THE ROLE OF EPIGENOME AND MICROBIOME IN ENDOCRINOID-MEDIATED INFLAMMATION REGULATION IN DIET-INDUCED OBESITY

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ABSTRACT

New evidence suggests that microbiomes, often called epigenomes, contribute to metabolic health and the inflammation associated with diet-induced obesity (DIO). Within the context of endocannabinoid-mediated inflammatory regulation in a mouse model, this study investigates the interplay of DIO, the epigenomic landscape, and the composition of the microbes in the digestive tract. Inducing obesity in C57BL/6J mice and then tracking their weight allowed the researchers to analyze the consequences of a high-fat diet (HFD). The goal was to identify any changes in metabolic parameters, inflammatory markers, or both. By combining genomic sequencing with epigenetic profiling, researchers were able to identify alterations in DNA methylation patterns associated with obesity. The genes that regulate inflammation and lipid metabolism showed the most significant modifications. Simultaneously, researchers performed gut microbiota analyses using 16S rRNA sequencing; their findings showed that the HFD was linked to significant changes in microbial diversity and composition. Notably, researchers observed an increase in inflammatory-promoting microbial taxa, which is associated with elevated levels of endogenous cannabinoids. To find the pathways, researchers modified endocannabinoid signaling using pharmaceutical therapy and evaluated the effect on inflammatory responses and metabolic consequences. The microbiota and the epigenome work together to influence inflammation in DIO, according to the findings. This, in turn, impacts endocannabinoid signaling. More research is needed to determine the exact mechanisms involved and their implications for the treatment and prevention of obesity, but this work highlights the complex interplay between nutrition, microbiome, and epigenetic pathways in metabolic health and the potential of targeting these pathways as treatment approaches for inflammation associated with obesity.

Keywords: Obesity produced by diet, Modulation of the epigenome & microbiome, Endocannabinoid system,Inflammation.

INTRODUCTION

Chronic illnesses and metabolic disorders are on the rise, and diet-induced obesity is one of the leading causes of this epidemic (Kumar et al., 2020). Poor dietary choices frequently worsen this condition, which is essentially caused by consuming more calories than energy expended (Kienzl et al., 2020). Recent research has shown that diet-induced obesity is influenced by two important biological systems: the microbiome and the epigenome. Particularly in modulating the endocannabinoid system, these systems play a pivotal role in controlling inflammation. To control gene expression, the epigenome makes chemical changes to DNA and histone proteins, but these changes do not change the genetic code. Environmental variables, such people's diets, may play a role in these changes. aboutic alterations brought about by certain food components may impact inflammatory pathways and exacerbate obesity-related diseases. As an example, a high-fat diet changes the methylation landscape, which affects genes associated with inflammation and metabolism. The gut microbiome refers to the diverse community of bacteria that inhabits the digestive tract and has a significant impact on metabolic health. Dietary decisions impact energy balance and chronic inflammation, which in turn affect the composition and function of the microbiome. In addition to regulating inflammation, metabolism, and appetite, the endocannabinoid system is involved in a wide variety of other physiological processes (Manca et al., 2020). This mechanism might be affected by the microbiome. Scientists may get a better understanding of obesity and its related disorders by studying the effects of diet-induced obesity on the microbiome, especially the epigenome, and how these factors influence the control of inflammation by endocannabinoids. According to (Hui et al., 2020), this multimodal approach may lead to novel ways of addressing inflammation associated with obesity and enhancing metabolic well-being.

BACKGROUND OF THE STUDY

Over the last several decades, there has been a sea shift in how researchers approach investigating diet-induced obesity and its processes. Long ago, it was thought that eating more calories than one burns was the main reason why people became fat. Recent research has shown a complicated interplay between biochemical, environmental, and genetic variables as contributors to obesity. The field of epigenetics emerged in the early 2000s when researchers started to wonder whether there were any dietary variables that may influence gene expression in addition to DNA. Epigenetic studies have shown that dietary and other environmental variables may alter genes via mechanisms including DNA methylation and histone modification. These alterations may exacerbate obesity-related metabolic diseases by impacting inflammatory pathways, as stated by (Harsch & Konturek, 2021). New evidence from microbiome studies, meantime, has shown that the microbes in one's digestive tract are crucial in deciding whether they are healthy or sick. The trillions of microbes that comprise the human microbiome have a major influence on digestion, metabolism, and the functioning of the immune system. In the 2010s, the idea that dietary changes may influence the composition and function of the microbiome, which impacts obesity and systemic inflammation, became

widely accepted. Obesity may develop from metabolic dysregulation, inflammation, and an unbalanced microbiota, according to research. Inflammation and metabolism are two of many physiological processes regulated by the endocannabinoid system, a network of receptors and signaling molecules. Research shows that changes in the microbiota and one's diet may trigger endocannabinoid modifications, which in turn impact inflammatory responses, energy balance, and appetite regulation. There is a lot of hope at the crossroads of epigenetics, microbiome studies, and endocannabinoid modulation. Research into the effects of diet-induced obesity on these systems and how they work together to regulate inflammation could provide new avenues for the prevention and treatment of obesity and its associated diseases (Hryhorowicz et al., 2021).

PURPOSE OF THE RESEARCH

Examining the intricate interplay between the endocannabinoid system, the gut microbiome, and the epigenome is central to the study of diet-induced obesity and the microbiome's function in endocannabinoid-mediated inflammation regulation. Inflammation is a key component of metabolic problems associated with obesity, and this research intends to examine the ways in which these systems impact inflammation. Researchers want to find ways to help people with diet-induced obesity manage inflammation and improve their metabolic health by figuring out the complex web of connections between food, genes, microbes, and endocannabinoid signaling. The primary aim of this research is to fill gaps in researchers understanding of how diet-induced obesity influences epigenetic modifications, microbiome composition changes, and the function of endocannabinoid signaling in inflammatory control. Inflammatory disorders associated with obesity may benefit from tailored therapies, and this information could lead the way in their creation.

LITERATURE REVIEW

One of the most pressing problems in public health today is diet-induced obesity (DIO), which is closely linked to metabolic disorders and inflammation (Harsch & Konturek, 2021). The intricate relationship between inflammation, obesity, and diet has sparked research into the roles of the microbiome and epigenome, including endocannabinoid signaling. The lipid-based neurotransmitters known as endocannabinoids play a pivotal role in the context of obesity by modulating inflammatory processes and metabolic activity. More and more research is connecting the complicated interplay between the epigenome, microbiota, and endocannabinoid system (ECS) to diet-induced obesity (DIO), a major problem in public health. Dietary components and inflammation associated with obesity have the potential to modify the epigenome, which is made up of heritable alterations in gene expression that do not affect DNA sequences. These alterations have the potential to worsen circuits related to inflammation and metabolic imbalance. Host

metabolism and immunological responses are both profoundly influenced by the gut microbiota. The ECS is a network of lipid signaling molecules that regulates inflammation, hunger, and energy balance; dysbiosis, which is common in DIO, may impact this network. Through their interactions with immune cells and cytokine signaling, the bioactive lipids found within this system known as endocannabinoids regulate inflammatory processes. New evidence reveals that changes in the microbiome and epigenome brought about by dietary changes might impact the ECS, which in turn can increase inflammation and metabolic diseases. Dietary therapies and microbiota modification are two potential therapeutic targets for reducing the inflammatory effects of obesity, and a better understanding of this complex interaction may provide light on these issues (Colosimo et al., 2019).

RESEARCH QUESTION

What is the impact of time constraints on the control of inflammation regulation?

METHODOLOGY

Laboratory methods were used to conduct the research for this study. An animal model called a mouse was used to carry out the experiment.

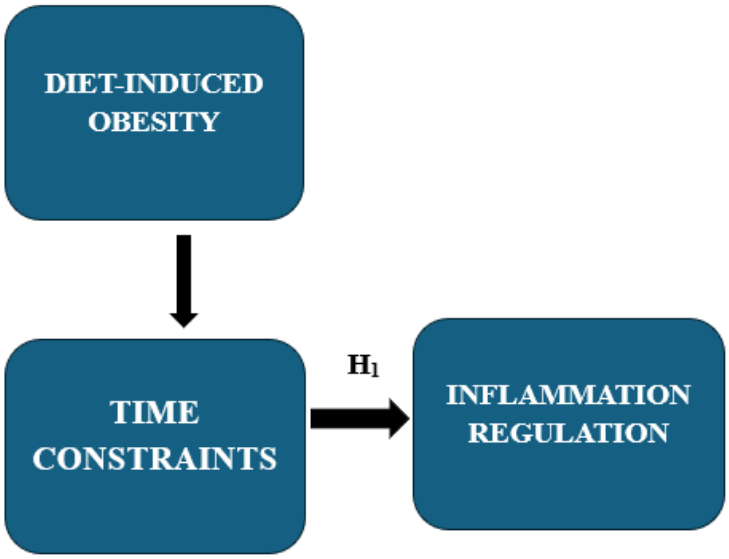
RESEARCH DESIGN

Nobody knows whether fat-fed obese mice experience changes in the endocannabinoid system, which is crucial for pain signals and emotion processing. In this study, obese mice will be used as a model to examine the nociceptive response and discover how dietary changes impact the endocannabinoid system. This project seeks to use a diet-induced obesity mice model to investigate the functions of the ECS in inflammation and metabolic control by genetic and pharmacological modification of the gut microbiota and cannabinoid receptors CB1 and CB2. Further testing of the CB1 antagonist was conducted on obese mice. The impact of HFD on leukocyte infiltration in the cecal-colonic lamina propria was better comprehended when CB1 and CB2 were studied. It is plausible to expect that alterations in the gut microbiota mediated by the ECS contribute to the obesity phenotype, given that inhibiting cannabinoid 1 (CB1) reduces intestinal inflammation. Researchers were looking at microbiota profiles using 16S rRNA gene sequencing to see whether CB1-/- or CB2-/-mice could withstand the effects of a high-fat diet on their gut flora.

MICE MODEL

All the male C57Bl/6J mice used in this investigation came from The University Laboratory. Adult mice were randomly assigned to either a 60% kcal HFD for 12 weeks or a 10% low-fat diet solely for 12 weeks, based on the findings. A variety of meals were introduced to the six- to eight-week-old mice. Experimental CB1-/- and CB2-/-mice were generated at the medical school of the University of South Carolina. From what the treatment group could tell, every experiment that wasn't the co-housing trial had cages with three or five mice each. Various litters and living conditions provided the mice used in this investigation. The aggressive behavior of mice led to their occasional isolation. To conduct the DIO intervention experiments, obese mice were split according to their average DEXA fat mass after 12 weeks of an unhealthy diet. The AM251 dosage for the treatment group was 10 mg/kg given orally in a 0.1% Tween 80 solution. They gave "Veh" valves to all the other experimental groups. When it comes to the PA feeding program, researchers make sure that the Pair-fed group gets the same amount of HFD every day by watching what they eat. Mice were induced to sleep by inhaling an excess of isoflurane once the experiment was complete.

CONCEPTUAL FRAMEWORK



RESULTS

The HFHS group of mice gained greater weight compared to the LFLS group when fed varied diets. The difference between the two groups became apparent as early as day 31, as seen in Figure 1A. The average weight increase for the HFHS group was 32.6 ± 1.8 g, whereas the LFLS group only managed 28.1 ± 1.6 g. As seen by a higher glucose area under the curve (Fig. 1B), the oral glucose tolerance test (OGTT) demonstrated a decrease in glucose tolerance. They started dropping on the third

day of HFHS feeding. Loss of this tolerance threshold was linked to a rise in body fat percentage. If the insulin area beneath the OGTT curves only showed a substantial improvement on day 56 of HFHS feeding, then the results in Figure 1C indicating insulin sensitivity decreases with increasing body weight throughout treatment must be believed.

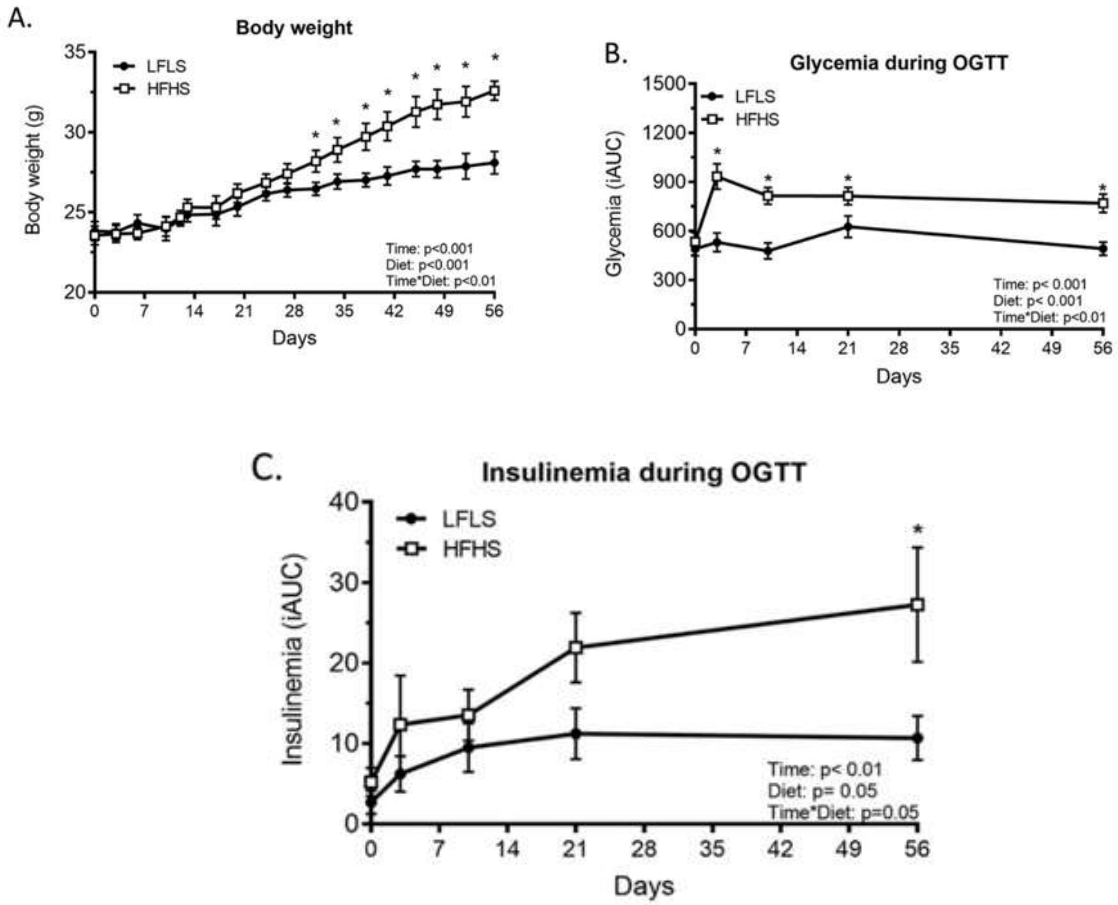


Figure 1. A low-fat diet rich in sugar and fat affects the phenotypic of rats after 56 days. Eleven mice had 56 days of sequential dosing with LFLS or HFHS. Here, the researchers have three variables: the individual's weight increase, their oral glucose tolerance test (OGTT) curve, and their insulin area under the plasma OGTT curve (iAUC). For this study, the researchers utilized mixed linear regression and extended linear regression models to find the correlations and effects of diet and time. Shown as mean ± SEM is the data (n = 9 to 12). A significant result was found, with a p-value of less than 0.05, when comparing the LFLS and HFHS groups using a Tukey HSD post hoc test.

SEGMENT-SPECIFIC GUT MICROBIOME COMMUNITY RESHAPING DURING HFHS DIET FEEDING

Prior to starting the HFHS diet, a principal component analysis (PCA) was conducted on the gut flora, which included segmenting the cecum and small intestine (Fig. 2A). These results agreed with projections made for gut flora populations. As shown in Figure 3, aerobes and facultative anaerobes, including Bacillales, Erysipelotrichales, and Lactobacillales, do better in the small intestine segments than obligatory anaerobes, such as Clostridiales, Bacteroidales, and Verrucomicrobiales, who do poorly in the cecum. There is a breakdown of the relative abundance of bacterial taxa and the number of genera for each location in Figure 4. There was a greater diversity of bacteria in the cecum (3.2 [3.0-3.3]) (represented as median [Q1-Q3]), indicating that different parts of the small intestine had different relative abundances of genera, in contrast to the jejunum (2.1 [1.8-2.8]) and ileum (2.2 [1.9-2.5], $P < 0.01$). While Bacteroidetes were more numerous in the cecum (1.46 [1.31-1.65] and 1.44 [1.40-1.64] respectively; p-value was less than 0.01), Firmicutes were more numerous in the ileum and jejunum. These results supported the researchers' decision to continue examining the HFHS diet on isolated intestinal sections.

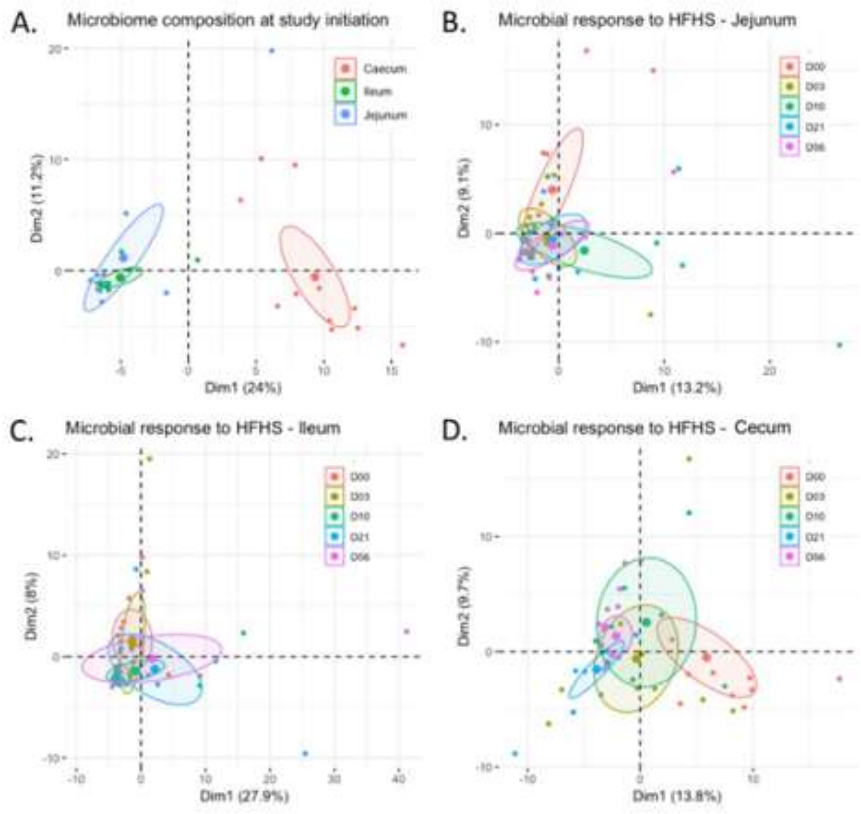


Fig 2: Gut microbiota composition because of HFHS diet. Using "principal component analysis (PCA)" before starting the HFHS diet, the researchers investigated the microbiota makeup in each section of the intestines (A). Impact of HFHS on microbiota structure in the jejunum (from A to D), ileum, and cecum (n= 6-12 for each time point).

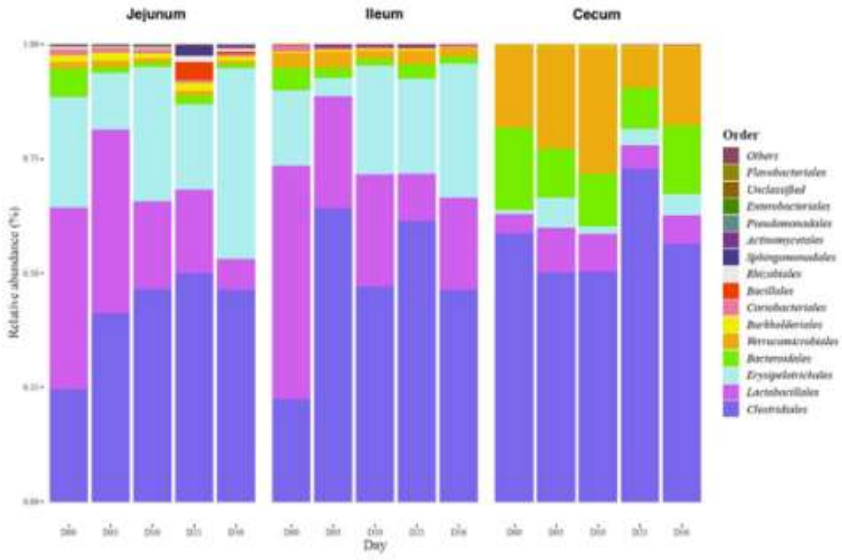
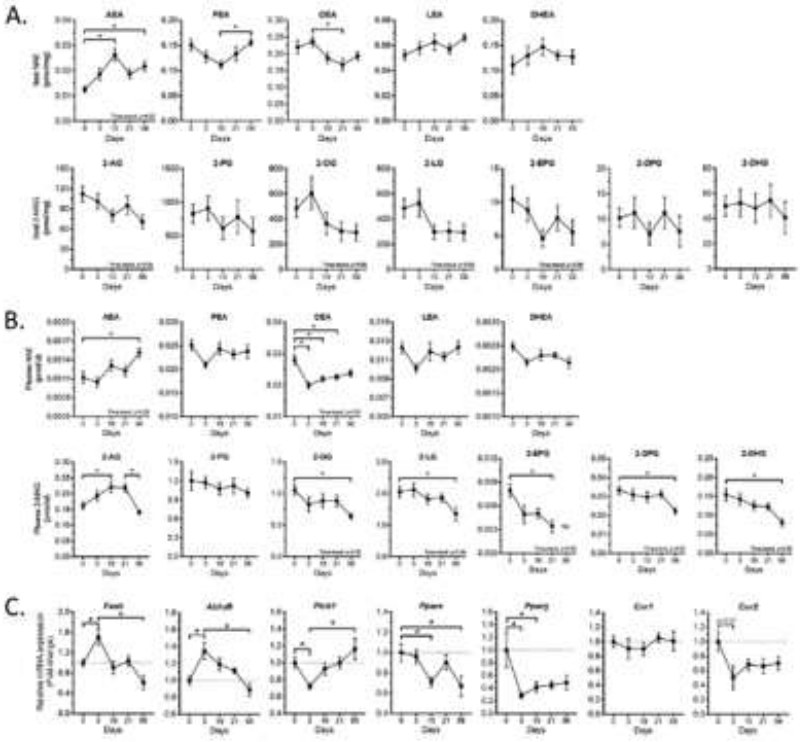


Fig 3: Influence of HFHS on the order-level relative abundance of microbes. In certain parts, the orders that made up the bacteria were jumbled up, even though they only made up 1% of the total.

BECOME MEDIATORS ARE MODIFIED IN RESPONSE TO THE HFHS DIET

The ability to regulate the behavior of target molecules is intricately linked to the modulation of metabolic processes, including CB1 (AEA and 2-AG), PPARα (N-oleoyl ethanolamine [OEA] and N-palmitoyl ethanolamine [PEA]), TRPV1 (which encompasses long-chain non-saturated N-acylethanolamines and 2-monoacylglycerols), the GPR technique (OEA, N-linoleoyl ethanolamine [LEA], 2-oleoyl-glycerol [2-OG], and 2-linoleoyl-glycerol [2-LG]), and GPR55 (PEA). Advances in the eCBome intermediary have been associated with the development of metabolic syndrome, obesity, and type 2 diabetes, as well as their potential interactions with gut microbiota. Researchers investigated ileal or plasma eCBome concentrations to determine the mediating influence of a high-fat, high-sugar meal. Upon evaluating AEA using analysis of variance (ANOVA) and linear comparability post hoc analysis, researchers identified a significant increase in the ileum 10 days after the initiation of the HFHS diet (+109 percent after 10 days, $P < 0.05$). Although PEA levels had reverted to baseline by day 56 of HFHS feeding, OEA and PEA, two AEA congeners, exhibited a decline after 10 days of HFHS feeding. The concentrations of the anti-inflammatory AEA congener N-docosahexaenoylethanolamine (DHEA) were unchanged by the HFHS diet. On day 56, there was a negligible decrease in the secondary principal endocannabinoid, 2-AG, which had been decreasing consistently throughout the period. GPR119 and TRPV1 activators, 2-OG and 2-LG, two congeners of 2-AG, have a distinct diminishing pattern in Figure 4A.

Figure 4. A reaction in the endocannabinoidome is triggered by consuming excessive amounts of sugar and fat.



In addition to A and B For each time point after the onset of HFHS feeding, this line chart displays the endocannabinoidome mediator in the ileum (A) and plasma (B). Notice the N-acylethanolamines (NAEs) in the upper row. In the next rows, the researchers could see 2-monoacylglycerols (2-MAGs). With the act approach, the FC of the ileum mRNA expression of the endocannabinoidome-related gene was recognized. With Tbp applied, the data were transformed into percentages as of day 0. There are 9–12 observations at each time point, and the data is presented as the mean plus or minus the average error of the mean. Statistically significant findings are shown in the bottom right corner by the P values from the post hoc nonlinear contrast analysis. Per time point, the Tukey HSD post hoc test is executed with a significance threshold of $P < 0.05$. Here, "not determined" (ND) is the designation.

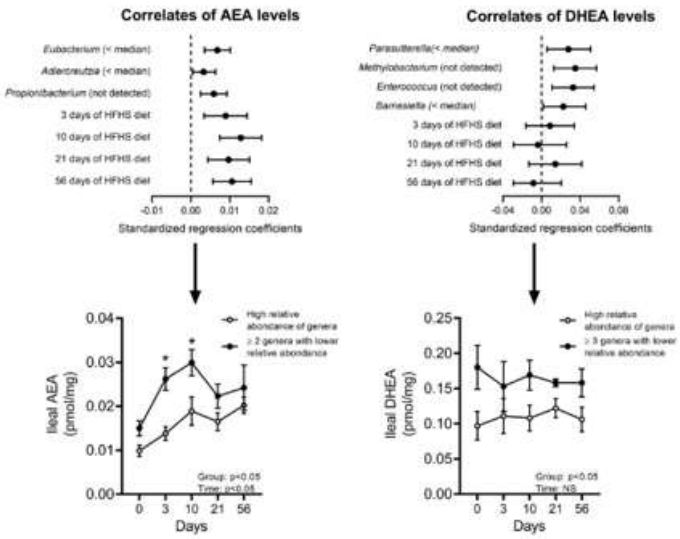
As seen in Figure 4B, there were also noticeable alterations to the eCBome mediators in the plasma. There was a 31% rise in ECBs containing arachidonic acid (+AG) and a 50% increase in 2-AG (+31%; $P < 0.05$). Plasma 2-AG levels peaked on days 10 and 21, and then declined significantly by day 56. Most eCBome mediators, including oleoyl "(OEA and 2-OG)-, linoleoyl (2-LG)-, and omega-3 [2-EPG, 2-DPG and 2-DHG]" were decreased when HFHS was administered, as shown in Figure 4B. Although the two diets were nutritionally identical, the HFHS diet purposefully consumed 4.5 times more total lipids than the LFLS diet. This made it possible to execute these changes in a controlled manner.

They were able to detect changes in the intestinal microbiome-eCBome axis by identifying the smallest collection of ileal microbiome taxa that adequately reflects the levels of each ileal eCBome mediator following HFHS consumption (Fig. 5).

Changes in the ileal levels of the ECB AEA and the PPARα/γ agonist DHEA prompted that

the regression models that were created showed that some bacterial species were either undetectable or had low levels, irrespective of the increased weight. The relative concentrations of Eubacterium, Adlercreutzia, and Pro bacterium in the ileum were either undetectable or very low. Subsequently, there was a distinct and considerable correlation between early HFHS feeding intervals and higher AEA levels (Fig. 5). On the third and tenth days, when glucose intolerance first began, the AEA levels were significantly higher in the mice whose ileum microbiota had decreased the relative abundance of two of these species, according to the findings of the analysis using this model (Fig. 5). Elevated ileal DHEA was independently and strongly correlated with undetectable levels and low relative numbers of Parasutterella, Methylobacterium, Enterococcus, or Barnesiella (Fig. 5). Ileal DHEA levels were typically highest at zero hours and lowest at the beginning of glucose intolerance. It was also not possible to adequately imitate the other eCBome mediators, such as 2-AG.

Figure 5. The ileum endocannabinoid me mediator engages in interactions with the gut flora in response to HFHS. The standardized regression coefficients of the intestinal flora are associated with the ileum's AEA and DHEA levels (top). The ileum microbiota profile was used to filter the AEA and DHEA levels at each time point. Species that have been shown to have strong ties to the eCBome as intermediaries were not included, nor were those with relative abundance levels that could not be detected. In this study, we looked at every species that may be affected by HFHS feeding as well as every species that was strongly linked to the mediator. The time spent HFHS feeding is taken into consideration by all models. The final models were computed using a stepwise selection technique. With n ranging from 3 to 8 per group at each point, The data is shown as an average with or without the standard error of the mean in parentheses. A significance threshold of $P < 0.05$ was used to conduct a Tukey HSD post hoc test at each time point.



DISCUSSION

Alterations to the gut microbiota and eCBome signaling may influence the host's metabolic response to environmental and dietary stimuli (Manca et al., 2020). The interdependent "omes" are still in the early stages of development, whether they are endogenous or symbiotic. Ultimately, this study set out to determine if there is a correlation between diet-induced obesity and the metabolic consequences that follow. At the onset of glucose intolerance, obesity, and hyperinsulinemia brought on by the HFHS diet, there is a correlation between changes in the relative abundance of certain genera in the gut microbiota and specific levels of eCBome mediators in the ileum or plasma. Past studies have shown that obesogenic meals may change the gut microbiome composition by affecting blood and gut levels of eCBome mediators. Time of day and segmentation influence the precise differences. Additionally, researchers found that certain bacterial species in the cecum and small intestine correlated with eCBome mediator levels in blood and tissues; this link remained constant regardless of changes in body weight. This class includes the genera Adlercreutzia, Barnesiella, Parasutterella, Propionibacterium, Enterococcus, and Methylobacterium. The gut microbiome-eCBome axis likely plays a role in the first host adaptation to the HFHS diet, as several concurrent modifications to the gut microbiota or eCBome were seen as early as three days into the diet.

Some commensal bacterial populations shift, and levels of 2-monoacylglycerol and N-acylethanolamine shift as a result of dietary changes that lead to obesity. Some species' stool abundance is reduced because of obesity, which has been shown in previous research to be caused by a high-fat diet. That is why it makes sense that the present finding of reduced Barnesiella numbers in the intestines during HFHS supports this. Parasutterella levels in the ileum and jejunum are lower in overweight people. The subsequent metabolic mayhem, inflammation, obesity, and Acinetobacter baumannii are inversely related to the decline of Akkermansia populations in this area after HFHS feeding. Lastly, studies on animals who are overweight have shown that the ileum has an increased population of Intestinimonas and Sphingomonas, but the jejunum remains unchanged. Obesity and impaired leptin signaling have been linked to these two bacteria in previous studies. Responses to variations in the availability of nutrients might account for these and other changes in gut flora. To determine how increasing sugar and fatty acid consumption affected weight gain, dysmetabolism, and gut microbiota, this research used HFHS and LFLS diets that were substantially similar in terms of fatty acid composition, fiber sources, and quantities.

Researchers discovered that plasma AEA and 2-AG levels were higher after HFHS eating, which is consistent with the large body of literature demonstrating that these intermediates are amplified in obese individuals and animal models of obesity. An inverse relationship between BMI and other 2-monoacylglycerol levels was also shown, which is in line with the declining plasma 2-OG and 2-LG levels. The existence of eCBome mediators may be associated with the gut microbiome's makeup. Possible explanation: the two systems' shared sensitivity to changes in food intake. This may be the case in certain contexts, as researchers still discovered several connections

despite controlling for changes in body mass index. Interactions between commensal bacteria and eCBome mediators may start long before obesity develops if the changes occur in the same tissue. The predicted preventive benefits of the ileal genera Barnesiella, Parasutterella, Akkermansia, and Coprobacillus against diet-induced dysmetabolism in mice were disproven by a prominent temporal adjustment of ileal AEA levels. Based on these results, it is reasonable to assume that the two effects are related. Reintroducing this beneficial species by probiotic usage reduces AEA levels instantly, and other studies have linked conditions causing increased AEA levels to a decrease in the predominance of A. muciniphila. Some genera are influenced by the ileum's n-3 polyunsaturated fatty acid eCBome mediators regardless of weight. It is possible that these mediators may alleviate inflammation to a certain degree. Also, the relative abundance of bacterial species in all three parts of the intestines was shown to be linked with plasma concentrations of eCBome mediators. Because plasma eCBome mediators' sources are still a mystery, more research is required to determine the significance of these correlations. It is worth considering that the small intestine could not be the main source of plasma mediators or becomes, as there seems to be little evidence connecting the genera identified in the ileal microbiome to these chemicals.

Researchers observed that mice given the HFHS diet had lower levels of many genera that are helpful to metabolism in their ileum, which was associated with higher levels of AEA and DHEA. It might be that this indicates the activation of CB1 and PPARα/γ. These results are associated with glucose intolerance and localized inflammation. Given the metabolic relevance of the relationships between gut microbiota and eCBome, our findings suggest that studying these links as a community, rather than concentrating on individual genera, would be the most fruitful approach. The findings suggest that further research on the effects of gut colonization on eCBome targets and mediators, as well as the relationship between eCBome modifications and these factors, should be feasible. Oddly enough, the data imply that the HFHS diet triggers bacterial responses that are time- and segment-specific. This highlights the need of studying various sections of the intestines, ideally in animal models where it is simpler to do so (Sharma & Tripathi, 2019).

CONCLUSION

The HFHS-related problems such as hyperinsulinemia, glucose intolerance, obesity, and others are mapped out in this work by documenting the microbiome, or eCBome, in different parts of the intestines throughout time. The research found that an endogenous signaling pathway that plays a significant role in metabolic regulation may be the starting point for metabolic issues caused by the HFHS diet and disharmony between the microbiome and the host. During this process, there is an interaction between the biome of the online community and the gut microbiome. Finally, further research into the molecular underpinnings of the gut microbiome-eCBome axis should be made possible by the present findings (Sudo, 2019).

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