

Vitamin A Attenuates High-Fat High-Sugar Diet-Associated Intestinal and Hippocampal Alterations in Wistar Rats: Implications for Gut-Brain Axis Dysfunction

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Abstract

High-fat high-sugar diets (HFHSD) are associated with metabolic disturbances, intestinal and hippocampal alterations, inflammation, and oxidative stress, which may contribute to gut-brain axis dysfunction. This study evaluated the effects of vitamin A on metabolic, inflammatory, and oxidative stress parameters and intestinal and hippocampal alterations in Wistar rats exposed to an HFHSD. Sixty Wistar rats (80–120 g) were randomly assigned to six groups (n = 10). Groups A–C received standard chow, whereas Groups D–F received an HFHSD containing 30% vegetable oil and 25% sucrose for 28 days. Vitamin A (500 or 1000 IU/kg/day) was administered orally to Groups B and C, respectively, and co-administered with the HFHSD in Groups E and F. Behavioural and cognitive performance were assessed using the Open Field Test, Y-maze, and Novel Object Recognition test. Blood glucose, adiponectin, leptin, TNF- α , IL-6, IL-10, malondialdehyde (MDA), and total antioxidant capacity (TAC), and hippocampal and ileal histopathology were evaluated. HFHSD increased body weight gain, feed intake, TNF- α , IL-6, and MDA, while blood glucose was numerically higher but not significantly different from controls. Adiponectin, leptin, IL-10, and TAC were reduced, accompanied by impaired locomotor and exploratory activity, cognitive deficits, and intestinal and hippocampal histopathological alterations. Vitamin A attenuated metabolic, inflammatory, oxidative, behavioural, and histopathological alterations. The 500 IU/kg dose showed greater improvement in several behavioural and histological outcomes, whereas 1000 IU/kg produced stronger effects on antioxidant status and glycaemic regulation. These findings indicate dose-specific differences in the effects of vitamin A, although the optimal dose and underlying mechanisms remain to be established.

KEYWORDS: Gut-brain axis, High-fat high-sugar diet, inflammation, Oxidative stress, Cognitive function

1. Introduction

Obesity and its associated metabolic disorders have become major global public health challenges, largely driven by increased consumption of energy-dense diets rich in saturated fats and refined sugars. Rapid urbanisation, industrialisation and advances in food processing have profoundly altered dietary habits, increasing the availability and consumption of highly

processed foods while contributing to the rising prevalence of obesity, type 2 diabetes mellitus, cardiovascular disease and other non-communicable disorders [1-3]. Among these dietary patterns, high-fat high-sugar diets (HFHSD) have emerged as major contributors to metabolic dysfunction through excessive energy intake, insulin resistance, dyslipidaemia and adipose tissue dysfunction [4, 5]. Beyond these peripheral metabolic

disturbances, growing evidence indicates that chronic HFHSD consumption adversely affects central nervous system function, resulting in impaired learning and memory, behavioural abnormalities and an increased susceptibility to neurodegenerative and neuropsychiatric disorders [6,7].

Increasing attention has focused on the gut-brain axis as a key mechanistic link between diet-induced metabolic dysfunction and neurological impairment. The gut-brain axis comprises an integrated bidirectional communication network connecting the gastrointestinal tract and the central nervous system through neural, endocrine, immune and metabolic pathways [8]. Maintenance of intestinal barrier integrity is fundamental to this communication because the intestinal epithelium regulates interactions between luminal contents and the systemic circulation. High-fat high-sugar diet has been associated with alterations in gut microbial balance, intestinal barrier function, and intestinal permeability, which may allow microbial-derived products and pro-inflammatory mediators to enter the circulation. These changes are linked to systemic inflammation, oxidative stress, and neuroinflammatory responses, and may contribute to metabolic and cognitive dysfunction through interrelated systemic and neural processes [9]. These events promote systemic inflammation, oxidative stress and neuroinflammation, ultimately impairing neuronal function and contributing to both metabolic and cognitive dysfunction [10]. However, disruption of gut-brain axis homeostasis is increasingly recognised as a central mechanism underlying the pathogenesis of obesity-associated metabolic and neurobehavioural disorders.

Among the brain regions affected by gut-brain axis dysfunction, the hippocampus is particularly vulnerable because of its critical roles in learning, memory consolidation and spatial navigation [11]. Experimental studies have demonstrated that prolonged exposure to HFHSD induces hippocampal neuroinflammation, oxidative stress, impaired neurogenesis, synaptic dysfunction and neuronal degeneration, resulting in deficits in cognition and behavioural performance [12-15]. These findings highlight the relationship between consumption of obesogenic diets and alterations in brain structure and function.

Vitamin A is an essential fat-soluble micronutrient whose biological functions extend well beyond its classical roles in vision, growth and epithelial maintenance. Through its biologically active metabolite, retinoic acid, Vitamin A regulates gene expression by activating nuclear retinoid receptors involved in cellular differentiation, immune regulation, inflammatory signalling and antioxidant defence [16, 17]. Emerging evidence indicates that Vitamin A possesses antioxidant, anti-inflammatory and neuroprotective properties by reducing oxidative damage, modulating microglial activation, promoting neuronal survival and preserving synaptic plasticity [18]. Furthermore, Vitamin A contributes to the maintenance of intestinal epithelial integrity, supports mucosal immune function

and influences metabolic regulation through modulation of glucose homeostasis, adipokine signalling and cellular antioxidant systems [19, 20].

Although previous studies have demonstrated beneficial effects of Vitamin A on metabolic regulation, inflammatory responses, oxidative stress, and intestinal barrier-related processes, its potential to attenuate concurrent intestinal and hippocampal alterations during HFHSD exposure remains poorly understood. In particular, few studies have comprehensively evaluated whether Vitamin A-mediated modulation of metabolic, inflammatory, and oxidative stress responses is accompanied by preservation of intestinal and hippocampal architecture and improvements in behavioural and cognitive outcomes. Addressing this knowledge gap is important for understanding whether Vitamin A can provide multi-system protection against diet-induced metabolic and neurobiological dysfunction. Therefore, the present study examined the effects of Vitamin A supplementation on behavioural performance, metabolic homeostasis, inflammatory cytokines, oxidative stress, and histopathological alterations in the hippocampus (CA1 and dentate gyrus) and ileum of HFHSD-fed Wistar rats. By integrating behavioural, biochemical, and histopathological assessments, the study evaluated the effects of Vitamin A supplementation on intestinal and hippocampal alterations associated with HFHSD exposure, with potential implications for gut-brain axis dysfunction.

2. Materials and Methods

2.1. Chemicals and Drugs

Vitamin A (Nature's Field® Vitamin A, 10,000 IU per softgel; retinyl palmitate) was purchased from GoMed Pharmacy (Lagos, Nigeria). The soft gel contents were extracted and dissolved in commercially available corn oil, which served as the vehicle for oral administration. The preparation was made in batches and stored in airtight containers until use. Vitamin A was administered orally at doses of 500 and 1,000 IU/kg/day. Standard rodent chow was obtained from Top Feeds Nigeria Ltd. (Ogbomoso, Nigeria). Commercial rat-specific ELISA kits for adipokines, inflammatory cytokines and oxidative stress biomarkers were procured from Cayman Chemical and Invitrogen (USA). All chemicals and reagents were of analytical grade and were stored and used in accordance with the manufacturers' instructions.

2.2 Animals

Sixty healthy male Wistar rats weighing 80-120 g were obtained from a certified breeder in Orioke-Ara, Ede, Osun State, Nigeria. Animals were acclimatised for seven days before the commencement of the experiment and maintained under standard laboratory conditions (22-25°C; relative humidity 50-60%; 12 h light/dark cycle), with free access to standard feed and water ad libitum [21]. All procedures were carried out

following approval from the Research Ethics Committee of the Faculty of Basic Clinical Sciences, LAUTECH, and in compliance with the European Council Directive (2010/63/EU) governing the use and care of laboratory animals.

2.3 Diet

All animals were maintained on commercially available standard rodent chow provided *ad libitum*. The high-fat high-sugar diet (HFHSD) was prepared according to the method described by Onaolapo [22]. Standard rodent chow containing 5% crude fat was supplemented with 25% (w/w) Commercial food grade vegetable oil to obtain the high-fat component, giving an intended total fat content of approximately 30%. Subsequently, the prepared high-fat component was mixed with 25% (w/w) sucrose to obtain the final HFHSD. The ingredients were thoroughly homogenised, prepared in batches, and stored in airtight containers until administration throughout the 28-day experimental period. According to the manufacturer's specifications, the standard rodent chow contained 16% crude protein, 5% crude fat, 8% crude fibre, 1% calcium, 0.4% available phosphorus, 0.9% lysine, 0.45% methionine, and 0.8% methionine plus lysine.

2.4 Experimental Design

Following the period of acclimatization, rats were randomly assigned to six groups (n = 10 per group) and treated for 28 days (Table 1). The HFHSD was formulated by supplementing standard chow with 30% vegetable oil and 25% sucrose. Vitamin A was administered orally by gavage at doses of 500 or 1000 IU/kg/day.

Table 1: Experimental Design

Group	Description	Diet	Treatment
A	Control	Standard chow	Corn oil
B	Vitamin A (Low dose)	Standard chow	Vitamin A (500 IU/kg/day)
C	Vitamin A (High dose)	Standard chow	Vitamin A (1000 IU/kg/day)
D	Induced model	HFHSD	Corn oil
E	HFHSD + Vitamin A (Low dose)	HFHSD	Vitamin A (500 IU/kg/day)
F	HFHSD + Vitamin A (High dose)	HFHSD	Vitamin A (1000 IU/kg/day)

HFHSD = high-fat high-sugar diet; Vit-A = Vitamin A

2.5 Assessment of Body Weight and Feed Intake

Body weight was measured weekly in grams using a calibrated electronic balance, and food intake was recorded daily in grams as the difference between the quantity of feed supplied and the amount remaining after 24 h [23]. Percentage change in body weight and food intake was calculated as:

Percentage change = $\frac{\text{Final value} - \text{Initial value}}{\text{Initial value}} \times 100$

2.6 Behavioural and Cognitive Assessments

Behavioural assessments were performed during the light phase (09:00 -14:00 h) towards the end of the experimental period. Animals were acclimatized to the testing room for 30 min before each session, and all apparatus were cleaned with 70% ethanol between trials.

2.6.1 Novel Object Recognition (NOR) Test

Recognition memory was assessed using the Novel Object Recognition (NOR) test as previously described [24, 25]. During the familiarization phase, rats were allowed to explore two identical objects for 5 min. The objects were positioned at opposite sides of the arena, slightly away from the corners, to allow free exploration. Following a 24 h retention interval, one familiar object was replaced with a novel object, and animals were allowed to explore for another 5 min. The objects were similar in size and general appearance but differed in colour. Object exploration was defined as nose-directed contact or sniffing of the object. All animals were included in the analysis, and no animals were excluded based on exploration time. The apparatus and objects were cleaned with 70% ethanol between trials to minimise odour cues. Recognition memory was expressed as the discrimination index (DI):

$$DI = \frac{T_{\text{novel}} - T_{\text{familiar}}}{T_{\text{novel}} + T_{\text{familiar}}}$$

where T_{novel} and F_{familiar} represent the time spent exploring the novel and familiar objects, respectively.

2.6.2 Y-maze Spontaneous Alternation Test

Spatial working memory was evaluated using the Y-maze spontaneous alternation paradigm as previously described [26, 27]. Animals were allowed to explore the maze freely for 5 min. Percentage spontaneous alternation was calculated as:

$$\% \text{ Alternation} = \frac{\text{Number of correct alternations}}{\text{Total Arm entries} - 2} \times 100$$

2.6.3 Open Field Test

Locomotor and exploratory activities were assessed using the Open Field Test [28]. Each rat was placed individually at the centre of the arena and allowed to explore for 10 min. The number of line crossings and rearing frequency behaviour were recorded manually.

2.7 Biochemical Analysis

Following an overnight fasting (12 h), blood glucose levels were determined by tail vein puncture using an Accu-Chek® glucometer (Roche Diagnostics, Germany). Animals were subsequently euthanized by cervical dislocation, and blood samples were collected via intracardiac puncture. Serum was separated by centrifugation at 2000 rpm for 10 min and stored at -80 °C until analysis [29]. Serum concentrations of adiponectin,

leptin, TNF- α , IL-6, and IL-10 were determined using commercially available rat-specific ELISA kits obtained from Cayman Chemical and Invitrogen (USA). Lipid peroxidation was assessed by measuring malondialdehyde (MDA) concentration using the thiobarbituric acid reactive substances (TBARS) method, while total antioxidant capacity (TAC) was determined using a commercially available assay kit.

2.8 Tissue Histology

At the end of the experiment, the hippocampus and ileum were excised, rinsed in normal saline, and fixed in 10% neutral buffered formalin for 24-48 h. Tissues were dehydrated in graded ethanol, cleared in xylene, [30] embedded in paraffin wax, sectioned at 5-7 μ m, and stained with haematoxylin and eosin (H&E). Histological evaluation focused on neuronal morphology within the hippocampal CA1 region and dentate gyrus, and mucosal architecture of the ileum, including epithelial integrity, villus morphology, and inflammatory cell infiltration. Histological sections were examined under a light microscope at $\times 400$ magnification and representative photomicrographs were captured.

2.9 Statistical Analysis

Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using EZANOVA software. Differences among groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. Statistical significance was accepted at $p < 0.05$.

3. Result

3.1 Effects of Vitamin A on Body Weight and Feed Intake

Figure 1a and 1b show the effect of vitamin A supplementation on body weight and feed intake. High fat high sugar diet (HFHSD) significantly increased percentage change in body weight and feed intake compared with the control group (Figure 1a, 1b; $p < 0.05$). These increases were significantly attenuated by Vitamin A supplementation in HFHSD-fed rats, with the 500 IU/kg/day dose producing a greater reduction than the 1000 IU/kg/day dose. Administration of Vitamin A alone reduced both parameters relative to control animals.

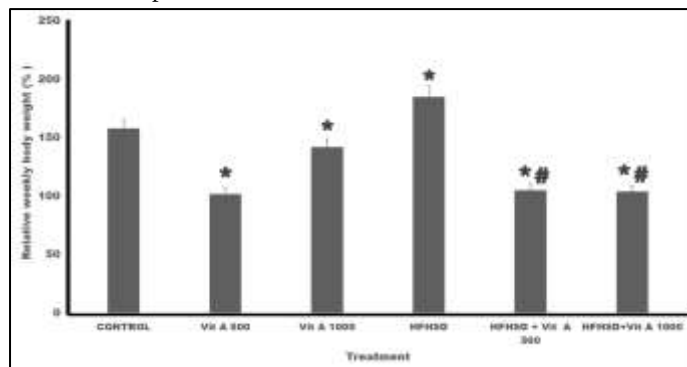


Figure 1a. Effects of Vitamin A on percentage change in body weight in HFHSD-fed Wistar rats. Values are presented as mean $\pm$ SEM ($n =$

10 per group). $P < 0.05$ versus the control group; # $P < 0.05$ versus the HFHSD group. HFHSD, high-fat high-sugar diet; Vit A, Vitamin A.

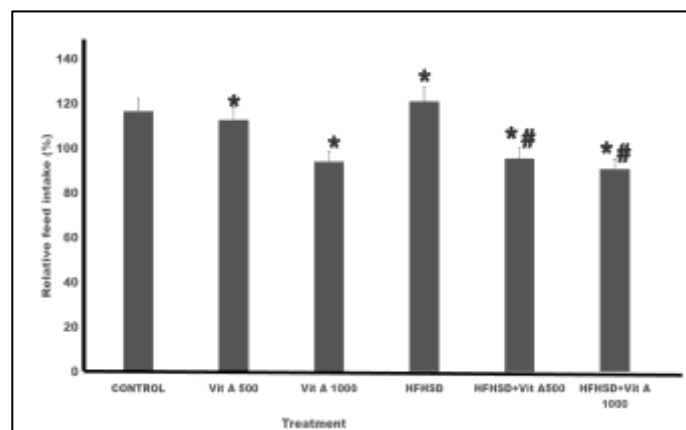


Figure 1b. Effects of Vitamin A on percentage change in feed intake in HFHSD-fed Wistar rats. Values are presented as mean $\pm$ SEM ($n = 10$ per group). $P < 0.05$ versus the control group; # $P < 0.05$ versus the HFHSD group. HFHSD, high-fat high-sugar diet; Vit A, Vitamin A.

3.2 Effects Of Vitamin A on Neurobehavioural Function

3.2.1 Effect of Vitamin A on Open Field Test

Figures 2a and 2b show the effect of vitamin A supplementation on open field behaviours including line crossing and rearing. Result showed that HFHSD significantly reduced line crossing and rearing behaviours compared with the control group ($p < 0.05$). These reductions were significantly attenuated by Vitamin A supplementation in HFHSD-fed rats, indicating improved locomotor and exploratory activity. The 500 IU/kg/day dose produced greater improvement in rearing behaviour than the 1000 IU/kg/day dose, while both doses improved line crossing. Administration of Vitamin A alone reduced line crossing relative to the control group but had no significant effect on rearing.

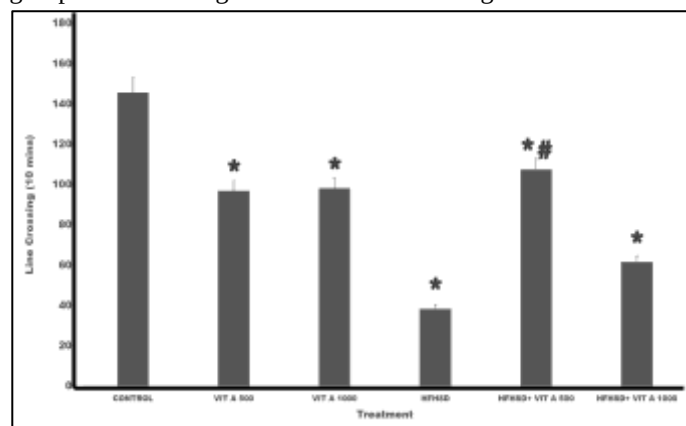


Figure 2a. Effects of Vitamin A on line crossing in HFHSD-fed Wistar rats. Values are presented as mean $\pm$ SEM ($n = 10$ per group). $P < 0.05$ versus the control group; # $P < 0.05$ versus the HFHSD group. HFHSD, high-fat high-sugar diet; Vit A, Vitamin A.

3.2.2 Effect of Vitamin A on Spatial Working Memory and Novel Object Recognition

Figures 2c and 2d show that HFHSD significantly impaired spatial working memory and novel object recognition compared with the control group ($p < 0.05$). These impairments were significantly attenuated by Vitamin A supplementation in HFHSD-fed rats, indicating improved learning and memory. The 500 IU/kg/day dose produced greater improvement in percentage correct alternation and novel object recognition than the 1000 IU/kg/day dose. Administration of Vitamin A alone improved percentage correct alternation at 500 IU/kg/day but reduced novel object recognition at 1000 IU/kg/day relative to the 500 IU/kg/day dose.

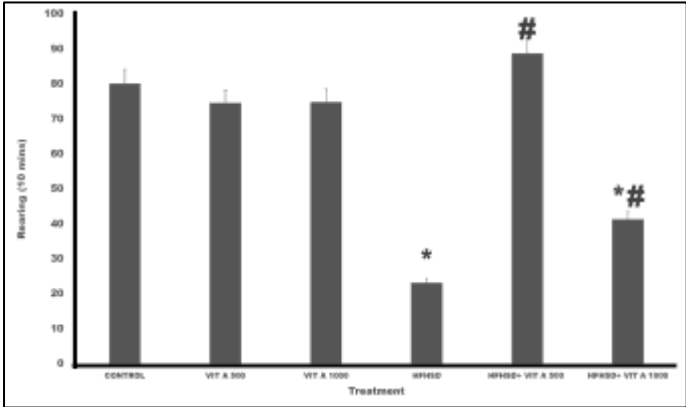


Figure 2b. Effects of Vitamin A on rearing in HFHSD-fed Wistar rats. Values are presented as mean $\pm$ SEM ($n = 10$ per group). $P < 0.05$ versus the control group; $\#P < 0.05$ versus the HFHSD group. HFHSD, high-fat high-sugar diet; Vit A, Vitamin A.

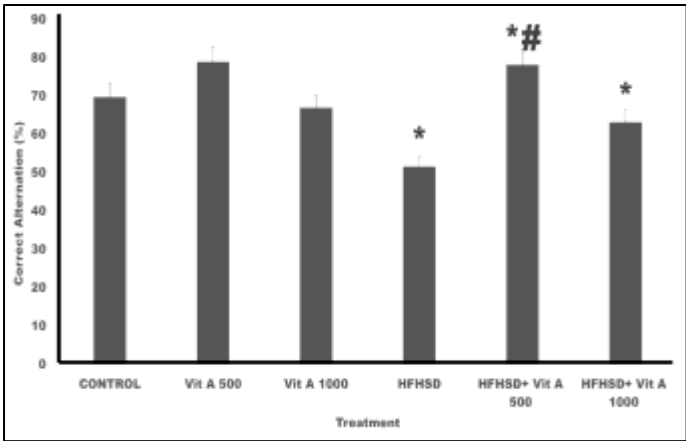


Figure 2c. Effect of vitamin A on spatial working memory assessed by percentage correct alternation in the Y-maze. Data are presented as mean $\pm$ SEM ($n = 10$ per group). $*p < 0.05$ compared with the control group; $\#p < 0.05$ compared with the HFHSD group. HFHSD: high-fat high-sugar diet; Vit A: vitamin A.

3.3 Effects Of Vitamin A on Metabolic Function

High fat, high sugar diet disrupted metabolic homeostasis, as evidenced by reduced serum adiponectin and leptin concentrations, while blood glucose was numerically higher than in the control group but did not differ significantly (Table

2). Vitamin A supplementation significantly ameliorated these metabolic alterations, increasing adiponectin and leptin concentrations while reducing blood glucose levels relative to untreated HFHSD-fed rats. The 500 IU/kg/day dose produced a greater increase in leptin concentration, whereas the 1000 IU/kg/day dose showed a greater numerical reduction in blood glucose.

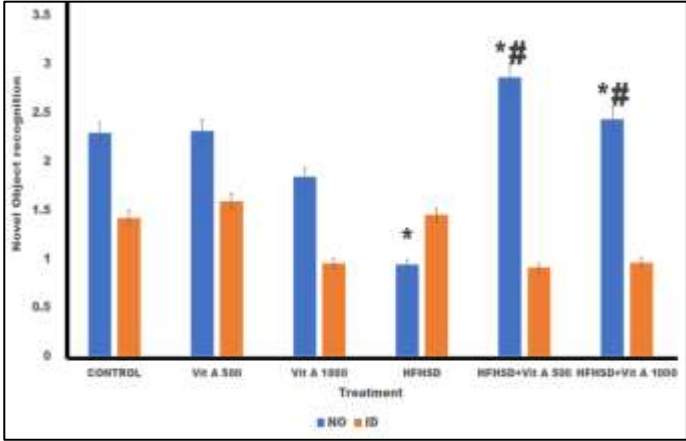


Figure 2d. Effect of vitamin A on Novel object recognition test. Data are presented as mean $\pm$ SEM ($n = 10$ per group). $*p < 0.05$ compared with the control group; $\#p < 0.05$ compared with the HFHSD group. HFHSD: high-fat high-sugar diet; Vit A: vitamin A.

Table 2. Effects of Vitamin A on serum adiponectin, leptin, and blood glucose levels in high-fat high-sugar diet (HFHSD)-fed Wistar rats

Group	Adiponectin (ng/mL)	Leptin (ng/mL)	Blood glucose (mg/dL)
Control	63.98 $\pm$ 15.65	9.02 $\pm$ 1.34	96.00 $\pm$ 3.58
Vitamin A 500 IU/kg	34.43 $\pm$ 2.64	8.02 $\pm$ 0.89	90.50 $\pm$ 2.34
Vitamin A 1000 IU/kg	67.98 $\pm$ 14.50	17.05 $\pm$ 1.34*	83.50 $\pm$ 2.91*
HFHSD	25.85 $\pm$ 3.64*	4.52 $\pm$ 0.67*	108.50 $\pm$ 6.04
HFHSD + Vitamin A 500 IU/kg	41.46 $\pm$ 1.46#	15.02 $\pm$ 1.34##	86.50 $\pm$ 4.25#
HFHSD + Vitamin A 1000 IU/kg	40.16 $\pm$ 5.85#	11.01 $\pm$ 1.79#	82.50 $\pm$ 6.93#

Data are presented as mean $\pm$ SEM ($n = 10$ rats per group). $p < 0.05$ versus the control group; $\#p < 0.05$ versus the HFHSD group. HFHSD, high-fat high-sugar diet; Vit A, Vitamin A.

3.4 Effects Of Vitamin A on Inflammatory Cytokines

HFHSD feeding significantly increased serum TNF- α and IL-6 concentrations while reducing IL-10 levels compared with the control group (Table 3; $p < 0.05$). Administration of Vitamin A alone significantly reduced TNF- α and increased IL-10 concentrations at the 500 IU/kg/day dose, whereas the 1000 IU/kg/day dose significantly increased IL-6 and reduced IL-10 relative to the control group ($p < 0.05$). In HFHSD-fed rats, co-administration of Vitamin A significantly attenuated the HFHSD-induced inflammatory response by reducing TNF- α and IL-6 concentrations while increasing IL-10 levels

compared with the untreated HFHSD group ($p < 0.05$). The reduction in $\text{TNF-}\alpha$ was more pronounced in rats treated with 500 IU/kg/day, whereas the 1000 IU/kg/day dose produced a greater reduction in IL-6 concentration

Table 3. Effects of Vitamin A on serum inflammatory cytokine concentrations in HFHSD-fed Wistar rats

Group	TNF- α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Control	42.10 $\pm$ 0.57	12.14 $\pm$ 0.70	7.83 $\pm$ 0.01
Vitamin A 500 IU/kg/day	33.18 $\pm$ 2.32*	12.94 $\pm$ 0.09	10.83 $\pm$ 0.24*
Vitamin A 1000 IU/kg/day	52.62 $\pm$ 6.37	24.37 $\pm$ 0.42*	1.58 $\pm$ 0.21*
HFHSD	77.33 $\pm$ 5.95*	45.88 $\pm$ 1.38*	0.18 $\pm$ 0.02*
HFHSD + Vitamin A 500 IU/kg/day	42.67 $\pm$ 8.47#	23.81 $\pm$ 1.61*#	11.99 $\pm$ 0.17*#
HFHSD + Vitamin A 1000 IU/kg/day	48.57 $\pm$ 1.95*#	19.23 $\pm$ 0.17*#	12.03 $\pm$ 0.11*#

Data are presented as mean $\pm$ SEM (n = 10 rats per group). $p < 0.05$ versus the control group; # $p < 0.05$ versus the HFHSD group. HFHSD, high-fat high-sugar diet; Vit A, Vitamin A.

3.5 Effects of Vitamin A on Oxidative Stress Markers

Feeding rats with HFHSD significantly increased serum malondialdehyde (MDA) concentration while reducing total antioxidant capacity (TAC) compared with the control group (Table 4; $p < 0.05$). Administration of Vitamin A alone at 500 IU/kg/day significantly reduced MDA concentration compared with the control group ($p < 0.05$), whereas no significant changes were observed in TAC. In HFHSD-fed rats, co-administration of Vitamin A significantly reduced MDA concentration compared with the untreated HFHSD group ($p < 0.05$). Moreover, the 1000 IU/kg/day dose significantly increased TAC relative to the HFHSD group ($p < 0.05$), while the 500 IU/kg/day dose produced only a modest, non-significant increase.

Table 4. Effects of Vitamin A on Oxidative Stress and Antioxidant Status in HFHSD-Fed Wistar Rats

Group	MDA (μM)	TAC (mM/L)
Control	1.25 $\pm$ 0.02	15.47 $\pm$ 1.32
Vitamin A 500	0.77 $\pm$ 0.03	11.30 $\pm$ 0.61
Vitamin A 1000	1.08 $\pm$ 0.14	13.47 $\pm$ 1.02
HFHSD	2.77 $\pm$ 0.04*	10.01 $\pm$ 2.09*
HFHSD +Vitamin A 500	0.85 $\pm$ 0.08#	11.46 $\pm$ 0.73
HFHSD +Vitamin A 1000	1.08 $\pm$ 0.17#	16.06 $\pm$ 0.85#

Data are presented as mean $\pm$ SEM (n = 10 rats per group). $p < 0.05$ versus the control group; # $p < 0.05$ versus the HFHSD group. HFHSD,

high-fat high-sugar diet; MDA, malondialdehyde; TAC, total antioxidant capacity; Vit A, Vitamin A.

3.6 Effect of Vitamin A on HFHSD-Induced Histopathological Changes in the Ileum

Plate 1 shows that HFHSD disrupted normal ileal architecture compared with the control group, characterised by epithelial erosion, fewer visible goblet cells, and distortion of the lamina propria. These histopathological changes were attenuated by Vitamin A supplementation in HFHSD-fed rats, with preservation of enterocytes, goblet cells, and lamina propria architecture appearing more pronounced in the 500 IU/kg/day group than in the 1000 IU/kg/day group.

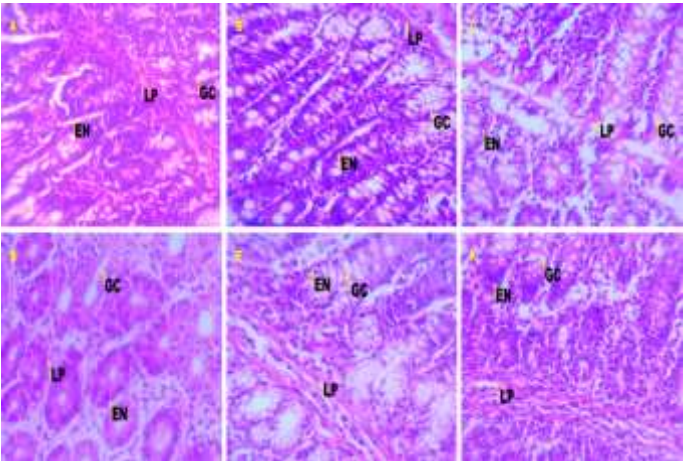


Plate 1. Representative photomicrographs of H&E-stained ileal sections ($\times 400$). (A) Control; (B) Vitamin A (500 IU/kg/day); (C) Vitamin A (1000 IU/kg/day); (D) HFHSD; (E) HFHSD + Vitamin A (500 IU/kg/day); and (F) HFHSD + Vitamin A (1000 IU/kg/day). The control and Vitamin A-treated groups exhibited preserved ileal architecture with intact enterocytes, abundant goblet cells, and a well-organised lamina propria. The HFHSD group showed epithelial erosion, fewer visible goblet cells, and distortion of the lamina propria. Vitamin A supplementation improved the structural organisation of the ileum, with preservation appearing more pronounced in the 500 IU/kg/day group than in the 1000 IU/kg/day group. EN, enterocytes; GC, goblet cells; LP, lamina propria.

3.7 Effects of Vitamin A on Hippocampal Histoarchitecture

Plate 2 shows that HFHSD disrupted the normal cytoarchitecture of the hippocampal CA1 region compared with the control group, characterised by pyramidal cell shrinkage, neuronal disorganisation, and neuropil vacuolation. These histopathological alterations were attenuated by Vitamin A supplementation in HFHSD-fed rats, with preservation of hippocampal architecture appearing more pronounced in the 500 IU/kg/day group than in the 1000 IU/kg/day group.

3.8 Effects of Vitamin A on Histopathological Alterations in the Dentate Gyrus

Plate 3 shows that HFHSD disrupted the normal cytoarchitecture of the dentate gyrus compared with the control group, characterised by disruption of the granule cell layer, less

densely arranged granule cells, and distortion of the dentate gyrus architecture. These histopathological alterations were attenuated by Vitamin A supplementation in HFHSD-fed rats, with preservation of the granule cell layer and dentate gyrus architecture appearing more pronounced in the 500 IU/kg/day group than in the 1000 IU/kg/day group.

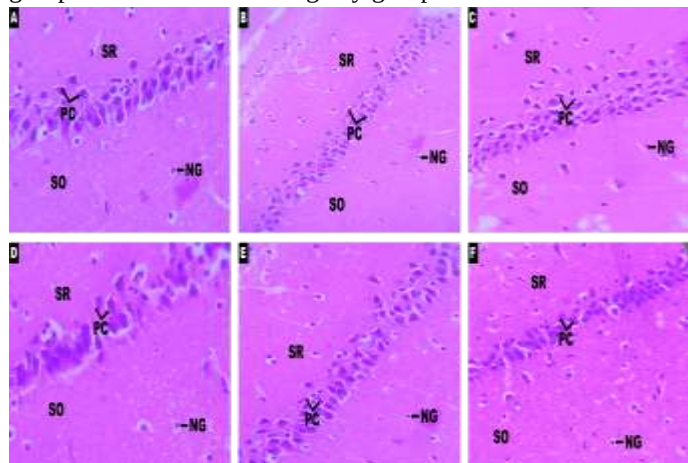


Plate 2. Representative H&E-stained photomicrographs of the hippocampal CA1 region (×400). (A) Control; (B) Vitamin A (500 IU/kg/day); (C) Vitamin A (1000 IU/kg/day); (D) HFHSD; (E) HFHSD + Vitamin A (500 IU/kg/day); and (F) HFHSD + Vitamin A (1000 IU/kg/day). HFHSD disrupted the CA1 cytoarchitecture, characterised by pyramidal cell shrinkage, neuronal disorganisation, and neuropil vacuolation. Vitamin A improved the preservation of hippocampal architecture, with preservation appearing more pronounced in the 500 IU/kg/day group. SR, stratum radiatum; PC, pyramidal cells; SO, stratum oriens; NG, neuroglial cells.

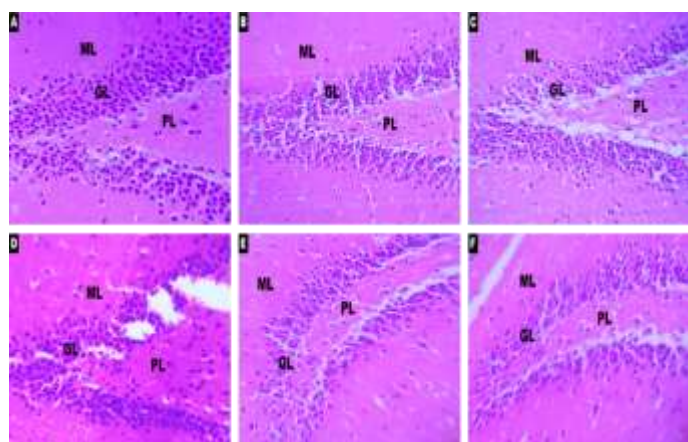


Plate 3. Representative photomicrographs of the dentate gyrus stained with haematoxylin and eosin (H&E, ×400). (A) Control; (B) Vitamin A (500 IU/kg/day); (C) Vitamin A (1000 IU/kg/day); (D) HFHSD; (E) HFHSD + Vitamin A (500 IU/kg/day); and (F) HFHSD + Vitamin A (1000 IU/kg/day). The control and Vitamin A-treated groups exhibited preserved dentate gyrus cytoarchitecture with well-organised granule cell layers. The HFHSD group showed disruption of the granule cell layer, less densely arranged granule cells, and distortion of the dentate gyrus architecture. Vitamin A improved the structural organisation of the dentate gyrus, with preservation appearing more pronounced in the 500 IU/kg/day group. ML, molecular layer; GL, granule cell layer; PL, polymorphic layer (hilus).

Discussion

Consumption of an energy-dense diet causes a series of harmful changes, including metabolic dysfunction, systemic inflammation, oxidative stress, and structural damage in both the

intestine and the brain. The present study identified concurrent intestinal, systemic and hippocampal alterations that were associated with behavioural and cognitive impairment. Vitamin A supplementation reduced many of these adverse effects, supporting its potential role in protecting against diet-induced metabolic and neurological disturbances.

The obesity-promoting effect of the high-fat, high-sugar diet (HFHSD) was evident from the marked increase in food intake and body weight. These findings are consistent with previous reports showing that diets rich in fat and refined sugar promote central leptin and insulin resistance, resulting in persistent overeating and weight gain [31, 32]. Vitamin A supplementation reduced both food intake and body weight, supporting earlier studies demonstrating that retinoids regulate fat metabolism, promote lipid oxidation and improve energy balance [33,34]. In the present study, animals treated with 500 IU/kg/day showed a greater reduction in weight gain than those receiving 1000 IU/kg/day. As only two doses were evaluated, the reason for this difference cannot be determined from the present findings.

The metabolic effects of the HFHSD extended beyond increased body weight. Reduced adiponectin and leptin levels, together with the numerically higher blood glucose level, suggest altered adipose tissue function and metabolic homeostasis [35, 36]. Vitamin A improved these metabolic disturbances. However, differences were observed between the two treatment groups. The 500 IU/kg/day dose was associated with greater improvement in leptin levels, whereas the 1000 IU/kg/day dose produced a greater reduction in blood glucose.

The metabolic disturbances were accompanied by a marked systemic inflammatory response, characterised by increased serum TNF- α and IL-6 and reduced serum IL-10. This cytokine profile is consistent with the chronic low-grade inflammation commonly associated with obesity [37, 38]. Vitamin A improved this systemic inflammatory imbalance by reducing serum pro-inflammatory cytokines while increasing serum IL-10, supporting previous reports of its immune-regulating properties [39, 40]. An interesting finding was that animals receiving 1000 IU/kg/day while maintained on a standard diet showed increased IL-6 and reduced IL-10 compared with controls. Although the biological basis of this observation remains unclear, it suggests that the immunological effects of Vitamin A may vary under different physiological conditions. The differential effects observed between the two Vitamin A doses further suggest that the responses were not uniform across the assessed outcomes. The 500 IU/kg/day dose was associated with greater improvement in TNF- α and several behavioural and histopathological outcomes, whereas the 1000 IU/kg/day dose produced greater improvement in IL-6, total antioxidant capacity and blood glucose in HFHSD-fed animals. However, the increased IL-6 and reduced IL-10 observed following administration of 1000 IU/kg/day in standard-diet animals

indicate that the higher dose may elicit different immunological effects in the absence of dietary stress. This pattern suggests that the effects of Vitamin A may depend on both dose and physiological context. Nevertheless, because only two doses were evaluated, a definitive dose-response relationship cannot be established from the present findings.

Oxidative stress also appeared to contribute substantially to the pathological changes induced by the HFHSD. Increased serum malondialdehyde (MDA) levels, together with reduced serum total antioxidant capacity (TAC), indicate systemic oxidative imbalance and increased oxidative stress [41, 42]. Vitamin A reduced serum MDA levels at both treatment doses, while the 1000 IU/kg/day dose produced a greater improvement in serum TAC. The behavioural findings should also be considered in relation to the observed reduction in locomotor and exploratory activity. Reduced line crossing and rearing in the Open Field Test may have influenced performance in the Y-maze and Novel Object Recognition tests, as both tasks require animals to explore their surroundings. Although the reduced spontaneous alternation and novel object discrimination suggest impairments in spatial working and recognition memory, respectively, the contribution of reduced exploratory activity cannot be completely ruled out. Therefore, the behavioural improvements observed following Vitamin A supplementation may reflect improvements in both general activity and cognitive performance.

The systemic effects of HFHSD were reflected in both intestinal and hippocampal histopathology. The concurrent occurrence of intestinal mucosal alterations, systemic inflammatory and oxidative disturbances, and hippocampal structural changes supports an association between intestinal injury, systemic inflammatory and oxidative imbalance, and hippocampal dysfunction, with potential implications for gut-brain axis disruption. In the ileum, HFHSD caused epithelial erosion, goblet cell depletion and distortion of the lamina propria, indicating disruption of intestinal mucosal architecture [43, 44]. These structural alterations may be associated with altered mucosal interactions with luminal contents and could contribute to systemic inflammatory signalling; however, microbial translocation or endotoxaemia was not directly assessed. Similar histopathological alterations were observed in the hippocampus, including pyramidal cell shrinkage, neuropil vacuolation, and reduced granule cell density within the dentate gyrus. These changes are consistent with the effects of chronic inflammation and oxidative stress on neural tissue and may provide a structural correlate for the observed impairments in learning and memory [45]. Although intestinal permeability, gut microbiota composition, and microbial translocation were not directly assessed, the combined histological, biochemical, and behavioural findings indicate concurrent intestinal, systemic,

and hippocampal alterations that may have implications for gut-brain axis dysfunction.

Vitamin A markedly improved the histological appearance of both the ileum and hippocampus. In the present study, tissue preservation appeared more pronounced in animals receiving 500 IU/kg/day than in those receiving 1000 IU/kg/day. The preservation of ileal architecture may be consistent with reduced intestinal exposure to luminal inflammatory stimuli, although intestinal permeability and microbial translocation were not directly measured. Thus, the observed preservation of hippocampal morphology and behavioural outcomes may reflect parallel protective effects of Vitamin A across intestinal, systemic, and neural compartments rather than a demonstrated gut-to-brain mechanism [34, 44, 46]. However, the mechanisms responsible for these protective effects were not examined in the present study.

A major strength of this study is the integrated evaluation of metabolic, inflammatory, oxidative, histological and behavioural outcomes within the same experimental model. This approach provides a broader understanding of the potential protective effects of Vitamin A against HFHSD-associated metabolic, inflammatory, oxidative, behavioural and tissue alterations, with implications for gut-brain axis dysfunction. Nevertheless, several limitations should be acknowledged. Only male Wistar rats were included, which limits the generalisability of the findings across sexes; the experimental period was limited to 28 days, and molecular mechanisms underlying the observed protective effects were not investigated. Furthermore, biochemical analyses were performed using serum samples; therefore, the observed inflammatory and oxidative changes reflect systemic rather than hippocampal biochemical alterations. Specifically, retinoid receptor signalling, tight-junction protein expression, gut microbiota composition and markers of hippocampal neurogenesis were not assessed. In addition, histological assessment was based on qualitative evaluation of representative H&E sections rather than quantitative morphometric analysis, which limits the ability to objectively compare the extent of tissue alterations and preservation between groups. Furthermore, because only two Vitamin A doses were evaluated, a definitive dose-response relationship could not be established. Therefore, caution is required when extrapolating these findings to humans. Future studies should include longer treatment periods, both male and female animals, a broader range of Vitamin A doses, quantitative histomorphometric analysis, and molecular analyses to clarify the mechanisms underlying the observed protective effects of Vitamin A.

Conclusion

The present findings demonstrate that Vitamin A reduced many of the harmful effects of an HFHSD, including metabolic

dysfunction, inflammation, oxidative stress, tissue injury and cognitive impairment. Differences were observed between the two Vitamin A doses across several outcome measures; however, because only two doses were evaluated, a definitive dose-response relationship or optimal dose could not be established. Further studies are therefore needed before specific dosing recommendations can be made. Overall, these findings support the potential of Vitamin A as an adjunct nutritional strategy for mitigating diet-associated metabolic, inflammatory, oxidative, behavioural and tissue abnormalities, with possible implications for gut-brain axis dysfunction.

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Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

Competing interests

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Declarations Ethics approval

Ethical approval was obtained from the Faculty of Basic Clinical Sciences LAUTECH with identification number CHS/REC/1103

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References

- 1 Tapsell, L. C., Neale, E. P., Satija, A., & Hu, F. B. (2016). Foods, nutrients, and dietary patterns: interconnections and implications for dietary guidelines. *Advances in Nutrition*, 7(3), 445-454.
- 2 Mendoza-Herrera, K., Florio, A. A., Moore, M., Marrero, A., Tamez, M., Bhupathiraju, S. N., & Mattei, J. (2021). The leptin system and diet: a mini review of the current evidence. *Frontiers in endocrinology*, 12, 749050.
- 3 Huang, Y., Chen, Z., Chen, B., Li, J., Yuan, X., Li, J., ... & Liu, L. (2023). Dietary sugar consumption and health: umbrella review. *Bmj*, 381.
- 4 Gupta, P., & Sachdev, H. S. (2022). The escalating health threats from ultra-processed and high fat, salt, and sugar foods: urgent need for tailoring policy. *Indian Pediatrics*, 59(3), 193-197.
- 5 Banerjee, D., & Mani, A. (2025). Obesity's systemic impact: exploring molecular and physiological links to diabetes, cardiovascular disease, and heart failure. *Frontiers in Endocrinology*, 16, 1681766.
- 6 Ibrahim, F. A., Bello, T. O., Fakolujo, I. Y., Oladele, J. O., & Oladiji, A. T. (2024). Nutrition and Mental Health. *Nutrition and Diet in Health*, 148-168.
- 7 Gupta, C., Byahut, A., Deka, C., Bag, A., & Khandelwal, B. (2024). The Gut-Brain Axis and the Human Microbiome. In *Microbial Ecology* (pp. 56-75). CRC Press.
- 8 Zhuang, M., Zhang, X., & Cai, J. (2024). Microbiota-gut-brain axis: interplay between microbiota, barrier function and lymphatic system. *Gut microbes*, 16(1), 2387800.
- 9 Lamichhane, G., Olawale, F., Liu, J., Lee, D. Y., Lee, S. J., Chaffin, N., ... & Kim, Y. (2024). Curcumin mitigates gut dysbiosis and enhances gut barrier function to alleviate metabolic dysfunction in obese, aged mice. *Biology*, 13(12), 955.
- 10 Bhalla, D., Dinesh, S., Sharma, S., & Sathisha, G. J. (2024). Gut-brain axis modulation of metabolic disorders: exploring the intertwined neurohumoral pathways and therapeutic prospects. *Neurochemical Research*, 49(4), 847-871.
- 11 Squire, L. R., Cohen, N. J., & Nadel, L. (2014). The medial temporal region and memory consolidation: A new hypothesis. In *Memory consolidation* (pp. 185-210). Psychology Press.
- 12 De Felice, F. G., & Lourenco, M. V. (2015). Brain metabolic stress and neuroinflammation at the basis of cognitive impairment in Alzheimer's disease. *Frontiers in Aging Neuroscience*, 7, 94.
- 13 Onaolapo, O. J., Olofinnade, A. T., Ojo, F. O., Falade, J., & Onaolapo, A. Y. (2023). Prepubertal continuous dietary folate fortification enhances the brain function of adult mice by modulating antioxidant status, inflammation, and brain neurotransmitter levels. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry*, 22(3), 198-209.
- 14 Popa-Wagner, A., Dumitrascu, D. I., Capitanescu, B., Petcu, E. B., Surugiu, R., Fang, W. H., & Dumbrava, D. A. (2020). Dietary habits, lifestyle factors and neurodegenerative diseases. *Neural Regeneration Research*, 15(3), 394-400.
- 15 Duarte, J. M. (2023). Loss of brain energy metabolism control as a driver for memory impairment upon insulin resistance. *Biochemical Society Transactions*, 51(1), 287-301.
- 16 Nau, H., & Blaner, W. S. (Eds.). (2012). Retinoids: the biochemical and molecular basis of vitamin A and retinoid action.
- 17 Roy, S., & Awasthi, A. (2019). Vitamin A and the immune system. In *Nutrition and immunity* (pp. 53-73). Cham: Springer International Publishing.
- 18 Lee, K. H., Cha, M., & Lee, B. H. (2020). Neuroprotective effect of antioxidants in the brain. *International Journal of Molecular Sciences*, 21(19), 7152.
- 19 Bos, A., van Egmond, M., & Mebius, R. (2022). The role of retinoic acid in the production of immunoglobulin A. *Mucosal immunology*, 15(4), 562-572.
- 20 Wang, Z. L., Pang, S. J., Zhang, K. W., Li, P. Y., Li, P. G., & Yang, C. (2024). Dietary vitamin A modifies the gut microbiota and intestinal tissue transcriptome, impacting intestinal permeability and the release of inflammatory factors, thereby influencing Aβ pathology. *Frontiers in Nutrition*, 11, 1367086.
- 21 Neville, V., Lind, J., Mendl, E., Cozma, N. E., Paul, E. S., & Mendl, M. (2023). A mapping review of refinements to laboratory rat housing and husbandry. *Lab Animal*, 52(3), 63-74.
- 22 Onaolapo, A. Y., Adebisi, E. O., Adeleye, A. E., Olofinnade, A. T., & Onaolapo, O. J. (2020). Dietary melatonin protects against behavioural, metabolic, oxidative, and organ morphological changes in mice that are fed high-fat, high-sugar diet. *Endocrine, Metabolic & Immune Disorders-Drug Targets*, 20(4), 570-583.
- 23 Allard, C., Zizzari, P., Quarta, C., & Cota, D. (2022). Food intake and body weight in rodent studies: the devil is in the details. *Nature Metabolism*, 4(11), 1424-1426.
- 24 Braid, D., Donzelli, A., Martucci, R., Ponzoni, L., Pauletti, A., Langus, A., & Sala, M. (2013). Mice discriminate between stationary and moving 2D shapes: application to the object recognition task to increase attention. *Behavioural Brain Research*, 242, 95-101.
- 25 Wahl, D., Coogan, S. C., Solon-Biet, S. M., De Cabo, R., Haran, J. B., Raubenheimer, D., ... & Le Couteur, D. G. (2017). Cognitive and behavioral evaluation of nutritional interventions in rodent models of brain aging and dementia. *Clinical Interventions in Aging*, 1419-1428.
- 26 Onaolapo, O. J., Onaolapo, A. Y., Mosaku, T. J., Akanji, O. O., & Abiodun, O. R. (2012). Elevated plus maze and Y-maze behavioral effects of subchronic, oral low dose monosodium glutamate in Swiss albino mice. *J Pharm Biol Sci*, 3(4), 21-27.

- 27 Krauter, A. K., Guest, P. C., & Sarnyai, Z. (2018). The Y-maze for assessment of spatial working and reference memory in mice. In *Pre-clinical models: Techniques and protocols* (pp. 105-111). New York, NY: Springer New York.
- 28 Castanon, N., Luheshi, G., & Layé, S. (2015). Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity. *Frontiers in Neuroscience*, 9, 229.
- 29 Xue, H., Hu, Z., Liu, S., Zhang, S., Yang, W., Li, J., ... & Lei, X. (2024). The mechanism of NF- κ B-TERT feedback regulation of granulosa cell apoptosis in PCOS rats. *PLoS One*, 19(10), e0312115.
- 30 Egbunah, U. P., Akinshipo, A. O., & Ajayi, O. F. (2022). Principles of Manual Tissue Processing in an Oral and Maxillofacial Pathology Laboratory in Lagos, Nigeria. *West African Journal of Medicine*, 39(1), 31-38.
- 31 Chang, Y. C., Lee, H. L., Yang, W., Hsieh, M. L., Liu, C. C., Lee, T. Y., ... & Chuang, L. M. (2023). A common East-Asian ALDH2 mutation causes metabolic disorders and the therapeutic effect of ALDH2 activators. *Nature Communications*, 14(1), 5971.
- 32 Kotzé-Hörstmann, L., Cois, A., Johnson, R., Mabasa, L., Shabalala, S., Van Jaarsveld, P. J., & Sadie-Van Gijzen, H. (2022). Characterization and comparison of the divergent metabolic consequences of high-sugar and high-fat diets in male Wistar rats. *Frontiers in Physiology*, 13, 904366.
- 33 Napoli, J. L. (2022). Retinoic acid: sexually dimorphic, anti-insulin and concentration-dependent effects on energy. *Nutrients*, 14(8), 1553.
- 34 Blaner, W. S. (2019). Vitamin A signaling and homeostasis in obesity, diabetes, and metabolic disorders. *Pharmacology & Therapeutics*, 197, 153-178.
- 35 Stern, J. H., Rutkowski, J. M., & Scherer, P. E. (2016). Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. *Cell metabolism*, 23(5), 770-784.
- 36 Osman, A. A., Laczkó, D., Vágvolgyi, M., Seres-Bokor, A., Sztojok-Ivanov, A., Kemény, K. K., ... & Ducza, E. (2025). Investigation of calonysterone and 20-hydroxyecdysone effects in high-fat, high-sugar diet-induced obesity rat model. *Heliyon*, 11(3).
- 37 Hotamisligil, G. S. (2017). Inflammation, metaflammation and immunometabolic disorders. *Nature*, 542(7640), 177-185.
- 38 Li, X., Chu, L., Liu, S., Zhang, W., Lin, L., & Zheng, G. (2022). *Smilax china* L. flavonoid alleviates HFHS-induced inflammation by regulating the gut-liver axis in mice. *Phytomedicine*, 95, 153728.
- 39 Cantorna, M. T., Snyder, L., & Arora, J. (2019). Vitamin A and vitamin D regulate the microbial complexity, barrier function, and the mucosal immune responses to ensure intestinal homeostasis. *Critical Reviews in Biochemistry and Molecular Biology*, 54(2), 184-192.
- 40 Wu, S., Wang, L., Cui, B., Wen, X., Jiang, Z., & Hu, S. (2023). Effects of vitamin A on growth performance, antioxidants, gut inflammation, and microbes in weaned piglets. *Antioxidants*, 12(12), 2049.
- 41 Mahmud, A. A., Anu, U. H., Foysal, K. A., Hasan, M., Sazib, S. M., Ragib, A. A., ... & Emran, T. B. (2022). Elevated serum malondialdehyde (MDA), insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH), and reduced antioxidant vitamins in polycystic ovarian syndrome patients. *Narra J*, 2(1), e56.
- 42 Liang, J. R., Dai, H., Yang, H. M., Yang, Z., & Wang, Z. Y. (2019). The effect of dietary vitamin A supplementation in maternal and its offspring on the early growth performance, liver vitamin A content, and antioxidant index of goslings. *Poultry Science*, 98(12), 6849-6856.
- 43 Rohr, M. W., Narasimhulu, C. A., Rudeski-Rohr, T. A., & Parthasarathy, S. (2020). Negative effects of a high-fat diet on intestinal permeability: a review. *Advances in Nutrition*, 11(1), 77-91.
- 44 Valdes, J., Gagné-Sansfaçon, J., Reyes, V., Armas, A., Marrero, G., Moyo-Muamba, M., ... & Menendez, A. (2023). Defects in the expression of colonic host defense factors associate with barrier dysfunction induced by a high-fat/high-cholesterol diet. *The Anatomical Record*, 306(5), 1165-1183.
- 45 Reichelt, A. C., Killcross, S., Hambly, L. D., Morris, M. J., & Westbrook, R. F. (2015). Impact of adolescent sucrose access on cognitive control, recognition memory, and parvalbumin immunoreactivity. *Learning & Memory*, 22(4), 215-224.
- 46 Iyer, N., Grizotte-Lake, M., Duncan, K., Gordon, S. R., Palmer, A. C., Calvin, C., ... & Vaishnava, S. (2020). Epithelium intrinsic vitamin A signaling co-ordinates pathogen clearance in the gut via IL-18. *PLoS Pathogens*, 16(4), e1008360.

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