. 97(1): p. 179-84.

44. Sánchez-Fuentes, A., et al., mGluR1/5 activation in the lateral hypothalamus increases food intake via the endocannabinoid system. Neurosci Lett, 2016. 631: p. 104-108.

45. Sarmento-Cabral, A., et al., Adipokines (Leptin, Adiponectin, Resistin) Differentially Regulate All Hormonal Cell Types in Primary Anterior Pituitary Cell Cultures from Two Primate Species. Scientific Reports, 2017. 7(1): p. 43537.

46. Sharkey, K.A. and J.W. Wiley, The Role of the Endocannabinoid System in the Brain-Gut Axis. Gastroenterology, 2016. 151(2): p. 252-266.

47. Sobrino Crespo, C., et al., Peptides and food intake. Frontiers in endocrinology, 2014. 5: p. 58-58.

48. Soria-Gómez, E., et al., The endocannabinoid system controls food intake via olfactory processes. Nat Neurosci, 2014. 17(3): p. 407-15.

49. Sticht, M.A., et al., Endocannabinoid regulation of homeostatic feeding and stress-induced alterations in food intake in male rats. Br J Pharmacol, 2019. 176(10): p. 1524-1540.

50. Tedesco, L., et al., Cannabinoid receptor stimulation impairs mitochondrial biogenesis in mouse white adipose tissue, muscle, and liver: the role of eNOS, p38 MAPK, and AMPK pathways. Diabetes, 2010. 59(11): p. 2826-2836.

51. Tucci, S.A., et al., The cannabinoid CB_1 receptor antagonist SR141716 blocks the orexigenic effects of intrahypothalamic ghrelin. British journal of pharmacology, 2004. 143(5): p. 520-523.

52. Verty, A.N., et al., The cannabinoid receptor antagonist SR 141716 attenuates overfeeding induced by systemic or intracranial morphine. Psychopharmacology (Berl), 2003. 168(3): p. 314-23.

53. Wang, J., et al., Overfeeding Rapidly Induces Leptin and Insulin Resistance. Diabetes, 2001. 50(12): p. 2786.

54. Zagorsky, J.L. and P.K. Smith, The association between socioeconomic status and adult fast-food consumption in the U.S. Econ Hum Biol, 2017. 27(Pt A): p. 12-25.

55. Arita, Y., et al., Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun, 1999. 257(1): p. 79-83.

56. Assifi, M.M., et al., AMP-activated protein kinase and coordination of hepatic fatty acid metabolism of starved/carbohydrate-refed rats. American Journal of Physiology-Endocrinology and Metabolism, 2005. 289(5): p. E794-E800.

57. Baur, R., J. Gertsch, and E. Sigel, The cannabinoid CB_1 receptor antagonists rimonabant (SR141716) and AM251 directly potentiate GABA(A) receptors. British journal of pharmacology, 2012. 165(8): p. 2479-2484.

58. Bouaboula, M., et al., Anandamide induced PPARγ transcriptional activation and 3T3-L1 preadipocyte differentiation. European Journal of Pharmacology, 2005. 517(3): p. 174-181.

59. Cordain, L., et al., Origins and evolution of the Western diet: health implications for the 21st century. The American Journal of Clinical Nutrition, 2005. 81(2): p. 341-354.

60. Cousin, B., et al., Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. Journal of Cell Science, 1992. 103(4): p. 931.

61. Hales CM, C.M., Fryar CD, Ogden and CL., Prevalence of obesity and severe obesity among adults: United States, 2017–2018. NCHS Data Brief, 2020. No. 360.

62. Hill, J.O., H.R. Wyatt, and J.C. Peters, Energy balance and obesity. Circulation, 2012. 126(1): p. 126-132.

63. Jager, G. and R.F. Witkamp, The endocannabinoid system and appetite: relevance for food reward. Nutrition Research Reviews, 2014. 27(1): p. 172-185.

64. Jo, J., et al., Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. PLoS computational biology, 2009. 5(3): p. e1000324-e1000324.

65. Juge-Aubry, C.E., et al., Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. Diabetes, 2003. 52(5): p. 1104-10.

66. Kopp, W., How Western Diet And Lifestyle Drive The Pandemic Of Obesity And Civilization Diseases. Diabetes Metab Syndr Obes, 2019. 12: p. 2221-2236.

67. Lihn, A.S., et al., AICAR stimulates adiponectin and inhibits cytokines in adipose tissue. Biochem Biophys Res Commun, 2004. 316(3): p. 853-8.

68. Osei-Hyiaman, D., et al., Endocannabinoid activation at hepatic CB_1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. The Journal of clinical investigation, 2005. 115(5): p. 1298-1305.

69. Pagano, C., et al., The Endogenous Cannabinoid System Stimulates Glucose Uptake in Human Fat Cells via Phosphatidylinositol 3-Kinase and Calcium-Dependent Mechanisms. The Journal of Clinical Endocrinology & Metabolism, 2007. 92(12): p. 4810-4819.

70. Petrovic, N., et al., Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. The Journal of biological chemistry, 2010. 285(10): p. 7153-7164.

71. Spoto, B., et al., Human adipose tissue binds and metabolizes the endocannabinoids anandamide and 2-arachidonoylglycerol. Biochimie, 2006. 88(12): p. 1889-1897.

72. Verty, A.N., et al., The Cannabinoid Receptor Agonist THC Attenuates Weight Loss in a Rodent Model of Activity-Based Anorexia. Neuropsychopharmacology, 2011. 36(7): p. 1349-1358.

73. Wu, J., et al., Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell, 2012. 150(2): p. 366-376.

74. Di Marzo, V., A. Ligresti, and L. Cristino, The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. International Journal of Obesity, 2009. 33(S2): p. S18-S24.

75. Organization, W.H., Taking Action on Childhood Obesity, W.O. Federation, Editor. 2018.

76. Monteleone, A., et al., Peripheral Endocannabinoid Responses to Hedonic Eating in Binge-Eating Disorder. Nutrients, 2017. 9(12): p. 1377.

77. Kola, B., et al., The Orexigenic Effect of Ghrelin Is Mediated through Central Activation of the Endogenous Cannabinoid System. PLoS ONE, 2008. 3(3): p. e1797.

78. Berg, A.H., T.P. Combs, and P.E. Scherer, ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends in Endocrinology & Metabolism, 2002. 13(2): p. 84-89.

79. Motaghedi, R. and T.E. McGraw, The CB_1 Endocannabinoid System Modulates Adipocyte Insulin Sensitivity. Obesity, 2008. 16(8): p. 1727-1734.

80. Skurk, T., et al., Relationship between Adipocyte Size and Adipokine Expression and Secretion. The Journal of Clinical Endocrinology & Metabolism, 2007. 92(3): p. 1023-1033.

81. Weyer, C., et al., Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts Type II diabetes independent of insulin resistance. Diabetologia, 2000. 43(12): p. 1498-1506.

82. Cota, D., CB_1 receptors: emerging evidence for central and peripheral mechanisms that regulate energy balance, metabolism, and cardiovascular health. Diabetes/Metabolism Research and Reviews, 2007. 23(7): p. 507-517.

83. Matias, I., et al., Regulation, Function, and Dysregulation of Endocannabinoids in Models of Adipose and β-Pancreatic Cells and in Obesity and Hyperglycemia. The Journal of Clinical Endocrinology & Metabolism, 2006. 91(8): p. 3171-3180.

84. Diraison, F., et al., Differences in the regulation of adipose tissue and liver lipogenesis by carbohydrates in humans. Journal of Lipid Research, 2003. 44(4): p. 846-853.

85. Teixeira, D., et al., Modulation of Adipocyte Biology by Δ9 -Tetrahydrocannabinol. Obesity, 2010. 18(11): p. 2077-2085.

86. Engeli, S., et al., Activation of the Peripheral Endocannabinoid System in Human Obesity. Diabetes, 2005. 54(10): p. 2838-2843.

87. Hu, E., P. Liang, and B.M. Spiegelman, AdipoQ Is a Novel Adipose-specific Gene Dysregulated in Obesity. Journal of Biological Chemistry, 1996. 271(18): p. 10697-10703.

88. Kola, B., et al., Cannabinoids and Ghrelin Have Both Central and Peripheral Metabolic and Cardiac Effects via AMP-activated Protein Kinase. Journal of Biological Chemistry, 2005. 280(26): p. 25196-25201.

89. Perwitz, N., et al., Cannabinoid type 1 receptor blockade induces transdifferentiation towards a brown fat phenotype in white adipocytes. Diabetes, Obesity and Metabolism, 2010. 12(2): p. 158-166.

90. Aziz, S.A., et al., Metabolic programming of a beige adipocyte phenotype by genistein. Molecular nutrition & food research, 2017. 61(2): p. 1600574.

91. Bartelt, A., et al., Brown adipose tissue activity controls triglyceride clearance. Nature Medicine, 2011. 17(2): p. 200-205.

92. Bjørndal, B., et al., Different Adipose Depots: Their Role in the Development of Metabolic Syndrome and Mitochondrial Response to Hypolipidemic Agents. Journal of Obesity, 2011. 2011: p. 490650.

93. Cohen, P., et al., Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. Cell, 2014. 156(1-2): p. 304-316.

94. Côté, M., et al., Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. Int J Obes (Lond), 2007. 31(4): p. 692-9.

95. Côté, M., et al., Adiponectinemia in visceral obesity: impact on glucose tolerance and plasma lipoprotein and lipid levels in men. J Clin Endocrinol Metab, 2005. 90(3): p. 1434-9.

96. Dodd, G.T., et al., Leptin and insulin act on POMC neurons to promote the browning of white fat. Cell, 2015. 160(1-2): p. 88-104.

97. Fayyad, A.M., et al., Rosiglitazone Enhances Browning Adipocytes in Association with MAPK and PI3-K Pathways During the Differentiation of Telomerase-Transformed Mesenchymal Stromal Cells into Adipocytes. International journal of molecular sciences, 2019. 20(7): p. 1618.

98. Herling, A.W., et al., Increased Energy Expenditure Contributes More to the Body Weight-Reducing Effect of Rimonabant than Reduced Food Intake in Candy-Fed Wistar Rats. Endocrinology, 2008. 149(5): p. 2557-2566.

99. Howlett, A.C., Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. Life Sciences, 1984. 35(17): p. 1803-1810.

100. Jbilo, O., et al., The CB_1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. The FASEB Journal, 2005. 19(11): p. 1567-1569.

101. Kajimura, S., B.M. Spiegelman, and P. Seale, Brown and Beige Fat: Physiological Roles beyond Heat Generation. Cell metabolism, 2015. 22(4): p. 546-559.

102. Lone, J. and J.W. Yun, Monoterpene limonene induces brown fat-like phenotype in 3T3-L1 white adipocytes. Life Sciences, 2016. 153: p. 198-206.

103. Perwitz, N., M. Fasshauer, and J. Klein, Cannabinoid receptor signaling directly inhibits thermogenesis and alters expression of adiponectin and visfatin. Horm Metab Res, 2006. 38(5): p. 356-8.

104. Russo, E.B., Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British journal of pharmacology, 2011. 163(7): p. 1344-1364.

105. Song, Z., A.M. Xiaoli, and F. Yang, Regulation and Metabolic Significance of De Novo Lipogenesis in Adipose Tissues. Nutrients, 2018. 10(10): p. 1383.

106. Tchkonia, T., et al., Mechanisms and Metabolic Implications of Regional Differences among Fat Depots. Cell Metabolism, 2013. 17(5): p. 644-656.

107. Vázquez-Vela, M.E.F., N. Torres, and A.R. Tovar, White Adipose Tissue as Endocrine Organ and Its Role in Obesity. Archives of Medical Research, 2008. 39(8): p. 715-728.

108. Verty, A.N.A., A.M. Allen, and B.J. Oldfield, The Effects of Rimonabant on Brown Adipose Tissue in Rat: Implications for Energy Expenditure. Obesity, 2009. 17(2): p. 254-261.

109. Di Marzo, V., The endocannabinoid system in obesity and type 2 diabetes. Diabetologia, 2008. 51(8): p. 1356-1367.

110. Le Foll, C., et al., FAT/CD36: A Major Regulator of Neuronal Fatty Acid Sensing and Energy Homeostasis in Rats and Mice. Diabetes, 2013. 62(8): p. 2709-2716.

111. Towler, M.C. and D.G. Hardie, AMP-Activated Protein Kinase in Metabolic Control and Insulin Signaling. Circulation Research, 2007. 100(3): p. 328-341.

112. Armani, A., et al., Phosphodiesterase type 5 (PDE5) in the adipocyte: a novel player in fat metabolism? Trends in Endocrinology & Metabolism, 2011. 22(10): p. 404-411.

113. Yoon, M.J., et al., Adiponectin Increases Fatty Acid Oxidation in Skeletal Muscle Cells by Sequential Activation of AMP-Activated Protein Kinase, p38 Mitogen-Activated Protein Kinase, and Peroxisome Proliferator–Activated Receptor α. Diabetes, 2006. 55(9): p. 2562-2570.

114. Bellocchio, L., et al., Cannabinoid Type 1 Receptor: Another Arrow in the Adipocytes' Bow. Journal of Neuroendocrinology, 2008. 20(s1): p. 130-138.

115. Blüher, M., et al., Dysregulation of the Peripheral and Adipose Tissue Endocannabinoid System in Human Abdominal Obesity. Diabetes, 2006. 55(11): p. 3053.

116. Fellous, T., et al., Phytocannabinoids promote viability and functional adipogenesis of bone marrow-derived mesenchymal stem cells through different molecular targets. Biochemical Pharmacology, 2020. 175: p. 113859.

117. Galve-Roperh, I., et al., Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. Progress in Lipid Research, 2013. 52(4): p. 633-650.

118. Hosogai, N., et al., Adipose Tissue Hypoxia in Obesity and Its Impact on Adipocytokine Dysregulation. Diabetes, 2007. 56(4): p. 901.

119. Kraus, N.A., et al., Quantitative assessment of adipocyte differentiation in cell culture. Adipocyte, 2016. 5(4): p. 351-358.

120. Kraynik, S.M., R.S. Miyaoka, and J.A. Beavo, PDE3 and PDE4 isozyme-selective inhibitors are both required for synergistic activation of brown adipose tissue. Molecular pharmacology, 2013. 83(6): p. 1155-1165.

121. Ould Amer, Y. and E. Hebert-Chatelain, Mitochondrial cAMP-PKA signaling: What do we really know? Biochimica et Biophysica Acta (BBA) - Bioenergetics, 2018. 1859(9): p. 868-877.

122. Viscarra, J.A., et al., 5'AMP-activated protein kinase activity is increased in adipose tissue of northern elephant seal pups during prolonged fasting-induced insulin resistance. The Journal of endocrinology, 2011. 209(3): p. 317-325.

123. Yang, H. and L. Yang, Targeting cAMP/PKA pathway for glycemic control and type 2 diabetes therapy. Journal of Molecular Endocrinology, 2016. 57(2): p. R93-R108.

124. Bielawiec, P., E. Harasim-Symbor, and A. Chabowski, Phytocannabinoids: Useful Drugs for the Treatment of Obesity? Special Focus on Cannabidiol. Frontiers in Endocrinology, 2020. 11.

125. Bays, H.E., et al., Obesity, adiposity, and dyslipidemia: A consensus statement from the National Lipid Association. Journal of Clinical Lipidology, 2013. 7(4): p. 304-383.

126. Gómez-Hernández, A., et al., Differential Role of Adipose Tissues in Obesity and Related Metabolic and Vascular Complications. International journal of endocrinology, 2016. 2016: p. 1216783-1216783.

127. Karschner, E.L., et al., Extended plasma cannabinoid excretion in chronic frequent cannabis smokers during sustained abstinence and correlation with psychomotor performance. Drug Testing and Analysis, 2016. 8(7): p. 682-689.

128. Longo, M., et al., Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. International journal of molecular sciences, 2019. 20(9): p. 2358.

129. Dibba, P., et al., Potential Mechanisms Influencing the Inverse Relationship Between Cannabis and Nonalcoholic Fatty Liver Disease: A Commentary. Nutr Metab Insights, 2019. 12: p. 1178638819847480.

130. Hirsch, S. and J. Tam, : From a Plant That Modulates Feeding Behaviors toward Developing Selective Inhibitors of the Peripheral Endocannabinoid System for the Treatment of Obesity and Metabolic Syndrome. Toxins (Basel), 2019. 11(5).

131. Järbe, T.U. and N.V. DiPatrizio, Delta9-THC induced hyperphagia and tolerance assessment: interactions between the CB_1 receptor agonist delta9-THC and the CB_1 receptor antagonist SR-141716 (rimonabant) in rats. Behav Pharmacol, 2005. 16(5-6): p. 373-80.

132. Morton, G.J., et al., Central nervous system control of food intake and body weight. Nature, 2006. 443(7109): p. 289-295.

133. Tam, J., et al., Peripheral CB_1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. The Journal of clinical investigation, 2010. 120(8): p. 2953-2966.

134. Van Eenige, R., et al., Regulation of Adipose Tissue Metabolism by the Endocannabinoid System. Trends in Endocrinology & Metabolism, 2018. 29(5): p. 326-337.

135. Ahima, R.S. and D.A. Antwi, Brain regulation of appetite and satiety. Endocrinology and metabolism clinics of North America, 2008. 37(4): p. 811-823.

136. Aung, M.M., et al., Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB(1) and CB(2) receptor binding. Drug Alcohol Depend, 2000. 60(2): p. 133-40.

137. Bancks, M.P., et al., Self-reported marijuana use over 25 years and abdominal adiposity: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Addiction, 2018. 113(4): p. 689-698.

138. Bentley-Hewitt, K.L., et al., Comparison of quantitative real-time polymerase chain reaction with NanoString® methodology using adipose and liver tissues from rats fed seaweed. New Biotechnology, 2016. 33(3): p. 380-386.

139. Camilleri, M., et al., Cannabinoid receptor 1 gene and irritable bowel syndrome: phenotype and quantitative traits. American Journal of Physiology-Gastrointestinal and Liver Physiology, 2013. 304(5): p. G553-G560.

140. Cirera, S., Highly efficient method for isolation of total RNA from adipose tissue. BMC research notes, 2013. 6: p. 472-472.

141. Clark, T.M., et al., Theoretical Explanation for Reduced Body Mass Index and Obesity Rates in Cannabis Users. Cannabis and cannabinoid research, 2018. 3(1): p. 259-271.

142. Cluny, N.L., et al., A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. British journal of pharmacology, 2010. 161(3): p. 629-642.

143. Collins, S., et al., Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol Behav, 2004. 81(2): p. 243-8.

144. Devane, W.A., et al., Determination and characterization of a cannabinoid receptor in rat brain. Molecular pharmacology, 1988. 34(5): p. 605-613.

145. DiPatrizio, N.V., et al., Fasting stimulates 2-AG biosynthesis in the small intestine: role of cholinergic pathways. (1522-1490 (Electronic)).

146. Dossou, K.S.S., et al., Development and preliminary validation of a plate-based CB_1/CB2 receptor functional assay. Analytical biochemistry, 2013. 437(2): p. 138-143.

147. Dunstan, W.R. and T.A. Henry. On oxycannabin from Indian hemp.

148. Fattore, L., et al., Intravenous self-administration of the cannabinoid CB_1 receptor agonist WIN 55,212-2 in rats. Psychopharmacology (Berl), 2001. 156(4): p. 410-6.

149. Flurkey, K., J. Currer, and D. Harrison, The Mouse in Aging Research. The Mouse in Biomedical Research, 2007. 3.

150. Foloppe, N., et al., Discovery of a novel class of selective human CB_1 inverse agonists. Bioorg Med Chem Lett, 2008. 18(3): p. 1199-206.

151. Fong, T.M. and S.B. Heymsfield, Cannabinoid-1 receptor inverse agonists: current understanding of mechanism of action and unanswered questions. Int J Obes (Lond), 2009. 33(9): p. 947-55.

152. Gaoni, Y. and R. Mechoulam, Isolation, structure, and partial synthesis of an active constituent of hashish. Journal of the American chemical society, 1964. 86(8): p. 1646-1647.

153. Gómez, R., et al., A peripheral mechanism for CB_1 cannabinoid receptor-dependent modulation of feeding. J Neurosci, 2002. 22(21): p. 9612-7.

154. Hayatbakhsh, M.R., et al., Cannabis use and obesity and young adults. Am J Drug Alcohol Abuse, 2010. 36(6): p. 350-6.

155. Herkenham, M., et al., Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci, 1991. 11(2): p. 563-83.

156. Ho, J., J.M. Cox, and E.J. Wagner, Cannabinoid-induced hyperphagia: correlation with inhibition of proopiomelanocortin neurons? Physiology & behavior, 2007. 92(3): p. 507-519.

157. Huestis, M.A., Human cannabinoid pharmacokinetics. Chemistry & biodiversity, 2007. 4(8): p. 1770-1804.

158. Juan-Picó, P., et al., Cannabinoid receptors regulate Ca(2+) signals and insulin secretion in pancreatic beta-cell. Cell Calcium, 2006. 39(2): p. 155-62.

159. Kalant, O.J., Report of the Indian Hemp Drugs Commission, 1893-94: a critical review. Int J Addict, 1972. 7(1): p. 77-96.

160. Kim, D., et al., Inverse association of marijuana use with nonalcoholic fatty liver disease among adults in the United States. PLoS One, 2017. 12(10): p. e0186702.

161. Koch, M., et al., Hypothalamic POMC neurons promote cannabinoid-induced feeding. Nature, 2015. 519(7541): p. 45-50.

162. Le Strat, Y. and B. Le Foll, Obesity and cannabis use: results from 2 representative national surveys. Am J Epidemiol, 2011. 174(8): p. 929-33.

163. Leonti, M., et al., Falcarinol is a covalent cannabinoid CB_1 receptor antagonist and induces pro-allergic effects in skin. Biochemical Pharmacology, 2010. 79(12): p. 1815-1826.

164. Long, J.Z., D.K. Nomura, and B.F. Cravatt, Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. Chem Biol, 2009. 16(7): p. 744-53.

165. LoVerme, J., et al., Synthesis and characterization of a peripherally restricted CB_1 cannabinoid antagonist, URB447, that reduces feeding and body-weight gain in mice. Bioorg Med Chem Lett, 2009. 19(3): p. 639-43.

166. Mahadevan, A., et al., Novel Cannabinol Probes for CB_1 and CB2 Cannabinoid Receptors. Journal of Medicinal Chemistry, 2000. 43(20): p. 3778-3785.

167. Martellotta, M.C., et al., Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. Neuroscience, 1998. 85(2): p. 327-30.

168. Mechoulam, R., et al., Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochemical pharmacology, 1995. 50(1): p. 83-90.

169. Meier, M.H., et al., Associations Between Cannabis Use and Cardiometabolic Risk Factors: A Longitudinal Study of Men. Psychosom Med, 2019. 81(3): p. 281-288.

170. Merroun, I., et al., Influence of intracerebroventricular or intraperitoneal administration of cannabinoid receptor agonist (WIN 55,212-2) and inverse agonist (AM 251) on the regulation of food intake and hypothalamic serotonin levels. (1475-2662 (Electronic)).

171. Munro, S., K.L. Thomas, and M. Abu-Shaar, Molecular characterization of a peripheral receptor for cannabinoids. Nature, 1993. 365(6441): p. 61.

172. Parton, L.E., et al., Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. Nature, 2007. 449(7159): p. 228-32.

173. Penner, E.A., H. Buettner, and M.A. Mittleman, The impact of marijuana use on glucose, insulin, and insulin resistance among US adults. Am J Med, 2013. 126(7): p. 583-9.

174. Perdikaris, P., et al., Long lasting effects of chronic WIN55,212-2 treatment on mesostriatal dopaminergic and cannabinoid systems in the rat brain. Neuropharmacology, 2018. 129: p. 1-15.

175. Perez, P.A. and N.V. DiPatrizio, Impact of maternal western diet-induced obesity on offspring mortality and peripheral endocannabinoid system in mice. PLoS One, 2018. 13(10): p. e0205021.

176. Pertwee, R.G., The diverse CB_1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. British journal of pharmacology, 2008. 153(2): p. 199-215.

177. Pertwee, R.G., Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 2012. 367(1607): p. 3353-3363.

178. Pertwee, R.G., et al., International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. Pharmacological reviews, 2010. 62(4): p. 588-631.

179. Pertwee, R.G., et al., The psychoactive plant cannabinoid, Delta9-tetrahydrocannabinol, is antagonized by Delta8- and Delta9-tetrahydrocannabivarin in mice in vivo. British journal of pharmacology, 2007. 150(5): p. 586-594.

180. Pontieri, F.E., et al., Metabolic mapping of the effects of WIN 55212-2 intravenous administration in the rat. Neuropsychopharmacology, 1999. 21(6): p. 773-6.

181. Potenzieri, C., C. Harding-Rose, and D.A. Simone, The cannabinoid receptor agonist, WIN 55, 212-2, attenuates tumor-evoked hyperalgesia through peripheral mechanisms. Brain research, 2008. 1215: p. 69-75.

182. Rajavashisth, T.B., et al., Decreased prevalence of diabetes in marijuana users: cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) III. BMJ Open, 2012. 2: p. e000494.

183. Randall, P.A., et al., The novel cannabinoid CB_1 antagonist AM6545 suppresses food intake and food-reinforced behavior. (1873-5177 (Electronic)).

184. Reggio, P.H., Endocannabinoid binding to the cannabinoid receptors: what is known and what remains unknown. Current medicinal chemistry, 2010. 17(14): p. 1468-1486.

185. Reggio, P.H., et al., The bioactive conformation of aminoalkylindoles at the cannabinoid CB_1 and CB2 receptors: insights gained from (E)- and (Z)-naphthylidene indenes. J Med Chem, 1998. 41(26): p. 5177-87.

186. Ren, M., et al., The origins of cannabis smoking: Chemical residue evidence from the first millennium BCE in the Pamirs. Science Advances, 2019. 5(6): p. eaaw1391.

187. Rhee, M.H., et al., Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. J Med Chem, 1997. 40(20): p. 3228-33.

188. Rinaldi-Carmona, M., et al., SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett, 1994. 350(2-3): p. 240-4.

189. Russo, E.B., Clinical Endocannabinoid Deficiency Reconsidered: Current Research Supports the Theory in Migraine, Fibromyalgia, Irritable Bowel, and Other Treatment-Resistant Syndromes. Cannabis and cannabinoid research, 2016. 1(1): p. 154-165.

190. Sassmann, A., S. Offermanns, and N. Wettschureck, Tamoxifen-inducible Cre-mediated recombination in adipocytes. Genesis, 2010. 48(10): p. 618-25.

191. Scopinho, A.A., et al., Cannabidiol inhibits the hyperphagia induced by cannabinoid-1 or serotonin-1A receptor agonists. Pharmacology Biochemistry and Behavior, 2011. 98(2): p. 268-272.

192. Showalter, V.M., et al., Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. Journal of Pharmacology and Experimental Therapeutics, 1996. 278(3): p. 989.

193. Simon, V. and D. Cota, MECHANISMS IN ENDOCRINOLOGY: Endocannabinoids and metabolism: past, present and future. Eur J Endocrinol, 2017. 176(6): p. R309-R324.

194. Simoneau, Isabelle I.M.D., et al., The Cannabinoid Agonist WIN55,212-2 Suppresses Opioid-induced Emesis in Ferrets. Anesthesiology: The Journal of the American Society of Anesthesiologists, 2001. 94(5): p. 882-887.

195. Spiller, K.J., et al., Cannabinoid CB_1 and CB2 receptor mechanisms underlie cannabis reward and aversion in rats. Br J Pharmacol, 2019. 176(9): p. 1268-1281.

196. Stern, J.H., J.M. Rutkowski, and P.E. Scherer, Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. Cell Metab, 2016. 23(5): p. 770-84.

197. Sykaras, A.G., et al., Duodenal enteroendocrine I-cells contain mRNA transcripts encoding key endocannabinoid and fatty acid receptors. PLoS One, 2012. 7(8): p. e42373.

198. Tam, J., et al., Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. Cell metabolism, 2012. 16(2): p. 167-179.

199. Tedesco, L., et al., Cannabinoid type 1 receptor blockade promotes mitochondrial biogenesis through endothelial nitric oxide synthase expression in white adipocytes. Diabetes, 2008. 57(8): p. 2028-36.

200. Tedesco, L., et al., Cannabinoid Receptor Stimulation Impairs Mitochondrial Biogenesis in Mouse White Adipose Tissue, Muscle, and Liver. Diabetes, 2010. 59(11): p. 2826.

201. Thomas, A., et al., Cannabidiol displays unexpectedly high potency as an antagonist of CB_1 and CB2 receptor agonists in vitro. British journal of pharmacology, 2007. 150(5): p. 613-623.

202. Todd, A.R., The chemistry of hashish. Scientific Journal of the Royal College of Science, London, 1942. 12: p. 37-45.

203. Turner, P.V., et al., Administration of substances to laboratory animals: routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science : JAALAS, 2011. 50(5): p. 600-613.

204. Vázquez-Bourgon, J., et al., Effect of cannabis on weight and metabolism in first-episode non-affective psychosis: Results from a three-year longitudinal study. J Psychopharmacol, 2019. 33(3): p. 284-294.

205. Vidot, D.C., et al., Metabolic Syndrome Among Marijuana Users in the United States: An Analysis of National Health and Nutrition Examination Survey Data. The American Journal of Medicine, 2016. 129(2): p. 173-179.

206. Wang, C.-Y. and J.K. Liao, A mouse model of diet-induced obesity and insulin resistance. Methods in molecular biology (Clifton, N.J.), 2012. 821: p. 421-433.

207. Weltens, N., et al., Effect of acute Δ9-tetrahydrocannabinol administration on subjective and metabolic hormone responses to food stimuli and food intake in healthy humans: a randomized, placebo-controlled study. Am J Clin Nutr, 2019. 109(4): p. 1051-1063.

208. Wood, T.B., W.T.N. Spivey, and T.H. Easterfield, XL.—Charas. The resin of Indian hemp. Journal of the Chemical Society, Transactions, 1896. 69: p. 539-546.

209. Agurell, S. and K. Leander, Stability, transfer and absorption of cannabinoid constituents of cannabis (hashish) during smoking. Acta Pharm Suec, 1971. 8(4): p. 391-402.

210. Argueta, D.A. and N.V. DiPatrizio, Peripheral endocannabinoid signaling controls hyperphagia in western diet-induced obesity. Physiol Behav, 2017. 171: p. 32-39.

211. Bagchi, D.P. and O.A. MacDougald, Identification and Dissection of Diverse Mouse Adipose Depots. Journal of visualized experiments : JoVE, 2019(149): p. 10.3791/59499.

212. Banni, S., et al., Vagus Nerve Stimulation Reduces Body Weight and Fat Mass in Rats. PLOS ONE, 2012. 7(9): p. e44813.

213. Frontini, A. and S. Cinti, Distribution and Development of Brown Adipocytes in the Murine and Human Adipose Organ. Cell Metabolism, 2010. 11(4): p. 253-256.

214. Hashimoto, O., et al., Activin E Controls Energy Homeostasis in Both Brown and White Adipose Tissues as a Hepatokine. Cell Reports, 2018. 25(5): p. 1193-1203.

215. Starowicz, K.M., et al., Endocannabinoid Dysregulation in the Pancreas and Adipose Tissue of Mice Fed With a High-fat Diet. Obesity, 2008. 16(3): p. 553-565.

216. Tchkonia, T., et al., Mechanisms and metabolic implications of regional differences among fat depots. Cell metabolism, 2013. 17(5): p. 644-656.

217. Dickson, L.M., et al., Protein kinase A induces UCP1 expression in specific adipose depots to increase energy expenditure and improve metabolic health. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2016. 311(1): p. R79-R88.

218. Han, W., et al., Influence of food texture on energy metabolism and adiposity in male rats. Experimental Physiology, 2018. 103(10): p. 1347-1356.

219. Cuttler, C., L.K. Mischley, and M. Sexton, Sex Differences in Cannabis Use and Effects: A Cross-Sectional Survey of Cannabis Users. Cannabis and cannabinoid research, 2016. 1(1): p. 166-175.

220. Hansen, H.S. and V. Vana, Non-endocannabinoid N-acylethanolamines and 2-monoacylglycerols in the intestine. British Journal of Pharmacology, 2019. 176(10): p. 1443-1454.

221. Darcy, J., C.-H. Wang, and Y.-H. Tseng, Analyzing Mitochondrial Function in Brown Adipocytes with a Bioenergetic Analyzer, in Clinical and Preclinical Models for Maximizing Healthspan: Methods and Protocols, P.C. Guest, Editor. 2020, Springer US: New York, NY. p. 289-296.

222. Elmes, M.W., et al., Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). The Journal of biological chemistry, 2015. 290(14): p. 8711-8721.

223. Fu, J., et al., Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. Nature, 2003. 425(6953): p. 90-93.

224. Green, H. and O. Kehinde, Sublines of mouse 3T3 cells that accumulate lipid. Cell, 1974. 1(3): p. 113-116.

225. Guzmán, M., et al., Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-alpha). J Biol Chem, 2004. 279(27): p. 27849-54.

226. Karastergiou, K., et al., Sex differences in human adipose tissues – the biology of pear shape. Biology of Sex Differences, 2012. 3(1): p. 13.

227. Mäuser, W., et al., Direct adipotropic actions of atorvastatin: differentiation state-dependent induction of apoptosis, modulation of endocrine function, and inhibition of glucose uptake. Eur J Pharmacol, 2007. 564(1-3): p. 37-46.

228. Palomares, B., et al., Tetrahydrocannabinolic acid A (THCA-A) reduces adiposity and prevents metabolic disease caused by diet-induced obesity. Biochem Pharmacol, 2020. 171: p. 113693.

229. Ramirez-Zacarias, J., F. Castro-Munozledo, and W. Kuri-Harcuch, Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry, 1992. 97(6): p. 493-497.

230. Rodbell, M., METABOLISM OF ISOLATED FAT CELLS. I. EFFECTS OF HORMONES ON GLUCOSE METABOLISM AND LIPOLYSIS. J Biol Chem, 1964. 239: p. 375-80.

231. Sharma, A.M. and B. Staels, Review: Peroxisome proliferator-activated receptor gamma and adipose tissue--understanding obesity-related changes in regulation of lipid and glucose metabolism. J Clin Endocrinol Metab, 2007. 92(2): p. 386-95.

232. Uppenberg, J., et al., Crystal structure of the ligand binding domain of the human nuclear receptor PPARγ. Journal of Biological Chemistry, 1998. 273(47): p. 31108-31112.

233. Yang, Y., et al., Emodin with PPARgamma ligand-binding activity promotes adipocyte differentiation and increases glucose uptake in 3T3-LI cells. Biochem Biophys Res Commun, 2007. 353(2): p. 225-30.

234. Esposito, G., et al., Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. PLoS One, 2011. 6(12): p. e28668.

235. Petersen, R.K., et al., Cyclic AMP (cAMP)-Mediated Stimulation of Adipocyte Differentiation Requires the Synergistic Action of Epac- and cAMP-Dependent Protein Kinase-Dependent Processes. Molecular and Cellular Biology, 2008. 28(11): p. 3804-3816.

236. Abreu-Vieira, G., et al., Cidea improves the metabolic profile through expansion of adipose tissue. Nature Communications, 2015. 6(1): p. 7433.

237. Jadoon, K.A., et al., Efficacy and Safety of Cannabidiol and Tetrahydrocannabivarin on Glycemic and Lipid Parameters in Patients With Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled, Parallel Group Pilot Study. Diabetes Care, 2016. 39(10): p. 1777.

238. Lefterova, M.I., et al., PPARγ and the global map of adipogenesis and beyond. Trends in endocrinology and metabolism: TEM, 2014. 25(6): p. 293-302.

239. Metna-Laurent, M., et al., Cannabinoid-Induced Tetrad in Mice: Cannabinoid-Induced Tetrad. 2017. p. 9.59.1-9.59.10.

240. Miller, J.A., et al., Adipose tissue accumulation of d-limonene with the consumption of a lemonade preparation rich in d-limonene content. Nutrition and cancer, 2010. 62(6): p. 783-788.

241. Nelson, N.G., et al., Joint and separate exposure to alcohol and ∆9-tetrahydrocannabinol produced distinct effects on glucose and insulin homeostasis in male rats. Scientific Reports, 2019. 9(1): p. 12025.

242. Rawitch, A.B., R. Rohrer, and R.M. Vardaris, Delta-9-tetrahydrocannabinol uptake by adipose tissue: Preferential accumulation in gonadal fat organs. General Pharmacology: The Vascular System, 1979. 10(6): p. 525-529.

243. Ricquier, D., Uncoupling Protein 1 of Brown Adipocytes, the Only Uncoupler: A Historical Perspective. Frontiers in Endocrinology, 2011. 2(85).

244. Seale, P. and M. Lazar, Brown Fat in Humans: Turning up the Heat on Obesity. Diabetes, 2009. 58: p. 1482-4.

245. Wargent, E.T., et al., The cannabinoid Δ(9)-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in two mouse models of obesity. Nutrition & diabetes, 2013. 3(5): p. e68-e68.

246. Krott, L.M., et al., Endocannabinoid regulation in white and brown adipose tissue following thermogenic activation. J Lipid Res, 2016. 57(3): p. 464-73.

247. Lahesmaa, M., et al., Cannabinoid Type 1 Receptors Are Upregulated During Acute Activation of Brown Adipose Tissue. Diabetes, 2018.

248. Tarragon, E. and J.J. Moreno, Role of Endocannabinoids on Sweet Taste Perception, Food Preference, and Obesity-related Disorders. Chemical Senses, 2017. 43(1): p. 3-16.

249. Argueta, D.A., et al., Cannabinoid CB(1) Receptors Inhibit Gut-Brain Satiation Signaling in Diet-Induced Obesity. Front Physiol, 2019. 10: p. 704.

250. Gunasekaran, N., et al., Reintoxication: the release of fat-stored delta(9)-tetrahydrocannabinol (THC) into blood is enhanced by food deprivation or ACTH exposure. Br J Pharmacol, 2009. 158(5): p. 1330-7.

251. Ma, H., et al., Peripheral CB_1 Receptor Neutral Antagonist, AM6545, Ameliorates Hypometabolic Obesity and Improves Adipokine Secretion in Monosodium Glutamate Induced Obese Mice. Front Pharmacol, 2018. 9: p. 156.

252. Ruiz de Azua, I., et al., Adipocyte cannabinoid receptor CB_1 regulates energy homeostasis and alternatively activated macrophages. The Journal of clinical investigation, 2017. 127(11): p. 4148-4162.

253. Scherma, M., et al., Adolescent Δ(9)-Tetrahydrocannabinol Exposure Alters WIN55,212-2 Self-Administration in Adult Rats. Neuropsychopharmacology, 2016. 41(5): p. 1416-26.

254. Sidibeh, C.O., et al., Role of cannabinoid receptor 1 in human adipose tissue for lipolysis regulation and insulin resistance. Endocrine, 2017. 55(3): p. 839-852.

255. Hillard, C.J., et al., Synthesis and Characterization of Potent and Selective Agonists of the Neuronal Cannabinoid Receptor (CB_1). Journal of Pharmacology and Experimental Therapeutics, 1999. 289(3): p. 1427.

256. Matias, I., I. Belluomo, and D. Cota, The Fat Side of the Endocannabinoid System: Role of Endocannabinoids in the Adipocyte. Cannabis and Cannabinoid Research, 2016. 1(1): p. 176-185.

257. Wang, Y., et al., Will All Americans Become Overweight or Obese? Estimating the Progression and Cost of the US Obesity Epidemic. Obesity, 2008. 16(10): p. 2323-2330.

258. Alshaarawy, O. and J.C. Anthony, Are cannabis users less likely to gain weight? Results from a national 3-year prospective study. Int J Epidemiol, 2019. 48(5): p. 1695-1700.

259. Huff, H.C., et al., Differential Interactions of Selected Phytocannabinoids with Human CYP2D6 Polymorphisms. Biochemistry, 2021. 60(37): p. 2749-2760.

260. Qi, M., et al., Chronic marijuana usage by human pancreas donors is associated with impaired islet function. PLOS ONE, 2021. 16(10): p. e0258434.

261. Vaughn, S., et al., The Impact of Marijuana on Antidepressant Treatment in Adolescents: Clinical and Pharmacologic Considerations. Journal of Personalized Medicine, 2021. 11(7): p. 615.

262. Lavigne, J.E., et al., Cannabis sativa terpenes are cannabimimetic and selectively enhance cannabinoid activity. Scientific Reports, 2021. 11(1).

263. Muniyappa, R., et al., Metabolic Effects of Chronic Cannabis Smoking. Diabetes Care, 2013. 36(8): p. 2415-2422.

264. Rodondi, N., et al., Marijuana Use, Diet, Body Mass Index, and Cardiovascular Risk Factors (from the CARDIA Study). The American Journal of Cardiology, 2006. 98(4): p. 478-484.

265. Smit, E. and C.J. Crespo, Dietary intake and nutritional status of US adult marijuana users: results from the Third National Health and Nutrition Examination Survey. Public Health Nutrition, 2001. 4(3): p. 781-786.

266. Baglot, S.L., et al., Pharmacokinetics and central accumulation of delta-9-tetrahydrocannabinol (THC) and its bioactive metabolites are influenced by route of administration and sex in rats. Scientific Reports, 2021. 11(1).

267. Abioye, A., et al., Δ9-Tetrahydrocannabivarin (THCV): a commentary on potential therapeutic benefit for the management of obesity and diabetes. Journal of Cannabis Research, 2020. 2(1).

268. Barré, T., et al., Cannabis use is associated with a lower risk of diabetes in chronic hepatitis C-infected patients (ANRS CO22 Hepather cohort). Journal of Viral Hepatitis, 2020. 27(12): p. 1473-1483.

269. Kim, W., et al., Cannabinoids induce pancreatic β-cell death by directly inhibiting insulin receptor activation. Science signaling, 2012. 5(216): p. ra23-ra23.

270. Ellermann, M., et al., Endocannabinoids Inhibit the Induction of Virulence in Enteric Pathogens. Cell, 2020. 183(3): p. 650-665.e15.

271. Annuzzi, G., et al., Differential alterations of the concentrations of endocannabinoids and related lipids in the subcutaneous adipose tissue of obese diabetic patients. Lipids in Health and Disease, 2010. 9(1): p. 43.

272. Porr, C.J., et al., The effects of recreational cannabis use on glycemic outcomes and self-management behaviours in people with type 1 and type 2 diabetes: a rapid review. Systematic Reviews, 2020. 9(1).

273. Ngueta, G., et al., Cannabis use in relation to obesity and insulin resistance in the inuit population. Obesity, 2015. 23(2): p. 290-295.

274. Ewell, T.R., et al., Pharmacokinetic Investigation of Commercially Available Edible Marijuana Products in Humans: Potential Influence of Body

Composition and Influence on Glucose Control. Pharmaceuticals, 2021. 14(8): p. 817.

275. Jin, L.Z., et al., Association Between Use of Cannabis in Adolescence and Weight Change into Midlife. PLOS ONE, 2017. 12(1): p. e0168897.

276. Murphy, T. and B. Le Foll, Targeting the Endocannabinoid CB_1 Receptor to Treat Body Weight Disorders: A Preclinical and Clinical Review of the Therapeutic Potential of Past and Present CB_1 Drugs. Biomolecules, 2020. 10(6): p. 855.

277. Farokhnia, M., et al., Effects of oral, smoked, and vaporized cannabis on endocrine pathways related to appetite and metabolism: a randomized, double-blind, placebo-controlled, human laboratory study. Translational Psychiatry, 2020. 10(1).

278. Livingston, S.J., et al., Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. The Plant Journal, 2020. 101(1): p. 37-56.

279. Adejumo, A.C., et al., Cannabis use is associated with reduced prevalence of non-alcoholic fatty liver disease: A cross-sectional study. PLOS ONE, 2017. 12(4): p. e0176416.

280. Charytoniuk, T., et al., Cannabidiol – A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance. Biomedicine & Pharmacotherapy, 2021. 142: p. 112057.

281. Danielsson, A.K., et al., Cannabis Use as Risk or Protection for Type 2 Diabetes: A Longitudinal Study of 18 000 Swedish Men and Women. Journal of Diabetes Research, 2016. 2016: p. 1-6.

282. Ibrahim, S., et al., Recreational Cannabis Usage Among Young Adults Living with Diabetes: Protocol for a Mixed Methods Study. Diabetes Therapy, 2020. 11(6): p. 1407-1417.

283. Ravi, D., et al., Associations Between Marijuana Use and Cardiovascular Risk Factors and Outcomes: A Systematic Review. Annals of internal medicine, 2018. 168(3): p. 187-194.

284. Assa-Glazer, T., et al., Cannabis Extracts Affected Metabolic Syndrome Parameters in Mice Fed High-Fat/Cholesterol Diet. Cannabis and cannabinoid research, 2020. 5(3): p. 202-214.

285. Le Foll, B., et al., Cannabis and Δ9-tetrahydrocannabinol (THC) for weight loss? Medical Hypotheses, 2013. 80(5): p. 564-567.

286. Silva, M.H., Chlorpyrifos and Δ(9) Tetrahydrocannabinol exposure and effects on parameters associated with the endocannabinoid system and risk factors for obesity. Current research in toxicology, 2021. 2: p. 296-308.

287. Ahmad, T.R. and R.A. Haeusler, Bile acids in glucose metabolism and insulin signalling - mechanisms and research needs. Nature reviews. Endocrinology, 2019. 15(12): p. 701-712.

288. Kim, J.-Y., et al., Obesity-associated improvements in metabolic profile through expansion of adipose tissue. Journal of Clinical Investigation, 2007. 117(9): p. 2621-2637.