Chronic Hypothalamic 2-Arachidonoylglycerol Infusion Drives Diet-Dependent Obesity, Metabolic Dysfunction, and Peripheral Tissue Remodeling in Rats

Rahul Deshmukh

drrahuld09@gmail.com

Central University of Punjab

Shilpa Kumari

Central Univeristy of Punjab

Biswanath Nayak

Central University of Punjab

Mahendra Bishnoi

National Agri-Food Biotechnology Institute

Article

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Chronic Hypothalamic 2-Arachidonoylglycerol Infusion Drives Diet-Dependent Obesity, Metabolic Dysfunction, and Peripheral Tissue Remodeling in Rats

Shilpa Kumari[1], Biswanath Nayak[1], Mahendra Bishnoi[2], Rahul Deshmukh[1*]

[1]Department of Pharmacology, Central University of Punjab, Bathinda-151401, Punjab, India

[2]BRIC-National Agri-Food and Biomanufacturing Institute (BRIC-NABI), S.A.S. Nagar, Mohali, Punjab, India

***Corresponding author**

Dr. Rahul Deshmukh, M. Pharm. Ph.D.
Professor
Department of Pharmacology
Dean, School of Health Sciences
Central University of Punjab, Ghudda, Bathinda-151001, Punjab, India
Mob: - (+91) 99889 04375
Email: - drrahuld09@gmail.com; rahul.deshmukh@cup.edu.in

Abstract

Background: The endocannabinoid system (ECS) is a critical regulator of feeding behaviour, energy homeostasis, and metabolic function, and its dysregulation has been strongly implicated in the pathogenesis of obesity. Increased endocannabinoid tone has been associated with obesity, while pharmacological inhibition of cannabinoid receptor signaling produces anti-obesity effects. However, the metabolic consequences of sustained elevation of endogenous cannabinoids within central regulatory circuits, particularly the hypothalamus, remain poorly characterized. The present study investigated the effects of chronic hypothalamic infusion of 2-arachidonoylglycerol (2-AG), the principal endogenous ligand of cannabinoid receptors, on metabolic, hormonal, inflammatory, and tissue-specific alterations under normal- and high-fat dietary conditions.

Methods: Chronic intra-hypothalamic 2-AG was infused using Alzet mini-osmotic pumps in normal chow and high-fat fed animals for 30 days. Food intake, dietary preference, body weight, and other biochemical parameters including gut hormones, glucose homeostasis, etc. were estimated at regular intervals. At the end, the hypothalamus, liver, pancreas, and adipose tissue were collected for gene expression and immunohistochemical analysis.

Results: Collectively, these findings demonstrate that chronic elevation of hypothalamic 2-AG is sufficient to promote obesity and metabolic dysfunction and both the cannabinoid receptors contribute differently in the development of obese phenotype.

The study identifies sustained central 2-AG signaling as a potential mechanistic link between hypothalamic endocannabinoid dysregulation and peripheral metabolic remodeling, providing new evidence for the role of central endocannabinoid signaling in the development and progression of obesity and associated metabolic disorders.

Conclusion: The hypothalamus is a major site at which endocannabinoid signaling influences energy homeostasis. Experimental studies have demonstrated that hypothalamic 2-AG levels are altered by fasting, feeding and dietary composition, supporting a role in the control of energy intake.

Keywords: Obesity; Metabolic dysfunction; Endocannabinoids; 2-Arachidonoylglycerol; Hypothalamus; High Fat Diet.

1. Introduction

Obesity and its metabolic outcomes remain major global health challenges across every household nowadays characterized by excessive adiposity, dyslipidemia, and impaired glucose homeostasis. According to the World Health Organization, obesity is defined as a body mass index (BMI) ≥ 30 kg/m². In 2022, approximately 13% of the global adult population nearly 890 million individuals-were classified as obese, with projections suggesting this number may exceed 1 billion by 2030 (1,2). This alarming trend suggests the urgent need for a deeper understanding of obesity pathophysiology, etiology, and therapeutic strategies. Despite advances in this area, current therapies largely target gut-derived hormonal pathways and fail to comprehensively address central neuromodulatory systems governing energy homeostasis (3). The Endocannabinoid System (ECS) has gained prominence as a key modulator of appetite and metabolism. In particular, the ECS, a critical regulator of feeding behavior, adiposity, and inflammation, remains underexplored. Given the conflicting roles of CB1 receptor and CB2 receptor in obesity, targeting their central interplay may offer a novel therapeutic strategy beyond existing treatments (5–7). Endocannabinoids such as 2-arachidonoylglycerol (2-AG) and anandamide (AEA) play a central role by stimulating orexigenic signaling in the hypothalamus while simultaneously amplifying reward pathways. This dual action promotes increased food intake to achieve the same reward threshold, thereby accelerating obesity progression (8). Despite these advances, the role of central 2-AG in modulating peripheral metabolic processes-including gut hormones, glucose homeostasis, and insulin dynamics-remains inadequately explored. Activation of central CB1 receptors increases appetite and promotes lipid storage, while pharmacological blockade of CB1 has been shown to reduce food intake and improve metabolic parameters in preclinical and clinical studies (9–11). However, the specific effects of sustained central elevation of 2-AG, and how these effects are modulated by dietary composition, are incompletely defined. High-fat diets (HFDs) not only drive weight gain and insulin resistance but also modify hypothalamic inflammation, neuronal responsiveness, and receptor expression. We hypothesized that chronic central infusion of 2-AG would exacerbate metabolic dysfunction and that these effects would be amplified in the context of an HFD than 2-AG administration with NPD.

Here, we used intrahypothalamically osmotic pumps to deliver 2-AG chronically and compared outcomes at 60 days in rats on normal chow (NPD) or High Fat Diet (HFD). We measured daily food intake, food preference, body weight trajectories, adipose depot mass

percentage, glucose tolerance (OGTT), and HbA1c. To characterize systemic and tissue-level changes we performed plasma lipid profiling and ELISA for metabolic hormones, histology of liver and adipose (H&E, Oil Red O), immunohistochemistry in different tissues including hypothalamic nuclei, liver, adipose tissue, pancreas and RT-qPCR for key neuropeptides and ECS components. This multi-level approach allowed us to determine whether central 2-AG drives hyperphagia and metabolic dysfunction directly and whether diet potentiates these effects.

2. Material & Methods

2.1 Experimental Animals

24 Male Sprague-Dawley rats weighing 200-250g were obtained from the animal House of Lovely Professional University, Jalandhar, Punjab, 144411, and kept at the Animal House Facility, Central University of Punjab, VPO- Guddha, Bathinda, Punjab. After arrival, the animals were housed in groups of 2 rats per polyacrylic cage and kept in well-maintained standard atmospheric conditions (Room Temperature: 22 ± 2ºC; Relative Humidity: 60-70%; 12:12 h Light-Dark cycle). The animal experiments were conducted between 9:00 am to 5:00 pm. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), having approval no. CUPB/IAEC/2025/10/36 and care was carried out adhering to the guidelines provided by the Committee for the Control and Supervision of Experiments on Animals (CCSEA) for the welfare of the animals.

2.2 Drugs and Chemicals

2-Arahidonoylglycerol (53847-30-6) was purchased from Cayman Chemical Company catalog No. 362162 and other diet chemicals were purchased from Central Drug House (P) Ltd. (CDH chemicals) and Sisco Research Laboratories Pvt. Ltd. (SRL)-India, Sigma Aldrich etc.

2.3 Experiments

I. Normal Pellet Diet: High Fiber (60-70%), Low Fat (5-10%), and low energy dense diet (15-20%).

II. High Fat Diet: High Fat (50-60%), Low Fiber (15-20%), and low energy dense diet (15-20%).

I. Differential Dietary Paradigms

When compared to regular NPD, the high-fat diet was designed to have a much higher percentage of fat, composition (12) listed in **Table-1**. The experimental rats were continuously fed with NPD and High Fat Diet (HFD) continued till 60 days categorised into

groups discussed in experimental grouping. The exclusion and inclusion of animals into further groups will be done on the basis of feed intake and food preference. OGTT and HbA1C levels were assessed at day 1, day 30, and day 60 for each group.

II. Osmotic Pump-Mediated Intrahypothalamic Delivery of 2-Arachidonoylglycerol

All control animals were made an incision to remove surgical bias and were made sham control i.e normal control with incision (NC), high fat fed rats with incision (HFD). After induction of disease and disease confirmation, the HFD fed rats on 30th day will be implanted with osmotic pumps prefilled with 2-AG in accordance with our hypothesis coined as HFD+2-AG group. The parameters assessment will be done on weekly and daily basis. Alzet® osmotic mini-pumps were surgically implanted using stereotaxic instrument into a 1 cm opening in the skin of experimental rats under ketamine/xylazine (80/5 mg/kg/i.p.) deep anaesthesia. The catheter tubing and brain guide cannula supplied with brain infusion kit (BIK) was implanted into paraventricular nucleus (PVN) at 0.8 mm anterior to central surface, 0.4 mm lateral to midline, and 7.5 mm below surface of skull according to stereotaxic coordinates given by Paxinos and Watson. Before implantation, the pumps were primed by filling them with 2-AG solution (200 µL solutions at the required dose of 2-AG i.e. 1µg/rat/day) and placing them into 37°C physiological saline for about 48 hrs at mean pump volume of 234.3 µL and mean pump flow rate of 0.22 µL. Preconditioning of all osmotic pumps with catheter applications is mandatory for minimizing the chances of occlusion. Following insertion of the mini-pumps into subcutaneous pockets, the skin was closed with surgical sutures. Surgery time was 10-15 min, and the rats received postoperative care as depicted in **supplementary file Fig. S1**. The pump provided continuous administration of 0.22 µL drug conc. per hour. To overcome the surgical biasness all the parameters are resumed after 7 days recovery period.

2.4 Experimental Groups and Experimental Timeline

The study comprised of 4 experimental groups with 6 animals in each group and total of 24 male S.D. rats. The groups were divided as animals fed with normal pellet diet and were surgically operated without any other intervention (NC). The animal groups fed with high fat diet and an incision is made to remove surgical biasness (HFD), and continued for 60 days. The rat group fed with HFD for 30 days and then implanted with osmotic pumps for intra-hypothalamic infusion of 2-Arachidonoylglycerol (HFD+2-AG) group at a dose of 1µg/rat/day implanted with osmotic pumps for enhanced endocannabinoid tone with dietary

modulation. Similarly the osmotic pumps are implanted to animals fed with NPD for continuous infusion of 2-AG at a dose of 1µg/rat/day (NC+2-AG). All the groups were sacrificed using higher dose of anaesthesia on Day 60 for isolation of brain, liver, white adipose tissue (WAT), brown adipose tissue (BAT), and pancreas or directly stored to -80 deep freezer for further molecular assessment.

The experimental timeline for the duration of study and the timeline for each parameter is discussed and given in detail in **supplementary file Fig. S2.**

2.5 Assessment of Food Intake, Adiposity, Glycemic Status, and Lipid Profile

Food preference was assessed by monitoring of NPD and HFD, with hopper positions alternated almost daily to minimize positional bias. Food intake was recorded after correcting for spillage, and HFD preference was expressed as a proportion of total intake. Daily food consumption was monitored at 12-h intervals. Body weight was recorded weekly, and percentage weight change was calculated from initial and final body weights. Relative liver, adipose tissue, and pancreatic weights were expressed as percentages of final body weight. Oral glucose tolerance tests (OGTT) were performed after 5-h fasting using glucose (2 g/kg), with blood glucose measured at 0, 30, 60, 90, and 120 min. HbA1c was quantified using a commercial assay kit. Plasma LDL, HDL, triglycerides, total cholesterol, ALT, AST, and ALP were measured using commercial diagnostic kits (Accurex) according to the manufacturer's instructions.

2.6 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA assays were performed for plasma and tissue homogenates for Brain, Liver, WAT, BAT and Pancreas in accordance with ELK biotechnology ELISA kits for AEA and 2-AG, while Elabscience ELISA kits for other parameters.

2.7 Analysis of metabolic markers mRNA gene expression by real-time PCR (RT-PCR)

A 50 µg of each tissue was homogenised in 250 µl of Trizol reagent and centrifuged at 10,000 rpm for 7 min. The supernatant was carefully collected, and procedure mentioned in the protocol (13) **Table 2.**

2.8 Histology

2.8.1 Haematoxylin and Eosin (H&E) Staining

H&E staining was done for all the tissues assessed in the current study i.e Brain, Liver, Adipose tissue and Pancreas to see the morphological changes among different groups and different tissues.

2.8.2 Oil Red O (ORO) Staining

ORO staining is specific to stain neutral fats and lipids Fresh frozen tissue samples of Liver and WAT were embedded in OCT compound and sectioned at 8–10 µm thickness using a cryostat. Sections were fixed in 10% neutral buffered formalin for 10 min, rinsed with distilled water, and immersed in 60% isopropanol. The sections were then stained with freshly prepared Oil Red O working solution for 10-15 min, followed by differentiation in 60% isopropanol and washing with distilled water. Nuclei were counterstained with hematoxylin, and sections were mounted using an aqueous mounting medium. Stained sections were examined under a light microscope.

2.9 Immunohistochemistry (IHC) Analysis

Immunohistochemical staining was performed to evaluate the expression of cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) in tissue sections. Paraffin-embedded tissue samples were sectioned at 4-5 µm thickness, deparaffinized in xylene, and rehydrated through a graded series of ethanol and further processed in accordance with IHC kit pathnsitu (PEH002). Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) chromogen, and nuclei were counterstained with hematoxylin. The stained sections were dehydrated, mounted, and examined under a light microscope. The expression of CB1 and CB2 receptors was assessed qualitatively and/or quantitatively by analyzing the intensity and distribution of brown DAB staining in the tissue sections.

3 Results

3.1 Dietary Intake and Adiposity Assessment Parameters

3.1.1 Effect of chronic central 2-Arachidonoylglycerol infusion on different dietary and adiposity indices

Chronic hypothalamic 2-AG infusion significantly influenced, body weight gain **Fig. 1 A**, feeding behavior **Fig. 1B**, Daily Feed Intake **Fig. 1C** and adiposity indicies & Fat Pad Mass %age **Fig. 1(D-F),** with the most pronounced effects observed in rats maintained on a high-fat diet (HFD).

3.1.2 Effect of chronic central 2-Arachidonoylglycerol infusion on plasma lipid profile and hepatic biomarkers assayed via diagnostic kits

Chronic hypothalamic 2-AG infusion significantly altered circulating lipid parameters, with the most pronounced effects observed in HFD-fed rats. Liver function markers revealed significant hepatic impairment following chronic hypothalamic 2-AG infusion as discussed in the above figure panel **Fig. 2 (A- B)**.

3.1.3 Effect of chronic central 2-Arachidonoylglycerol infusion on Glycemic Parameters via OGTT and HbA1C assays

In the given figure **Fig. 2** The OGTT **(A)** showed a pattern of increase in HFD and HFD+2-AG group at 30 min with delayed restoration to normal levels at $p<0.001$. While the other groups showed restoration in peak glucose levels after 120 min. The HFD group exhibited a significant in both HbA1C levels **(B)** and avg. blood glucose levels **(C)** when compared with the NC group. Furthermore infusion with 2-AG further enhanced blood glucose levels relative to HFD animals.

3.2 Enzyme-Linked Immunosorbent Assay (ELISA)

3.2.1 Effect of High-Fat Diet and 2-Arachidonoylglycerol on endocannabinoid tone, glycemic index, gut hormone levels and inflammatory markers in blood plasma (Fig. 3)

Chronic HFD feeding significantly increased circulating endocannabinoids (AEA and 2-AG), glucose, insulin, leptin, ghrelin, GLP-1, GIP, PYY, and pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) compared with the NC group, while anti-inflammatory IL-10 levels were significantly reduced ($p < 0.05$). These effects were further exacerbated by 2-AG administration, with the HFD+2AG group exhibiting the highest levels of metabolic and inflammatory markers and the lowest IL-10 levels, indicating that endocannabinoid over-activation aggravates obesity-associated metabolic dysfunction and systemic inflammation.

3.2.2 Effect of High-Fat Diet and 2-Arachidonoylglycerol on endocannabinoid tone, glycemic index, gut hormone levels, and inflammatory markers in the different tissue homogenates (Fig. 4)

In **Fig. 4** The above results showed significant increase in 2-AG and AEA levels with HFD and aggravated with chronic central infusion of 2-AG. Proinflammatory markers (IL-6, IL-1β, TNF-α) showed marked increase in HFD and HFD +2-AG. The Gut hormones and insulin, leptin showed a state of resistance with marked increased levels in HFD+2-AG group ($p<0.001$) and decrease in anti-inflammatory markers (IL-10) in HFD and HFD +2-AG ($p<0.001$) in similar pattern among all the tissues.

3.3 Effect of chronic intracerebroventricular 2-arachidonoylglycerol (2-AG) infusion on tissue-specific mRNA expression of genes regulating endocannabinoid signaling, lipid metabolism, insulin signaling, and thermogenesis assayed using RT-PCR.

The relative mRNA expression of genes associated with endocannabinoid signaling, energy homeostasis, lipid metabolism, and thermogenesis in the hypothalamus, liver, pancreas, white adipose tissue (WAT), and brown adipose tissue (BAT) is shown in **Fig. 5.** Collectively, the data demonstrate that chronic central 2-AG administration markedly potentiates HFD-induced alterations in tissue-specific gene expression. The combined treatment enhanced **CB1/CB2 receptor expression**, stimulated **lipogenic pathways** (SREBF and PPAR-γ), increased **insulin receptor expression**, and simultaneously suppressed genes involved in **mitochondrial biogenesis, fatty acid oxidation, and thermogenesis** (PGC-1α, UCP-1, and PPAR-α), thereby favoring metabolic dysfunction and adiposity.

3.4 Histopathological Analysis

3.4 Effect of HFD and 2-AG on morphological variations among different tissues via using Haematoxylin and Eosin stain

Representative hematoxylin and eosin (H&E)-stained sections of the brain, liver, pancreas, and white adipose tissue (WAT) from normal chow (NC), NC + 2-AG, high-fat diet (HFD), and HFD + 2-AG groups are presented in **Fig. 6.**

3.5 Effect of HFD and 2-AG on fat deposition among white adipose tissues and liver tissues via oil red o stain

Representative **Oil Red O-stained** sections of the **liver** and **white adipose tissue (WAT)** from normal chow (NC), NC + 2-AG, high-fat diet (HFD), and HFD + 2-AG groups are shown in **Supplementary figure S3**. Oil Red O-positive lipid droplets appear as red/orange staining and indicate neutral lipid accumulation. Oil Red O staining provides direct morphological evidence of lipid accumulation and corroborates the biochemical and molecular findings observed in the present study.

3.6 Immunohistochemistry

3.6.1 Representative immunohistochemical staining of CB1 receptor and CB2 receptor expression in brain sections from NC, NC + 2-AG, HFD, and HFD + 2-AG groups is presented in Fig. S4. Positive immunoreactivity is indicated by brown DAB staining in **Supplementary figure S4** for Brain, Liver, Pancreas and WAT in the given figure panel.

4. Discussion

Obesity is a multifactorial metabolic disorder across every household characterized by excessive adipose tissue accumulation, insulin resistance, dyslipidaemia, chronic low-grade inflammation, and impaired energy homeostasis (14). The present study demonstrates that chronic hypothalamic administration of 2-arachidonoylglycerol (2-AG), particularly in the context of a high-fat diet (HFD), markedly aggravates obesity-associated metabolic dysfunction. The integration of physiological, biochemical, molecular, histopathological, and immunohistochemical findings provides compelling evidence that excessive endocannabinoid signaling accelerates obesity progression through coordinated alterations in central and peripheral metabolic pathways.

A major finding of the study was the pronounced increase in feed intake, preference for energy-dense food, body weight gain, and adiposity following HFD feeding, all of which were further enhanced by chronic 2-AG administration with fat intake whilst no significant effects were observed in 2-AG alone administration. These observations support previous reports demonstrating that endocannabinoids regulate both homeostatic and hedonic feeding through activation of cannabinoid receptor type 1 (CB1R) within hypothalamic and mesolimbic reward circuits (16–18). Increased hypothalamic Cnr1 gene expression, accompanied by elevated AgRP and suppression of anorexigenic signaling, indicates a shift toward an orexigenic phenotype that promotes sustained hyperphagia. Since AgRP neurons stimulate feeding behavior whereas POMC neurons promote satiety, this reciprocal regulation provides a mechanistic explanation for the persistent energy balance observed in animals infused with 2-AG continued with HFD feeding animals. These findings reinforce the concept that enhanced hypothalamic ECS activity is a critical driver of obesity development when accompanied with high fat diet dietary paradigms.

The increase in caloric intake was accompanied by significant elevations in adiposity index, liver index, and body weight, indicating successful establishment of an obese phenotype. Notably, animals receiving both HFD and 2-AG exhibited the greatest adiposity, suggesting that elevated endocannabinoid tone enhances the energy storage. This observation is consistent with evidence showing that CB1 activation stimulates adipogenesis, promotes triglyceride synthesis, and suppresses energy expenditure (15,19,20). Thus, the obesity-promoting effects of 2-AG appear to arise not only from increased food consumption but also from direct metabolic reprogramming that favors lipid storage.

The current study suggests ECS activation was the disruption of lipid homeostasis. HFD feeding produced marked dyslipidaemia characterized by elevated triglycerides, total cholesterol, and LDL levels, together with reduced HDL concentrations. These biochemical alterations were accompanied by hepatic steatosis, adipocyte hypertrophy, and extensive lipid accumulation demonstrated by H&E histopathology and Oil Red O staining. At the molecular level, increased expression of hepatic Cnr1, SREBF, and PPARγ indicates activation of lipogenic pathways. SREBF is a master regulator of de novo fatty acid synthesis, while PPARγ promotes adipogenesis and lipid storage. Previous studies have shown that CB1 activation stimulates SREBP-1c-dependent lipogenesis, contributing to hepatic triglyceride accumulation and non-alcoholic fatty liver disease (21,22). Therefore, enhanced ECS activity with high fat food likely contributes directly to hepatic steatosis and dyslipidaemia through activation of lipogenic gene networks.

Concurrently, alterations in LIPE expression suggest impaired lipolytic capacity and reduced mobilization of stored triglycerides. Since hormone-sensitive lipase plays a central role in intracellular lipid breakdown, its dysregulation indicates a reduction in lipid utilization (23). The combined increase in lipogenesis and impairment of lipolysis creates a metabolic environment favoring excessive triglyceride storage and adipose tissue expansion. These findings indicate that ECS overactivation promotes obesity through coordinated stimulation of lipid synthesis and suppression of lipid turnover.

Beyond lipid metabolism, the present study identified mitochondrial dysfunction and impaired thermogenesis as important downstream consequences of chronic ECS activation. Brown adipose tissue exhibited significant alterations in PGC-1α and UCP-1 expression, particularly following 2-AG administration. PGC-1α serves as a master regulator of mitochondrial biogenesis and oxidative metabolism, whereas UCP-1 is essential for adaptive thermogenesis and energy dissipation. Suppression of these genes suggests impaired mitochondrial function, reduced oxidative phosphorylation, and diminished energy expenditure. Previous studies have demonstrated that chronic CB1 activation inhibits AMPK signaling and mitochondrial respiration while promoting lipid accumulation (24,25). Thus, the present findings suggest that enhanced endocannabinoid signaling simultaneously increases energy intake and decreases energy expenditure, thereby amplifying positive energy balance and accelerating obesity progression.

Disturbances in glucose homeostasis constituted another major feature of ECS-mediated metabolic dysfunction. HFD-fed animals displayed impaired oral glucose tolerance, elevated blood glucose concentrations, increased HbA1c levels, and hyperinsulinaemia, all indicative

of insulin resistance then in 2-AG alone group. These abnormalities were further aggravated following 2-AG administration. Excessive ECS activation has been shown to impair insulin receptor signaling through disruption of IRS-PI3K-Akt pathways, thereby reducing glucose uptake and promoting insulin resistance (21). Collectively, these findings indicate that elevated endocannabinoid tone disrupts both lipid and glucose metabolism, thereby contributing to the development of metabolic syndrome.

Chronic low-grade inflammation, or metaflammation, is widely recognized as a hallmark of obesity and a major contributor to metabolic dysfunction. Consistent with this concept, HFD feeding significantly increased TNF-α, IL-1β, and IL-6 expression across the hypothalamus, liver, pancreas, white adipose tissue, and brown adipose tissue, while disrupting anti-inflammatory IL-10 signaling. These findings are consistent with previous reports demonstrating that obesity promotes macrophage recruitment, innate immune activation, and cytokine production, ultimately driving insulin resistance and tissue injury (26). Importantly, inflammatory responses were further intensified by 2-AG administration, indicating that excessive ECS activation contributes to obesity-associated inflammation.

The current study showed simultaneous upregulation of both Cnr1 and Cnr2 across metabolically active tissues. While CB1 signaling was closely associated with hyperphagia, lipogenesis, insulin resistance, and tissue injury, increased Cnr2 expression appeared to correlate primarily with inflammatory changes. Since CB2 receptors are predominantly expressed on immune cells and generally mediate anti-inflammatory and immunomodulatory actions, their increased expression likely reflects an adaptive response to chronic metabolic stress (27,28). However, despite enhanced Cnr2 expression, inflammatory cytokine levels remained elevated, suggesting that endogenous CB2-mediated protective mechanisms were insufficient to counteract persistent CB1-driven metabolic and inflammatory injury. Thus, the present findings support a model in which CB1 signaling drives disease progression, whereas CB2 activation may represents a compensatory but ultimately inadequate attempt to restore tissue homeostasis.

The observed increase in AgRP expression, together with enhanced Cnr1 signaling, likely contributed to persistent hyperphagia and weight gain. These findings suggest that chronic ECS activation exacerbates hypothalamic dysfunction, thereby perpetuating metabolic imbalance. Similarly, pancreatic alterations, including disrupted islet architecture, increased inflammatory signaling, and altered expression of metabolic regulatory genes, indicate substantial endocrine stress. Excessive CB1 activation has previously been associated with impaired β-cell function and reduced glucose responsiveness (31). The pancreatic changes

observed in the present study therefore likely contribute to the development of glucose intolerance and insulin resistance.

Collectively, gene expression analyses, biochemical measurements, histopathology, and immunohistochemistry represents a major strength of this study. Increased CB1 and CB2 immunoreactivity in the hypothalamus, liver, pancreas, and adipose tissue confirmed activation of ECS signaling at the protein level, while histological evidence of steatosis, adipocyte hypertrophy, inflammatory infiltration, and neuronal degeneration validated the functional consequences of molecular dysregulation.

Collectively, the present findings support a comprehensive mechanistic model in which chronic HFD elevates endogenous endocannabinoid tone, while exogenous 2-AG further amplifies ECS activity across metabolically active tissues than when administered alone without HFD. Although both CB1 and CB2 pathways are activated, their biological roles appear distinct. CB1 signaling predominantly promotes hyperphagia, lipogenesis, impaired lipolysis, mitochondrial dysfunction, reduced thermogenesis, insulin resistance, and tissue remodeling, whereas CB2 signaling beyond inflammatory regulation may promote obesity as seen via increased mRNA and IHC expression. The findings suggest that chronic endocannabinoid infusion may shift CB2 immunoprotective actions towards detrimental effects supported by recent study upon Q63R variant of CB2 activated upon chronic 2-AG infusion with high fat diet stimulations promoting obesity and leading to metabolic dysfunctions.

5. Conclusion

In conclusion, this study identifies excessive 2-AG-mediated CB1R/CB2R activation as a critical contributor to obesity pathogenesis. These results provide mechanistic evidence linking ECS dysregulation to obesity and highlight CB2 receptor signaling as a key therapeutic target. Furthermore the Q63R variant may be responsible for pro obesity effects of CB2R upon chronic enhancement of endocannabinoid signaling with chronic high fat diet exposure.

Figures and Figure Captions

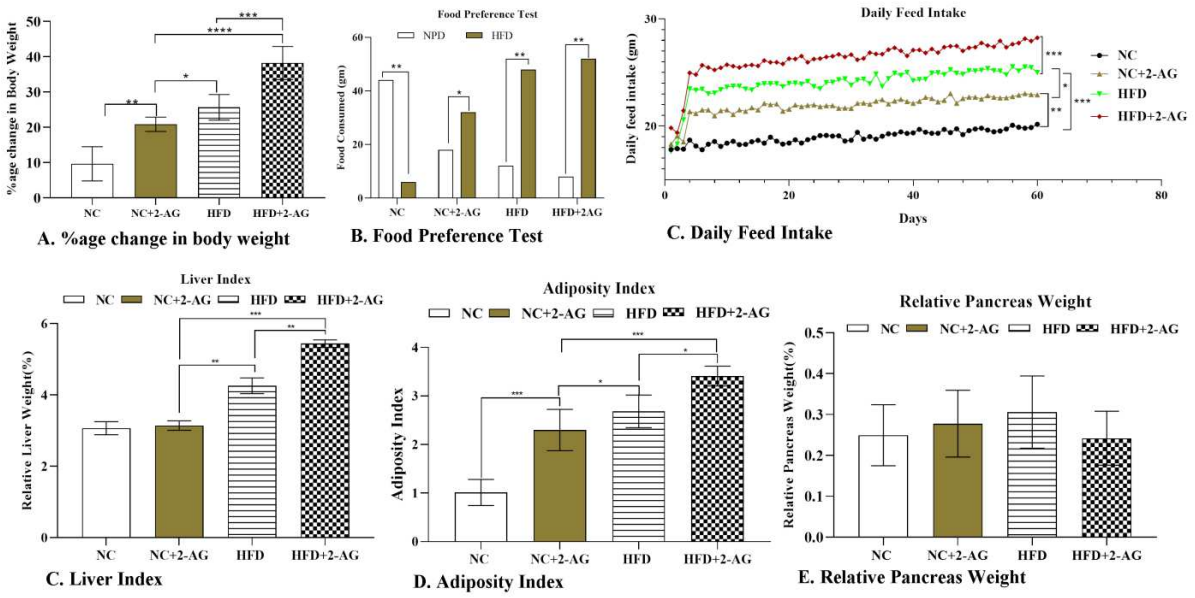

Fig. 1 Effects of chronic hypothalamic 2-arachidonoylglycerol (2-AG) infusion on body weight, feeding behavior, and organ indices in rats.

(A) Percentage change in body weight. **(B)** Food preference test showing consumption of NPD and HFD. **(C)** Daily feed intake recorded over the 60-day experimental period. **(D)** Relative liver weight (liver index). **(E)** Adiposity index. **(F)** Relative pancreas weight. Chronic hypothalamic 2-AG infusion increased food intake, preference for HFD, body weight gain, liver index, and adiposity, with the greatest effects observed in HFD-fed rats. Data are presented as mean ± SEM (n = 6/group). Statistical significance is indicated as ***p*<0.05 (*), *p<0.01 (**), *p*<0.001(***), and *p*<0.0001 (****).

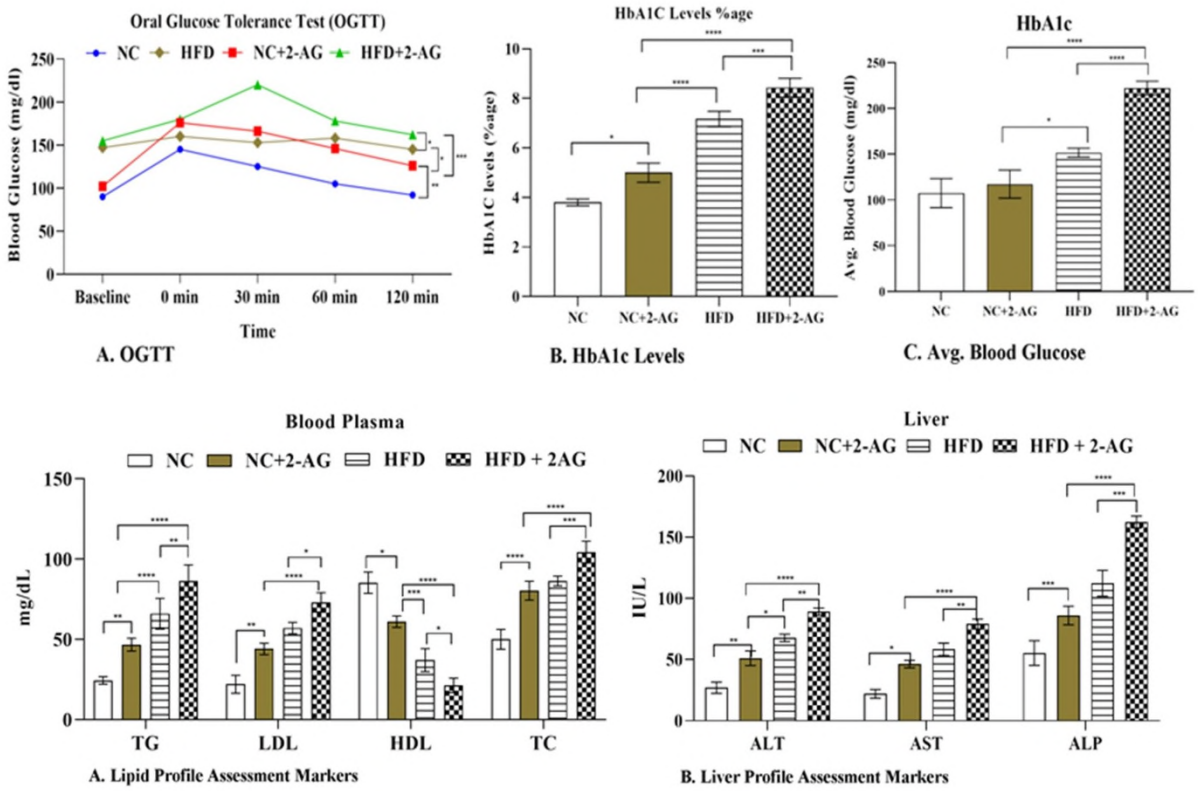

Fig.2 Effects of chronic hypothalamic 2-arachidonoylglycerol (2-AG) infusion on plasma lipid profile, liver function markers and glycemic index in HFD fed rats. In the above figure panel A. HFD feeding induced significant impairments in glucose homeostasis, lipid metabolism, and liver function, as evidenced by elevated OGTT responses (Panel A), HbA1c (B), average blood glucose (C), TG, LDL, TC, ALT, AST, and ALP levels, along with reduced HDL concentrations (panel A & B) ($p < 0.05–0.0001$). Chronic hypothalamic 2-AG infusion further intensified these metabolic abnormalities, with the HFD+2-AG group consistently exhibiting the most severe phenotype ($p < 0.01–0.0001$*), indicating that enhanced central endocannabinoid signaling promotes insulin resistance, dyslipidemia, and hepatic dysfunction under obesogenic conditions.

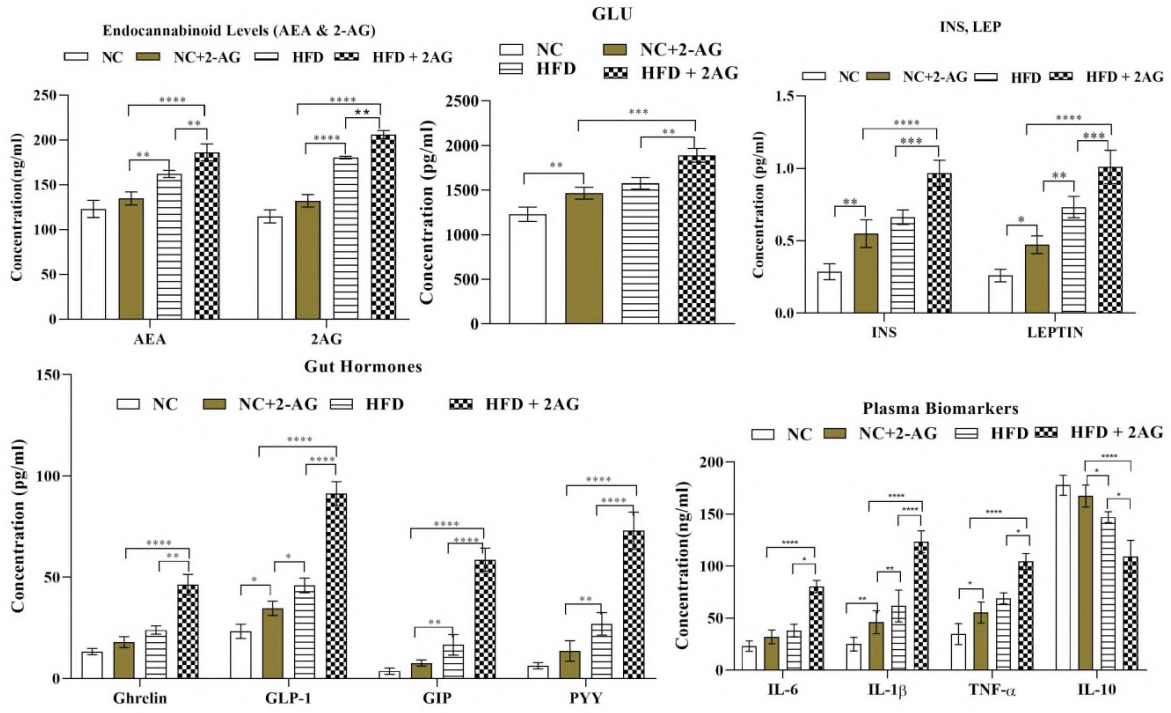

Fig. 3 Effect of High-Fat Diet and 2-Arachidonoylglycerol on endocannabinoid tone, glycemic index, gut hormone levels and inflammatory markers in blood plasma. Chronic HFD feeding significantly increased circulating endocannabinoids (AEA and 2-AG), glucose, insulin, leptin, ghrelin, GLP-1, GIP, PYY, and pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) compared with the NC group, while anti-inflammatory IL-10 levels were significantly reduced ($p < 0.05$). These effects were further exacerbated by 2-AG administration, with the HFD+2AG group exhibiting the highest levels of metabolic and inflammatory markers and the lowest IL-10 levels, indicating that endocannabinoid over-activation aggravates obesity-associated metabolic dysfunction and systemic inflammation. Data are presented as mean ± SEM (n = 6/group). Statistical significance is indicated as $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), and $p<0.0001$ (****).

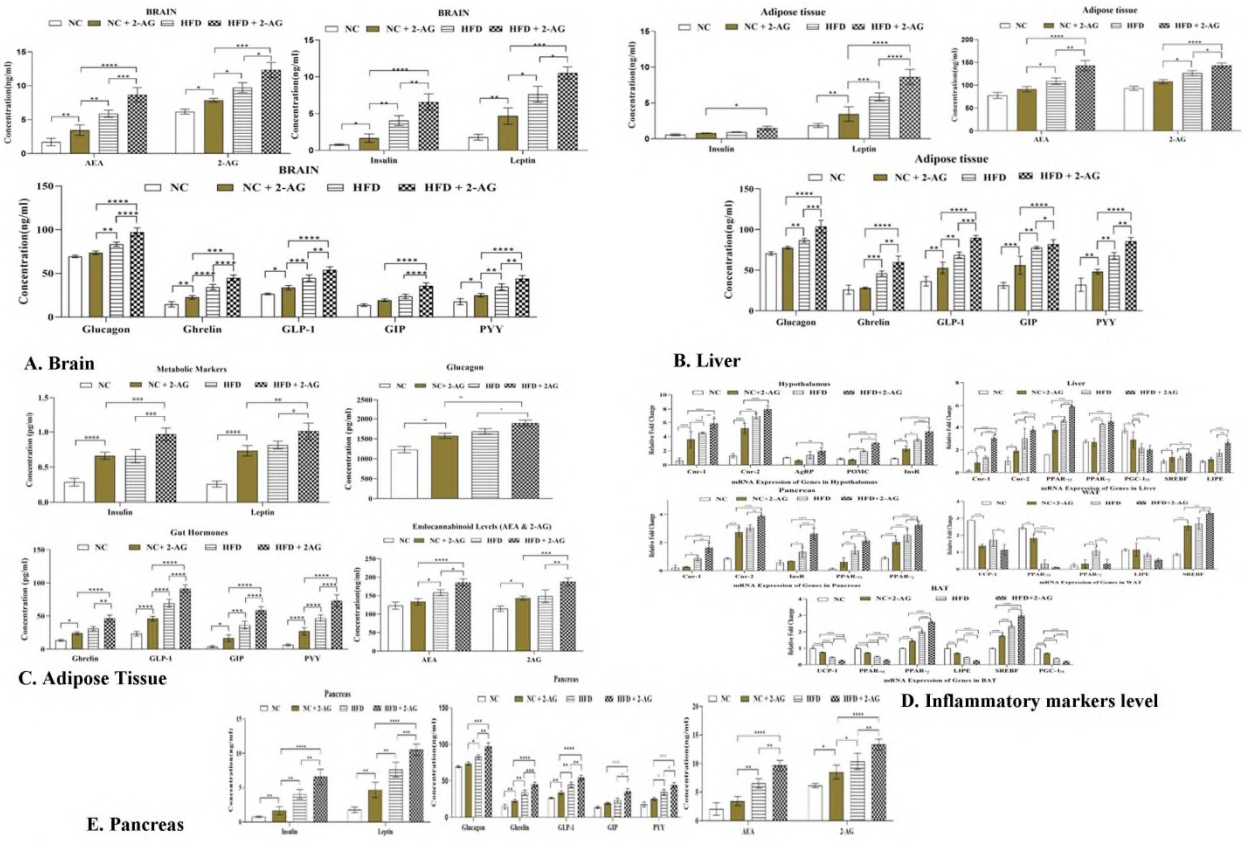

Fig. 4 Effect of High-Fat Diet and 2-Arachidonoylglycerol on endocannabinoid tone, glycemic index, gut hormone levels and inflammatory markers in different tissue homogenates.

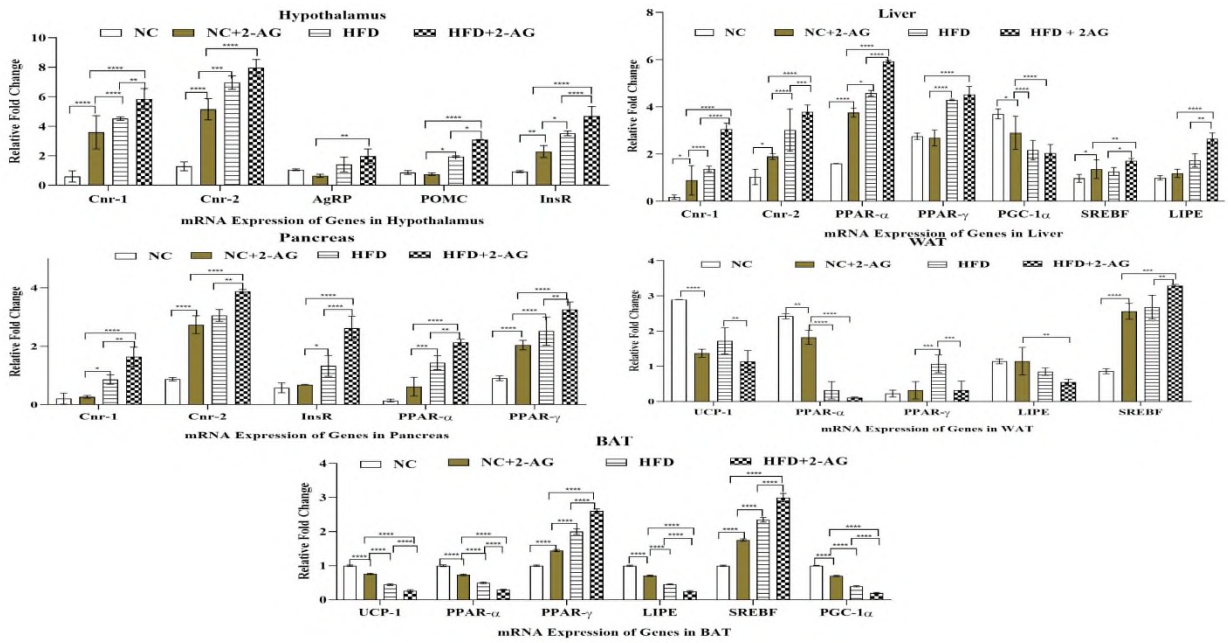

Fig. 5 Effect of chronic intrahypothalamic 2-AG infusion on tissue-specific mRNA expression of genes regulating endocannabinoid signaling, lipid metabolism, insulin

signaling, and thermogenesis in normal chow (NC) and high-fat diet (HFD)-fed rats. Relative mRNA expression was determined by quantitative real-time PCR in the **hypothalamus** (Cnr-1, Cnr-2, AgRP, POMC, and InsR), **liver** (Cnr-1, Cnr-2, PPAR-α, PPAR-γ, PGC-1α, SREBF, and LIPE), **pancreas** (Cnr-1, Cnr-2, InsR, PPAR-α, and PPAR-γ), **white adipose tissue (WAT)** (UCP-1, PPAR-α, PPAR-γ, LIPE, and SREBF), and **brown adipose tissue (BAT)** (UCP-1, PPAR-α, PPAR-γ, LIPE, SREBF, and PGC-1α) in four experimental groups: NC, NC + 2-AG, HFD, and HFD + 2-AG. Gene expression was normalized to the housekeeping gene and expressed as relative fold change using the 2^-ΔΔCt method. Data are presented as **mean ± SEM** (n = 6 animals per group). Statistical significance was determined using **one-way ANOVA followed by Tukey's multiple comparison test**. Significance is indicated as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Central 2-AG infusion potentiated HFD-induced activation of endocannabinoid receptor signaling and lipogenic gene expression while suppressing thermogenic and mitochondrial regulatory genes, indicating exacerbation of obesity-associated metabolic dysfunction. Data are presented as mean ± SEM (n = 6/group). Statistical significance is indicated as $p<0.05$ (*), $p<0.01$ (**), $p<0.001$(***), and $p<0.0001$ (****).

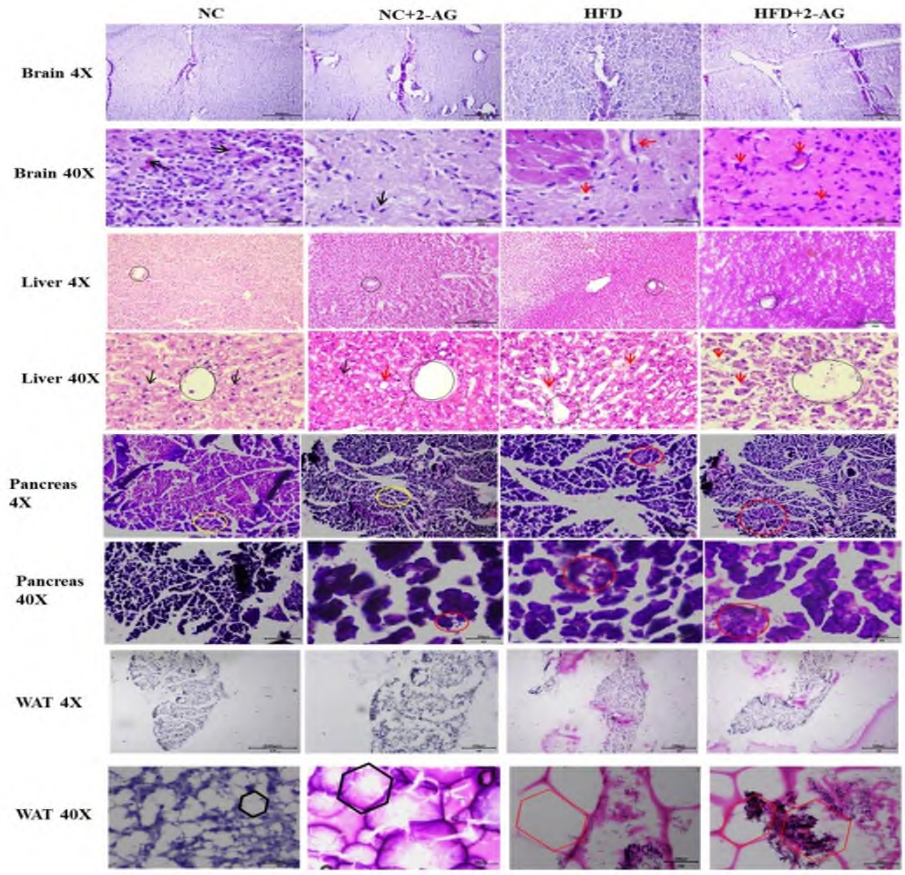

Fig. 6 Representative hematoxylin and eosin (H&E)-stained sections showing the histopathological effects of chronic intrahypothalamic 2-AG infusion in normal chow (NC) and high-fat diet (HFD) fed rats. Brain, liver, pancreas, and white adipose tissue (WAT) sections from the four experimental groups (NC, NC + 2-AG, HFD, and HFD + 2-AG) are shown at low (4×) and high (40×) magnification. Brain sections demonstrate neuronal morphology, vascular changes, and inflammatory cell infiltration (arrows). Liver sections illustrate hepatocellular architecture, lipid vacuolation, steatosis, and inflammatory changes (arrows). Pancreatic sections highlight the morphology of the islets of Langerhans (circled) and associated endocrine alterations. WAT sections depict adipocyte size (outlined), hypertrophy, and inflammatory remodeling. Chronic HFD induced tissue-specific pathological alterations that were further aggravated by central 2-AG infusion, resulting in enhanced neuronal injury, hepatic steatosis, pancreatic islet hypertrophy, and adipocyte hypertrophy, consistent with exacerbated obesity-associated metabolic dysfunction.

Supplementary Files

Fig. S1Osmotic Pump-Mediated Intrahypothalamic Delivery of 2-Arachidonoylglycerol

Fig. S2 Experimental Timeline of the study

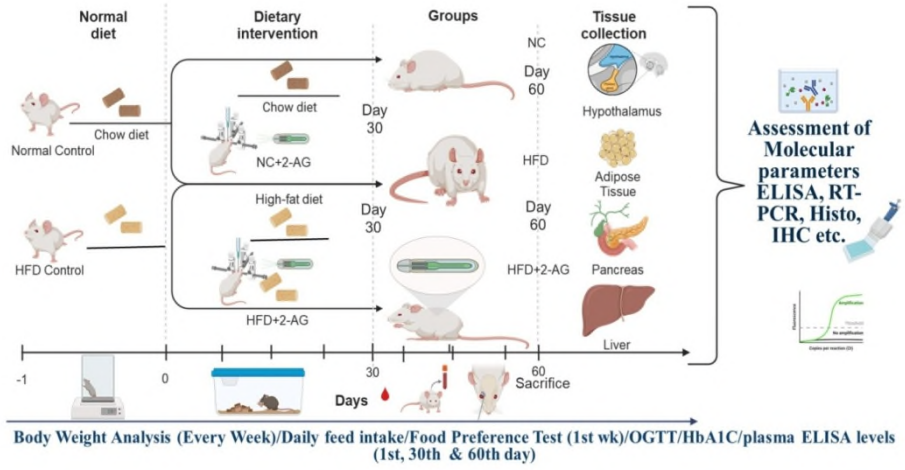

Fig. S3: Oil Red O staining or neutral lipids for Liver and Adipose Tissue

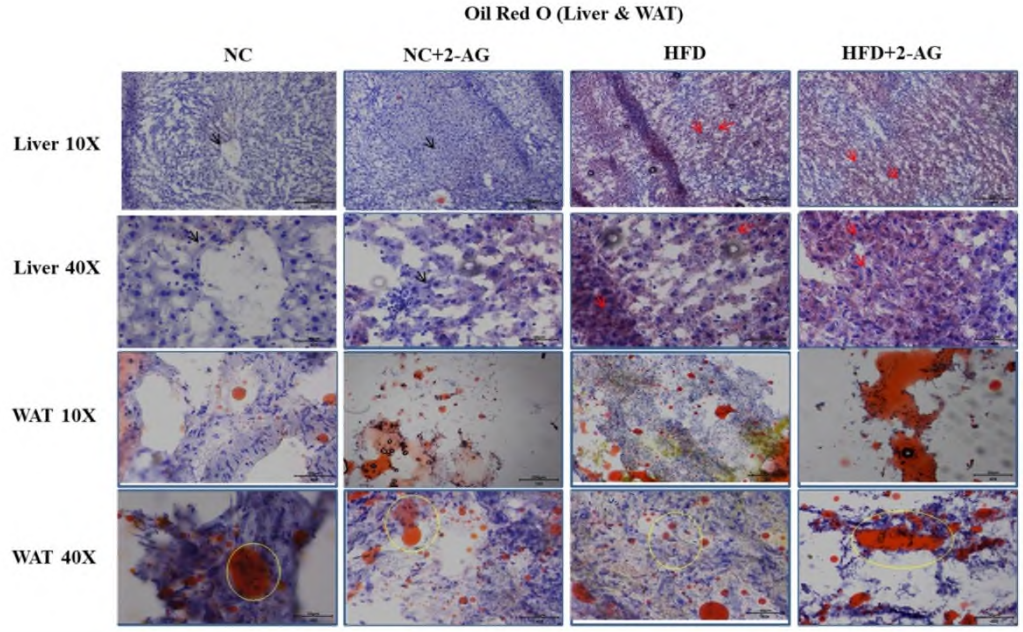

Fig. S3 Representative Oil Red O staining of liver and white adipose tissue (WAT) showing neutral lipid accumulation following chronic intrahypothalamic 2-AG infusion in normal chow (NC) and high-fat diet (HFD) fed rats. Liver sections are shown at 10× and 40× magnification, while WAT sections are presented at 10× and 40× magnification. Oil Red O-positive neutral lipid droplets appear as red/orange staining (indicated by arrows and circles), whereas nuclei are counterstained blue. The NC and NC + 2-AG groups exhibited minimal lipid deposition. HFD feeding induced marked hepatic steatosis and increased adipose lipid accumulation, which were further exacerbated by central 2-AG infusion. The HFD + 2-AG group demonstrated the most extensive lipid deposition, characterized by diffuse hepatic lipid accumulation and enlarged lipid droplets in WAT, indicating enhanced

lipogenesis and triglyceride storage under combined HFD and endocannabinoid activation. Data are representative of n = 6 animals per group.

Fig. S4 Immunostaining for CB1 and CB2 across different tissues and groups

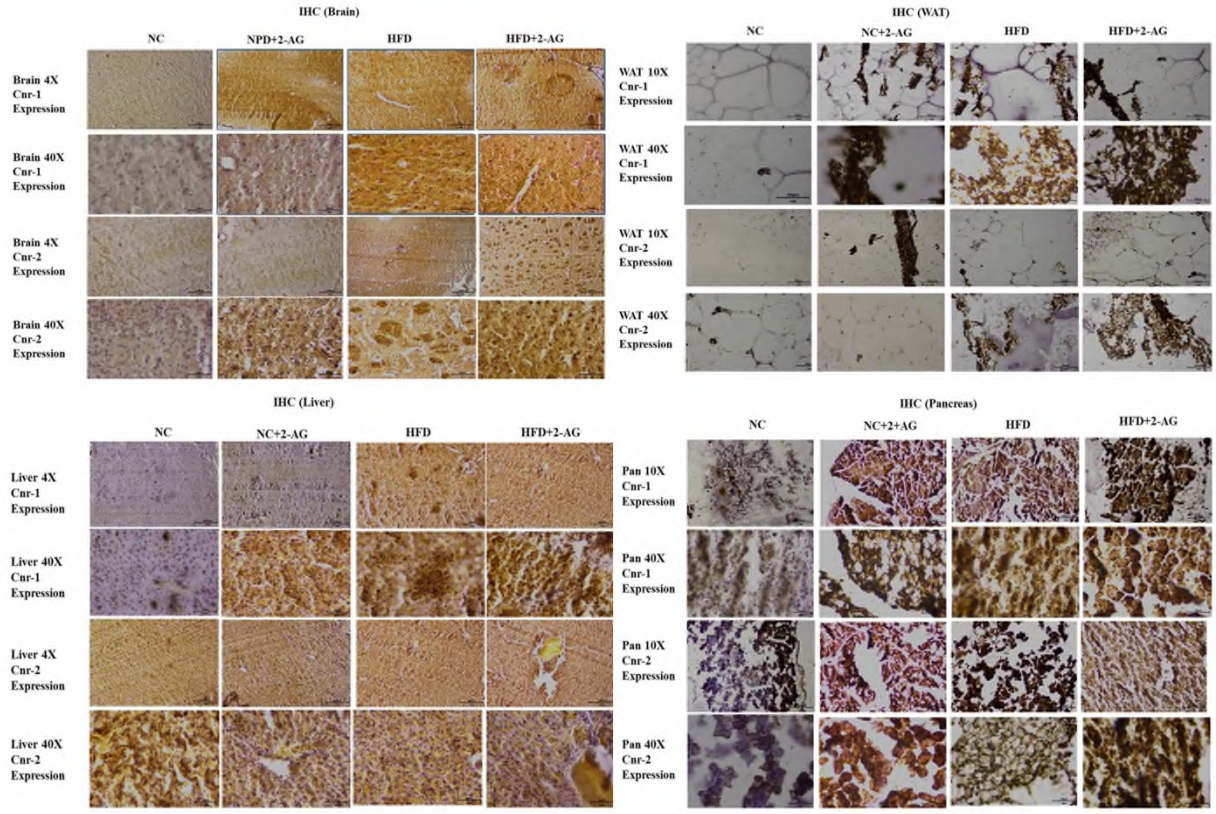

Fig. S4 Representative immunohistochemical (IHC) staining of cannabinoid receptor type 1 (Cnr-1/CB1) and cannabinoid receptor type 2 (Cnr-2/CB2) in pancreatic sections from normal chow (NC), NC + 2-AG, high-fat diet (HFD), and HFD + 2-AG rats. Pancreatic tissues were immunostained using antibodies against **Cnr-1** and **Cnr-2** and examined at **10× and 40× magnification**. Positive immunoreactivity is visualized as **brown DAB staining**, indicating receptor expression within pancreatic acinar cells and islets of Langerhans. The NC group exhibited weak basal CB1 and CB2 expression, whereas central 2-AG administration moderately increased receptor immunoreactivity. HFD feeding markedly enhanced pancreatic Cnr-1 and Cnr-2 expression, and chronic central 2-AG infusion further intensified staining, with the **HFD + 2-AG** group displaying the strongest and most extensive immunoreactivity. These findings corroborate the qRT-PCR data and demonstrate enhanced pancreatic endocannabinoid signaling associated with islet remodeling, impaired insulin homeostasis, and obesity-induced metabolic dysfunction.

Author Contribution Statement

Shilpa Kumari conducted the research experiments, data analysis, compilation and writing of original manuscript.

Biswanath Nayak helped during experiments and compilation of data of the current research

Mahendra Bishnoi helped in data curation and formal analysis of the manuscript.

Rahul Deshmukh has done the conceptualization, data curation, formal analysis, supervision and funding acquisition.

All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Conflict of Interest

The authors do not have any conflict of interest in publication of this manuscript.

Ethical Declaration

The Institutional Animal Ethical Committee (IAEC) approved the animals' experimental protocol with the approval number CUPB/IAEC/2025/10/36.

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6. References

1. Jeong S-M, Jung J-H, Yang YS, Kim W, Cho IY, Lee Y-B, et al. 2023 obesity fact sheet: prevalence of obesity and abdominal obesity in adults, adolescents, and children

in Korea from 2012 to 2021. J Obes Metab Syndr. 2024;33(1):27.

2. Dixon JB, Abdul Ghani R, Sbraccia P. Perceptions of obesity among healthcare professionals and policy makers in 2023: results of the global OPEN survey. Obes Sci Pract. 2025;11(1):e70033.
3. Kendall DA, Yudowski GA. Cannabinoid receptors in the central nervous system: Their signaling and roles in disease. Front Cell Neurosci. 2017;10(January):1–10.
4. Rosi A, Bragazzi NL, Bertolotti E, Biasini B, Grosso G, Del Rio D, et al. Dietary Interventions Based on the Mediterranean Diet to Treat Obesity in Children and Adolescents: A Systematic Review with Meta-analysis and Meta-regressions. Nutr Rev. 2026;nuaf191.
5. Boubertakh B, Silvestri C, Di Marzo V. Obesity: The fat tissue disease version of cancer. Cells. 2022;11(12):1872.
6. Rakotoarivelo V, Mayer TZ, Simard M, Flamand N, Di Marzo V. The impact of the CB2 cannabinoid receptor in inflammatory diseases: an update. Molecules. 2024;29(14):3381.
7. Sagredo O, Marzo V Di, Valdeolivas S, Bisogno T, Piscitelli F, Iannotti FA, et al. The inhibition of 2-arachidonoyl-glycerol (2-AG) biosynthesis , rather than enhancing striatal damage , protects striatal neurons from malonate-induced death : a potential role of cyclooxygenase-2-dependent metabolism of 2-AG. 2013;
8. Ratano P, Petrella C, Forti F, Passeri PP, Morena M, Palmery M, et al. Pharmacological inhibition of 2-arachidonoilglycerol hydrolysis enhances memory consolidation in rats through CB2 receptor activation and mTOR signaling modulation [Internet]. Vol. 138, Neuropharmacology. Elsevier Ltd; 2018. 210–218 p. Available from: https://doi.org/10.1016/j.neuropharm.2018.05.030
9. Mahmoud MF, Swefy S El, Hasan RA, Ibrahim A. Role of cannabinoid receptors in hepatic fibrosis and apoptosis associated with bile duct ligation in rats. Eur J Pharmacol. 2014 Nov;742:118–24.
10. Alonso M, Serrano A, Vida M, Crespillo A, Hernandez-Folgado L, Jagerovic N, et al. Anti-obesity efficacy of LH-21, a cannabinoid CB1 receptor antagonist with poor brain penetration, in diet-induced obese rats. Br J Pharmacol. 2012;165(7):2274–91.
11. Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand J-P, et al. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. Am J Physiol Integr Comp Physiol. 2003;284(2):R345–53.
12. Udomkasemsab A, Prangthip P. High fat diet for induced dyslipidemia and cardiac

pathological alterations in Wistar rats compared to Sprague Dawley rats. Clínica e Investig en Arterioscler (English Ed . 2019;31(2):56–62.

13. Aleixandre De Artiñano A, Miguel Castro M. Experimental rat models to study the metabolic syndrome. Br J Nutr. 2009;102(9):1246–53.
14. Suárez R, Sarno G, Andrade C, Gomez-Correa D, Jima D, Matos A. Global trends in obesity-related cancer: risk factors, prevention, and policy interventions. Food Agric Immunol. 2026;37(1):2649422.
15. Miralpeix C, Reguera AC, Fosch A, Zagmutt S, Casals N, Cota D, et al. Hypothalamic endocannabinoids in obesity: an old story with new challenges. Cell Mol Life Sci [Internet]. 2021;78(23):7469–90. Available from: https://doi.org/10.1007/s00018-021-04002-6
16. Kirkham TC, Williams CM, Fezza F, Marzo V Di. 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):550–7.
17. 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.
18. Deshmukh R, Sharma PL. Activation of central cannabinoid CB1 receptors by WIN 55, 212-2 induces hyperphagia and facilitates preferential increase in palatable diet consumption in Wistar rats. Int J Recent Adv Pharm Res. 2012;2:62–9.
19. Zizzari P, He R, Falk S, Bellocchio L, Allard C, Clark S, et al. CB1 and GLP-1 Receptors Cross Talk Provides New Therapies for Obesity. Diabetes. 2021;70(2):415–22.
20. Osei-Hyiaman D, Liu J, Zhou L, Godlewski G, Harvey-White J, Jeong W Il, et al. Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. J Clin Invest. 2008;118(9):3160–9.
21. Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátkai S, et al. Endocannabinoid activation at hepatic CB 1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest. 2005;115(5):1298–305.
22. Bátkai S, Osei-Hyiaman D, Pan H, El-Assal O, Rajesh M, Mukhopadhyay P, et al. Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. FASEB J Off Publ Fed Am Soc Exp Biol. 2007;21(8):1788.
23. Nascimento AR, Machado M, De Jesus N, Gomes F, Lessa MA, Bonomo IT, et al.

Structural and functional microvascular alterations in a rat model of metabolic syndrome induced by a high-fat diet. Obesity. 2013;21(10):2046–54.

24. Silvestri C, Di Marzo V. Second generation CB1 receptor blockers and other inhibitors of peripheral endocannabinoid overactivity and the rationale of their use against metabolic disorders. Expert Opin Investig Drugs. 2012;21(9):1309–22.
25. Tam J, Szanda G, Drori A, Liu Z, Cinar R, Kashiwaya Y, et al. Peripheral cannabinoid-1 receptor blockade restores hypothalamic leptin signaling. Mol Metab. 2017;6(10):1113–25.
26. Tong Q, Dalgin G, Xu H, Ting C-N, Leiden JM, Hotamisligil GS. Function of GATA transcription factors in preadipocyte-adipocyte transition. Science (80-). 2000;290(5489):134–8.
27. Mounsey RB, Mustafa S, Robinson L, Ross RA, Riedel G, Pertwee RG, et al. Increasing levels of the endocannabinoid 2-AG is neuroprotective in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine mouse model of Parkinson's disease. Exp Neurol. 2015;273:36–44.
28. Turcotte C, Blanchet M-R, Laviolette M, Flamand N. The CB2 receptor and its role as a regulator of inflammation. Cell Mol Life Sci. 2016;73(23):4449–70.
29. Thaler JP, Yi C-X, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest. 2012;122(1):153–62.
30. Thaler JP, Guyenet SJ, Dorfman MD, Wisse BE, Schwartz MW. Hypothalamic inflammation: marker or mechanism of obesity pathogenesis? Diabetes. 2013;62(8):2629–34.
31. Bermudez-Silva FJ, Sanchez-Vera I, Suárez J, Serrano A, Fuentes E, Juan-Pico P, et al. Role of cannabinoid CB2 receptors in glucose homeostasis in rats. Eur J Pharmacol. 2007;565(1–3):207–11.

Tables

Table-1: Composition of Normal Diet and High-Fat Diet

Ingredients	**Normal Diet (g/Kg Diet)**	**High-Fat Diet (g/Kg Diet)**
Normal Pellet Diet	875	365
Lard	-	310
Casein	100	250
Cholesterol	-	10
Vitamin Mixture	10	60
DL-Methionine	2	30
Yeast Powder	10	1
NaCL	3	1
Total	1000	1000

Table-2 List of Primer

S. No.	**Gene**	**Primer sequence**
1.	Lipe	Forward: **5′-** CCCTCGGATGTCAACTTTTT-**3′**
		Reverse: **3′-**AGTAGAAGGCAGTGGTAGAGTG-**5′**
2.	Srebf-1	Forward: **5′-** TCTGGGCTCCTCTCTGGAAG-**3′**
		Reverse: **3′-** CCTCTTTGATTCCAGGCCCA-**5′**
3.	PPAR-γ	Forward: **5′-** ACCCTTTACCACGGTTGATT-**3′**
		Reverse: **3′-**ATTGGGTCAGCTCTTGTGAA-**5′**
4.	PPAR-α	Forward: **5′-** CCATCTGTCCTCTCTCCCCA-**3′**
		Reverse: **3′-**CCTCTCCGAGGGACTGAGAA-**5′**
5.	Cnr-2	Forward: **5′-** GGAAGCCCTCGTACCTGTTC-**3′**
		Reverse: **3′-**TGGAGTCCACACCGTGAAAG-**5′**
6.	UCP-1	Forward: **5′-** CCTCTACGATACGGTCCAAG-**3′**
		Reverse: **3′**GCATTGTAGGTCCCAGTGTA-**5′**

7.	PGC-1α	Forward: **5′-** GGAGTGACATAGAGTGTGCT-**3′**
		Reverse: **3′-**CTGTCTGTCCAGGTCATT-**5′**
8.	Cnr-1	Forward: **5′-** TTCTGAATCCCAGCCAGCAG-**3′**
		Reverse: **3′-**GGAGTGCAGGATGACACACA-**5′**
9.	POMC	Forward: **5′-** CCATAGACGTGTGGAGCTGG-**3′**
		Reverse: **3′-**AGGGCTGTTCATCTCCGTTG **-5′**
10.	NRF2	Forward: **5′-** ACAAGAGATGAGCTTAGGGC-**3′**
		Reverse: **3′-**GCTTCGTTGAATTGCTCCTT **-5′**
11.	Insr	Forward: **5′-** TCTGACGATTCTCAAAAGCA-**3′**
		Reverse: **3′-**GAAGAGGTTTTTCTGGGAAC-**5′**
12.	AgRP	Forward: **5′-** TTTACTCTGAAGCTGAATGCC-**3′**
		Reverse: **3′-**TGCCCTGAGCTTATATGGC-**5′**
13.	GAPDH	Forward: **5′-** GTCGGTGTGAACGGATTTG-**3′**
		Reverse: **3′-**AGAATGGGAGTTGCTGTTGA-**5′**

Supplementary Files

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