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Research report

# Effect of high-fat diets on mood and learning performance in adolescent mice

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## HIGHLIGHTS

- High fat diets trigger a rapid antidepressive-like behaviour.
- Cognitive deficits induced by high fat diets appear after 4 weeks of treatment.
- Memory impairments induced by high fat diets are not related to the alleged obesity-induced depression neither to anxiety-like behaviors.

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## ABSTRACT

Recent studies point to dietary factors as important effectors in the brain and epidemiological studies suggest a direct relationship between mood and anxiety disorders, cognitive impairment and obesity. Nevertheless the link between the consumption of high-fat diets (HFD) and emotional disorders still remains unclear. This issue is of particular interest during adolescence, which is an important period for shaping learning and memory acquisition that can be particularly sensitive to the detrimental effects of HFD. Otherwise, major depressive disorder and anxiety crisis often emerge in adolescence. In the current study we have characterized in adolescent mice i) the onset of HFD-induced memory impairment using the novel location recognition (NLR) paradigm, and ii) the effect of HFD on depression- and anxiety-related behaviors by using the forced swimming and the elevated plus maze tests, respectively. Here we report that memory impairments induced by HFD were already perceptible after 4-weeks HFD whereas HFD induced already antidepressant-like effects after 48-h, that remained after long-term treatment (8 weeks). No effects in anxiety were found. These data indicate that the antidepressant-like effect of HFD is independent of memory deficits as it was already present after 48-h HFD, while no effects in memory were still observed at this time.



## 1. Introduction

The consumption of calorie-dense foods has dramatically increased over the last years in westernized societies [1]. The incidence of depressive symptoms is much higher in obese subjects than in the normal weight age-matched population [2,3]. In fact, obesity has been related to anxiety and depression both in humans and animal models [4,5]. Similarly, clinical studies have revealed a predictive longitudinal association between obesity and the development of age-related cognitive deficits [6–8]. Compatible with that, displeasured situations have been shown to promote the consumption of palatable diets in humans [9], supporting the concept of comfort food as a tool to self-manage mood disorders [10]. Interestingly, comfort food consumption has been observed in mice displaying lower basal corticosterone signaling [11].

Many authors have suggested that molecular events related to the management of neural energy metabolism and synaptic plasticity [12] evoked by dietary factors might trigger mood and anxiety disorders [13,11]. In this sense, we have recently reported a number of negative consequences of high-fat diets (HFD) in learning and memory processes that could be related to the impairment of hippocampal synaptic plasticity, changes in neurogenesis and decrease of glutamatergic transmission in this area [14,15]. Previous studies carried out in our laboratory and others have demonstrated that adolescent exposure to HFD impair hippocampal-dependent learning and memory processes [14,15,16], revealing adolescence as a period particularly sensitive to HFD in terms of hippocam-

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pal function. Our studies have shown that HFD consumption during adolescence impairs learning and memory hippocampal-dependent memory in adult age, which is not reversed by further caloric restriction or body weight (BW) loss [17]. Regarding this effect of HFD, an important issue that merits a careful analysis deals with the contribution of HFD themselves vs the contribution of obesity and metabolic-related alterations. Diabetes and hyperglycemia have been associated with the alteration of hippocampus structure as well as with impaired long-term spatial memory [18–20] revealing that metabolic alterations can account for memory deficits. In fact, previous studies evaluating the effect of long-term HFD on memory do not allow discriminate between the contribution of HFD itself and that of obesity and its related metabolic alterations, including inflammation [21,14,15]. Therefore, a main interest in the current study has been to know the effects of HFD before the obesity process starts. In addition we wanted to know whether HFD-induced memory deficits could be explained by a decrease in mood or even by an increase in anxiety behaviors. In this sense, some authors have shown the anxiolytic and antidepressant effects of highly palatable diets [14,15,22], whereas other studies rather suggest the opposite [23–25]. Differences between experimental conditions could likely explain these discrepancies.

Because our previous results show that adolescent mice are more sensitive than adult individuals to HFD in terms of learning performance, we aimed at characterizing the influence of HFD consumption during adolescence on anxiety, depression and memory. Moreover, we have studied the interaction of this type of diets with two representative drugs used for the management of anxiety (diazepam) and depression (fluoxetine).

## 2. Material and methods

### 2.1. Dietary treatment and experimental design

Five-week old C57BL/6J male mice (CRIFA, Barcelona, Spain) weighing 16–18 g were housed under a 12-h:12-h light-dark cycle in a temperature-controlled room (22 °C), with standard food and water available *ad libitum*, in accordance with the European Communities Council Directive (86/609/EEC) for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Committee on Animal Research and Ethics of the CEU-San Pablo University (SAF2009-09714). Animals were divided in two groups with similar average BW, housed five per cage, and assigned either to a standard diet (SD; Teklad Global 18% Protein Rodent Diet; 18% kcal from fat, 58% kcal from carbohydrates and 24% kcal from protein; 3.3 kcal/g) or to a high-fat diet (HFD; TestDiet, England, D12451; 45% kcal from fat, 35% kcal from carbohydrates and 20% kcal protein; 4.73 kcal/g). Both BW and food intake were monitored once a week until the end of the treatment.

### 2.2. Behavioral testing

#### 2.2.1. Novel object location recognition task

Animals used in this test (NLR) received the dietary treatment either during 48 h (SD, n = 10; HFD, n = 10), 1 (SD, n = 7; HFD, n = 9), 4 (SD, n = 7; HFD, n = 8) or 8 weeks (SD, n = 10; HFD, n = 10). Different cohorts for each length of treatment were used. The NLR was performed following a previously described protocol [16]. Briefly, the assay was carried out in a black open-field box made of wood (25 cm length, 25 cm width, 25 cm high). The stimuli presented were two identical copies of an object composed of Lego pieces (Lego UK, Slough, UK), which were heavy enough to avoid displacement during testing. The test was organized in two phases: (1) Exploration session: animals were allowed (10 min) to freely explore the box that contained the two objects, each placed 5 cm from top left and right corners. These objects will be referred from now as familiar (F) and novel (N) location, respectively. Animals were always introduced in the box with one's back to the object and were returned to their home cage after the session; (2) Retention session: Five-min retention sessions were performed 1 h and 24 h after the exploration session. During the first retention session (1 h after exploration session), F remained in identical position than in the exploration session while N was presented 5 cm from the bottom right corner. In the second retention session (24 h after the first retention session) N was presented 5 cm from the bottom left corner. Mice activity was evaluated by i) measuring the total time spent in the objects area, and ii) counting the number of contacts with objects. In all sessions, time interacting with objects (IT) was quantified. A single interaction was counted when the animal approached at least 2 cm to the object. Discrimination ratios (ITDr) were calculated by using the algorithm $ITDr = IT_N/(IT_N + IT_F)$, where $IT_N$ corresponds to IT with N, and $IT_F$ corresponds to the IT with F. Spatial discrimination was considered to be consistent when ITDr > 0.5 in the retention sessions. Animals showing preference for any location (more than 50% of time exploring one of the objects) during the exploration session were eliminated.

#### 2.2.3. Open field test

After eight weeks of dietary treatment, cohorts of SD and HFD-treated mice (n = 20 each) were tested in the open field test (OFT). Mice were placed individually in the middle of the open field apparatus consisting of a wooden box (40 cm width × 60 cm length × 50 cm height) divided into 12 equal squares and containing four different objects, and allowed to freely explore the field during 5 min. The number of squares crossed, the number of times that mice contacted with objects in the field, which reflects the exploratory activity, and the frequency of rearing and grooming behavior were measured. Crossings were recorded only when both hind paws entered one of the squares. The total distance run by the animals was estimated from the number of crossings. After each test, the open-field was cleaned with 10% ethanol, and then dried with a dry cloth.

#### 2.2.4. Elevated plus-maze test

Two weeks after the OFT, mice were tested in the elevated plus maze (EPM). In this case, mice were randomly assigned either to saline or to diazepam (DZ; 2 mg/kg, i.p.; Sigma-Aldrich, Spain) treatment 30 min before the assay. The experimental groups were SD/saline (n = 8), SD/DZ (n = 6), HFD/saline (n = 8), and HFD/DZ (n = 9). The EPM apparatus consisted of two opposing open arms (10 cm width × 50 cm length each) and two perpendicular opposing walled arms (10 width × 50 length × 16 cm height each) and was made of high-density black polyethylene. The apparatus was mounted 50 cm above the floor. The assay is based on the natural conflict of rodents to explore novel environments together with their innate aversion to open spaces. As a consequence, animals spend more time in the closed arms [26]. Behavior was scored by counting the time-in-open and the number of open entries during a 5-min period. An arm was scored as entered if the animal's head and the two front feet crossed a boundary line between the center region and that arm.

#### 2.2.5. Forced swimming test

Other cohorts of SD and HFD mice were subject to the forced swimming test (FST) after 48-h HFD and retested after eight additional weeks of dietary treatment. The FST is commonly used to test antidepressant drugs [27]. In both cases, SD and HFD animals received either saline or fluoxetine (FX; 10 mg/kg, i.p.; Tocris, USA) 30 min before the assay. Animals that received fluoxetine in the first task were saline-administered in the second and animals that received saline in the first task were fluoxetine-administered in