DIET-INDUCED OBESITY AND THE EPIGENOME/MICROBIOME'S ROLE IN ENDOCANNABINOID-MEDIATED INFLAMMATION REGULATION

Zhang Jiawei[1], Nurul Azmir Bin Amir Hashim[1]

[1]Lincoln University College, Petaling Jaya, Malaysia.

ABSTRACT

Microbiomes, also known as epigenomes, have a role in metabolic wellness and the inflammation linked to diet-induced obesity (DIO), according to recent research. Within the framework of endocannabinoid-mediated inflammatory control in a mouse model, this research explores the interaction between DIO, the epigenomic landscape, or gut microbiota composition. Researchers set out to induce obesity in C57BL/6J mice and track their weight over time to see how a high-fat diet (HFD) affected them, metabolic parameters, or inflammatory markers. Researchers found changes in DNA methylation patterns linked to obesity using genomic sequencing and epigenetic profiling. These changes were most pronounced in genes that control inflammation and lipid metabolism. At the same time, researchers used 16S rRNA sequencing to examine the gut microbiota, which revealed that the HFD was associated with substantial changes in the variety and composition of microbes. It is worth mentioning that researchers saw a rise in microbial taxa that promotes inflammation, which is linked to higher levels of endogenous cannabinoids. Researchers used pharmacological therapies to modify endocannabinoid signalling and assessed their impact on inflammatory responses & metabolic outcomes to uncover the underlying pathways. Our research indicates that inflammation in DIO is modulated by a combination of the microbiota and the epigenome, which in turn affect endocannabinoid signalling. This work emphasises the intricate relationships among nutrition, microbiome, and epigenetic pathways in metabolic health and the possibility of focusing on these pathways as treatment approaches for inflammation associated with obesity. The precise processes at work and their consequences for the treatment and prevention of obesity need more investigation.

Keywords: Diet-induced obesity, Epigenome/Microbiome's, Endocannabinoid, Inflammation regulation.

INTRODUCTION

A major issue in global health, diet-induced obesity, is linked to a growing number of metabolic disorders and chronic diseases. An excess of calories consumed relative

to energy expended is the fundamental reason for this illness (Wozniak et al., 2021), which is often exacerbated by poor dietary choices. Two major biological systems, the microbiome and the epigenome, have recently been revealed to have a complex interaction with diet-induced obesity (Miranda, 2019). These systems are crucial for regulating inflammation, particularly the modulation of the endocannabinoid system. The epigenome regulates gene expression by chemical modifications to DNA and histone proteins; these modifications do not alter the genetic code itself. These alterations might be influenced by environmental factors, such what people eat. Certain dietary components may induce epigenetic changes that influence inflammatory pathways and contribute to obesity-related illnesses. To illustrate the point, (Srivastava et al., 2022) found that a high-fat diet alters the methylation landscape, which impacts inflammation and metabolism-related genes.

Metabolic health is also greatly affected by the complex bacterial population in the digestive system, which is called the gut microbiome. Energy balance and chronic inflammation are influenced by dietary choices, which in turn alter the microbiome's makeup and function (Schulz et al., 2021). The endocannabinoid system controls inflammation via a complex network of receptors or signalling molecules, metabolism, and hunger, among other physiological functions (Manca et al., 2020). The microbiota may impact this system. Researchers can learn more about obesity and its associated diseases if researchers investigate the ways in which diet-induced obesity impacts the microbiome, particularly epigenome, as well as how these elements impact the regulation of inflammation by endocannabinoids. This multimodal strategy has the potential to open up new avenues for treating obesity-related inflammation and improving metabolic health (Cuddihey et al., 2022).

BACKGROUND OF THE STUDY

Researchers' approaches to studying diet-induced obesity and its underlying mechanisms have seen a dramatic change over the last several decades. It was formerly believed that the primary cause of obesity was a caloric imbalance, defined as consuming more energy than one expends. Scientific progress has shown that the complex causes of obesity include genetic, environmental, and biochemical factors (Argueta et al., 2019). Epigenetics is a relatively new discipline that arose in the early 2000s when scientists began to question the potential effects of dietary factors on gene expression beyond the effects of DNA. Epigenetic research has demonstrated that environmental factors, including diet, could cause modifications to be incorporated into genes via processes such as DNA methylation or histone modification. According to (Rakotoarivelo et al., 2021), these changes have the potential to worsen metabolic disorders linked to obesity by influencing inflammatory pathways. Meanwhile, fresh data from microbiome research has highlighted the significance of gut microorganisms in determining health or disease. Metabolism, immunological system function, or digestion are all significantly impacted by the billions of microorganisms that make up the human microbiome. Changing one's diet may affect one's microbiome's make-up and function, which in

turn affects obesity and systemic inflammation, gained widespread acceptance in the 2010s. Researchers have shown that an imbalance in the microbiome might exacerbate metabolic dysregulation and inflammation, which in turn can cause obesity (Chevalier et al., 2020).

A system of receptors and signalling substances called the endocannabinoid system regulates a number of physiological functions, including inflammation and metabolism. Inflammatory responses, energy balance, or appetite control are profoundly affected by endocannabinoid alterations caused by changes in the microbiota and one's diet, according to research. The intersection of epigenetics, microbiome research, and endocannabinoid regulation holds great promise. Finding out how these systems are impacted by diet-induced obesity as well as how they all collaborate to control inflammation might lead to novel approaches to preventing and treating obesity and related disorders (Forte et al., 2020).

PURPOSE OF THE STUDY

The main objective of this study is to provide information on the ways in which diet-induced obesity affects epigenetic alterations, changes in microbiome composition, and the role of endocannabinoid signalling to inflammation regulation. This knowledge may help guide the development of targeted treatments for inflammatory diseases linked to obesity.

LITERATURE REVIEW

Inflammation and metabolic disorders are strongly associated with diet-induced obesity (DIO), which has recently emerged as a key issue in public health. Research into the functions of the microbiome and epigenome, in particular endocannabinoid signalling, has been stimulated by the complex interplay between inflammation, obesity, and food. Crucial mediators in the setting of obesity are endocannabinoids, lipid-based neurotransmitters that influence inflammatory processes and metabolic activities (Sharma & Tripathi, 2019).

According to research, DIO has the potential to cause substantial epigenetic changes, such as changes to DNA methylation or histone modifications, by which metabolic and inflammatory gene expression is modulated. For example, studies show that fat promotes inflammation by inducing hypermethylation of genes related to the immune response. Dietary factors may have an effect on this epigenetic reprogramming, which raises the possibility that dietary therapies might undo these alterations. When it comes to DIO, gut microbiota is crucial for controlling inflammation and metabolism. There is evidence that suggests a link between high-fat diets and an increase in pro-inflammatory bacteria via reducing microbial diversity. The host's endocannabinoid system may be impacted by these changes in

microbiota composition, leading to a worsening of inflammation. New research suggests that some metabolites produced by microbes may affect levels of endogenous cannabinoids, further supporting the microbiome's function in inflammatory control (Lacroix et al., 2019)

Additionally, new research highlights how the microbiome and epigenome work together to influence endocannabinoid signalling pathways. Scientists hope to find new ways to treat inflammation caused by obesity by studying these relationships. To unravel the mysteries of DIO and the inflammatory reactions it triggers, researchers must examine the complex relationship between the microbiota, the epigenome, and the diet. Inflammation caused by obesity might be mitigated via epigenetic and endocannabinoid pathways; future studies should investigate this possibility using microbiome-targeted treatments and dietary changes (Manca et al., 2020).

RESEARCH QUESTIONS

How to examine diet-induced obesity alters epigenetic modifications and their impact on gene expression related to inflammation?.

RESEARCH METHODLOLOGY

The research for this study was carried out using laboratory procedures. The investigation was conducted using a mouse model, an animal model.

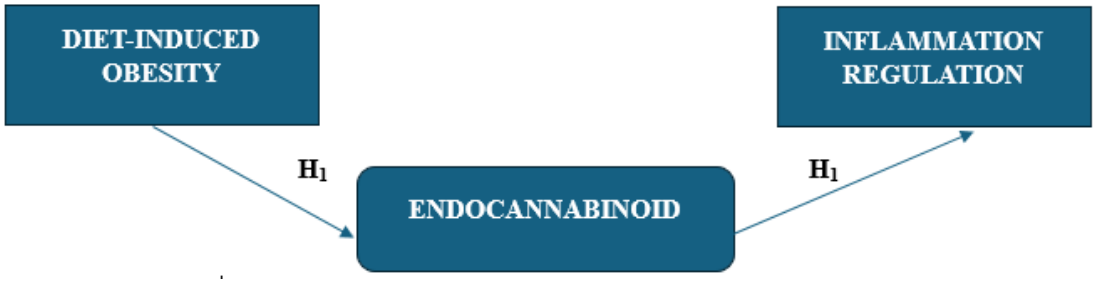
RESEARCH DESIGN

It is unclear whether mice that become obese due to a high-fat diet experience changes in the endocannabinoid system, which is crucial for the processing of emotions and pain signals. The purpose of this study is to investigate the nociceptive response of obese mice as a model for studying the impact of dietary changes on the endocannabinoid system. The purpose of this work is to identify the functions of the ECS in regulating inflammation, metabolism, either manipulating the gut microbiome or the cannabinoid receptors CB1 and CB2 by genetic and pharmacological means in a diet-induced obesity mice model. Moreover, the CB1 antagonist was also tested on obese mice. Investigating CB1 and CB2 helped researchers comprehend the impact of HFD on leukocyte infiltration in the cecal-colonic lamina propria. Hypothesised involvement of ECS-mediated alterations in the gut microbiota in the obesity phenotypic is supported by the fact that CB1-blockade reduces intestinal inflammation. The researchers were evaluating microbiota profiles using 16S rRNA gene sequencing to determine whether CB1-/- or CB2-/-mice were resistant to developing intestinal dysbiosis caused by a high-fat diet.

MICE MODEL

This research used male C57Bl/6J mice sourced from The University Laboratory. Aged mice were either fed a 60% kcal HFD for twelve weeks or a pure 10% low-fat diet for a total of twelve weeks, based on the indications. Mice were given different diets depending on whether they were six to eight weeks old. At the University of South Carolina School of Medicine's animal facility, researchers developed CB1-/- and CB2-/-mice in-house. According to the treatment group, all experiments except for the one including co-housing used cages with between three and five mice each. Research mice were selected from a wide range of litter and housing conditions. Mice were sometimes housed alone because of their fighting behaviour. Obese mice were divided into groups in the DIO intervention experiments according to their mean DEXA fat mass after 12 weeks of a high-fat diet. Oral administration of 10 mg/kg of AM251 in a 0.1% Tween 80 solution was used for the treatment group. In all the other experimental groups, valves marked "Veh" were given. Researchers keep tabs on the Pair-fed group's food consumption as part of the PA feeding program to make sure they get the identical amount of HFD every day. The trial ended with the mice being euthanised by inhaling an overdose of isoflurane.

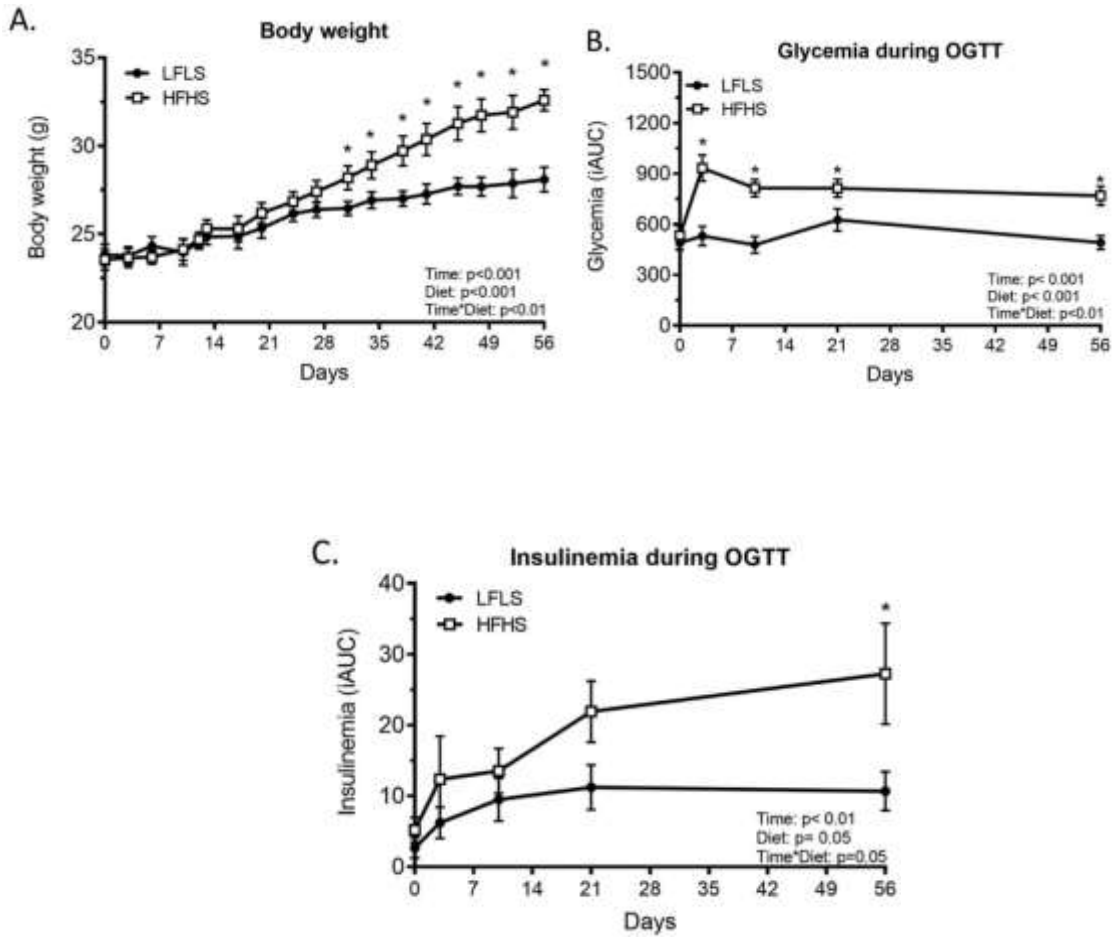
CONCEPTUAL FRAMEWORK



RESULTS

Weight increase was greater in the HFHS diet group compared to the LFLS diet group in mice. This difference was statistically significant beginning on day 31, with 32.6 ± 1.8 g of weight gain in the HFHS group and 28.1 ± 1.6 g of weight gain in the LFLS group (Fig. 1A). The oral glucose tolerance test (OGTT) showed an increased glucose area under the curve, which indicates a decline in glucose tolerance (Fig. 1B). This decline began on day 3 of HFHS feeding. Following this decrease in tolerance, there was an increase in weight gain. Insulin sensitivity seems to decline during the course of therapy, in line with rising body weight (Fig. 1C), as the insulin region under the OGTT curves only significantly improved on day 56 of HFHS feeding.

Figure 1. Rat phenotypic as affected by 56 days of low-fat, high-sugar, high-fat diets. Eleven mice were given a 56-day regimen of either LFLS or HFHS. First, the increase in weight; second, the surface area under the “oral glucose tolerance test (OGTT)” curve for plasma glucose; and third, the surface region under "the OGTT curve for plasma insulin (iAUC)". Researchers used generalised linear regression & mixed linear regression models to determine the impacts and relationships of time or diet. The data is shown as mean ± SEM (n = 9 to 12). When comparing the LFLS & HFHS groups, a significant result was obtained using a Tukey HSD post hoc test (*, P < 0.05).



SEGMENT-SPECIFIC GUT MICROBIOME COMMUNITY RESHAPING DURING HFHS DIET FEEDING

Precise component analysis (PCA) of the gut microbiota prior to beginning the HFHS diet demonstrated segmentation of the cecum & small intestine (Fig. 2A). They were consistent with predictions for the populations of gut microbiota. Figure 3 shows that, in comparison to the cecum, the small intestine segments are more favourable to aerobes and facultative anaerobes (such as Bacillales, Erysipelotrichales, &

Lactobacillales) than obligatory anaerobes (such as Clostridiales, Bacteroidales, and Verrucomicrobiales). Figure 4 also displays the relative abundance of bacterial taxa and the number of genera in each area. The bacterial diversity in the cecum was greater (3.2 [3.0-3.3]) (shown as median [Q1-Q3]) than in the jejunum (2.1 [1.8-2.8]) & ileum (2.2 [1.9-2.5], $P < 0.01$), suggesting that various sections of the small intestine had varying relative abundances of genera. Unlike the microbes in the cecum, the jejunum and ileum had a higher Firmicutes-to-Bacteroidetes ratio (1.46 [1.31-1.65] and 1.44 [1.40-1.64] respectively, with a p-value of less than 0.01. In light of these findings, researchers conducted further tests segment-by-segment to isolate the effects of the HFHS diet on the various parts of the intestines.

Figure 2. Gut microbiota composition as a consequence of HFHS eating. (A) The gut microbiota composition was explored in each part of the intestines using "principal component analysis (PCA)" prior to beginning the HFHS diet. The impact of HFHS on the composition of the Jejunum microbiota (from A to D), ileum, and cecum. n= 6-12 for each time point.

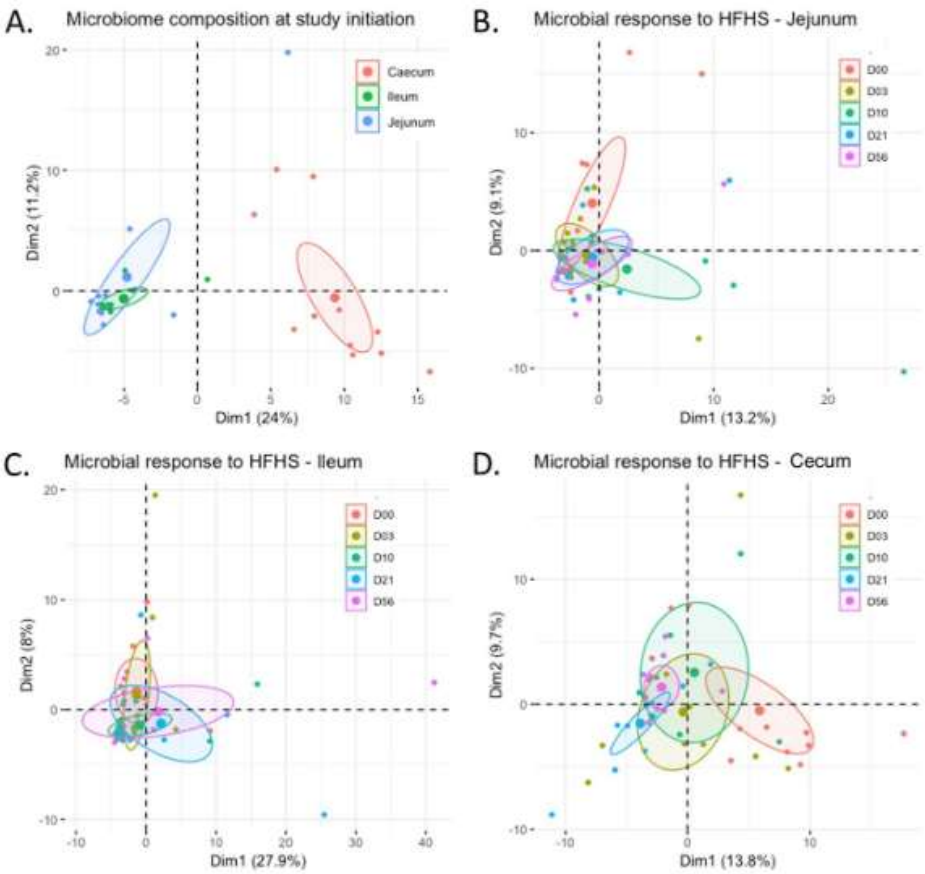
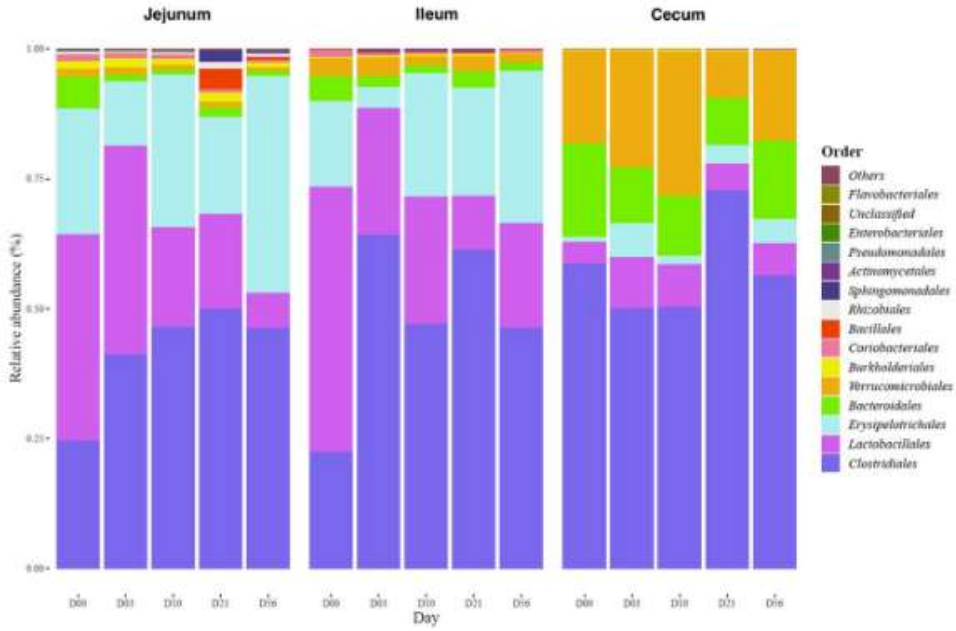


Figure 3. Influence of HFHS on order-level bacterial relative abundance. In at least one section, orders that made up less than 1% of the overall bacterial abundance were combined.

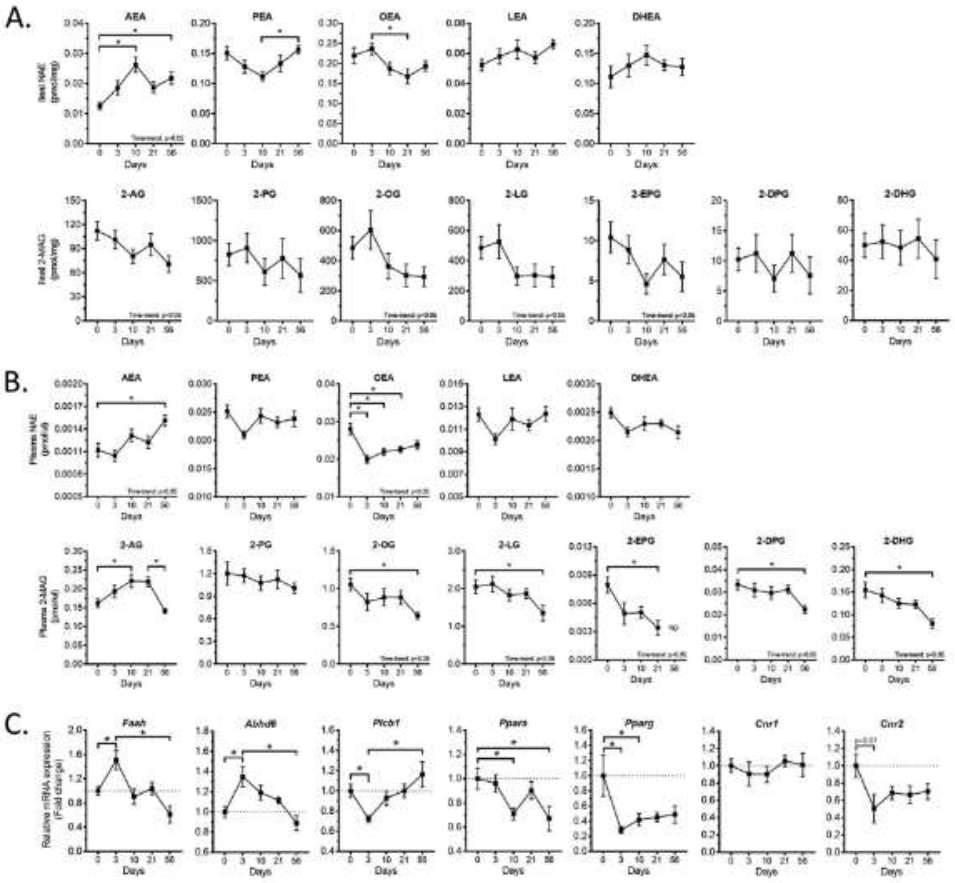


eCBome MEDIATORS ARE MODIFIED IN RESPONSE TO THE HFHS DIET

Through their capability of modulating the activity of molecular targets deeply involved in the regulation of metabolism, such as CB1 (AEA and 2-AG), PPARα (N-oleoyl ethanol amine [OEA] or N-palmitoyl ethanol amine [PEA]), TRPV1 (all long-chain non saturated N-acylethanolamines as well as 2-monoacylglycerols), GPR119 (OEA, N-linoleoyl ethanol amine [LEA], 2-oleoyl-glycerol [2-OG], and 2-linoleoyl-glycerol [2-LG]), or GPR55 (PEA), changes in eCBome mediators have been linked to growth of the metabolic syndrome, obesity, and type 2 diabetes, as well as their potential bidirectional interplay with gut microbiota has been highlighted. Researchers measured ileal and plasma eCBome mediator effects of HFHS feeding. When AEA was evaluated using analysis of variance (ANOVA) linear comparison post hoc analysis, researchers discovered a significant positive trend in the ileum 10 days after beginning the HFHS diet (+109 percent after 10 days, $P < 0.05$). Two AEA congeners, OEA and PEA, tended to fall after 10 days of HFHS feeding; however, PEA levels returned to baseline by day 56 of HFHS feeding. The anti-inflammatory AEA congener N-docosa hexa enoyleth anolamine (DHEA) levels were unchanged by the HFHS diet. A negative temporal trend was seen for 2-AG, the second main eCB, even if the reduction on day 56 was not statistically significant. Fig. 4A shows a significant negative trend in the 2-AG congeners 2-OG and 2-LG, which are agonists for GPR119 and TRPV1, respectively.

Figure 4. The endocannabinoidome's response to the high-fat, high-sum diet. Both A and B The line chart displays the endocannabinoidome mediator in the ileum (A) and plasma (B) at every single point after the start of HFHS feeding. In the top row, you can see N-acylethanolamines (NAEs). The rows below include 2-

monoacylglycerols (2-MAGs). The fold change (FC) of the endocannabinoidome-related gene's ileum mRNA expression was calculated using the ŔCT approach. After being normalised to Tbp, the results were presented as percentages as of day 0. With 9–12 observations per time point, the data are shown as the mean plus or minus the average error of the mean. On the lower right corner, you can see the P values of the nonlinear contrast post hoc analysis, which are shown when the results are significant. For every time point, a significance level of $P < 0.05$ is used for the Tukey HSD post hoc test. This is marked as "not determined" (ND).

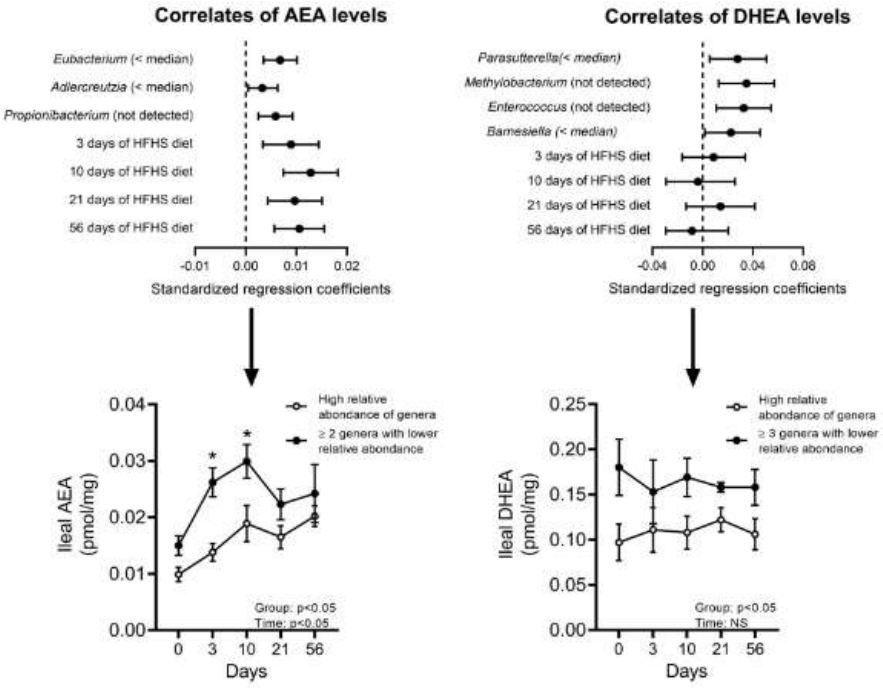


The eCBome mediators in the plasma also underwent notable changes, as shown in Figure 4B. ECBs containing arachidonic acid (+AG) increased by 31% and 2-AG (+31%; $P < 0.05$) by 50%. On days 10 and 21, plasma 2-AG levels were highest, and by day 56, they had dropped considerably. Feeding HFHS decreased most of the eCBome mediators made from oleoyl "(OEA and 2-OG)-, linoleoyl (2-LG)-, and omega-3 [2-EPG, 2-DPG and 2-DHG]" ($P < 0.05$, Fig. 4B). The HFHS diet purposefully consumed 4.5 times more total lipids than the LFLS diet, while maintaining the same composition and omega-3 to omega-6 ratio. The regulated rollout of these changes was made possible by this.

In order to understand changes in the intestinal microbiome-eCBome axis, they had to find the smallest group of ileal microbiome taxa that accurately represents the

amounts of each ileal eCBome mediator after HFHS consumption (Fig. 5). The regression models that were developed demonstrated that some bacterial species were either not detectable or had low levels in response to changes in the ileal levels for the eCB AEA and the PPARα/γ agonist DHEA, regardless of the increase in weight. Eubacterium, Adlercreutzia, and Pro bacterium relative concentrations in the ileum were low or no detectable, then early HFHS feeding periods were separately and substantially linked with larger AEA levels (Fig. 5). Curiously, when the mice were analysed using this model, the results showed that on days 3 and 10, when glucose intolerance first started, the AEA levels were considerably greater in mice whose ileum microbiota had reduced the relative abundance of at least two of these species (Fig. 5). Ileal DHEA levels were normally greatest at zero hours and lowest at the start of glucose intolerance; there was a strong and independent correlation between elevated ileal DHEA and undetectable levels and low relative numbers of Parasutterella, Methylobacterium, Enterococcus, or Barnesiella (Fig. 5). The other eCBome mediators, including 2-AG, were also unable to be well simulated.

Figure 5. The gut flora is associated with the reaction of the ileum endocannabinoidome mediator to HFHS feeding. (Top) The ileum AEA and DHEA levels are associated with the intestinal flora's standardised regression coefficients. In each time point, the ileum microbiome profile was used to filter the AEA and DHEA levels. Genera with a low relative abundance and nondetectable levels were excluded, and genera with high relative abundance that have been identified as important eCBome mediator correlations. This study considered all genera that were substantially connected with the mediator and all genera that were affected by the HFHS feeding. Included in all models is the length of the HFHS feeding. In order to calculate the final models, a stepwise selection approach was used. With n ranging from 3 to 8 per group at each point, the results are shown as the average plus or minus the standard error of the mean. *, At each time point, a Tukey HSD post hoc test was performed, with a significance level of $P < 0.05$.



DISCUSSION

The metabolic response of the host to dietary and environmental variables may be impacted by changes in the gut microbiota and signalling from the eCBome, according to new data. However, the interplay between the two primary "omes" the endogenous or the exogenous/symbiotic has only just begun to take shape. The primary objective of this research was to establish a causal relationship between the metabolic consequences of diet-induced obesity and its onset. The findings show that specific eCBome mediator levels in the ileum or plasma are correlated with changes in the relative abundance of specific genera in the gut microbiota during the early stages of glucose intolerance, obesity, and hyperinsulinemia induced by the HFHS diet. Evidence from prior research suggests that the composition of the gut microbiome may be changed by obesogenic diets via alterations in blood and gut levels of eCBome mediators. These changes depend on the time of day and are segment specific. Finding connections between certain bacterial species in the cecum and small intestine and regional and circulation levels of eCBome mediators, such AEA and DHEA, was also discovered by the researchers, and this link was independent of changes in body weight. Adlercreutzia, Barnesiella, Parasutterella, Propionibacterium, Enterococcus, and Methylobacterium are all genera that fall under this category. As early as three days into the diet, several concurrent alterations to the gut microbiota or eCBome were seen, indicating that the gut microbiome-eCBome axis contributes to the first host adaptation to the HFHS diet.

Obesity caused by food changes has been linked to changes in the amounts of 2-monoacylglycerol and N-acylethanolamine, as well as changes in the number of certain commensal bacteria. For example, the current discovery of decreased Barnesiella numbers during HFHS in all regions of the intestines is consistent with prior reports of decreased faecal abundance of these species in obesity caused by a high-fat diet. Similarly, obesity was correlated with a reduction in Parasutterella counts, as shown in the ileum and jejunum. This region's decreased Akkermansia populations following HFHS feeding are consistent with the negative correlation between this genus of mucin-degrading bacteria specifically, Inflammation throughout the body, A. muciniphila, obesity, and the metabolic havoc that follows. Finally, animal models of obesity revealed an increase in Intestinimonas in the ileum and Sphingomonas in the Jejunum. Past research has connected these two bacteria to obesity and poor leptin signalling. Possible adaptation to changes in nutrition availability accounts for these and other observed changes in the gut microbiota. It should be noted that the HFHS and LFLS diets used here had identical fatty acid compositions, fibre sources, and levels in order to differentiate between the impacts of increased sugar and fatty acid intake on weight gain, dysmetabolism, or gut microbiota.

Following HFHS feeding, researchers found elevated plasma AEA and 2-AG levels, consistent with the large body of evidence showing that these intermediates are magnified in fat people and in animal models of obesity. Consistent with the decreasing plasma 2-OG and 2-LG levels shown here, the levels of other 2-monoacylglycerols were instead inversely associated to body mass index. We may

have found associations between eCBome mediators and the relative abundance of certain species of microbes in the intestines. This might be due to the fact that both systems respond similarly to diet-induced weight gain. This could be the case in some situations, but when researchers accounted for variations in body mass index, they still found a plethora of associations. Particularly if the alterations take place in the same tissue, interactions between commensal bacteria or eCBome mediators may begin long before obesity occurs. Specifically, there was a remarkable temporal modulation of ileal AEA levels that ran counter to the predicted protective effects of the ileal genera Barnesiella, Parasutterella, Akkermansia, as well as Coprobacillus against diet-induced dysmetabolism in mice. This finding raises the possibility that one effect causes the other, or vice versa. In fact, prior research has shown that situations causing elevated AEA levels are associated with a decline in the prevalence of A. muciniphila, and that restoring this beneficial species by the use of probiotics simultaneously lowers AEA levels. It is interesting to note that some genera have associations with eCBome mediators generated from n-3 polyunsaturated fatty acids in the ileum that are not reliant on body weight. These mediators have the potential to have anti-inflammatory actions. Additionally, plasma concentrations of eCBome mediators were shown to be linked with the proportional richness of bacterial species in each of all three regions of the intestines. Since the source of plasma eCBome mediators is still a mystery, more research into the relevance of these associations is required. There may not be a significant source of these mediators in the small intestine, as studies did not discover the same relationships between plasma mediators and genera of ileal microbiome as there were between ileal mediators or plasma eCBomes.

The absence of more than one metabolically advantageous genera in the ileum of mice given the HFHS diet was shown by researchers to be associated with higher levels of AEA & DHEA. This, in turn, might be an indication of CB1 and PPARα/γ activation. Glucose intolerance & local inflammation are linked to these values. These findings suggest that, instead of focussing on individual genera, it would be more fruitful to investigate potential links between gut microbiota & eCBome on a community basis, since these interactions are likely to have metabolic relevance. In addition, these findings should pave the way for future research into the relationship between eCBome changes and the effects of gut colonisation on eCBome mediators and targets. Curiously, our findings point to bacterial responses to the HFHS diet that are time- and segment-specific. This highlights the need to explore various segments of the intestines, ideally in animal models where this is more practical.

CONCLUSION

This work documents the characterisation of the microbiome or eCBome in several parts of the intestines over time such as glucose intolerance, obesity, as a result of the HFHS diet, hyperinsulinemia develops. The gut microbiome or the electronic community biome interact with one another, according to the research, an endogenous signalling pathway that is highly implicated in metabolic regulation, may be pivotal in the onset of metabolic abnormalities caused by the HFHS diet and host-

microbiota dysbiosis. Lastly, studies aimed at determining the molecular bases of the gut microbiome-eCBome axis should be made possible by the current results.

REFERENCES

1. 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. doi:10.3389/fphys.2019.00704
2. Chevalier G, Siopi E, Guenin-Mace L, Pascal M, Laval T, Rifflet A, et al. The effect of gut microbiota on depressive-like behaviors in mice is mediated by the endocannabinoid system. Nat Commun. 2020;11:6363. doi:10.1038/s41467-020-19931-2
3. Cuddihey H, MacNaughton WK, Sharkey KA. Role of the endocannabinoid system in the regulation of intestinal homeostasis. Cell Mol Gastroenterol Hepatol. 2022;14(4):947–63. doi:10.1016/j.jcmgh.2022.05.015
4. Forte N, Fernández-Rilo AC, Palomba L, Di Marzo V, Cristino L. Obesity affects the microbiota–gut–brain axis and the regulation thereof by endocannabinoids and related mediators. Int J Mol Sci. 2020;21(5):1554. doi:10.3390/ijms21051554
5. Lacroix S, Pechereau F, Leblanc N, Boubertakh B, Houde A, Martin C, et al. Rapid and concomitant gut microbiota and endocannabinoidome response to diet-induced obesity in mice. mSystems. 2019;4:e00407-19. doi:10.1128/mSystems.00407-19
6. Manca C, Boubertakh B, Leblanc N, Deschenes T, Lacroix S, Martin C, et al. Germ-free mice exhibit profound gut microbiota-dependent alterations of intestinal endocannabinoidome signaling. J Lipid Res. 2020;61:70–85. doi:10.1194/jlr.RA119000424
7. Miranda K. Role of epigenome and microbiome in endocannabinoid-mediated regulation of inflammation during diet-induced obesity [doctoral dissertation]. University of South Carolina; 2019. Available from: https://scholarcommons.sc.edu/etd/5391
8. Rakotoarivelo V, Sihag J, Flamand N. Role of the endocannabinoid system in the adipose tissue with focus on energy metabolism. Cells. 2021;10(6):1279. doi:10.3390/cells10061279
9. Schulz P, Hryhorowicz S, Rychter AM, Zawada A, Słomski R, Dobrowolska A, et al. What role does the endocannabinoid system play in the pathogenesis of obesity? Nutrients. 2021;13(2):373. doi:10.3390/nu13020373
10. Sharma S, Tripathi P. Gut microbiome and type 2 diabetes: Where we are and where to go? J Nutr Biochem. 2019;63:101–8. doi:10.1016/j.jnutbio.2018.10.003
11. Srivastava RK, Lutz B, De Azua IR. The microbiome and gut endocannabinoid system in the regulation of stress responses and metabolism. Front Cell Neurosci. 2022;16:867267. doi:10.3389/fncel.2022.867267

12. Wozniak D, Cichy W, Przysławski J, Drzymała-Czyż S. The role of microbiota and enteroendocrine cells in maintaining homeostasis in the human digestive tract. Adv Med Sci. 2021;66:284–92. doi:10.1016/j.advms.2021.05.003