Food & Function

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Journal:	*Food & Function*
Manuscript ID	FO-ART-02-2020-000486.R1
Article Type:	Paper
Date Submitted by the Author:	03-May-2020
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(-)-Epicatechin mitigates high fat diet-induced neuroinflammation and altered behavior in mice

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Running title: (-)-Epicatechin mitigates neuroinflammation

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Abbreviations:

BDNF, brain-derived neurotrophic factor; CNS, central nervous system; EC, (-)-epicatechin; HFD, high fat diet; LPS, lipopolysaccharides; iNOS, inducible nitric oxide synthase; Iba-1, ionized calcium binding adaptor molecule 1; NOX, NADPH oxidase; NOR, novel object recognition; MWM, Morris water maze; OLM, object location memory; TLR4, Toll-like receptor 4; TNFα, tumor necrosis factor alpha;

Keywords: obesity, hippocampus, inflammation, memory, epicatechin, high fat diet

Abstract

Obesity is characterized by a condition of low-level chronic inflammation that can lead to altered cognition and behavior. The flavanol (-)-epicatechin (EC) has been shown to have anti-inflammatory actions in mouse models of diet-induced obesity. This study investigated the capacity of dietary EC to mitigate hippocampal inflammation and impaired memory in high fat diet (HFD)-fed mice. Healthy 6 weeks old male C57BL/6J mice (10 mice/group) were fed for 13 weeks either: a control diet (10% total calories from fat), a high fat diet (60% total calories from fat), or the control and high fat diets supplemented with 20 mg EC/kg body weight. Short-term object recognition memory was evaluated by the novel object recognition (NOR) task and spatial memory by the object location memory (OLM) task and the Morris water maze (MWM). After 13 weeks on the dietary treatments, HFD-fed mice developed obesity, which was not affected by EC supplementation. HFD consumption caused metabolic endotoxemia, and increases in parameters of hippocampal inflammation, i.e. mRNA levels of TLR4, Iba-1, and NOX4. All these changes were mitigated by EC supplementation. EC supplementation also significantly improved recognition memory in HFD-fed mice while neither HFD consumption nor EC supplementation affected mouse spatial memory. Overall, EC supplementation prevented short-term recognition memory impairment in HFD-induced obese mice, which could be in part due to the capacity of EC to mitigate metabolic endotoxemia and associated hippocampal inflammation and oxidative stress.

Introduction

Obesity has become a worldwide epidemic, and its incidence is rising at an alarming rate [1]. Chronic low-grade inflammation is an important characteristic of obesity. This chronic inflammatory condition contributes to the development of obesity-associated comorbidities, including cardiovascular disease, type 2 diabetes, insulin resistance, and cancer, resulting in serious health burdens and incalculable social and medical costs [2-5]. Moreover, in humans and rodents, obesity has been associated with increased occurrence of disorders in the central nervous system (CNS), such as mild cognitive impairment, dementia, and Alzheimer's disease [6-11]. Altered structure and function of the hippocampus, a region of the brain critical for learning and memory, is observed in obese humans and rodents [12-16]. For instance, a higher body mass index (BMI) was linked to hippocampal atrophy in males and females (60-64 y age), with those with higher BMI experiencing greater hippocampal atrophy upon an 8 year follow up [14].

Obesity and consumption of high fat/high sugar diets are contributing factors for metabolic endotoxemia, which is defined as 0.5- to 2-fold increase in circulating bacterial lipopolysaccharides (LPS) in the circulation [17, 18]. Endotoxemia is a potent trigger of inflammation that can affect both the periphery and the CNS. LPS can induce neuroinflammation by compromising the function of the blood-brain barrier, a barrier that tightly regulates exchanges of molecules between the peripheral blood and the CNS [19, 20]. In obesity, chronic inflammation and increased circulating levels of metabolites and proinflammatory molecules (e.g. fatty acids, glucose, cytokines and LPS) can affect the functioning of the barrier [13, 21-24]. In fact, the blood-brain barrier at the hippocampus is disrupted in mice chronically fed a high fat diet (HFD), which is proposed to cause neuroinflammation and impaired memory [13, 24].

While consumption of HFD and obesity can have a detrimental impact on the brain, dietary components may be able to mitigate these harmful effects. Epidemiological evidence suggests that consumption of cocoa flavanols can improve cognitive function including hippocampal-dependent

memory in humans [25-27]. (-)-Epicatechin (EC) is one of the most abundant flavanols found in cocoa products and one of the most widely consumed flavanols by humans [28]. EC consumption mainly derives from tea and cocoa products, and fruits such as grapes, berries, and apples. In humans, the effect of pure EC on cognition has not yet been investigated. In mice, EC improved cerebrovascular function, hippocampal angiogenesis, neuronal spine density, and spatial memory [29]. Thus, EC emerges as a dietary bioactive that may have beneficial effects on behavior and cognition.

Although there are studies implicating a relationship between obesity and cognitive impairment; and indicating beneficial neuroprotective effects of EC, there are no previous studies investigating the neuroprotective actions of EC in both obese humans and animal models of obesity. In humans, improvements in cognition in an elderly population was attributed in part to an improvement in insulin sensitivity [30]. We previously showed that dietary EC supplementation mitigates obesity- and high fructose/high fat diet-induced inflammation and insulin resistance in mice [31-35]. However, the anti-inflammatory capacity of EC in mitigating obesity-induced neuroinflammation in the hippocampus and in improving obesity-associated cognitive changes have not yet been characterized. This paper investigated if dietary supplementation with EC can mitigate hippocampal neuroinflammation and improve behavior in mice fed a HFD. EC supplementation prevented HFD-induced metabolic endotoxemia and increases in parameters of inflammation and oxidative stress, improving mouse performance on the novel object recognition task.

Materials and methods

Animals and animal care

All procedures were in agreement with standards for the care of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals. All procedures were administered under the

auspices of the Animal Resource Services of the University of California, Davis. Experimental protocols were approved before implementation by the University of California, Davis Animal Use and Care Administrative Advisory Committee.

Healthy male C57BL/6J mice (20-25 g) (10 mice/group) were fed for 13 weeks either: A- a diet containing approximately 10% total calories from fat (C) (TD.06416, Envigo, Indianapolis, IN), B- a diet containing approximately 60% total calories from fat (lard) (HFD) (TD.06414, Envigo, Indianapolis, IN), C- the control diet supplemented with 20 mg EC/kg body weight (CE), and D- the HFD supplemented with 20 mg EC/kg body weight (HFE). The composition of the control and the high fat diets is listed in **Supplemental Table 1**. The EC-containing diet was prepared every two weeks to account for changes in body weight and food intake, and to prevent potential EC degradation. All diets were stored at -20°C until use. The highest amount of EC supplemented has been found to improve insulin resistance in rats fed high fructose levels [34] and in mice fed a HFD [31]. In comparison to EC intake in human populations [36], the amount of EC supplementation is relatively high. However, it can be reached by supplementation or consumption of select EC-rich fruits/vegetables and derivatives [28].

Body weight and food intake were measured weekly throughout the study as previously described [31]. After 13 weeks on the dietary treatments, and after 4 h fasting, mice were euthanized by cervical dislocation. Blood was collected from the submandibular vein into tubes containing EDTA, and plasma collected after centrifugation at 3,000 x g for 10 min at room temperature. Tissues were dissected and flash frozen in liquid nitrogen and then stored at -80°C for further analysis.

Determination of plasma metabolic parameters.

Plasma LPS levels were determined using a kit from Abbexa (Abbexa, Cambridge, UK) and following the manufacturer's protocol. Triglyceride concentrations were determined using kits purchased from Wiener

Lab Group (Rosario, Argentina) and glucose concentrations using a kit from Sigma-Aldrich Co (St. Louis, MO), following the manufacturer's protocols.

RNA isolation and real-time PCR (RT-PCR)

For quantitative RT-PCR studies, RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA was generated using high-capacity cDNA Reverse Transcriptase (Applied Biosystems, Grand Island, NY). Expressions of β-Actin, BDNF (brain-derived neurotrophic factor); Iba-1 (ionized calcium binding adaptor molecule 1); iNOS (inducible nitric oxide synthase), NOX (NADPH oxidase) 2 and 4; TLR4 (Toll-like receptor 4) and TNFα (tumor necrosis factor alpha) were assessed by quantitative real-time PCR (iCycler, Bio-Rad, Hercules, CA) with the primers listed in **Table 1**.

Cognitive Function Test

Behavioral tests were performed between week 10 and 12 of the dietary intervention. After being exposed to the diets for 10 weeks, each animal was habituated in a white, square arena (40x40 cm) where the animal was naïve to, for 15 minutes each day for two consecutive days. Next day, short-term object recognition memory was evaluated using the novel object recognition (NOR) task. On the following day, short-term spatial memory was evaluated with the object location memory (OLM) task. At week 11, animals started training for the Morris water maze (MWM) to be evaluated for spatial learning and reference memory. Animals were acclimated to a behavioral testing room separate from the housing room at least 1 hour prior to all handlings and behavioral tests. All objects and arena were cleaned with 70% ethanol after each trial. The pool used for the MWM was emptied and cleaned daily.

Novel object recognition (NOR) and object location memory (OLM) tasks. For both tasks, each animal was allowed to explore two identical unfamiliar objects (*A, A'*) in the square arena described above for 5 minutes (sample phase). After being placed in the home cage for 1 hour (retention phase), mice were reintroduced to the arena for 5 minutes (test phase). For NOR task, one of the objects was changed to a novel object during the test phase (*A, B*). For OLM task, location of one of the objects was changed to a novel location (*A, B*) and each arena had spatial cues made with construction papers mounted on the north and west side of walls. The time that each animal spent directly sniffing or whisking towards the familiar and the novel objects or locations was analyzed by blinded investigators. A preference index, a ratio of the amount of time spent exploring one of the identical object (*A'*) in the sample phase or the novel object/location (*B*) in the test phase over the total amount of time spent exploring both objects was used to determine preference for novelty (*A'/(A + A'*) x 100% or *B/(A + B*) x 100% respectively)[37, 38]. A preference index above 50% indicates preference for novel object or location, below 50% for familiar object or location, and 50% null preference. Animals that did not spend more than 10 seconds total exploring both objects during the sample and the testing phases were excluded from analysis.

Morris water maze (MWM). Spatial learning and reference memory were assessed in a circular pool of 120 cm diameter containing water to a depth of 40 cm. The water temperature was controlled at 23±1°C. After every training and trial, each animal was gently scooped out of the pool, placed in a heated holding cage, and returned to the home cage. The pool was virtually divided into four quadrants: northeast (NE), northwest (NW), southeast (SE), and southwest (SW).

(1) Handling (MWM day 0): mice were introduced to water for the first time. Each animal was allowed to swim in a clear plastic cage (23.5 × 14 × 13 cm) containing water to a depth of 0.5 cm for 20 seconds. Afterwards, the animal was transferred to a cage filled with a depth of 1 cm water for 20 seconds and then to a cage filled with a depth of 2 cm water for 20 seconds.

(2) Pre-training (MWM day 1): mice were introduced to the pool (diameter 120 cm) described above and a plexiglass platform (10 cm top diameter). Each animal was placed on the platform, which was located in the center of the pool and 1 cm above the surface of the water, for 15 seconds. Afterwards, the animal was allowed to swim freely for 30 seconds. Then, the animal was guided to climb on the platform and to stay there for 30 seconds.

(3) Visible platform task (MWM day 2-3): non-spatial training was conducted to ensure that non-cognitive effects were not interfering with upcoming water maze performance. White curtains were hung around the pool to obscure any spatial cues in the room. Both locations of starting point of mice and platform were moved to new locations in each trial. The platform was 1 cm above the surface of the water and mounted with a flag that reached a height of 13 cm. Each animal was gently placed into the pool and allowed to swim freely for 60 seconds. Once the animal located the platform, the animal was allowed to stay on there for 20 seconds. If the animal failed to locate the platform within 60 seconds, experimenters gently scooped the animals with a net and placed the animal on the platform for 20 seconds. Visible platform task was conducted 5 times daily with a 1 hour intertrial interval.

(4) Hidden platform task (MWM days 4-7): large and high-contrast geometrical patterns made with construction papers were mounted on the walls of the testing room to serve as distant spatial landmarks. The platform was hidden from the mice; it was submerged 1 cm below the surface of the water, which was rendered opaque with non-toxic, white, powdered tempera paint. Starting point was moved to a new location for each trial while the location of the platform stayed in the center of the southwest (SW) quadrant throughout all trials. Hidden platform task was conducted 5 times daily with a 1 hour intertrial interval. Learning curves of the animals were analyzed by measuring time spent to reach the platform (escape latency) using EthoVision XT 13 (Noldus, Wageningen, The Netherlands).

(5) Probe trial (MWM day 8): the testing environment for probe trial was the same as the hidden platform task except there was no platform placed in the pool. For this one-time trial, each animal was allowed to swim freely for 60 seconds. Spatial memory was analyzed by measuring the time spent by the animals in the target quadrant (SW) using EthoVision XT 13.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.04 (GraphPad Software, Inc., San Diego, CA). Pearson correlation analyses were conducted to assess relationships between plasma endotoxin concentration and a-TLR4 mRNA and b-TNFα mRNA levels, and between TLR4 mRNA and TNFα mRNA levels. Tests for interaction were performed by two-way analysis of variance (ANOVA) and post-tested using Fisher's Least Significant Difference (LSD). Data were subsequently analyzed by one-way analysis of variance (ANOVA), and the Fisher's LSD was used to examine differences between group means. Within group performance of NOR and OLM was evaluated with two-tailed paired t-test. Differences were considered statistically significant at $p<0.05$. Data are shown as mean ± SEM.

Results

Animal outcome

Daily food intake in the groups fed the HFD was significantly lower than in those fed the control diets (**Fig. 1A**) while the calorie intake was similar among groups (**Fig. 1C**). Compared to the control diet, consumption of the HFD caused a higher increase in body weight gain, which became significant after only one week on the diet (**Fig. 1B**). After 13 weeks on the HFD, body weight was 24% higher than in the controls. This was accompanied by a 2.3- and 2.6-fold increase in the visceral fat pad weight in mice fed

the HFD or the HFD supplemented with EC, respectively (**Fig. 1C**). Supplementation with EC did not affect body weight gain neither in mice fed the control nor the HFD. Hippocampal tissue weights were similar among groups. Ratio of hippocampus weight to body weight was similar for the HF and HFE groups (0.50 ± 0.05mg and 0.50 ± 0.03mg respectively).

EC supplementation attenuates parameters of neuroinflammation in the hippocampus from HFD-fed mice

To evaluate neuroinflammation, we measured the mRNA levels of TLR4, Iba-1, and TNFα in the hippocampus by RT-PCR (**Fig. 2A**). Consumption of the HFD did not affect hippocampal TNFα mRNA content. On the other hand, Iba-1 mRNA levels were 16% higher in HF mice than in controls, and EC supplementation prevented these increases. A significant interaction between EC supplementation and diet on TLR4 mRNA levels was observed (interaction from two-way ANOVA: $p<0.04$). TLR4 mRNA levels were 52% higher in HF mice than in controls, and EC supplementation also fully prevented these increases.

We also measured enzymes involved in inflammation and reactive nitrogen/oxygen species (nitric oxide, superoxide anion, H_2O_2) production, i.e. NOX2, NOX4, and iNOS (**Fig. 2B**). NOX2 and iNOS mRNA levels were similar among groups, while 44% higher NOX4 mRNA levels were observed in the HF group compared to controls, which was prevented by EC supplementation.

EC supplementation prevents HFD-induced metabolic endotoxemia

Consumption of the HFD caused metabolic endotoxemia in mice. LPS concentration in plasma was 32% higher in HF than in control mice, and EC supplementation prevented this increase (**Fig. 3A**). There was a strong positive correlation (r: 0.57, p=0.001) between plasma endotoxin concentration and TLR4 mRNA levels in the hippocampus (**Fig. 3B**). Although hippocampal TNFα mRNA levels were similar among

groups, a significant correlation was observed between TNFα mRNA levels and i) plasma endotoxin (r: 0.41, p<0.03) (**Fig. 3C**) and ii) TLR4 (r: 0.58, p<0.0004) (**Fig. 3D**) mRNA levels.

EC supplementation improves novel object recognition memory in HFD-fed mice while did not affect spatial memory and learning

The NOR task was conducted to assess the short-term recognition memory of mice. During the sample phase, all groups spent a comparable amount of time exploring each of the two identical objects (**Fig. 4A**). Comparing within group preference index of the sample and test phase, all groups significantly preferred the novel object (**Supplemental Fig. 1A**). During the test phase, a significant interaction between EC supplementation and diet on NOR performance was observed (interaction from two-way ANOVA: p<0.03). EC supplemented HF group (HFE) exhibited greater novel object preference compared to the HF group as measured by the preference index (**Fig. 4A**). This demonstrates the greater ability of the EC supplemented group in recognizing the novel object from the familiar object.

EC supplementation did not affect spatial memory and learning. OLM task was conducted to assess short-term spatial memory of mice. All groups performed similarly in both sample and test phases as measured by the preference index (**Fig. 4B**). Interestingly, no significant within group changes in the preference index were observed in all C, CE, HF, HFE groups, indicating that there was no overall novel location preference (**Supplemental Fig. 1B**). Spatial learning was also evaluated with Morris water maze with the hidden platform task. Comparing the first (MWM day 4) and the last day (MWM day 7) of the hidden platform task, all groups found the hidden platform more quickly (**Fig. 5A**). On the last day of the hidden platform task, all groups had similar escape latencies exhibiting comparable spatial learning (**Fig. 5A**). Similarly, all groups spent a comparable amount of time in the target southwest quadrant zone during the probe trial, indicating no differences in spatial reference memory among groups (**Fig. 5B**).

EC supplementation increases the expression of BDNF

We next measured mRNA levels of BDNF, a promoter of neuronal differentiation and survival and important mediator of synaptic plasticity [39], in the hippocampus. Consumption of the HFD did not affect hippocampal BDNF mRNA content. However, BDNF mRNA levels were 90 and 76% higher in the CE and HFE groups compared to the control group (**Fig. 5C**).

Discussion

This work showed that supplementation with EC improves parameters of neuroinflammation and impaired behavior in HFD-induced obese mice. Thus, consumption of the HFD caused metabolic endotoxemia and upregulation of hippocampal neuroinflammatory markers, i.e. TLR4, Iba-1, and NOX4, in association with impaired recognition memory. EC supplementation prevented all these changes, supporting a potential benefit of an EC-rich diet on obesity-induced inflammation and altered behavior.

The HFD induced significant increase in body weight and fat pads weight in mice after 13 weeks which was not prevented by EC supplementation. The increase in adiposity in the HF and HFE groups despite of the similar caloric intake among all four groups could be explained by the fact that different macronutrients exert differential effects on the thermic effects of food [40]. Fat is less thermogenic than carbohydrates and proteins and thus can prompt greater positive energy balance in the body compared to carbohydrates and proteins when consumed over time [41-45].

EC supplementation prevented the metabolic endotoxemia associated with HFD consumption. This finding is in agreement with a similar observation in mice fed the HFD for 15 weeks [33], in which the metabolic endotoxemia was associated with an increase in intestinal permeability. EC protects the intestinal barrier from permeabilization by inhibiting HFD-associated down regulation of tight junction

proteins and by modulating signaling pathways that promote tight junction opening. In this regard, EC prevents both TNFα and bile acid-induced Caco-2 monolayer permeabilization by inhibiting NF-κB and ERK1/2 [33, 46, 47]. On the other hand, other mechanisms could be involved in HFD-induced metabolic endotoxemia. In this regard, LPS is also transported through intestinal epithelial cells and into the lymph, upon incorporation and secretion into chylomicrons [48]. Once in the circulation, endotoxins can reach different organs and initiate pro-inflammatory responses. Endotoxins bind to the TLR4 to initiate a cascade of events that leads to the activation of, among other signals, transcription factors NF-κB and AP-1 that increase the expression of proinflammatory molecules [49]. The increased expression of TLR4 in the hippocampus of HFD-mice suggests the activation of this pathway. This is paralleled by a pro-inflammatory condition as evidenced by an increased expression of Iba-1, a protein participating in microglia endocytosis, and a trend for higher TNFα expression. This is further supported by positive correlations among plasma endotoxin levels, TLR4 and TNFα expression. Overall, the capacity of EC to prevent metabolic endotoxemia and suppress TLR4 and Iba-1 upregulation supports a link between metabolic endotoxemia and neuroinflammation. It also underscores the health relevance of the actions of EC at the gastrointestinal tract, where it prevents endotoxin transport into the circulation [33].

While EC-mediated decrease in metabolic endotoxemia can be a relevant mechanism in EC's capacity to mitigate high fat diet-induced neuroinflammation, other potential contributing mechanism is a direct action of EC and/or EC metabolites at the level of the brain. In humans, 95% of EC is absorbed either as EC, structurally related EC metabolites (SREM) (glucuronyl, methyl and/or sulphated EC derivatives) or as smaller metabolites generated after EC metabolism by the microbiota[50]. SREM, mainly catechin and EC glucuronidated derivatives, were measured in the brain of Tg2576 AD transgenic mice consuming a flavan-3-ol-rich grape powder [51]. When a synthetic SREM (3-O-Me-EC-5-O-β-glucuronide) was added to brain slices from Tg2576 AD mice, it improved basal synaptic transmission and long-term potentiation[51]. In mice fed EC for 13 days, both EC and 3'-O-methyl-(-) EC were detected in perfused

brains [52]. This was associated to an improvement in the retention of spatial memory that was attributed to increased angiogenesis[52]. Recently, the main EC microbiota-derived metabolite 5-(hydroxyphenyl)-γ-valerolactone-sulfate was found in mouse brain after 5-(hydroxyphenyl)-γ-valerolactone i.p. injection, and in rat and pig brain upon consumption of EC-rich foods[53]**.** Overall, although evidence is limited, EC and SREM can reach the brain where they could have direct protective effects against neuroinflammation.

NADPH oxidase is one of the key producers of cell reactive oxygen species, in particular of superoxide anion and H_2O_2. Although adequate amounts of select oxidants are critical for normal brain function such as hippocampal long-term potentiation [54], excessive generation of oxidants can result in oxidative stress [55]. Oxidative damage to cellular components may play a role in the development of cognitive impairment associated with CNS disorders [56-58]. For instance, postmortem brain tissue analysis of Alzheimer's disease patients showed an upregulation of the NOX cytosolic subunits p67phox, p47phox, and p40phox and an increase in NOX enzymatic activity [56]. In our study, EC mitigated HFD-induced increased expression of NOX4 in the hippocampus while neither HFD nor EC supplementation affected iNOS and NOX2 mRNA levels. Consumption of a HFD increases the expression of Iba-1 and NOX subunits and the activity of NOX in mouse brain compared to their age-matched controls [59]. Also, a HFD induces protein oxidation in the hippocampus of aged mice and impairs their cognitive performance in a T-maze [58]. The observed capacity of EC to regulate hippocampal regulation of NOX4 expression is in agreement with its capacity to modulate the expression and activity of several NOX isoforms and mitigate oxidative stress [60]. Collectively, current evidence suggests a relationship between oxidative stress and neuroinflammatory status in obesity that can be mitigated by consumption of EC.

Growing evidence indicates that diet-induced obesity contributes to neuroinflammation, as well as to cognitive dysfunction in rodent models [13, 16, 24, 61-63]. Both NOR and OLM tasks are based on rodents' innate tendency to explore a novel stimulus. The increased time spent exploring the replaced or relocated

object during the test phase suggests their ability to remember what type of object they were previously exposed to or where the object was previously located during the sample phase. All C, CE, HF, HFE groups had no significant novel location preference shown in OLM task, while novel object preference was observed in NOR task in all groups. It is possible that the spatial cues chosen for OLM were not recognizable or distinguishable by mice to spatially differentiate the two identical objects or the animals were simply not interested in the chosen objects [38, 64]. It is also plausible that they needed a shorter retention phase or longer time to explore the objects in the arena[64]. In the current study, consumption of the HFD did not cause large effects on learning and memory. In terms of HFD-mediated cognitive impairment, it is possible that in our study the HFD did not induce cognitive impairment due to its duration. Indeed, it is suggested that specific effects of a HFD on cognition are dependent on the duration of dietary exposure [22]. For instance, while 5 weeks on a HFD (60% kcal from fat) did not impair object recognition memory in mice [65], 21 weeks on a HFD (40% kcal from fat) impaired recognition memory [62]. In terms of the MWM, we observed that HFD-fed mice performed similarly to the control group in both the hidden platform task and the probe trial. It has been reported that mice fed a HFD (60% kcal from fat) display impaired spatial memory compared to mice fed control diets, but the duration of the HFD was longer than in our study. For instance, impaired spatial memory was observed in mice after consumption of HFD for 19 weeks [61], 20 weeks [13], and 5 months [66]. Therefore, future studies with longer durations of HFD consumption are warranted to investigate the effects of EC on HFD-induced impaired learning and memory in mice.

EC supplementation significantly improved recognition memory in HFD-fed mice. Several studies have characterized the effects of EC-rich cocoa on cognition in humans [30, 67]. In elderly individuals with mild cognitive impairment, consumption of cocoa flavanols improved cognitive functions [30, 67]. So far, most of the effects of EC on cognition have been attributed to improvements in blood flow [68]. However, EC and/or its metabolites could also act through the mitigation of HFD/obesity-induced neuroinflammation

and through the observed increase in hippocampal BDNF. BDNF is a member of the neurotrophin family of growth factors crucial for the differentiation and survival of neurons. BDNF plays a critical role in the induction of hippocampal long-term potentiation, a form of synaptic plasticity considered to underlie learning and memory [39]. BDNF deletion within dorsal hippocampus of mouse impairs learning and memory in novel object recognition task and Morris water maze [69]. In humans, it has been proposed that circulating BDNF may be used as a biomarker for select psychiatric disorders [70]. Circulating and brain BDNF levels are found to be increased upon flavanol supplementation in rodents and humans. An effect of EC on BDNF metabolism is supported by findings in humans consuming EC-rich cocoa [71]. High serum levels of BDNF were found in a population of males and females (62-75 y of age) consuming 494 mg flavanols/d for 12 weeks. The increase in serum BDNF was correlated with an improvement in global cognitive performance. Adult male mice fed a control diet and administered with 4 mg EC/day for 14 weeks show a decrease in anxiety assessed in open field and elevated plus maze tests, and an increase in BDNF hippocampal levels [72]. Although we also observed an increase in hippocampal BDNF mRNA levels in the EC supplemented groups, we did not observe significant changes in the open field behavior among groups (data not shown). On the other hand, the 7-times lower EC intake in our experimental model compared to this previous study [72], can explain the differential response. Overall, given the relevant role of BDNF in neurogenesis and in supporting brain physiology, future studies investigating the mechanisms of brain BDNF increase by EC will be of outmost relevance. It is important to mention that other flavonoids can have neuroprotective actions. However, rather than on obesity-associated altered behavior, most studies were focused on ageing-related cognitive dysfunction[73]. In this regard, high consumption of green tea, which contains catechin, epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG), was associated with a lower prevalence of cognitive impairment in Japanese subjects aged ≥70 y, as assessed by Mini-Mental State Examination[74, 75].

In summary, EC supplementation improved recognition memory and mitigated neuroinflammation in a model of diet (HFD)-induced obesity in mice. Among the underlying mechanisms, we provide evidence that EC can in part act by promoting BDNF upregulation and by preventing obesity-induced metabolic endotoxemia and the associated activation of pro-inflammatory responses and oxidative stress in the hippocampus. Further studies, particularly in obese humans, will be essential to support the concept that consumption of EC-rich foods could contribute to improve behavior and cognition in obesity.

Acknowledgements

This work was supported by H.E. Jastro awards to J.K. and grant NIFA-USDA (CA-D*-NTR-7244-H) to P.O. P.O. is a correspondent researcher from CONICET, Argentina.

Author contributions

J.K. and Z.W. ran all experiments. P.I.O. designed the study. J.K. and P.I.O. wrote the manuscript. All authors revised the article, critically reviewed it for intellectual content, and approved the final version.

Legend to Figures

Figure 1. Effects of supplementation with EC on mice general outcome. A- Food intake and **B-** body weight gain. Mice were fed a control diet (empty circles), the control diet supplemented with 20 mg EC/kg body weight (empty triangles), a HFD (black circles), or the HFD supplemented with 20 mg EC/kg body weight (blue triangles). Body weight was measured weekly. * Differences between the HF and control body weight gain values are significant between week 1 and 13 on the diets ($p<0.05$, two-way ANOVA with Fisher's LSD). **C**- Mice general outcome parameters after 13 weeks on the diets. Results are shown as means ± SEM and are the average of 6-10 animals/group. Values having different superscripts are significantly different ($p<0.05$, two-way ANOVA with Fisher's LSD).

Figure 2. EC supplementation improves parameters of inflammation and oxidant production in the hippocampus. A, B- TLR4, Iba-1, TNFα, iNOS, NOX2 and NOX4 mRNA levels in the hippocampus were determined by RT-PCR and the relative gene expression was normalized to β-actin as housekeeping gene. Determinations were done after 13 weeks on the respective diets. Results are shown as mean ± SEM of 6-10 animals/group Data were normalized to control values. Values having different superscripts are significantly different ($p<0.05$, two-way ANOVA with Fisher's LSD).

Figure 3. Effects of EC supplementation on HFD-induced endotoxemia. A- Plasma endotoxin concentration on week 13 on the respective diets. Results are shown as mean ± SEM of 6-10 animals/group. Values having different superscripts are significantly different ($p<0.05$, two-way ANOVA with Fisher's LSD). **B-D-** Correlations between **B, C**-plasma endotoxin concentration and **B**- TLR4 mRNA,

C-TNFα mRNA levels. **D**- Correlation between TLR4 and TNFα mRNA levels. Solid line represents the regression line and dashed lines delineate the 95% confidence band.

Figure 4. Effects of EC supplementation on short-term recognition memory and spatial memory. For both novel object recognition and object location memory tasks, animals explored two identical unfamiliar objects for 5 minutes (sample phase). After being placed in the home cage for 1 hour (retention phase), they were reintroduced to the arena for 5 minutes (test phase). **A-** EC supplemented HF group (HFE) exhibited greater novel object preference compared to the HF group as measured by the preference index. **B-** All groups performed similarly in both sample and test phases as measured by the preference index. Neither HFD nor EC supplementation affected spatial memory in the object location memory task. Dashed lines delineate 50% null preference. Results are shown as mean ± SEM of 8-10 animals/group. Values having different superscripts are significantly different ($p<0.05$, two-way ANOVA with Fisher's LSD).

Figure 5. Effects of EC supplementation on spatial learning and memory and levels of BDNF in the hippocampus. A- Learning curves of mice in the hidden platform task and **B-** Time spent in each quadrant during the probe trial. **C-** BDNF mRNA levels were determined by RT-PCR and the relative gene expression was normalized to β-actin as housekeeping gene. Results are shown as mean ± SEM of 6-10 animals/group. Data were normalized to control values. Values having different superscripts are significantly different ($p<0.05$, two-way ANOVA with Fisher's LSD).

Supplemental Figure 1. Within group performance of NOR and OLM. A- C, CE, HF, and HFE groups all significantly preferred the novel object during the testing phase. **B-** No significant within group changes in the preference index was observed in all groups, indicating no overall novel location preference. Dashed lines delineate 50% null preference. Results are shown as mean ± SEM of 8-10 animals/group (*p<0.05, paired t-test).

References

1. N. R. F. C. (NCD-RisC), Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants, *The Lancet*, 2016, **387**, 1377-1396.
2. R. Monteiro and I. Azevedo, Chronic inflammation in obesity and the metabolic syndrome, *Mediators Inflamm*, 2010, **2010**, 289645.
3. M. S. Ellulu, I. Patimah, H. Khaza'ai, A. Rahmat and Y. Abed, Obesity and inflammation: the linking mechanism and the complications, *Arch Med Sci*, 2017, **13**, 851-863.
4. X. Pi-Sunyer, The medical risks of obesity, *Postgrad Med*, 2009, **121**, 21-33.
5. D. Withrow and D. A. Alter, The economic burden of obesity worldwide: a systematic review of the direct costs of obesity, *Obes Rev*, 2011, **12**, 131-141.
6. L. B. Hassing, A. K. Dahl, N. L. Pedersen and B. Johansson, Overweight in midlife is related to lower cognitive function 30 years later: a prospective study with longitudinal assessments, *Dement Geriatr Cogn Disord*, 2010, **29**, 543-552.

7. E. Pedditzi, R. Peters and N. Beckett, The risk of overweight/obesity in mid-life and late life for the development of dementia: a systematic review and meta-analysis of longitudinal studies, *Age Ageing*, 2016, **45**, 14-21.

8. R. A. Whitmer, D. R. Gustafson, E. Barrett-Connor, M. N. Haan, E. P. Gunderson and K. Yaffe, Central obesity and increased risk of dementia more than three decades later, *Neurology*, 2008, **71**, 1057-1064.

9. M. F. Elias, P. K. Elias, L. M. Sullivan, P. A. Wolf and R. B. D'Agostino, Obesity, diabetes and cognitive deficit: The Framingham Heart Study, *Neurobiol Aging*, 2005, **26 Suppl 1**, 11-16.

10. M. Cournot, J. C. Marquie, D. Ansiau, C. Martinaud, H. Fonds, J. Ferrieres and J. B. Ruidavets, Relation between body mass index and cognitive function in healthy middle-aged men and women, *Neurology*, 2006, **67**, 1208-1214.

11. S. Sabia, M. Kivimaki, M. J. Shipley, M. G. Marmot and A. Singh-Manoux, Body mass index over the adult life course and cognition in late midlife: the Whitehall II Cohort Study, *Am J Clin Nutr*, 2009, **89**, 601-607.

12. P. L. Yau, M. G. Castro, A. Tagani, W. H. Tsui and A. Convit, Obesity and metabolic syndrome and functional and structural brain impairments in adolescence, *Pediatrics*, 2012, **130**, e856-864.

13. B. T. Jeon, E. A. Jeong, H. J. Shin, Y. Lee, D. H. Lee, H. J. Kim, S. S. Kang, G. J. Cho, W. S. Choi and G. S. Roh, Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet, *Diabetes*, 2012, **61**, 1444-1454.

14. N. Cherbuin, K. Sargent-Cox, M. Fraser, P. Sachdev and K. J. Anstey, Being overweight is associated with hippocampal atrophy: the PATH Through Life Study, *Int J Obes (Lond)*, 2015, **39**, 1509-1514.

15. J. M. Moreno-Navarrete, G. Blasco, J. Puig, C. Biarnes, M. Rivero, J. Gich, F. Fernandez-Aranda, J. Garre-Olmo, L. Ramio-Torrenta, A. Alberich-Bayarri, F. Garcia-Castro, S. Pedraza, W. Ricart and J.

M. Fernandez-Real, Neuroinflammation in obesity: circulating lipopolysaccharide-binding protein associates with brain structure and cognitive performance, *Int J Obes (Lond)*, 2017, **41**, 1627-1635.

16. A. A. Miller and S. J. Spencer, Obesity and neuroinflammation: a pathway to cognitive impairment, *Brain Behav Immun*, 2014, **42**, 10-21.
17. P. D. Cani, J. Amar, M. A. Iglesias, M. Poggi, C. Knauf, D. Bastelica, A. M. Neyrinck, F. Fava, K. M. Tuohy, C. Chabo, A. Waget, E. Delmee, B. Cousin, T. Sulpice, B. Chamontin, J. Ferrieres, J. F. Tanti, G. R. Gibson, L. Casteilla, N. M. Delzenne, M. C. Alessi and R. Burcelin, Metabolic endotoxemia initiates obesity and insulin resistance, *Diabetes*, 2007, **56**, 1761-1772.
18. N. E. Boutagy, R. P. McMillan, M. I. Frisard and M. W. Hulver, Metabolic endotoxemia with obesity: Is it real and is it relevant?, *Biochimie*, 2016, **124**, 11-20.
19. L. Qin, X. Wu, M. L. Block, Y. Liu, G. R. Breese, J. S. Hong, D. J. Knapp and F. T. Crews, Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration, *Glia*, 2007, **55**, 453-462.
20. R. Daneman and M. Rescigno, The gut immune barrier and the blood-brain barrier: are they so different?, *Immunity*, 2009, **31**, 722-735.
21. O. Guillemot-Legris, J. Masquelier, A. Everard, P. D. Cani, M. Alhouayek and G. G. Muccioli, High-fat diet feeding differentially affects the development of inflammation in the central nervous system, *J Neuroinflammation*, 2016, **13**, 206.
22. O. Guillemot-Legris and G. G. Muccioli, Obesity-Induced Neuroinflammation: Beyond the Hypothalamus, *Trends Neurosci*, 2017, **40**, 237-253.
23. C. N. Lumeng and A. R. Saltiel, Inflammatory links between obesity and metabolic disease, *J Clin Invest*, 2011, **121**, 2111-2117.

24. P. J. Pistell, C. D. Morrison, S. Gupta, A. G. Knight, J. N. Keller, D. K. Ingram and A. J. Bruce-Keller, Cognitive impairment following high fat diet consumption is associated with brain inflammation, *J Neuroimmunol*, 2010, **219**, 25-32.

25. S. T. Francis, K. Head, P. G. Morris and I. A. Macdonald, The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people, *J Cardiovasc Pharmacol*, 2006, **47 Suppl 2**, S215-220.

26. D. T. Field, C. M. Williams and L. T. Butler, Consumption of cocoa flavanols results in an acute improvement in visual and cognitive functions, *Physiol Behav*, 2011, **103**, 255-260.

27. A. M. Brickman, U. A. Khan, F. A. Provenzano, L. K. Yeung, W. Suzuki, H. Schroeter, M. Wall, R. P. Sloan and S. A. Small, Enhancing dentate gyrus function with dietary flavanols improves cognition in older adults, *Nat Neurosci*, 2014, **17**, 1798-1803.

28. J. M. Harnly, R. F. Doherty, G. R. Beecher, J. M. Holden, D. B. Haytowitz, S. Bhagwat and S. Gebhardt, Flavonoid content of U.S. fruits, vegetables, and nuts, *J Agric Food Chem*, 2006, **54**, 9966-9977.

29. H. van Praag, M. J. Lucero, G. W. Yeo, K. Stecker, N. Heivand, C. Zhao, E. Yip, M. Afanador, H. Schroeter, J. Hammerstone and F. H. Gage, Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice, *J Neurosci*, 2007, **27**, 5869-5878.

30. G. Desideri, C. Kwik-Uribe, D. Grassi, S. Necozione, L. Ghiadoni, D. Mastroiacovo, A. Raffaele, L. Ferri, R. Bocale, M. C. Lechiara, C. Marini and C. Ferri, Benefits in cognitive function, blood pressure, and insulin resistance through cocoa flavanol consumption in elderly subjects with mild cognitive impairment: the Cocoa, Cognition, and Aging (CoCoA) study, *Hypertension*, 2012, **60**, 794-801.

31. E. Cremonini, A. Bettaieb, F. G. Haj, C. G. Fraga and P. I. Oteiza, (-)-Epicatechin improves insulin sensitivity in high fat diet-fed mice, *Arch Biochem Biophys*, 2016, **599**, 13-21.

32. E. Cremonini and P. I. Oteiza, (-)-Epicatechin and its metabolites prevent palmitate-induced NADPH oxidase upregulation, oxidative stress and insulin resistance in HepG2 cells, *Arch Biochem Biophys*, 2018, **646**, 55-63.

33. E. Cremonini, Z. Wang, A. Bettaieb, A. M. Adamo, E. Daveri, D. A. Mills, K. M. Kalanetra, F. G. Haj, S. Karakas and P. I. Oteiza, (-)-Epicatechin protects the intestinal barrier from high fat diet-induced permeabilization: Implications for steatosis and insulin resistance, *Redox biology*, 2018, **14**, 588-599.

34. A. Bettaieb, M. A. Vazquez-Prieto, C. R. Lanzi, R. M. Miatello, F. G. Haj, C. G. Fraga and P. I. Oteiza, (-)-Epicatechin mitigates high fructose-associated insulin resistance by modulating redox signaling and endoplasmic reticulum stress, *Free radical biology & medicine*, 2014, DOI: 10.1016/j.freeradbiomed.2014.04.011.

35. A. Bettaieb, E. Cremonini, H. Kang, J. Kang, F. G. Haj and P. I. Oteiza, Anti-inflammatory actions of (-)-epicatechin in the adipose tissue of obese mice, *The international journal of biochemistry & cell biology*, 2016, **81**, 383-392.

36. A. Vogiatzoglou, A. A. Mulligan, M. A. Lentjes, R. N. Luben, J. P. Spencer, H. Schroeter, K. T. Khaw and G. G. Kuhnle, Flavonoid intake in European adults (18 to 64 years), *PloS one*, 2015, **10**, e0128132.

37. M. Antunes and G. Biala, The novel object recognition memory: neurobiology, test procedure, and its modifications, *Cogn Process*, 2012, **13**, 93-110.

38. D. Wang, Y. Noda, Y. Zhou, A. Mouri, H. Mizoguchi, A. Nitta, W. Chen and T. Nabeshima, The Allosteric Potentiation of Nicotinic Acetylcholine Receptors by Galantamine Ameliorates the Cognitive Dysfunction in Beta Amyloid25–35 I.c.v.-Injected Mice: Involvement of Dopaminergic Systems, *Neuropsychopharmacology*, 2007, **32**, 1261-1271.

39. C. Cunha, R. Brambilla and K. L. Thomas, A simple role for BDNF in learning and memory?, *Front Mol Neurosci*, 2010, **3**, 1-1.

40. K. R. Westerterp, Diet induced thermogenesis, *Nutr Metab (Lond)*, 2004, **1**, 5-5.

41. A. Astrup, The role of dietary fat in the prevention and treatment of obesity. Efficacy and safety of low-fat diets, *Int J Obes Relat Metab Disord*, 2001, **25 Suppl 1**, S46-50.

42. A. Golay and E. Bobbioni, The role of dietary fat in obesity, *Int J Obes Relat Metab Disord*, 1997, **21 Suppl 3**, S2-11.

43. E. Jéquier, Is Fat Intake a Risk Factor for Fat Gain in Children?, *The Journal of Clinical Endocrinology & Metabolism*, 2001, **86**, 980-983.

44. C. Maffeis, Y. Schutz, A. Grezzani, S. Provera, G. Piacentini and L. Tatò, Meal-Induced Thermogenesis and Obesity: Is a Fat Meal a Risk Factor for Fat Gain in Children?1, *The Journal of Clinical Endocrinology & Metabolism*, 2001, **86**, 214-219.

45. P. Schrauwen and K. R. Westerterp, The role of high-fat diets and physical activity in the regulation of body weight, *British Journal of Nutrition*, 2000, **84**, 417-427.

46. T. C. Contreras, E. Ricciardi, E. Cremonini and P. I. Oteiza, (-)-Epicatechin in the prevention of tumor necrosis alpha-induced loss of Caco-2 cell barrier integrity, *Arch Biochem Biophys*, 2015, **573**, 84-91.

47. Z. Wang, M. C. Litterio, M. Muller, D. Vauzour and P. I. Oteiza, (-)-Epicatechin and NADPH oxidase inhibitors prevent bile acid-induced Caco-2 monolayer permeabilization through ERK1/2 modulation, *Redox Biol*, 2020, **28**, 101360.

48. S. Ghoshal, J. Witta, J. Zhong, W. de Villiers and E. Eckhardt, Chylomicrons promote intestinal absorption of lipopolysaccharides, *J Lipid Res*, 2009, **50**, 90-97.

49. E. M. Pålsson-McDermott and L. A. J. O'Neill, Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4, *Immunology*, 2004, **113**, 153-162.

50. G. Borges, J. I. Ottaviani, J. J. J. van der Hooft, H. Schroeter and A. Crozier, Absorption, metabolism, distribution and excretion of (-)-epicatechin: A review of recent findings, *Mol Aspects Med*, 2017, DOI: 10.1016/j.mam.2017.11.002.

51. J. Wang, M. G. Ferruzzi, L. Ho, J. Blount, E. M. Janle, B. Gong, Y. Pan, G. A. Gowda, D. Raftery, I. Arrieta-Cruz, V. Sharma, B. Cooper, J. Lobo, J. E. Simon, C. Zhang, A. Cheng, X. Qian, K. Ono, D. B. Teplow, C. Pavlides, R. A. Dixon and G. M. Pasinetti, Brain-targeted proanthocyanidin metabolites for Alzheimer's disease treatment, *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 2012, **32**, 5144-5150.

52. H. van Praag, M. J. Lucero, G. W. Yeo, K. Stecker, N. Heivand, C. Zhao, E. Yip, M. Afanador, H. Schroeter, J. Hammerstone and F. H. Gage, Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice, *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 2007, **27**, 5869-5878.

53. D. Angelino, D. Carregosa, C. Domenech-Coca, M. Savi, I. Figueira, N. Brindani, S. Jang, S. Lakshman, A. Molokin, J. F. Urban, Jr., C. D. Davis, M. A. Brito, K. S. Kim, F. Brighenti, C. Curti, C. Bladé, J. M. Del Bas, D. Stilli, G. I. Solano-Aguilar, C. N. D. Santos, D. Del Rio and P. Mena, 5-(Hydroxyphenyl)-γ-Valerolactone-Sulfate, a Key Microbial Metabolite of Flavan-3-ols, Is Able to Reach the Brain: Evidence from Different in Silico, In Vitro and In Vivo Experimental Models, *Nutrients*, 2019, **11**.

54. L. T. Knapp and E. Klann, Role of reactive oxygen species in hippocampal long-term potentiation: contributory or inhibitory?, *J Neurosci Res*, 2002, **70**, 1-7.

55. H. Sies, C. Berndt and D. P. Jones, Oxidative Stress, *Annu Rev Biochem*, 2017, **86**, 715-748.

56. M. A. Ansari and S. W. Scheff, NADPH-oxidase activation and cognition in Alzheimer disease progression, *Free radical biology & medicine*, 2011, **51**, 171-178.

57. M. A. Ansari and S. W. Scheff, Oxidative stress in the progression of Alzheimer disease in the frontal cortex, *J Neuropathol Exp Neurol*, 2010, **69**, 155-167.

58. C. D. Morrison, P. J. Pistell, D. K. Ingram, W. D. Johnson, Y. Liu, S. O. Fernandez-Kim, C. L. White, M. N. Purpera, R. M. Uranga, A. J. Bruce-Keller and J. N. Keller, High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: implications for decreased Nrf2 signaling, *J Neurochem*, 2010, **114**, 1581-1589.

59. A. J. Bruce-Keller, C. L. White, S. Gupta, A. G. Knight, P. J. Pistell, D. K. Ingram, C. D. Morrison and J. N. Keller, NOX activity in brain aging: exacerbation by high fat diet, *Free radical biology & medicine*, 2010, **49**, 22-30.

60. C. G. Fraga, P. I. Oteiza and M. Galleano, Plant bioactives and redox signaling: (-)-Epicatechin as a paradigm, *Mol Aspects Med*, 2018, DOI: 10.1016/j.mam.2018.01.007.

61. J. Lu, D. M. Wu, Y. L. Zheng, B. Hu, W. Cheng, Z. F. Zhang and Q. Shan, Ursolic acid improves high fat diet-induced cognitive impairments by blocking endoplasmic reticulum stress and IkappaB kinase beta/nuclear factor-kappaB-mediated inflammatory pathways in mice, *Brain Behav Immun*, 2011, **25**, 1658-1667.

62. D. Camer, Y. Yu, A. Szabo, F. Fernandez, C. H. L. Dinh and X. F. Huang, Bardoxolone methyl prevents high-fat diet-induced alterations in prefrontal cortex signalling molecules involved in recognition memory, *Prog Neuropsychopharmacol Biol Psychiatry*, 2015, **59**, 68-75.

63. S. Wang, X. F. Huang, P. Zhang, H. Wang, Q. Zhang, S. Yu and Y. Yu, Chronic rhein treatment improves recognition memory in high-fat diet-induced obese male mice, *J Nutr Biochem*, 2016, **36**, 42-50.

64. L. M. Lueptow, Novel Object Recognition Test for the Investigation of Learning and Memory in Mice, *J Vis Exp*, 2017, DOI: 10.3791/55718, 55718.

65. S. Krishna, M. M. Keralapurath, Z. Lin, J. J. Wagner, C. B. de La Serre, D. A. Harn and N. M. Filipov, Neurochemical and electrophysiological deficits in the ventral hippocampus and selective behavioral alterations caused by high-fat diet in female C57BL/6 mice, *Neuroscience*, 2015, **297**, 170-181.

66. A. N. Carey, S. M. Gomes and B. Shukitt-Hale, Blueberry supplementation improves memory in middle-aged mice fed a high-fat diet, *J Agric Food Chem*, 2014, **62**, 3972-3978.

67. D. Mastroiacovo, C. Kwik-Uribe, D. Grassi, S. Necozione, A. Raffaele, L. Pistacchio, R. Righetti, R. Bocale, M. C. Lechiara, C. Marini, C. Ferri and G. Desideri, Cocoa flavanol consumption improves cognitive function, blood pressure control, and metabolic profile in elderly subjects: the Cocoa, Cognition, and Aging (CoCoA) Study--a randomized controlled trial, *Am J Clin Nutr*, 2015, **101**, 538-548.

68. C. F. Haskell-Ramsay, J. Schmitt and L. Actis-Goretta, The Impact of Epicatechin on Human Cognition: The Role of Cerebral Blood Flow, *Nutrients*, 2018, **10**.

69. S. A. Heldt, L. Stanek, J. P. Chhatwal and K. J. Ressler, Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories, *Mol Psychiatry*, 2007, **12**, 656-670.

70. A. Cattaneo, N. Cattane, V. Begni, C. M. Pariante and M. A. Riva, The human BDNF gene: peripheral gene expression and protein levels as biomarkers for psychiatric disorders, *Transl Psychiatry*, 2016, **6**, e958.

71. S. Neshatdoust, C. Saunders, S. M. Castle, D. Vauzour, C. Williams, L. Butler, J. A. Lovegrove and J. P. Spencer, High-flavonoid intake induces cognitive improvements linked to changes in serum brain-derived neurotrophic factor: Two randomised, controlled trials, *Nutr Healthy Aging*, 2016, **4**, 81-93.

72. T. P. Stringer, D. Guerrieri, C. Vivar and H. van Praag, Plant-derived flavanol (-)epicatechin mitigates anxiety in association with elevated hippocampal monoamine and BDNF levels, but does not influence pattern separation in mice, *Transl Psychiatry*, 2015, **5**, e493.

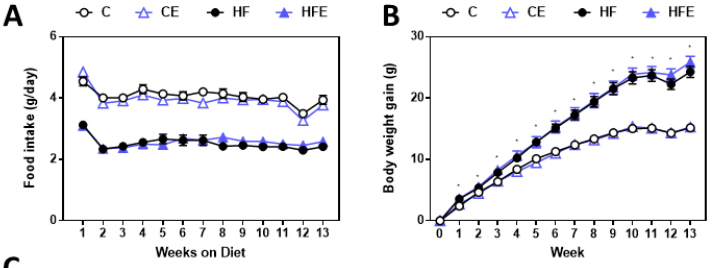
73. M. Ayaz, A. Sadiq, M. Junaid, F. Ullah, M. Ovais, I. Ullah, J. Ahmed and M. Shahid, Flavonoids as Prospective Neuroprotectants and Their Therapeutic Propensity in Aging Associated Neurological Disorders, 2019, **11**.

74. S. Kuriyama, A. Hozawa, K. Ohmori, T. Shimazu, T. Matsui, S. Ebihara, S. Awata, R. Nagatomi, H. Arai and I. Tsuji, Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project, *The American Journal of Clinical Nutrition*, 2006, **83**, 355-361.

75. R. A. Riemersma, C. A. Rice-Evans, R. M. Tyrrell, M. N. Clifford and M. E. J. Lean, Tea flavonoids and cardiovascular health, *QJM: An International Journal of Medicine*, 2001, **94**, 277-282.

Table 1. Primers used in the study.

Gene	Forward Primer (5'→ 3')	Reverse Primer (5'→ 3')
β-Actin	TCATGAAGTGTGACGTGGACATCCGC	CCTAGAAGCATTTGCGGTGCACGATG
BDNF	ATGGGACTCTGGAGAGCCTGAA	CGCCAGCCAATTCTCTTTTTGC
Iba-1	GTCCTTGAAGCGAATGCTGG	CATTCTCAAGATGGCAGATC
iNOS	CGAAACGCTTCACTTCCAA	TGAGCCTATATTGCTGTGGCT
NOX2	AACTGTATGCTGATCCTGCTGC	GTTCTCATTGTCACCGATGTCAG
NOX4	TGAGGAGTCACTGAACTATGAAGTTAATC	TGACTGAGGTACAGCTGGATGTTCACA
TLR4	GGAAGTTCACATAGCTGAATGAC	CAAGGCATGTCCAGAAATGAGA
TNFα	CCCCTCAGCAAACCACCAAGT	CTTGGGCAGATTGACCTCAGC



C

Parameter	C	CE	HF	HFE
Food intake (g/d)	4.07 ± 0.07^a	3.95 ± 0.09^a	2.80 ± 0.08^b	2.87 ± 0.07^b
Caloric intake	15.1 ± 0.2	14.6 ± 0.3	14.4 ± 0.3	14.7 ± 0.3
Body weight (g)	37.0 ± 0.7^a	36.2 ± 0.8^a	46.0 ± 1.1^b	47.5 ± 1.0^b
Brain (mg)	432 ± 5	432 ± 6	421 ± 4	428 ± 6
Hippocampus (mg)	23.8 ± 1.0	25.8 ± 1.7	22.8 ± 2.5	24.2 ± 1.2
Visceral Fat (g)	0.66 ± 0.05^a	0.63 ± 0.04^a	1.51 ± 0.19^b	1.70 ± 0.06^b
Epididymal Fat (g)	1.65 ± 0.08^a	1.52 ± 0.07^a	2.15 ± 0.17^b	2.41 ± 0.16^b
Retroperitoneal Fat (g)	0.69 ± 0.05^a	0.66 ± 0.09^a	1.74 ± 0.14^b	1.78 ± 0.08^b
Subcutaneous fat (g)	2.3 ± 0.2^a	2.4 ± 0.2^a	4.9 ± 0.3^b	5.5 ± 0.3^b
Brown Fat (mg)	0.23 ± 0.02	0.24 ± 0.02	0.24 ± 0.03	0.28 ± 0.02
Plasma glucose (mg/dl)	190 ± 9^a	179 ± 5^a	247 ± 12^b	208 ± 9^a
Plasma triglycerides (mg/dl)	68 ± 5^a	81 ± 4^a	98 ± 5^b	101 ± 4^b

Figure 1

Figure 1

254x190mm (96 x 96 DPI)

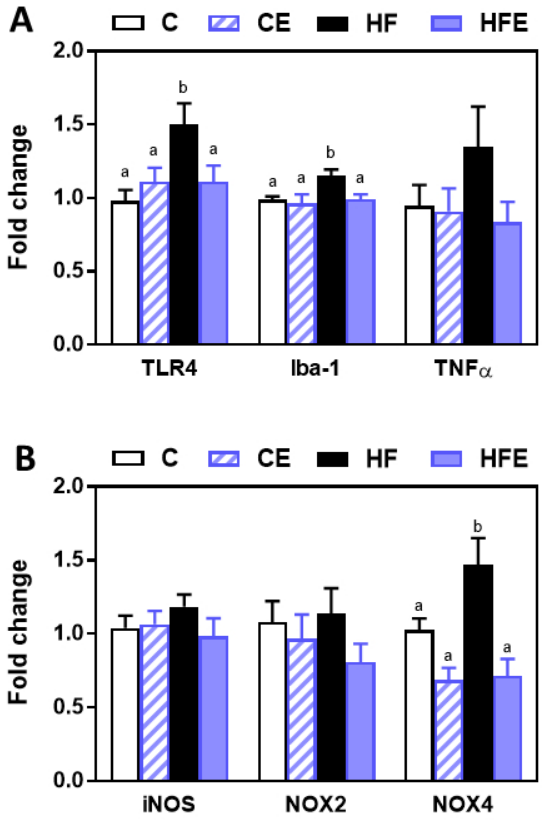


Figure 2

Figure 2

190x254mm (96 x 96 DPI)

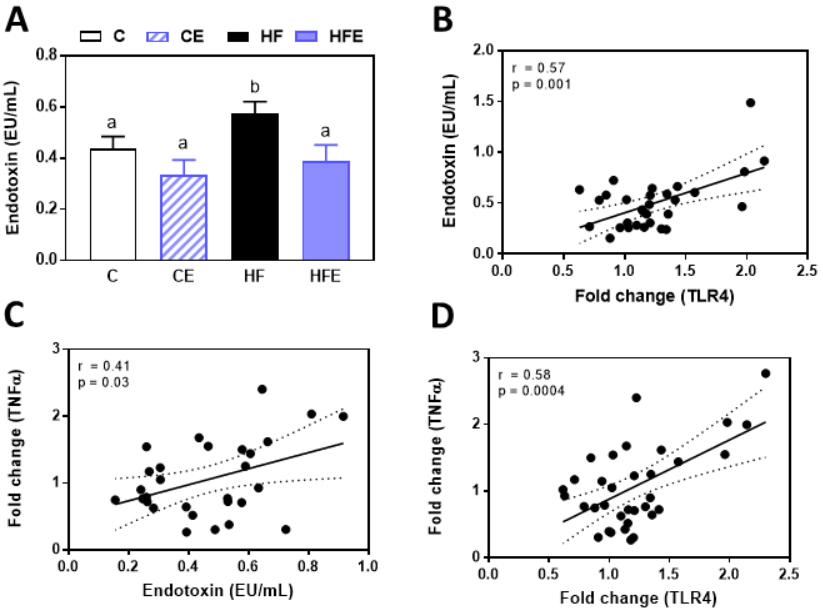


Figure 3

Figure 3

190x254mm (96 x 96 DPI)

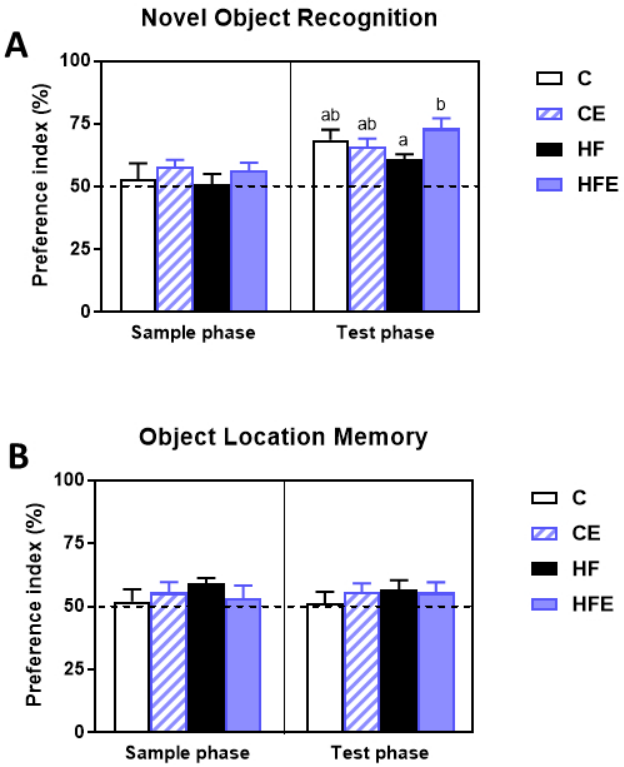


Figure 4

Figure 4

190x254mm (96 x 96 DPI)

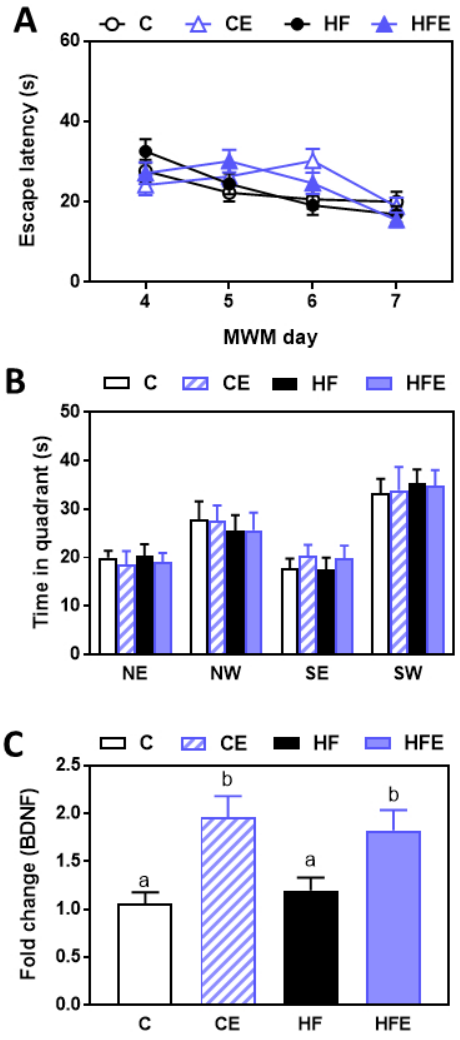
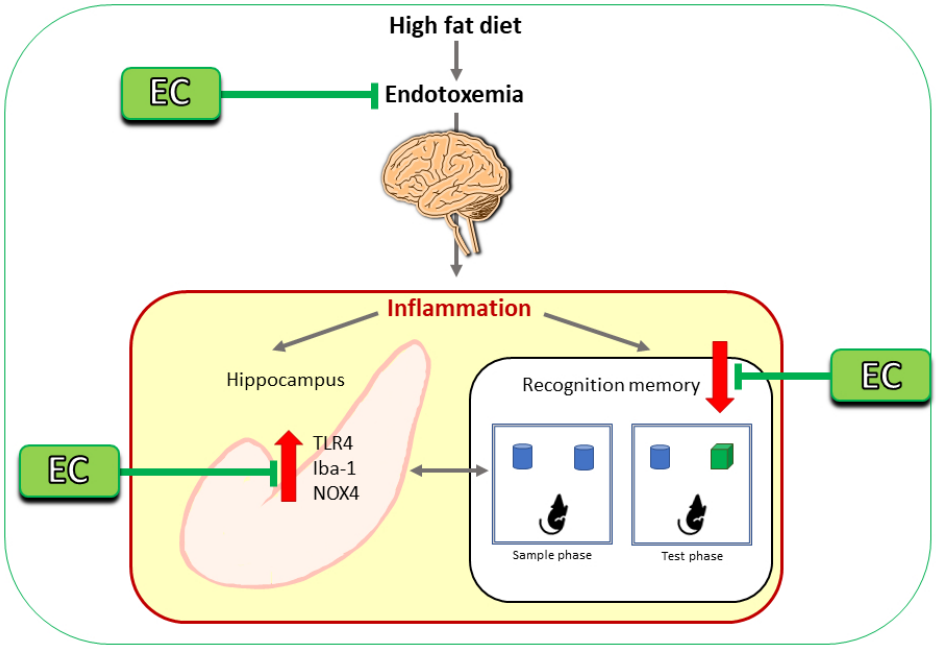


Figure 5

Figure 5

190x254mm (96 x 96 DPI)



(-)-Epicatechin improves memory in high fat diet-induced obese mice in association with prevention of endotoxemia and mitigation of neuroinflammation.

254x190mm (96 x 96 DPI)