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Cafeteria-diet induced obesity results in impaired cognitive functioning in a rodent model

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Abstract

Objectives: This study seeks to characterize the progressive course of physiological and behavioural outcomes in rodents following excessive caloric intake through the chronic consumption of a highly palatable diet, the cafeteria (CAF) diet.

Methods: Male Sprague Dawley rats were maintained on either CAF or chow (CON) diets for 20 weeks. Metabolic and physiological parameters were monitored throughout the feeding period. From week 18, rats were subjected to behavioural testing, which included the Morris water maze (MWM) and novel object recognition (NOR) tasks.

Results: CAF rats consistently showed higher food intakes and consumed six times the energy of chow-fed rats, being significantly heavier by week 5. CAF rats further exhibited greater abdominal widths, fat pads, and larger fatty livers, as well as compromised glucose tolerance. Hyperinsulinemia and dyslipidaemia with elevated serum cholesterol and triglyceride levels and reduced HDL cholesterol were also evident along with a pro-inflammatory profile in the CAF rats. Cognitive decline in CAF rats manifested as a decline in long-term retention memory in the MWM. Further, CAF rats exhibited deficits in recognition

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memory as they spent less time exploring the novel object than chow-fed rats in the NOR task.

Discussion: This model of obesity is a robust paradigm for producing an obese animal phenotype that closely mimics the evolution of human obesity, complete with metabolic dysfunctions that are indicative of pre-diabetes. Additionally, chronic CAF-diet induced obesity promotes cognitive impairments in hippocampal-dependent reference and working memory.

Keywords: Biochemistry, Neuroscience, Physiology

1. Introduction

Obesity is 'the epidemic of the 21st century' with worldwide rates having more than doubled since 1980. The term 'globesity' describes the growing world population of some 1.9 billion overweight and 600 million obese adults [1]. Obesity refers to an excessive or abnormal accumulation of fat, as a result of energy intake exceeding energy expenditure due to disruptions in the homeostatic and hedonic control axes of food intake [2].

The rapid increase in the incidence of obesity, and associated diseases (e.g. metabolic syndrome, diabetes, cardiovascular disease), during only the past few generations is primarily attributed to excessive consumption of palatable energy-dense foods which are high in saturated fats, refined sugars and sodium, combined with sedentary lifestyles [3]. Many diet-induced obesity (DIO) animal studies have attempted to model this unhealthy lifestyle through regimens of obesogenic diets, of which a single nutritional component, usually fat or sugar (e.g. fructose, fat from lard) is isolated in a pelleted or powdered formulation [4]. However, these regimens lack the full recapitulation of the nutritionally varied, energy-dense and highly palatable grocery store-purchased foods at the core of the current global obesity epidemic.

Cafeteria (CAF) diets are the closest equivalent to the ultra-processed food diet of humans. This diet type provides animals with free access to 'cafeteria-type foodstuffs' (ultra-processed, energy dense foods) along with laboratory chow ad libitum, so that they have a free choice in what they consume [5]. It provides a robust model of obesity as it mirrors the key obesogenic features of the human diet, maintaining its nutritional and sensorial diversity, and induces similar behavioural and physiological responses associated with human obesity [5, 6]. In addition to the cascade of physiological changes that lead to increased chronic metabolic dysfunction, the obese phenotype has now been shown to stimulate a cascade of changes in neurochemical signalling which can modify hypothalamic regulation of food consumption [7], as well as behaviours dependent on other susceptible structures in the brain, such as the hippocampus [8]. While the metabolic dysfunctions resulting from obesity are

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well documented; less is known about the impact on the brain. Clinical, epidemiological and rodent studies suggest that there is an association between obesity and the development of cognitive decline. This manifests as impairments in cognition through deficits in learning and memory, executive functioning and potentially brain atrophy [8, 9, 10].

Considering this along with the growing obese world population, there is an apparent need for a better understanding of the pathophysiological mechanisms underlying obesity and its impact on cognition. The body's defense response to harmful stimuli, inflammation has been proposed to be a principal pathophysiological mechanism underpinning cognitive impairment and dementia [10, 11]. Obesity is characterized by a state of chronic low-grade inflammation in peripheral tissues and the circulation of increased levels of free fatty acids, pro-inflammatory cytokines, chemokines, and immune cells [12]. This may lead to neuroinflammation which appears to be central mechanism through which obesity and cognitive decline intertwine [3]. Indeed, the vagal and humoral systems of the immune pathways are also traversed by nutritional signals that are responsible for maintaining energy homeostasis [13]. These systemic nutritional and immune signals eventually reach the brain where they alter feeding behaviour under the control of the hypothalamus. The extra-hypothalamic brain regions – hippocampus and perirhinal cortex – responsible for learning and memory are simultaneously affected through modulators of synaptic plasticity, neuronal apoptosis and impaired neurogenesis. These ultimately contribute to neurodegeneration which may manifest as cognitive dysfunction, and even initiation of brain atrophy [3, 11].

It remains to be demonstrated whether long-term CAF-diet induced obesity induces concurrent physiological and behavioural deficits. Thus, the objective of this study was to evaluate the effect of a chronic 20-week CAF feeding period on metabolic and cognitive functioning in a Sprague-Dawley rats. We assessed the time course of metabolic and inflammatory alterations in the periphery of the rodents followed by an examination of the effects of hippocampal-dependent spatial and non-spatial learning and memory using the Morris water maze and novel object recognition tasks, respectively.

2. Methods

2.1. Animals

All experimental procedures were approved by the Campus Ethics Committee, Faculty of Medical Sciences, The University of the West Indies (UWI), Trinidad and Tobago and followed the guidelines established by the Laboratory Animal House, School of Veterinary Medicine, UWI. Male Sprague Dawley rats (~6–8 weeks old) (Laboratory Animal House, Champs Fleurs, Trinidad and Tobago) were housed three per polypropylene/polycarbonate cage (47 cm × 29 cm × 15 cm) in a

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temperature controlled (18−20 °C) on a 12 h light/dark cycle (6 am on/6 pm off). Rats were acclimated to the housing facility for one week during which they were provided with regular chow and water *ad libitum*. Prior to diet commencement rats were weight matched across groups and home cages were randomly assigned diet groups.

2.2. Diets

Upon initiation of experimental diets rats (avg. weight 358.1 ± 6.947 g) were maintained on a regular chow (CON) diet (n = 21) or cafeteria (CAF) diet (n = 21) for 20 weeks. CON diet-fed rats received regular chow (National Feed Mills, Wrightson Road, Port-of-Spain, Trinidad and Tobago), with an average macronutrient kcal% content of 75.9% carbohydrates, 14.1% protein and 10% fat and access to one 500 ml bottle of water. CAF diet-fed rats received regular chow, and access to one 250 ml bottle containing commercial soft drink and one 250 ml water bottle in each cage. This was supplemented with a pre-selection of grocery store items − cheese, bread, sausages, corned beef, crackers, cookies − with an average macronutrient kcal% content of 38% carbohydrates, 14% protein and 48% fat (Table 1).

All food, water and soft drink were available *ad libitum* and replaced daily. Food and liquid consumption were recorded daily to carefully monitor intake. Items were weighed before presentation to rats, and the remainder weighed after 24 h. The amount of chow or CAF items consumed per cage was converted to energy (kcal) using data provided by the product manufacturers and average intake was calculated using the assumption of equal intake for each rat. Body weights were measured weekly using a triple beam animal scale (Ohaus, NJ, USA).

2.3. Blood sampling

Following an overnight (~16 h) fast, whole blood was collected into uncoated microcentrifuge tubes via the tail clip method from all rats in the study. The animal was restrained, ~2 mm of the distal tail was clipped and ~300 L of blood was collected. All whole blood samples were clotted, centrifuged (10 min, 3000g) at room temperature and serum stored at −20 °C until subsequent batch analysis. Blood was sampled in experimental weeks 0, 6, 12 and 18.

2.4. Intraperitoneal glucose tolerance test (IPGTT)

In experimental week 20, rats were fasted overnight (~16 h) and an initial blood sample was taken via the tail clip method. Rats were then intraperitoneally injected (i.p.) with a glucose solution (2 g/kg body weight; 2 mg/g; Sigma-Aldrich, ON, Canada). Blood was then drawn every 30 min thereafter for a period of 120 min, and glucose levels were measured using the Hemocue Glucose Analyser (Hemocue AB, Ängelholm, Sweden).

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Table 1. Nutritional composition of the cafeteria food items as given by the product manufacturers.

Values are per 1g of food item	Total kcal	Total fat g	Total Carb g	Total Protein g	Dietary fibre g	Sugars g	Saturated fat g	Cholesterol mg	Sodium mg
Cheese	4.2	0.35	0.05	0.25	0	0	0.25	1.05	6.25
Vienna sausage (MacFood ®)	2.88	0.25	0.05	0.1	0	0	0.07	0.93	4.92
Corned beef (Naisa ®)	2.32	0.16	<0.01	0.21	0	0	0.07	1.39	9.82
Cookies (Festival ®)	4.69	0.19	0.72	0.03	<0.03	0.34	0.09	0	2.81
Cookies (TeaTime ®)	5	0.2	0.7	0.05	0	0.33	0.13	0	3.13
White hops bread	2.23	0.02	0.52	0.07	0.02	2	0.01	0	3.1
Crackers (Crix ®)	4.29	0.11	0.71	0.11	<0.04	<0.04	0.04	0	8.21
Values are per 1ml									
Soft drink (Fanta ®)	0.48	0	0.12	0	0	0.12	0	0	0.06

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2.5. Serum analyses

A subset of the blood of collected, CON (n = 9) and CAF (n = 9) were used for serum analyses. Glucose, triglycerides (TAGs), total cholesterol (T-CHOL) and high-density lipoprotein (HDL-) cholesterol levels were analysed using colorimetric kits according to manufacturer's instructions (Human Diagnostics, Wiesbaden, Germany). LDL- and VLDL-cholesterol were calculated according to Friedewald, Levy, and Fredrickson [14]. Insulin was assessed using a Rat Insulin Enzyme Linked Immunosorbent Assay (ELISA) kit (Thermo Scientific, MD, USA). Inflammatory status − cytokines and/or chemokines, namely interleukin (IL) 1α and β, IL2, IL4, IL6, IL10, IL12, IL13, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and regulated on activation, normal T cell expressed and secreted (RANTES) − was profiled using a Rat Inflammatory Cytokines Multi-Analyte ELISArray kit (Qiagen, MD, USA) for the week 18 blood samples only. The absorbance was read and the fold changes were assessed by determining the ratio between the absorbance values of the two groups of each cytokine/chemokine.

2.6. Terminal tissue collection

At the experimental endpoint, rats were deeply anaesthetized by i.p. administration of urethane (1.5 g/kg body weight; 25g/100ml dH2O; Sigma-Aldrich, Oakville, ON, Canada). Body weight, naso-anal length and abdominal circumference at point of greatest girth were measured. Rats were then sacrificed by decapitation. Organs (liver, kidney, heart, adrenals, pancreas) and white adipose tissue (WAT) (retroperitoneal and gonadal deposits) were collected and weighed. All tissues collected were snap frozen in liquid nitrogen and stored at -80C, pending further analyses.

2.7. Behavioural testing

2.7.1. Morris water maze (MWM)

Rats were tested for spatial learning and reference memory (CON: n = 18, CAF: n = 21) using the MWM. The water maze consisted of a black circular tank (165 cm diameter, 80 cm height), containing clear water to a depth of 60 cm. The water temperature was maintained at ~22 ± 1 °C. Surrounding the tank were white walls to occlude the sight of extra cues. A number of spatial cues − large, graphic posters − were placed around the pool, and features inherent to room served as additional cues. The pool was virtually divided into four quadrants, i.e., north-east (NE), south-east (SE), south-west (SW), and north-west (NW), and a transparent platform (10 cm^2) was hidden 2 cm below the surface of water at the midpoint of one quadrant, rendering it invisible to the rats. During the experiment, rats were kept in their usual room and moved to the maze room in their home cages. At the start of each trial, a rat

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was placed into the tank at one of four entry points, N, E, SE or NW. The entry point for each trial was followed as outlined in Vorhees and Williams [15], with the order being constant across rats. Entry point was the same for all rats on any given trial number, but no trial and the subsequent one shared the same entry point. The activities of the rats were recorded by video camera.

For the first phase of the protocol (hidden phase), rats were trained to locate the hidden platform (in the SW quadrant) for 5 consecutive days, 4 trials per day in correspondence to the entry points. Each trial lasted until either the rat had found the platform or for a maximum of 2 min. All rats were allowed to rest and observe on the platform for 10 sec. A probe trial was performed on the sixth day to assess the extent of memory consolidation. The single-probe trial consisted of a 30 sec free swim in the tank without the platform. The degree of memory consolidation after learning was assessed by the time spent in the target quadrant during the probe trial.

The reversal phase, immediately followed, with the platform now moved to the opposite quadrant of the tank (NE). All procedures of the hidden phase were repeated. This phase tested the rats' ability to learn a new direct path to the moved goal position by extinguishing their previous knowledge of the initial platform position.

The final phase, cued phase, served as a control condition to test the rats for their ability to learn to swim to a cued goal. All distal cues were removed and the platform was the same as in the hidden phase but a flag (proximal cue) was mounted on it that extended above the water surface by $\sim$12 cm so that there was a direct line-of-sight to the location of the platform. The platform's location and the rat's entry point were both shifted to novel positions during each trial as outlined Vorhees and Williams [15]. Each rat underwent 4 trials per day for 2 consecutive days, immediately following the reversal phase.

2.7.2. Novel object recognition (NOR) test

A subset of rats from MWM testing were allowed to rest for 5 days and then subjected to the NOR test to assess their recognition working memory (CON: n = 12, CAF: n = 12). The apparatus consisted of an empty box (60 cm 60 cm 50 cm) constructed from black-painted plywood, divided equally into 16 quadrants. The activities of the rats were recorded by video camera. Rats were acclimatised to the empty box for 5 min on day 1, and testing commenced the next day. For the NOR task, each rat was placed into the box with two identical objects (located in two of the middle four quadrants) and allowed to explore for 5 min (familiarisation phase). The rat was then removed for 10 min (retention phase) and the box and objects were cleaned with 70% ethanol to ensure the absence of olfactory cues. In the

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test phase, two objects were placed into the same positions as the familiarisation phase. One of the objects was identical to the sample object previously presented and the other was a novel object. The test phase lasted 5 min.

Rats were tested at the same time of day and the object order and location were counterbalanced between rats and across trials. Exploration was defined as sniffing, licking, chewing, touching or by moving vibrissae while directing the nose towards and less than 2 cm from the object as outlined in Antunes and Biala [16]. The number of explorations and time spent in exploration of each object by each animal was assessed.

2.8. Statistical analyses

Statistics were performed using Prism software (V8.0.1, GraphPad, La Jolla, CA). Data are expressed as mean ± SEM. Repeated measures analyses were used to analyse mean differences between experimental groups when measurements were repeated longitudinally. t-tests and/or one-or two-way ANOVAs followed by Tukey's multiple comparisons post-hoc test, where necessary were used to analyse group differences among all other variables. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Effect of diet on energy intake and body weight − CAF diet induced hyperphagia and weight gain

There were no differences in body weight and food intake between experimental groups at the start of the study. All rats consistently gained weight across the feeding period, with the CAF rats gaining weight more rapidly than the CON rats, with a significant ($p < 0.05$) divergence seen after 5 weeks (CON: 418.62 ± 13.077 g, CAF: 495.86 ± 17.77 g). CAF rats gained more than twice the weight gained by CON rats, for final body weights of CON: 507.90 ± 24.11g and CAF: 670.30 ± 26.01 g (Fig. 1D). Total energy intake over the feeding period was significantly ($p < 0.0001$) greater in the CAF rats as they consumed on average of ×6 kcal as CON rats, for total kcal consumption of 641.71 ± 9.165 and 116.36 ± 4.038, respectively (Fig. 1A, B, C). At sacrifice, body variables were measured and CAF rats were found to have significantly ($p < 0.001$) greater central adiposity as noted by the larger body widths. Fat pad and liver weights were also significantly higher ($p < 0.0001$ and $p < 0.001$ respectively) in CAF rats (Table 2). Upon gross examination of the livers of the CAF rats, they appeared pale and diffusely yellow with blunt and friable edges. Those of the CON rats were dark red and flexible with sharp edges (Data Not Shown).

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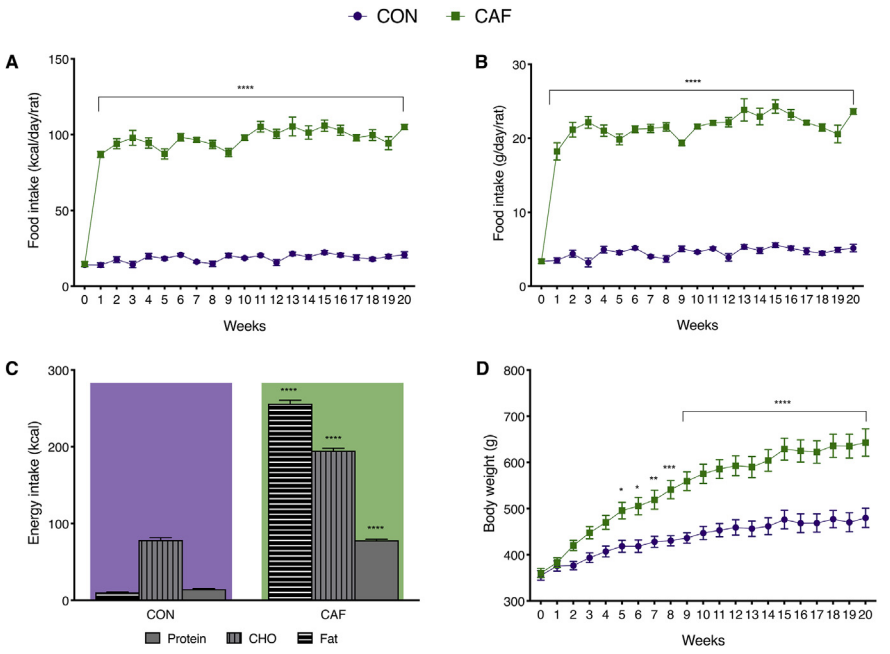

Fig. 1. Food consumption and body weight changes over feeding period. Daily average energy intake, in kcal/day/rat chow and CAF diet food items. (B) Daily average food intake, in g/day/rat. (C) Weekly nutritional caloric intake of diets, in kcal protein, CHO and fats. (D) Body weight progression (g) over 20 weeks. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) (CON, n = 21; CAF, n = 21).

3.2. Effect of diet on blood glucose and insulin − CAF diet induced hyperinsulinemia and impaired glucose tolerance

Across the feeding period, glucose homeostasis was maintained for both CON and CAF rats, as fasted overnight blood glucose levels were not significantly different at weeks 0, 6, 12 or 18 (Fig. 2A). However, insulin levels were significantly ($p < 0.0001$) elevated in CAF rats at these time points, more than tripling by the end

Table 2. Body weight (bwt.), length and width and weight of adrenals, fat pads, liver and spleen of the dietary groups. (***$p < 0.001$, ****$p < 0.0001$) (CON $n = 21$; CAF $n = 21$).

	CON	CAF
Initial bwt. (g)	355.50 ± 10.33	360.70 ± 9.83
Final bwt. (g)	507.90 ± 24.11	670.30 ± 26.01 ***
Body length (cm)	28.81 ± 0.80	29.90 ± 0.33
Body width (cm)	10.06 ± 0.38	12.76 ± 0.39 ***
Adrenal glands (g)	0.06 ± 0.03	0.07 ± 0.03
Retroperitoneal and gonadal fat pads (g)	12.72 ± 0.98	54.4 ± 6.58 ****
Liver (g)	13.37 ± 0.53	17.68 ± 0.74 ***
Spleen (g)	0.73 ± 0.13	0.76 ± 0.11

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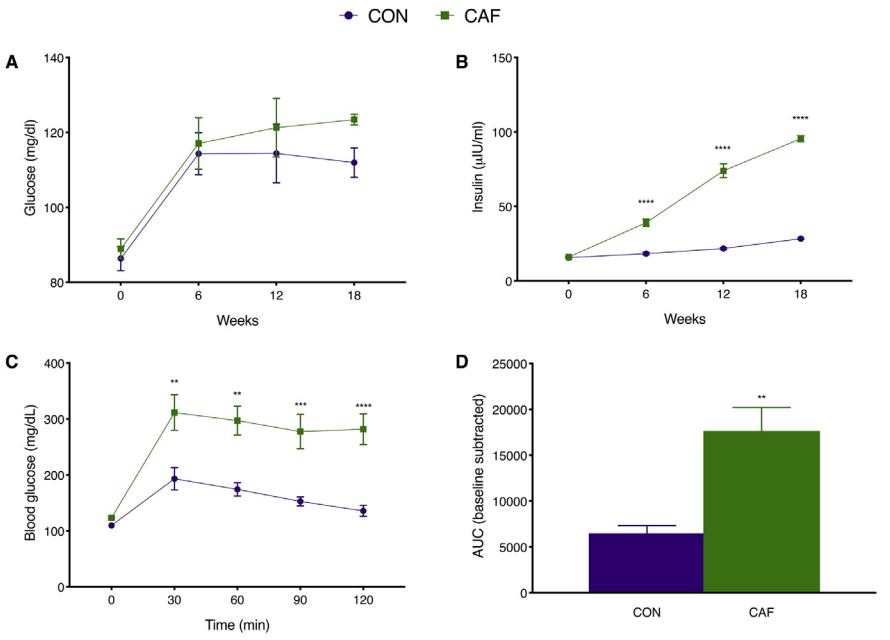

Fig. 2. CAF feeding causes hyperinsulinemia and glucose intolerance but does not lead to elevated fasted glucose levels. (A) Fasted blood glucose (mg/dl) and (B) serum insulin (µlU/ml) in CON and CAF rats over the feeding period. (C) Fasted blood glucose (mg/dl) levels following a glucose challenge in week 20. (D) Area under the curve (AUC) quantifications for IPGTT of averaged individual animals. (**$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) (CON, $n = 9$; CAF, $n = 9$).

of the feeding period (18 weeks − CON: 28.33 ± 0.559 µIU/ml, CAF: 95.50 ± 2.054 µIU/ml) (Fig. 2B). To determine the effects of the diet on glucose tolerance, IPGTTs was performed and CAF rats displayed decreased recovery when challenged with a glucose load, as blood glucose levels remained elevated (120 min: 281.67 ± 27.285 mg/dl) from normal (0 min: 123.11 ± 1.82 mg/dl). CON rats quickly returned to normal after the challenge, 0 min: 109.57 ± 3.644 mg/dl vs. 120 min: 135.714 ± 9.920 mg/dl) (Fig. 2C). The total area under the curve representative of the degree of the glucose tolerance impairment was also significantly ($p < 0.001$) different between groups, CON: 6486 ± 831.8 mg/dl/min and CAF: 17628 ± 2578.0 mg/dl/min (Fig. 2D).

3.3. Effect of diet on blood lipids − CAF diet induced dyslipidaemia

During the feeding period, CAF rats were dyslipidemic, with serum triglycerides significantly ($p < 0.0001$) increasing one and a half times relative to the CON rats by the end of the feeding period (18 weeks − CON: 109.67 ± 7.702 mg/dl, CAF: 190.91 ± 14.343 mg/dl) (Fig. 3A). CAF rats showed significantly increased total cholesterol levels from week 12 (CON: 82.68 ± 4.309 mg/dl, CAF: 99.16 ±

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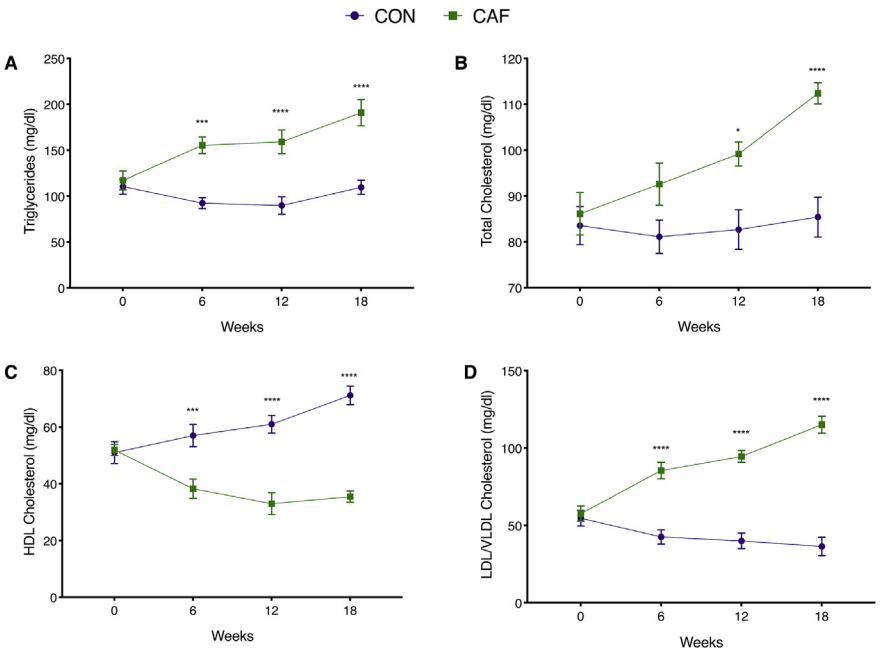

Fig. 3. CAF diet causes dyslipidemia. Elevated (A) triglycerides, (C) LDL-C, (D) T-CHOL and VLDL (not shown) with reduced (B) HDL cholesterol levels (mg/dl) compared to CON rats. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) (CON, n = 9; CAF, n = 9).

2.628 mg/dl) and onwards (18 weeks − CON: 85.41 ± 4.349 mg/dl, CAF: 112.36 ± 2.311 mg/dl) (Fig. 3B). HDL cholesterol was significantly lower in CAF rats, being approximately half of that of CON rats throughout the feeding period, showing final values of CON: 71.17 ± 3.279 mg/dl, CAF: 35.46 ± 1.980 mg/dl (Fig. 3C). LDL/VLDL cholesterol was increased in CAF rats across the feeding period, resulting in a final level of three times as much as CON rats (LDL − CON: 36.43 ± 5.941 mg/dl, CAF: 115.12 ± 5.449 mg/dl; VLDL − CON: 22.18 ± 1.539 mg/dl, CAF: 38.16 ± 2.864 mg/dl) (Fig. 3D).

3.4. Effect of diet on inflammatory status − CAF diet induced pro-inflammatory profile

The inflammatory profile of the CAF-fed rats indicated that they had an overall increased expression of pro- and anti-inflammatory cytokines and chemokines relative to the CON rats based on changes in expression levels after 18 weeks of consuming the experimental diets. Those that increased included IL1α and β, IL2, IL6, IL10, IL12, IFN-γ, TNF-α, GM-CSF and RANTES while the levels of IL4 and IL13 decreased. Statistical significance was only seen in the expression levels of IL4, IL6, IL10 and TNF- α (Fig. 4).

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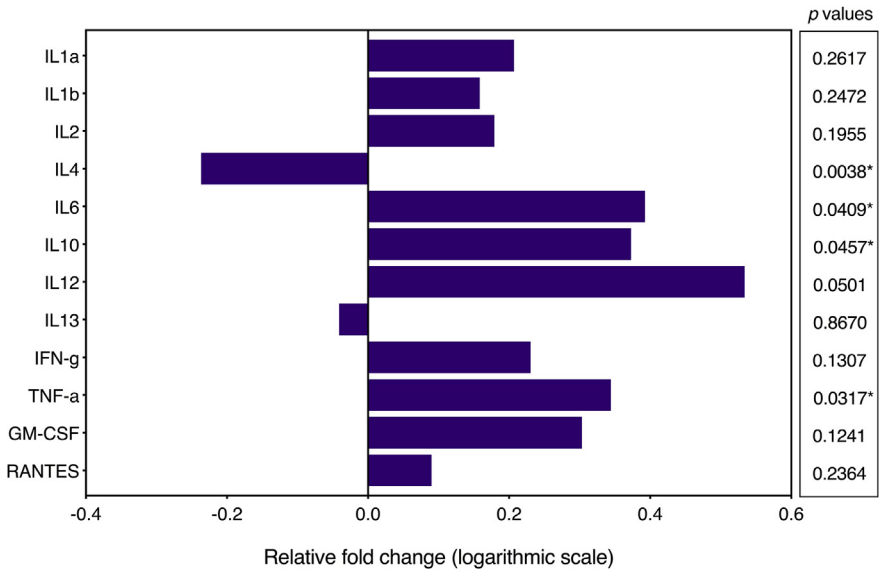

Fig. 4. Fold changes in cytokine/chemokine levels in CAF rats relative to CON rats. Data are ratio of absorbance values of protein levels of CAF rats relative to CON rats converted to a logarithmic scale. A positive log fold change indicates increased levels while a negative fold change indicates decreased levels in CAF compared to CON. (CON, n = 6; CAF, n = 6).

3.5. Behavioural data

3.5.1. Morris water maze (MWM)

Spatial learning and memory was assessed in the MWM after rats had been on their respective diets for 18 weeks. CAF-diet induced obesity led to cognitive dysfunction through a decline in long-term retention memory, but not acquisition and learning in the MWM task. Both CAF- and CON-fed rats showed similar escape latencies during training trials for locating a hidden platform in the hidden and reversal phases, indicating that they were learning the task similarly, as the time taken to find the platform decreased on consecutive days (Fig. 5A). However, in probe 1 when the platform was removed, the CON rodents returned to the quadrant (south-west) where it was previously located for a greater time than that of the CAF-fed rodents (Fig. 5B). Similar results were seen when the reversal probe trial (probe 2) was conducted, with CON rodents returning to the target quadrant (north-east) for a much greater time (Fig. 5C). Differences were not detected in the cued phase which served as a control procedure to determine that ability of diet groups to complete the cognitive task.

3.5.2. Novel object recognition (NOR)

Non-spatial learning and memory was assessed in the NOR after MWM testing. CAF-diet induced obesity led to cognitive dysfunction through a decline working recognition memory. CAF- and CON-fed rats showed similar percentages of exploration times when placed in an open field with two identical objects (Object A and

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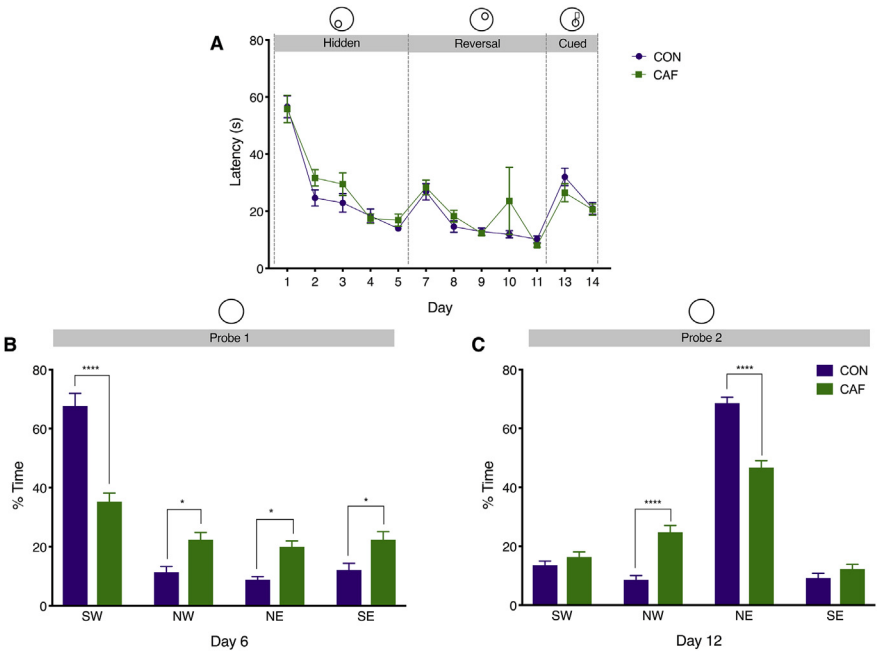

Fig. 5. MWM results. (A) Escape latencies (s) over the hidden, reversal and cued phases. (B) Time (%) spent in the target quadrant (SW) for probe trial 1. (C) Time (%) spent in the target quadrant (NE) for probe trial 2. (*p $<$ 0.05, ****p $<$ 0.0001) (CON, n $=$ 18; CAF, n $=$ 21).

Object A') (CON: object A 55.58 $\pm$ 9.424% time vs. object A' 44.42 $\pm$ 9.424% time; CAF: object A 53.03 $\pm$ 10.43% time vs. object A' 46.97 $\pm$ 10.43% time) (Fig. 6A). However, when one of the objects was replaced with a novel object, CON-fed rats spent significantly ($p < 0.0001$) more percentage time exploring the novel object compared to CAF-fed rats spent (CON: object A 26.22 $\pm$ 4.36% time vs. novel object 73.78 $\pm$ 4.36% time; CAF: object A 64.02 $\pm$ 12.05% time vs. novel object 35.98 $\pm$ 12.05% time) (Fig. 6B).

4. Discussion

We comprehensively examined the biochemical, physiological and behavioural changes following sustained consumption of a CAF diet in male rats. Our results confirm that 20 weeks of CAF feeding produces a robust model of the human obese phenotype by causing voluntary hyperphagia and excessive energy consumption resulting in rapid and significant weight gain, abdominable width and visceral adiposity. CAF feeding also produced rats with a prediabetic condition characterized by glucose intolerance, hyperinsulinemia and dyslipidaemia with elevated serum triglyceride and LDL cholesterol levels and reduced HDL cholesterol when compared to CON rats. Larger, fatty livers and adipose depots were also present in CAF rats. It was further shown that CAF-diet induced obesity is associated with perpetuating a peripheral pro-inflammatory status in these rats. Finally, our results indicate that

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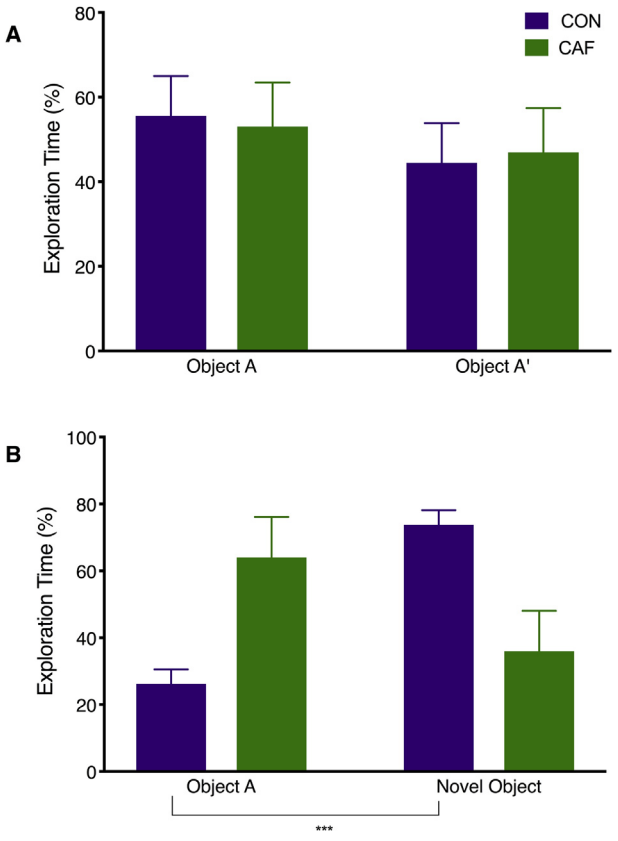

Fig. 6. NOR results. (A) Exploration time (%) when challenged with two identical objects. (B) Exploration time (%) when one of the identical objects was replaced with a novel object. (***p $<$ 0.001, ****p $<$ 0.0001). (CON, n $=$ 12; CAF, n $=$ 12).

rats maintained on this high-energy diet displayed impaired memory retention, evidenced by deficits in long-term retention memory and working recognition memory.

CAF feeding has been shown to lead to overconsumption of the high-energy dense foods of the diet [3, 6]. This is in alignment with human obesity which is centred on poor eating habits. In our study, CAF rats consumed six times the energy of CON rats, mainly from fat and refined sugars. This reflects the high palatability of the diet. Rats fed a high fat diet, unlike CAF-fed rats have been revealed to decrease total food intake to maintain caloric intake comparable rats fed a control diet [6]. The normal homeostatic mechanism for energy balance appears to be disabled in CAF feeding through activation of the reward system due to the variation of flavour and textures in the CAF diet [7]. The CAF-fed rats in spite of rapid weight gain, continuously ate excessive amounts of CAF foods, and when offered the choice of these foods and chow, complete in macronutrient requirements, selected chow least (~3% of total energy intake).

Resultant from the CAF-feeding was higher body weights and central adiposity. CAF and CON rats gained 91% and 44% of their initial body weights, respectively.

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The observed weight gain was associated with deteriorated parameters of metabolic function. CAF rats were normoglycaemic throughout the feeding period as fasting blood glucose levels were not different when compared to CON rats. However, insulin levels were elevated from week 6 and diverged even further as the feeding period continued. Insulin sensitivity was reduced in CAF rats, as although basal glucose levels were similar, when both groups were challenged with a glucose load, glucose levels remained comparatively lower in CON rats. In the CON rats, glucose levels quickly returned to the basal value over the 2 h period while CAF rats remained hyperglycaemic. These rats had more than twice the basal glucose level at the end of the 2 h period. These alterations in glucose metabolism are indicative of an early, pre-diabetic condition [6, 17]. The insulin tolerance test could be used as an additional measure to supplement these findings in order to further detect this observed insulin resistance.

Insulin insensitivity is thought to intrinsic to dyslipidaemia [18] which is consistent with the findings of elevated blood triglycerides and total cholesterol. When lipid profiles were examined, CAF rats showed lower HDL cholesterol and higher LDL/VLDL cholesterol. Similar effects of the CAF diet on blood lipids were previously described [3, 5, 6]. Dyslipidaemia was probably evident due to the overall higher consumption of the CAF diet which while being high in fat, is also high in saturated fatty acids, trans-fatty acids and cholesterol. Insulin resistance is strongly linked to central depots of fat, particularly visceral adiposity which are thought to cause increased hepatic fatty acid esterification, which forms triglycerides that are released through the portal vein supplying the liver ultimately resulting in hypertriglyceridaemia [18]. This is linked with the concurrent systemic release of inflammatory cytokines [19]. Indeed, it was found that insulin resistance in CAF rats was also compounded with wider abdominal circumference. Furthermore, these rats also had a four-fold increase in combined retroperitoneal and gonadal fat depots.

A key component of obesity and subsequent metabolic syndrome is chronic low-grade inflammation with the secretion of adipokines from white adipose contributing to this process [12, 20]. It remains to be determined whether this pro-inflammatory status is solely a cause or consequence of obesity. CAF-diet induced obesity promoted a pro-inflammatory milieu with an overall simultaneous increased expression of plasma cytokines and chemokines (IL1α and β, IL2, IL6, IL10, IL12, IFN-γ, TNF-α, GM-CS, RANTES). Of these, statistical significance was only seen for IL6 and IL10. These findings are consistent with other studies [21, 22], though to the best of our knowledge, these have not been comprehensively related with CAF-diet induced obese rodents. Decreased expression levels were seen for IL4 and IL13, with statistical significance for IL4 only, which contradict a prior study [20] that showed increased levels of these anti-inflammatory cytokines. This overall inflammatory profile may have been as a result of dietary factors such as the increased fatty acids stimulating immune cells and imitating an inflammatory

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cascade. Although, histologic analyses of white adipose, liver and pancreas were not carried out for macrophage-infiltration, this process is required for perpetuation of the obese state and systemic insulin resistance. This macrophage proliferation leads to an increase in pro-inflammatory cytokines such as TNF and IL-6 [13]. These cytokines along with fatty acids and adipokines can then contribute to systemic insulin resistance due to downstream effects on the liver and muscle, where they inhibit intracellular signalling from the insulin receptor. This inflammation can also further propagate disruption of feeding-related pathways in the hypothalamus, decreasing the ability of insulin and leptin to suppress hunger and feeding, thus maintaining the CAF diet-induced obesity [23]. These can further affect extra-hypothalamic regions in a similar manner, influencing synaptic remodelling and neurodegeneration, resulting in cognitive impairment.

CAF-diet induced obesity was also found to affect some of the organs when rats were dissected. Liver size significantly increased in CAF rats and upon gross morphological examination, the tissue was found to be softer, paler and diffusely yellow in colour when compared to those of the CON rats which were firmer and of a dark red colour. Additionally, the edges of livers of the CON rats were sharp while those of the CAF rats were blunt and friable. Taken together, these may be indicative of fat infiltrating the livers in the CAF rats, although histological diagnosis is need for a definitive determination. If infiltration of fat in the liver is prolonged, steatitis or activation of inflammatory cells in the liver can follow [24]. This is parallel to what occurs in obesity, lipid accumulation followed by chronic inflammation.

The adrenal glands and spleen were also examined as they produce hormones and cytokines, respectively as they both directly and indirectly affect the progression of obesity. It was found that there was no significant size difference of the adrenals and spleen in the two groups of rats. Obesity is implicated in changing adrenal endocrine function, either through an adaptive response to adipose tissue hormones or being part of the pathogenesis of obesity itself [25]. No change in the size of adrenal glands possibly indicates that the endocrine functioning of the adrenal glands was similar between the diet groups and the feeding period was not sufficient to alter adrenal functioning. Future work here requires the determination of the circulating levels of adrenal hormones. The spleen is another important organ in the pathogenesis of obesity as it plays a significant role in immune functioning, producing increased pro-inflammatory cytokines in obese rats [26]. Like the adrenal glands, the spleen was thought to be functioning similarly in the two groups of rats. The overall pro-inflammatory status that was noted in CAF rats was therefore overall resultant from those circulating in the blood, which would have been produced from multiple sources in the body.

In addition to these physiological findings, we also report that CAF diet-induced obesity is associated with neurobehavioural alterations. Several epidemiological

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studies in humans suggest that disruptions in cognitive ability are evident in obese individuals, even those that are considered otherwise healthy. However, human studies show a lack of direct evidence while animal studies show contradictory findings. Thus, to determine if CAF-diet induced obesity could affect spatial learning and memory, rats were tested in the MWM task after 18 weeks of feeding. No diet effect was seen in the acquisition (learning) phase of the task as both the CAF and CON groups showed similar escape latencies when trained to locate a hidden platform.

Further, both groups learned the task in the same manner as escape latencies decreased steadily over the consecutive training days, with the exception of day 9−10 for the CAF rats. However, this was rectified by day 11 when an even lower escape latency was observed relative to day 9. This could have possibly been resultant of the somewhat stressful nature of the test. Additionally, performing two phases, hidden and reversal reinforced spatial learning as the later revealed that the rats had the ability to form a direct path to the new position of the platform and extinguished the platform's position that they had initially learnt.

An impact of diet on reference memory was detected when the platform was removed on days 6 and 12 for the probe trial which was conducted 24 hours after the last spatial acquisition day in the hidden and reversal phases to test retention. The rat's preference for the quadrant in which the platform had been previously located was being assessed. It was expected that the rats would be employing reference memory to remember the platform's original location in the specific quadrant (target quadrant). Therefore, even if the rats recognized the platform was no longer in the target quadrant they would continuously return to it, instead of focusing on the other three quadrants. CAF rats showed impairment as they spent less time in the target quadrant relative to CON rats. These results were bolstered in the reversal phase when the hidden platform was moved to the quadrant opposite to its previous location as similar results were seen.

This capability of CAF-diet induced obesity to produce deficits in spatial memory adds to the paradoxical findings in this area. It has been suggested that variability in this area may be due to the duration of the feeding protocol, the age of the animals and the type of feeding protocol, particularly the fat and sugar content and composition [8]. These factors have all been shown to have some bearing on spatial tasks beyond the MWM, including the eight-arm radial maze [27, 28], where performance was impaired and recently, the Barnes maze, where performance remained intact [3]. A spatial variant of the NOR task, novel place recognition task further showed impaired performance of CAF-fed rats [29]. Of these behavioural tests, the MWM is described as most stress inducing to the animals and this can confound the measure of behavioural outcome. Human research methods typically employ positive rewards in learning tasks so that the highly stressful nature of MWM may show some discordance, if these results are to be translated to human populations.

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In light of this, the issue was raised as to whether the neurobehavioural task itself may have some role to play in the outcome of testing. To test this hypothesis as well as to serve as a control procedure for the MWM task, a cued learning phase was carried out. In the cued phase the spatial requirements are removed from the task since rats were required to swim to a non-hidden platform, confirming that place learning was the only factor being used to navigate the maze. This phase removed the distal cues that would have been previously used by the rats instead giving the rats a direct line-of-sight to the platform's location through a flag being elevated on the platform. No differences were found between the CON and CAF rats in the cued phase and thus, all rats possessed the capacity of learning using distal cues in the spatially dependent hidden and reversal phases. This is so as spatial versions and cued learning (non-spatial) required "the same basic abilities (intact eyesight, motoric ability (swimming)), basic strategies (learning to swim away from the wall, learning to climb on the platform)) and the same motivation (escape from water)" [15]. Therefore, differences detected in the spatial hidden/reversal phase were truly indicative of a memory deficit.

Further to this, we employed the use of the NOR task to test non-spatial, working memory. This test primarily relies on the innate exploratory behaviour of a rodent in the absence of externally applied rules or reinforcement e.g. food or electric shock making it comparable to memory tests used in humans. As such, it is completely free of the reference memory component of the MWM and is considered as a 'pure' working-memory test.

Our study has shown that CAF rats do show delay in the NOR task compared to their CON counterparts, implying that they displayed impaired working memory retention. This was so as in the familiarisation phase of the task both groups of rats showed similar exploration percentage time when with two identical objects. When one of these objects was replaced with a novel object, CON rats spent a greater percentage of time exploring the novel object as opposed to the CAF rats. Similar findings where shown by Francis et al [30] who demonstrated impaired performance in both the MWM and NOR tasks when rats were fed a diet high in saturated fat and refined sugar for 8 weeks. Again, deficits in non-spatial performance appear to only be seen in long term feeding protocols as Kanoski and Davidson [27] found a 3-day exposure was enough for rats fed a high glucose and fat diet to perform worse on a spatial task compared to chow fed control while non-spatial memory impairments could only be detected after 30 days. This raises the issue of precisely when cognitive dysfunctions are evident in diet-induced obesity and if one type of memory impairment may precede another, possibly due to some interconnectedness of memory formation and consolidation.

These behavioural findings have indeed shown that cognition can be influenced by obesity. The brain, specifically, the hippocampus – a region important in cognitive

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processing, learning and memory − is sensitive to changes in dietary energy intake [11]. The region may also be susceptible to time-dependent changes in these functions when compounded with high-energy diets in a similar manner to normal brain ageing due to the influence of obesity-related peripheral inflammation that was detected in the rats. It is speculated that peripheral inflammation precipitates central inflammation which in turn disrupts cognitive function.

Some limitations existed within our study. It was a requirement of the study that activity levels of the CON and CAF groups be minimal in order to mimic the sedentary lifestyle that accompanies diet-induced obesity. It was an assumption that size of the cages as well housing the animals in groups of three would ensure minimal activity; however a definitive measure of general activity should have been examined. This housing pattern also assumes that each of the rat in a group showed equal food consumption, though it was found in practice that weight gain variability within cages was minimal and corrected itself by the end of the experimental period. The mixed presentation of the CAF food items could not reveal if CAF rats showed preferences for specific food items, as is the case with humans which could have possibly affected the nutrition intake and by extension the calories consumed by these rats. It is also noted that the MWM test imposes some stress on the animals and as such other spatial tasks e.g. Barnes maze could be used in conjunction to the MWM to solidify that the effects seen in the MWM were due to cognitive impairments, though it is postulated that these other tests may not have the same strong motivating factor (escape from the water) to complete the task.

Future work will seek to bolster the findings of this study and address limitations that arose. Since cognitive impairments were found to be evident, additional measures will be used to explore the depth of these impairments, that is, if impairments outside of long-term and working memory exists and if we can pinpoint whether these impairments are time-dependent. Other functional measures would be used to explore the some of the physiological findings, for example, whether fatty livers are indicative of non-alcoholic fatty liver disease, and histologic analyses of fat and liver tissue for macrophage-infiltration, as this process is necessary for the maintenance of the obese state. Of interest is the pathophysiological mechanisms that underlie the relationship between diet-induced obesity and cognition. Our study has shown that peripheral inflammation does exist in the obese rats and it is postulated that this influences central inflammation. This will be explored by assessing the hippocampus of the obese rats for such changes as well as changes in memory-related genes.

5. Conclusion

The current staggering and projected world population of overweight and obese adults has prompted the need for basic research into obesity and its associated

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comorbidities that can be translated to the clinic. A key factor for this translation is the use of animal models that successfully capture the two primary attributions of the current global obesity epidemic −excessive consumption of palatable energy-dense foods and sedentary lifestyles. To address these concerns, we used a human diet of nutritionally varied, energy-dense and highly palatable grocery store-purchased foods and demonstrated that the CAF diet produces a robust model of the human obese phenotype in as little as twenty weeks in Sprague Dawley rats.

This was characterized both by metabolic and physiologic parameters that further elucidated a prediabetic condition with pro-inflammatory alterations in the rats. Concurrent impairments in cognitive functioning were also present as CAF-diet induced obesity led to deficits in hippocampal-dependent spatial and non-spatial memory. The diet-induced obesity model described herein has great utility for providing a rapid means for inducing all relevant physiological factors related to the condition. As a relationship has been proven to exist between diet-induced obesity and cognition, this model can further be used to gain a better understanding of the pathophysiological mechanisms underlying this relationship.

Declarations

Author contribution statement

Aneisha Lewis, Shamjeet Singh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Farid Youssef: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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