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RESEARCH

Ketogenic Diet Mitigates Age-Related Cognitive Decline and Neuroinflammation in Rats, While Antibiotics Exacerbate Brain Health Risks

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Abstract

The ketogenic diet (KD), a high-fat, low-carbohydrate regimen, has been shown to exert neuroprotective effects in various neurological models. This study explored how KD—alone or combined with antibiotic-induced gut microbiota depletion—affects cognition and neuroinflammation in aging. Thirty-two male rats (22 months old) were assigned to four groups ($n = 8$): control diet (CD), ketogenic diet (KD), antibiotics with control diet (AB), and antibiotics with KD (KDAB). Diets were maintained for 10 weeks; during the final week, AB and KDAB groups received a broad-spectrum antibiotic cocktail (ampicillin 1 g/L, vancomycin 0.5 g/L, neomycin 1 g/L, and metronidazole 1 g/L) in drinking water. Cognitive abilities were evaluated using the Morris Water Maze and Novel Object Recognition Test. BDNF and inflammatory cytokines (TNF-α, IL-1β, IL-10) were measured in the hippocampus and prefrontal cortex. KD and KDAB groups exhibited increased β-hydroxybutyrate and reduced glucose levels, enhanced cognitive performance, elevated BDNF and IL-10, and decreased TNF-α and IL-1β compared to non-KD groups. Although antibiotic treatment alone caused only a transient impairment in spatial memory and was associated with reduced TNF-α levels, the ketogenic diet—irrespective of microbiota status—consistently improved cognitive performance and elevated neuroprotective markers. These findings suggest that KD appears to promote brain resilience during aging, even in the presence of microbiota disruption.

Keywords Ketogenic diet · Antibiotic · Aging · Cognitive function · BDNF · Neuroinflammation

Introduction

Aging is characterized by a gradual, progressive deterioration of biological systems that leads to functional decline. In the brain, advancing age is accompanied by significant neuroanatomical and neurophysiological changes closely associated with impairments in learning and memory (Li et al. 2020; Partridge 2001). The mechanisms underlying age-related cognitive decline are complex and not yet fully understood. Recent evidence indicates that alterations in the gut microbiota and a state of chronic, low-grade neuroinflammation may contribute to cognitive deficits in normal aging (Li et al. 2020).

The gut microbiome is known to influence energy metabolism, immune responses, and central nervous system function, and its composition shifts markedly with age (Claesson et al. 2011; Li et al. 2020). The gut-brain axis plays a key role in modulating behavior, including learning and memory (Berding et al. 2021; Schroeder and Backhed 2016). However, studies on microbiota-brain interactions often rely on specific models like germ-free mice or antibiotic-induced dysbiosis, which may not fully account for biological aging (Frohlich et al. 2016; Gareau et al. 2011). Dysbiosis can affect mental health, and antibiotics are a valuable tool to explore gut-brain interplay, influencing brain structure and cognition (Braniste et al. 2014; Desbonnet et al. 2014). Antibiotics often lead to dysbiosis by reducing microbial diversity, altering taxa abundance, gene expression, protein

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activity, and gut metabolism, and decreasing resistance to harmful bacteria while promoting antibiotic-resistant microbes. Researchers use antibiotics to induce dysbiosis, with common regimens including a combination of ampicillin, neomycin, metronidazole, and vancomycin in drinking water for 1 to 4 weeks (Kennedy et al. 2018; Tirelle et al. 2020). Short-term antibiotic treatments, such as 1 week, are effective in reducing gut microbiota and observing changes without long-term disruption, minimizing side effects and resistance risks (Tirelle et al. 2020). These findings underscore the potential contribution of microbiota changes to age-related neuroinflammation and cognitive decline.

As the brain ages, its immune system becomes more reactive, and even a mild peripheral challenge—such as exposure to lipopolysaccharide (LPS)—can trigger an abnormally heightened inflammatory response within the central nervous system, even in otherwise healthy older individuals (Godbout et al. 2005; Rosczyk et al. 2008). Alongside elevated levels of proinflammatory mediators such as IL-1β, IL-6, and TNF-α in the brain, older animals experience intensified disease symptoms and cognitive impairments when the immune system is activated (Abraham et al. 2008; Chen et al. 2008). Normal aging is believed to prime the central cytokine system so that, upon immune stimulation, a heightened and prolonged inflammatory reaction occurs in the brain (Rosczyk et al. 2008). This excessive neuroinflammation may contribute to the neurological and behavioral disorders observed in aged patients with infections and may also accelerate the progression of neurodegenerative conditions like Alzheimer's disease (Perry et al. 2007).

Diet significantly influences gut microbiota composition throughout life, impacting brain function via microbial metabolites, immune, neuronal, and metabolic pathways (Berding et al. 2021; Rinninella et al. 2019). The microbiota-gut-brain axis highlights the role of nutritional interventions in brain health (Diaz Heijtz et al. 2011; Luo et al. 2024).

The ketogenic diet (KD), a high-fat, low-carbohydrate, moderate-protein diet mimicking fasting, shifts metabolism from carbohydrate to fat utilization, offering potential benefits such as weight loss, cognitive enhancement, and neuroprotection (Ma et al. 2018). KD interventions have been associated with improved neural plasticity and cognitive outcomes in aged models, pointing toward possible benefits for neurodegenerative disorders common in advanced age (Hernandez et al. 2023; Newman et al. 2017; Wang and Mitchell 2016).

Although the benefits of KD in neurodegenerative diseases such as Alzheimer's and Parkinson's are well-documented, its impact on healthy aging and its interaction with microbiota changes induced by antibiotics remain unclear. To investigate the combined effects of a KD and antibiotic-induced alterations in gut microbiota on cognitive functions in aged rats, we focused on two key brain regions involved in cognition: the hippocampus (HC) and the prefrontal cortex (PFC). This study examined how the intervention influences learning and memory, as well as the levels of brain-derived neurotrophic factor (BDNF) and inflammatory biomarkers, including IL-10, IL-1β, and TNF-α.

Materials and Methods

Animals

All animals were age matched at 22 months to ensure that group differences were not confounded by age-related factors. Thirty-two male Sprague–Dawley rats were obtained from the Dokuz Eylul University Medical School Experimental Animal Laboratory (İzmir, Turkey). Twenty-two-month-old rats were chosen to reflect advanced aging (~65–70 years in humans), a stage at which cognitive vulnerability becomes pronounced and therapeutic interventions are most clinically relevant (Li et al. 2020). Upon arrival, the rats were housed in groups under controlled conditions: ambient temperature of 22 ± 1 °C, relative humidity of ~60%, and a 12-h light/dark cycle. Food and water were provided ad libitum. All animal procedures adhered to institutional guidelines and were approved by the Dokuz Eylul University Animal Care and Use Committee (approval no. 35/2023). All efforts were made to ensure animal welfare throughout the experiment. All animals were age matched at 22 months to ensure that group differences were not confounded by age-related factors. Thirty-two male Sprague–Dawley rats were obtained from the Dokuz Eylul University Medical School Experimental Animal Laboratory (İzmir, Turkey).

Study Design

The rats were randomly assigned to one of four experimental groups ($n = 8$ per group): control diet (Con), ketogenic diet (KD), control diet + antibiotics (AB), and ketogenic diet + antibiotics (KDAB). Each group designation reflects the diet and treatment received (Fig. 1). Over a 10-week period, the rats were weighed weekly to monitor changes. During the final week of the study, the groups receiving antibiotics were treated with an antibiotic cocktail, whose composition is detailed under the "Antibiotic Administration" section.

Experimental Diet

The control groups were fed a standard rat diet containing 20.6% fat, 16.5% protein, and 62.9% carbohydrates. In contrast, the ketogenic diet provided to experimental groups

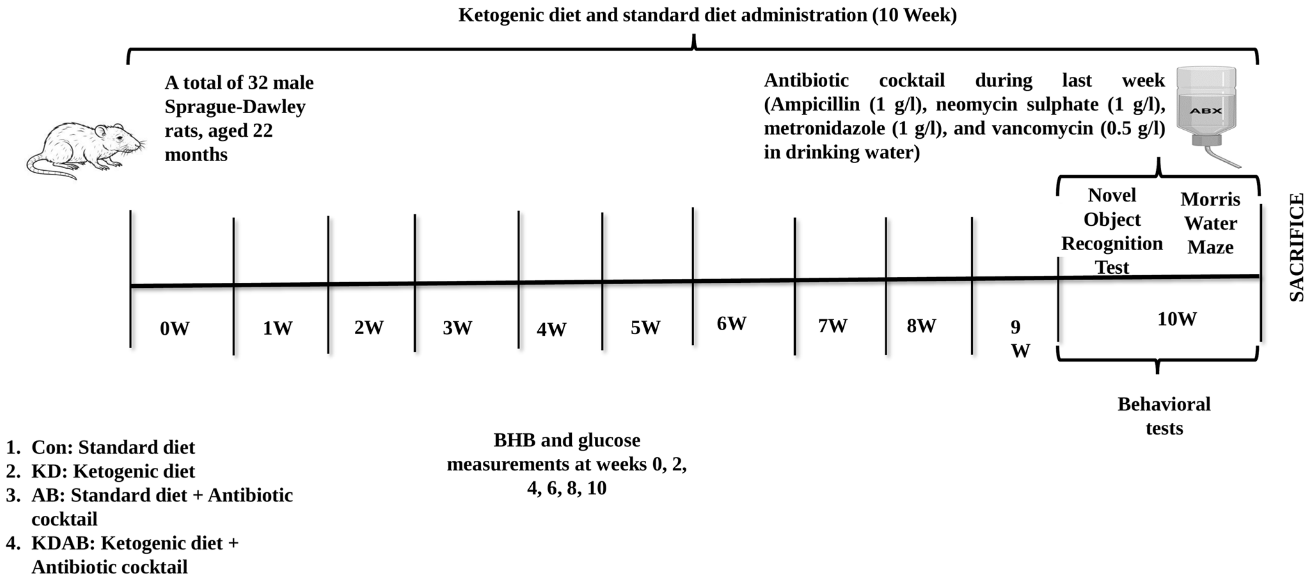

Fig. 1 Experimental procedure schedule

consisted of 89.30% fat, 10.20% protein, and 0.50% carbohydrates. The KD was formulated using a commercially available diet from Arden Research & Experiment.

Antibiotic Administration

We used a validated antibiotic cocktail (ampicillin, vancomycin, neomycin, and metronidazole) that ensures effective depletion of a broad range of gut microbes, including Gram-positive, Gram-negative, and anaerobic taxa (Tirelle et al. 2020). A 1-week regimen was selected based on prior studies showing that short-term treatment is sufficient to induce microbiota depletion while minimizing systemic side effects in aged rodents (Minter et al. 2016). To minimize handling-induced stress and ensure consistent voluntary intake, antibiotics were administered ad libitum via drinking water (Huang et al. 2022). The cocktail was freshly prepared each day by dissolving ampicillin (1 g/L), neomycin sulfate (1 g/L), metronidazole (1 g/L), and vancomycin (0.5 g/L) in 1 L of tap water. This solution was provided to the AB and KDAB groups throughout the final 7 days of the experiment.

Morris Water Maze Test (MWM)

Spatial learning and memory were assessed using the Morris water maze (MWM), a circular pool (140 cm diameter, 50 cm depth) filled with water (temperature ~ 24 °C). A platform (10 cm diameter) was submerged ~ 1 cm below the water surface in a fixed location within one quadrant, hidden from the rats' view. Visual cues (distinct geometric patterns) were placed on the walls around the pool to aid spatial orientation. Each rat underwent four training trials per day for four consecutive days, starting from different quadrants per trial. In each trial, the rat was released into the water and allowed up to 60 s to find the hidden platform. The escape latency (time to reach the platform) was recorded for each trial. Daily latency values were averaged for each rat. On day 5, a single probe trial was conducted with the platform removed to evaluate memory retention. Each rat was placed in the pool for 60 s, and the time spent in the target quadrant (where the platform used to be) versus the opposite quadrant was measured. Performance was tracked using an automated video tracking system (Noldus EthoVision XT). A shorter escape latency over training days and a greater proportion of time in the target quadrant during the probe trial were taken as indicators of better spatial learning and memory. The MWM procedure followed standard protocols as in our previous work (Karakilic et al. 2021).

Novel Object Recognition Test (NORT)

Recognition memory was evaluated by the Novel Object Recognition Test (NORT). The test was conducted in an open-top square arena (50 × 50 × 50 cm) with opaque black walls. To habituate the animals, each rat was first allowed to explore the empty arena for 3 min on two consecutive days. Twenty-four hours later, the first trial was conducted by placing two identical objects in the rear left and right corners of the platform. Each rat was positioned facing the wall opposite the objects and allowed to explore the platform for 3 min. After a 1-h retention interval, one of the familiar objects was replaced with a novel one, and a second trial was conducted under the same conditions. Between trials, the

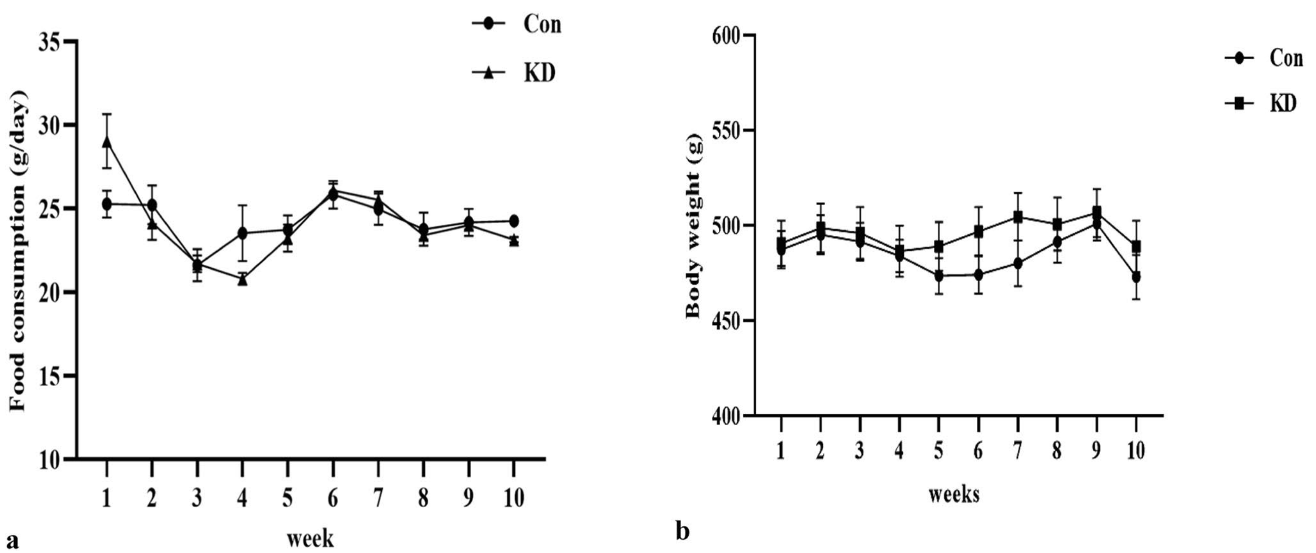

Fig. 2 Comparison of food consumption and body weight between aged rats given control diet or ketogenic diet. Time course of **a** the amount of food consumption and **b** body weight in an ad libitum control diet and ketogenic diet groups. Significant differences are shown by $^{*}p < 0.05$ and $^{**}p < 0.01$ ($n = 16$ in each group, Con, control; KD, ketogenic diet)

arena and objects were cleaned with 70% ethanol to eliminate odor cues. A higher percentage reflects better recognition memory. The time spent exploring both the familiar and the novel objects was recorded, and the percentage of novel object exploration was calculated as: (time spent exploring the novel object/total object exploration time) × 100 (Antunes and Biala 2012).

Biochemical Measurements

Blood samples were obtained through right heart ventricle puncture using 25-gauge needles under CO2 anesthesia. Following decapitation, the brain was extracted en bloc via craniotomy, and HC and PFC regions were carefully separated. Tissue samples were stored at − 80 °C until they underwent homogenization and measurement. Blood samples were centrifuged at 1000 g for 10 min to isolate serum, which was then preserved at − 80 °C for subsequent analysis.

Serum glucose (mg/dL) and β-hydroxybutyrate (BHB, mmol/L) levels were measured using a handheld dual glucometer/ketometer (On Call® Dual, ACON Laboratories) according to the manufacturer's instructions. These measures confirmed the metabolic state of each group (normoglycemic vs. ketotic).

Levels of BDNF, IL-10, TNF-α, and IL-1β in the HC and PFC supernatants were quantified using enzyme-linked immunosorbent assay (ELISA) kits specific for rat proteins. The following ELISA kits were used: BDNF, TNF-α, IL-1β, and IL-10 (Bioassay Technology Lab, China). All assays were performed in duplicate and according to the manufacturers' protocols.

Statistical Analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical analyses were carried out using IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY) and GraphPad Prism version 9.0.0 (GraphPad Software, San Diego, CA). Body weight data over time were analyzed with a GLM-repeated-measures ANOVA to detect group × time interactions across the 10 weeks. For endpoint measures (e.g., escape latency on final day, probe trial performance, novel object exploration percentage, BDNF, and cytokine levels), two-way ANOVA was used to test the effects of diet (control vs. ketogenic), antibiotic treatment (absent vs. present), and their interaction. When a significant main effect or interaction was found, Bonferroni post-hoc tests were applied for pairwise group comparisons. A significance criterion of $p < 0.05$ was adopted for all statistical tests.

Results

Food Consumption and Body Weight

To evaluate general health and metabolic effects of the diets, we monitored food consumption and body weight over the study. As shown in Fig. 2a, food consumption was generally comparable between the control and KD groups across the 10-week period. While a slight reduction in consumption was observed in the KD group during the early weeks, no statistically significant differences were detected at any time point. Body weight trajectories across the entire study period

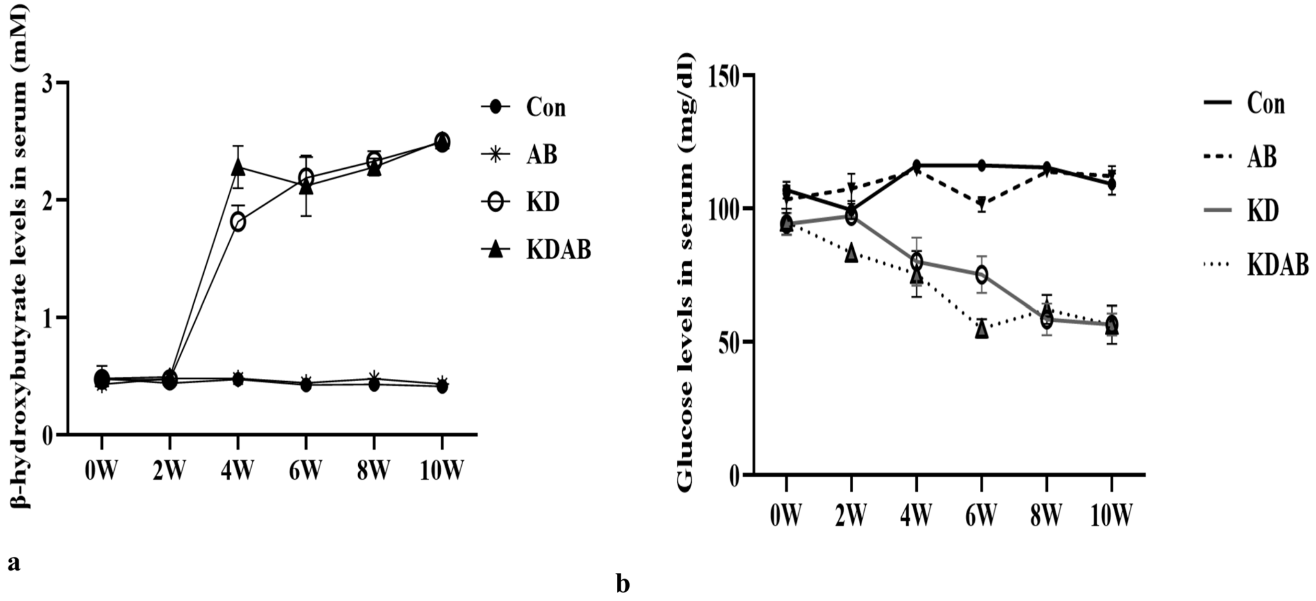

Fig. 3 The serum β-hydroxybutyrate and glucose levels at different time points. Values are demonstrated as means ± SEM. Significant differences are shown by $^{*}p < 0.05$ and $^{**}p < 0.01$ versus the control and $^{\#}p < 0.05$ and.$^{\#\#}p < 0.01$ versus AB groups ($n = 8$ for each group)

are shown in Fig. 2b. Both control and KD groups exhibited a gradual decline in body weight over the first 5 weeks, consistent with the rats' advanced age and adaptation to diet. After week 5, body weights in both groups plateaued, with control-diet rats maintaining a relatively steady weight and KD rats showing a slight upward adjustment toward PR baseline. By the end of 10 weeks, mean body weights did not differ significantly among the groups. No significant group × time interaction was found for weight change during the experiment, indicating that KD did not cause abnormal weight loss or gain compared to controls. At no time point was there a significant difference in body weight between KD-fed and control-fed aged rats ($p > 0.05$ at all weeks). Overall, the ketogenic diet was well-tolerated by the aged rats, with only modest early effects on feeding behavior and no adverse impact on weight maintenance.

Glucose and Ketone Levels

As expected, the ketogenic diet induced a clear shift in metabolic biomarkers. Figure 3a shows the serum β-hydroxybutyrate (BHB) concentrations measured at the start of the experiment and at weeks 2, 4, 6, 8, and 10. Rats on the KD and KDAB regimens exhibited markedly elevated BHB levels relative to those on the control diet. By week 4, mean BHB in KD and KDAB groups was significantly higher than in both Con and AB groups (which remained near baseline levels; $p < 0.01$ for KD vs. Con, KD vs. AB, KDAB vs. Con, KDAB vs. AB). This difference persisted at weeks 6, 8, and 10 ($p < 0.01$ at all-time points). By week 10, KD and KDAB rats reached serum BHB concentrations indicative of sustained nutritional ketosis, whereas Con and AB rats had minimal ketone levels.

Figure 3b presents the serum glucose measurements over the same time points. In contrast to ketones, blood glucose showed an opposite trend. KD and KDAB groups had significantly lower glucose concentrations than the control and AB groups at weeks 4, 6, 8, and 10 ($p < 0.01$ for all comparisons). By week 4, KD and KDAB rats were mildly hypoglycemic relative to controls. This reduction in glucose was maintained through week 10. There were no significant differences between the Con and AB groups' glucose levels at any time, indicating that antibiotic treatment alone did not alter baseline glucose metabolism. Taken together, these results confirm that the ketogenic diet successfully induced a metabolic state of ketosis (high BHB, low glucose) in both antibiotic-treated and non-treated rats, whereas the standard diet (with or without antibiotics) kept animals in a carbohydrate-dependent metabolic state.

Finally, there were no significant differences in serum glucose or β-hydroxybutyrate levels between AB and control groups at any time point ($p > 0.05$), suggesting that the antibiotic treatment alone did not affect systemic metabolic markers.

Cognitive Performance in Learning and Memory Tasks

Despite potential gut microbiota disruptions, KD had a notable positive impact on cognitive performance in aged

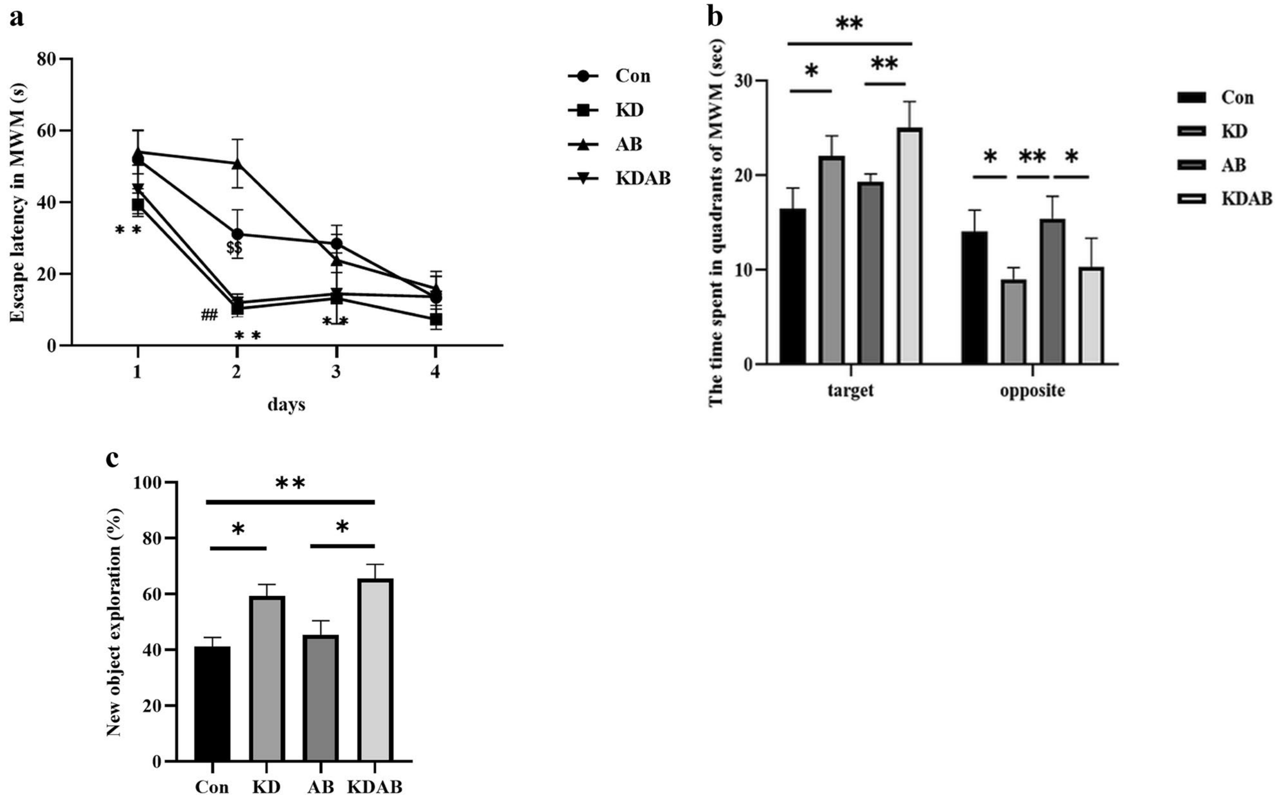

Fig. 4 Cognitive performance assessed via Morris water maze and novel object recognition tests. The MWM measured **a** escape latency and **b** time spent in the target and opposite quadrants, while **c** novel object recognition evaluated new object exploration (%) (new object exploration (%) = (exploration time on new object/total exploration time) × 100) ($n = 8$) per group. Values were presented as mean ± standard error, ** $p < 0.01$ compared with Con group; $^{\#\#}$ $p < 0.01$ compared with AB group; $^{\$\$}$ $p < 0.01$ compared with KDAB group (**a**) and ** $p < 0.01$ and *$p < 0.05$ (**b**, **c**)

rats. We first assessed spatial learning in the Morris water maze. Over the course of training (days 1–4), the escape latency (time to find the hidden platform) progressively decreased in all groups, indicating learning. However, the rate of improvement differed by group. Rats on the KD showed faster learning: by the end of the third training day, their average latency was significantly shorter than that of control rats ($p < 0.01$, KD vs. Con). Similarly, the KDAB group demonstrated improved learning compared to the AB group ($p < 0.01$, KDAB vs. AB). There was no significant difference in escape latency between the KD and KDAB groups across the training days, indicating that short-term antibiotic treatment did not diminish the cognitive improvements associated with the ketogenic diet. By the fourth day of training, all groups had converged to similar latencies (no significant intergroup differences on day 4), indicating that even the slower-learning groups eventually acquired the task (Fig. 4a). Memory retention was evaluated in the probe trial on day 5 (with the platform removed). Results are shown in Fig. 4b. KD-fed rats spent a greater proportion of the probe trial swimming in the target quadrant (where the platform had been) compared to control rats, indicating better spatial memory. Specifically, the KD group spent significantly more time in the target quadrant than the control group ($p < 0.05$), and the KDAB group spent even more time in the target quadrant than both control ($p < 0.01$) and AB ($p < 0.01$) groups. Although the AB group spent the least amount of time in the target quadrant, this difference was not statistically significant compared to the control group. However, both KD and KDAB groups performed significantly better than AB in this task, indicating a beneficial effect of the ketogenic intervention. Moreover, KDAB rats significantly outperformed AB rats on this measure ($p < 0.01$), suggesting that ketosis decreased the memory deficit caused by antibiotics. As an additional metric, time in the opposite quadrant was analyzed. Interestingly, KD rats showed a tendency to search more broadly, spending slightly more time in the opposite quadrant than control or AB rats (KD vs. Con $p < 0.05$; KD vs. AB $p < 0.01$). In contrast, KDAB rats showed focused searching, spending significantly less time in the opposite quadrant than AB rats ($p < 0.05$, KDAB vs. AB) These findings imply that the KD regimen enhanced spatial memory retention, even under conditions of microbiome disturbance, whereas antibiotics alone impaired memory search strategy. KD group spent significantly more time in the opposite quadrant compared with the control and AB group ($p < 0.05$ and $p < 0.01$, respectively). Additionally, KDAB group spent less time than the AB group in the opposite quadrant compared to AB group ($p < 0.05$). The AB group exhibited modest cognitive impairment, specifically showing increased escape latency on day 2 of the MWM and significantly reduced time in the target quadrant during the probe trial ($p < 0.01$).

We next examined recognition memory via the NORT. Figure 4c summarizes the novel object exploration results. In the training phase, all groups explored the identical objects for similar lengths of time (no initial preference, indicating no inherent object bias). In the testing phase, clear differences emerged. KD-fed rats demonstrated a significantly higher preference for the novel object compared to control-diet rats ($p < 0.05$). This indicates that KD rats remembered the familiar object and spent more time investigating the new object, reflecting better recognition memory. KDAB group showed an even more pronounced effect, exploring the novel object far more than either control or AB groups ($p < 0.05$ vs. Con; $p < 0.01$ vs. AB). Notably, AB group did not differ significantly from the control group in novel object exploration, implying that antibiotic treatment alone did not cause a measurable deficit in object recognition memory within the timeframe of this task. Overall, both KD and KDAB treatments enhanced recognition memory in aged rats, whereas antibiotics without KD did not substantially affect performance in this test.

BDNF Levels in the Hippocampus (HC) and Prefrontal Cortex (PFC)

BDNF is a key neurotrophic factor that supports learning and memory. We measured BDNF protein concentrations in the hippocampus and PFC to determine whether dietary and antibiotic interventions modulated neurotrophic support in these regions. In the hippocampus, aged rats on the ketogenic diet had significantly higher BDNF levels than those on the control diet. Specifically, the HC BDNF of KD group was elevated compared to controls ($p < 0.01$) and was also higher than that of AB group ($p < 0.01$, KD vs. AB). The KDAB group showed an increase in hippocampal BDNF as well, although this did not reach statistical significance versus control or AB (KDAB vs. Con and KDAB vs. AB, $p > 0.05$). In other words, while the KD alone significantly increased BDNF in HC, the combination of KD with antibiotics resulted in a higher BDNF that did not differ significantly from the other groups due to variability. Thus, KD's positive effect on hippocampal BDNF may have been partially attenuated by the antibiotic treatment, though the KDAB level was still numerically above control. Importantly, KD-treated rats (with or without AB) had higher hippocampal BDNF than antibiotic-only rats, underlining the neurotrophic benefit of the diet (Fig. 5a).

In the PFC, both KD and KDAB regimens led to significantly increased BDNF levels relative to control groups. The PFC BDNF in KD rats was greater than that in both control ($p < 0.05$) and AB ($p < 0.05$) rats. The KDAB group showed an even larger increase: its PFC BDNF was significantly higher than in control ($p < 0.01$) and AB ($p < 0.01$) groups. Notably, there was no significant difference between the KD and KDAB groups' PFC BDNF levels ($p > 0.05$), showing that adding antibiotics did not reduce the BDNF elevation induced by KD in the PFC. AB treatment did not significantly alter BDNF in either brain region compared to controls. Therefore, the data suggest that KD improves central BDNF levels in aged rats, especially in the PFC, and maintains this effect even when the gut microbiota is disrupted by antibiotics. An overall pattern is that KD (with or without AB) provided a neurotrophic advantage in both HC and PFC, which likely contributed to the better cognitive outcomes observed in those groups (Fig. 5b).

Additionally, BDNF concentrations in both the hippocampus and PFC did not differ significantly between AB and control groups, indicating that antibiotics alone did not alter neurotrophic support in aged rats (Fig. 5a and b).

Inflammatory Cytokine Levels in Hippocampus (HC) and Prefrontal Cortex (PFC)

We assessed pro-inflammatory (TNF-α, IL-1β) and anti-inflammatory (IL-10) cytokines in the brain to gauge neuroinflammatory status under each condition. Tumor necrosis factor-alpha (TNF-α): In the HC, TNF-α levels were significantly lower in KD and KDAB groups compared to both control and AB groups ($p < 0.01$ vs. Con; $p < 0.05$, vs. AB). Similarly, the KDAB group's TNF-α in HC was markedly reduced relative to control ($p < 0.01$) and AB ($p < 0.01$). Interestingly, AB group itself showed a slight decrease in HC TNF-α compared to controls ($p < 0.05$, AB vs. Con), despite the cognitive impairment seen in AB group. This suggests that antibiotic-induced microbiota depletion might reduce certain inflammatory signals in the brain. However, AB treatment was not as effective as KD in lowering TNF-α: the AB group's TNF-α remained significantly higher than that of KD rats ($p < 0.05$, AB vs. KD). There was no significant difference between KD and KDAB groups (their TNF-α levels were comparably low, $p > 0.05$) (Fig. 6a.1). In the PFC, a broadly similar pattern was observed. KD, KDAB, and AB groups all had significantly lower PFC TNF-α than the control group ($p < 0.01$ for KD and KDAB; $p < 0.05$ for AB vs. Con). Notably, the PFC TNF-α in the AB group, while lower than control, was still significantly higher than in the KD ($p < 0.01$) and KDAB ($p < 0.05$) groups (Fig. 6a.2). Thus, KD (with or

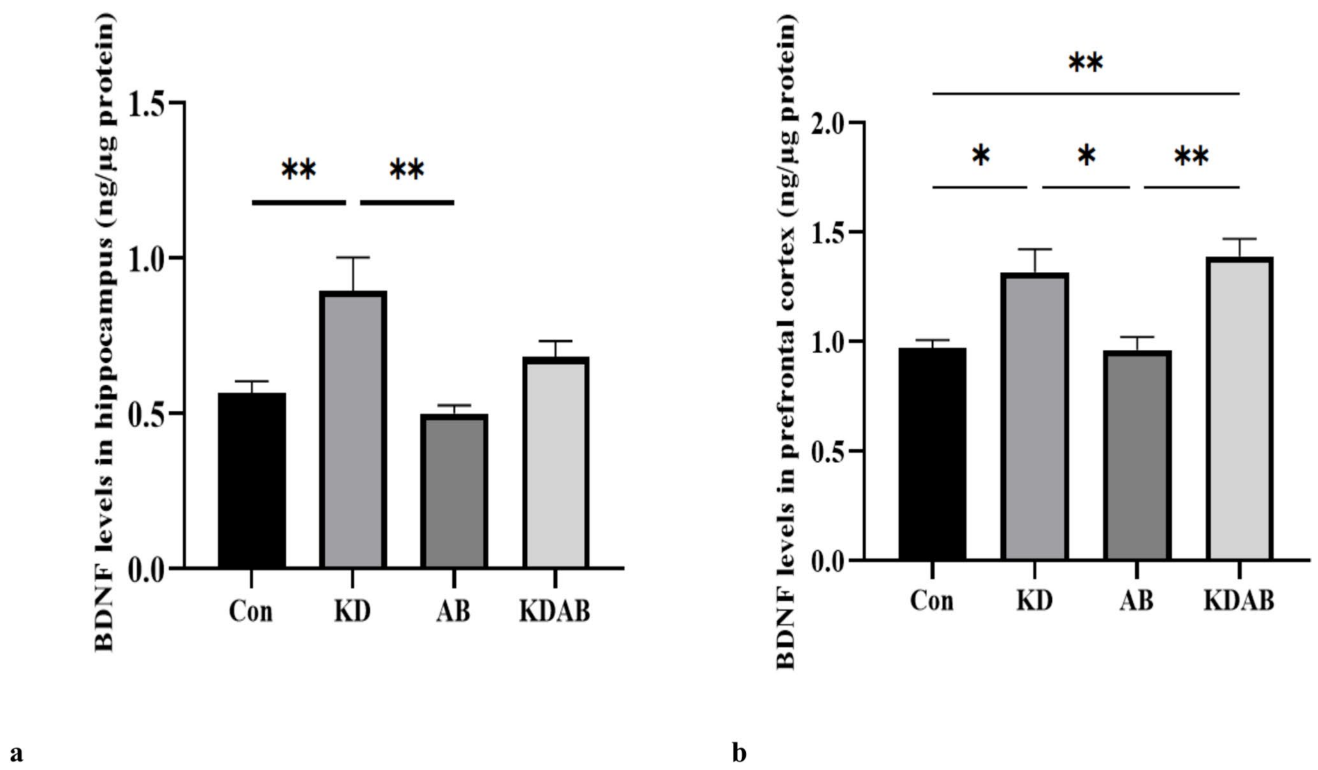

Fig. 5 The effects of ketogenic diet and antibiotic-treatment on BDNF levels in the hippocampus (**a**) and prefrontal cortex (**b**) in aged rats. Values were presented as mean ± standard error; ** $p < 0.01$ and * $p < 0.05$. BDNF, brain derived neurotrophic factor

without antibiotics) most effectively suppressed TNF-α in both brain regions, whereas antibiotics alone had a moderate and incomplete effect.

HC IL-1β levels showed significant group differences. The KDAB group had the lowest IL-1β in the HC, significantly below both control ($p < 0.05$) and AB ($p < 0.01$) groups. KD rats also tended to have lower HC IL-1β than controls, but in our sample, this did not reach significance ($p > 0.05$, KD vs. Con). However, importantly, the KD group's IL-1β was significantly lower than that of the AB group ($p < 0.01$, KD vs. AB). The AB group showed the highest hippocampal IL-1β, slightly exceeding control levels (though not statistically significantly, $p > 0.05$) (Fig. 6b.1). In the PFC, IL-1β levels were relatively low in all groups, and no significant differences were detected between any of the groups ($p > 0.05$ for all) (Fig. 6b.2). This indicates that PFC IL-1β did not vary markedly with diet or antibiotic treatment in our aged rats possibly because baseline IL-1β in the aged PFC was already minimal or the interventions selectively affected hippocampal inflammation. Summarizing IL-1β: KDAB provided the strongest reduction in HC IL-1β, while KD alone also helped reduce IL-1β (especially compared to antibiotic-treated animals).

IL-10 is an anti-inflammatory cytokine that can counteract the effects of TNF-α and IL-1β. In aged control rats, IL-10 levels were low in both brain regions. As shown in Fig. 6c.1, the ketogenic diet significantly increased IL-10 in HC. KD group's HC IL-10 was significantly higher than that of control ($p < 0.05$) and AB ($p < 0.01$) groups. KDAB group similarly showed elevated HC IL-10, with levels significantly above both control and AB groups ($p < 0.01$ for KDAB vs. Con; $p < 0.01$ for KDAB vs. AB). There was no significant difference between KD and KDAB in HC IL-10; both achieved roughly a two-fold increase over controls.

In the PFC (Fig. 6c.2), we found a consistent trend: IL-10 was significantly higher in KD and KDAB groups compared to control and AB ($p < 0.01$ for KD or KDAB vs. Con or AB). KDAB rats had the highest IL-10 concentrations in PFC, followed closely by KD rats, whereas AB rats had IL-10 levels similar to or slightly below controls. The AB group did not differ significantly from controls in IL-10 (in either region), indicating that antibiotics alone did not induce a compensatory anti-inflammatory response. This suggests that antibiotics may selectively suppress certain pro-inflammatory markers without broadly altering the inflammatory profile. Meanwhile, the ketogenic diet, with or without antibiotic co-treatment, greatly enhanced IL-10 in both HC and PFC. However, these results suggest that KD appears to exert anti-inflammatory effects in the aging brain, which may help lower proinflammatory cytokine levels and enhance cognitive function.

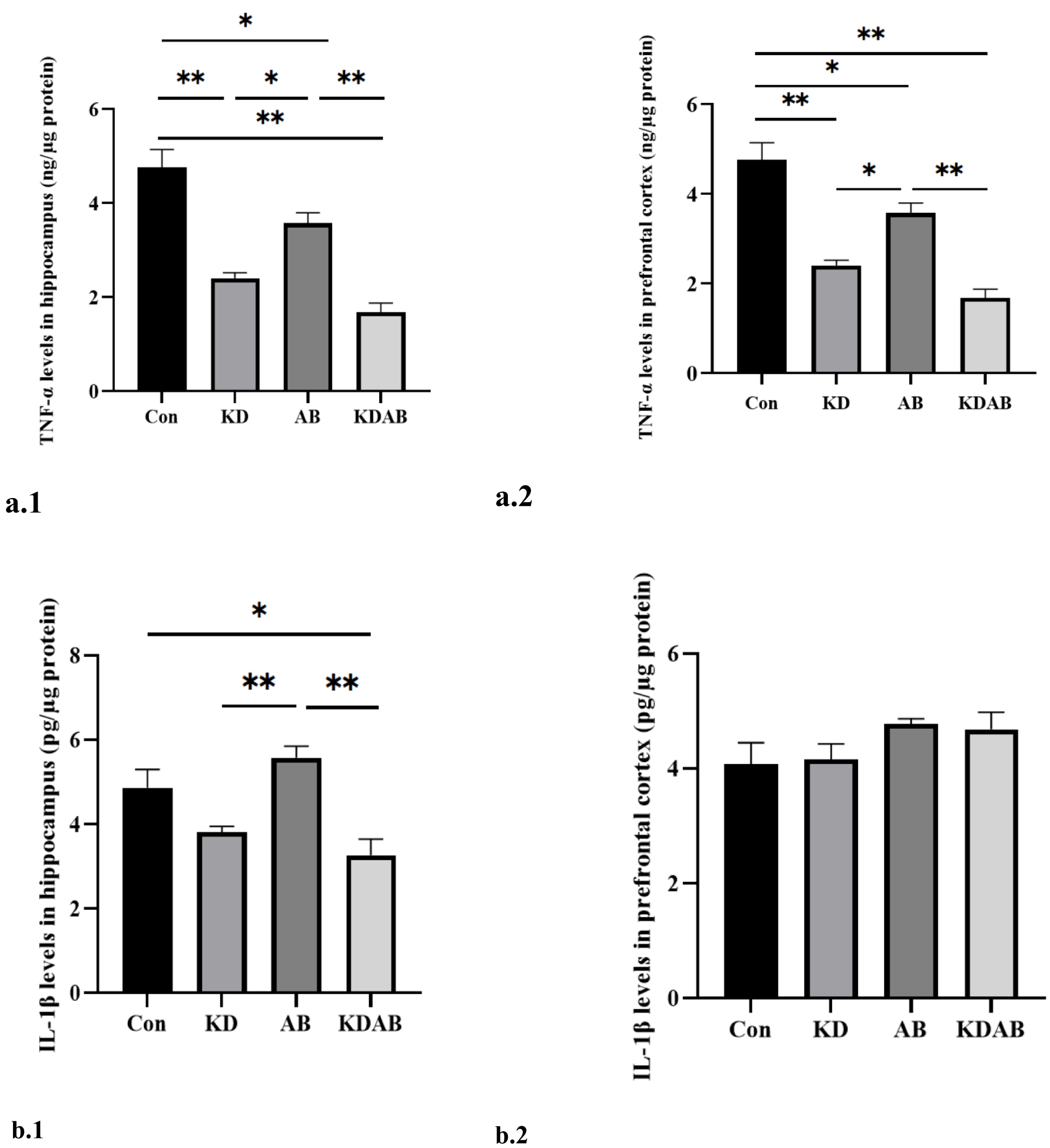

Fig. 6 Effects of the ketogenic diet and antibiotic treatment on pro- and anti-inflammatory biomarkers in aged rats. This figure illustrates the impact of dietary and antibiotic interventions on inflammatory marker levels in different brain regions. Tumor necrosis factor-alpha (TNF-α) concentrations were measured in both the hippocampus (**a.1**) and PFC (**a.2**). Likewise, interleukin-1 beta (IL-1β) levels were quantified in the hippocampus (**b.1**) and PFC (**b.2**). In contrast, interleukin-10 (IL-10), a key anti-inflammatory cytokine, was assessed in the hippocampus (**c.1**) and PFC (**c.2**). All values are presented as mean ± standard error, with statistical significance indicated as $p < 0.01$ and $*p < 0.05$

Discussion

In this study, we investigated the interactive effects of ketogenic diet and antibiotic-induced microbiota depletion on cognitive function and neuroinflammation in naturally aged rats. Our findings suggest that the application of a ketogenic diet during the ageing process can significantly improve learning and memory performance despite gut microbiota disruption and modulate brain inflammatory profiles. Conversely, the administration of broad-spectrum antibiotics alone (to mimic gut dysbiosis) tended to impair cognitive performance and did not provide the neurochemical benefits observed with ketosis. Although drinking water administration of antibiotics is less robust in depleting gut flora compared to gavage, it offers a non-invasive and stress-free alternative, making it suitable for studies on aged animals (Frohlich et al. 2016; Staley et al. 2017). Prior studies indicate that 1–4 weeks of antibiotic treatment effectively depletes gut microbiota, and extending this beyond 7 days

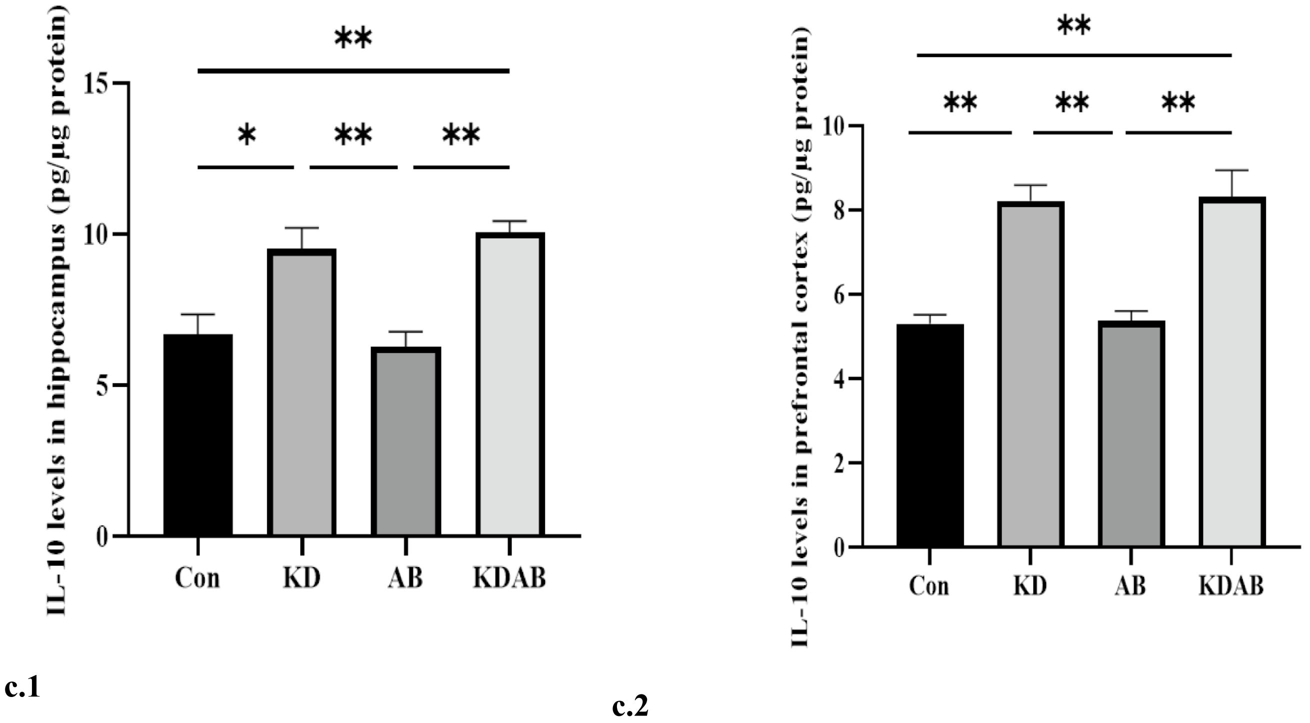

Fig. 6 (continued)

may lead to fungal overgrowth without further bacterial depletion (Goulding et al. 2021; Tirelle et al. 2020). This approach minimizes stress and potential antibiotic resistance, consistent with protocols in earlier research (Battson et al. 2018; Josefsdottir et al. 2017). Although a 7-day antibiotic regimen is widely used in rodent models to induce microbiota depletion, the translational relevance of this duration must be carefully considered. Rodent-to-human age conversion models suggest that one rat day corresponds to approximately 35 human days, implying that a 7-day intervention in rats equates to nearly 6–8 months of human exposure (Sengupta 2013). Therefore, while our experimental design aimed to model a transient and reversible perturbation of the gut microbiota, it may also serve as a proxy for chronic microbiota disruption in humans. This duality presents both a strength—in terms of modeling time-dependent effects—and a limitation in terms of direct clinical equivalence. Future studies may benefit from comparing short- and long-term antibiotic regimens across species-relevant timeframes. Thus, our model may simultaneously reflect both a short-term mechanistic probe and a proxy for sustained microbial dysbiosis, depending on the translational context. By using this method, we aimed to examine its combined impact with KD on cognitive processes during aging.

Aged rats on the ketogenic diet (with or without antibiotics) showed superior performance in both spatial memory (MWM) and recognition memory (NORT) tasks compared to aged-matched controls on a standard diet. KD-fed rats learned the water maze task faster and remembered the platform location better during the probe trial, indicating enhanced spatial learning and memory retention. These results are consistent with previous reports that ketogenic diets can improve cognitive outcomes in aging and disease models (Krikorian et al. 2012; Newman and Verdin 2017; Maalouf et al. 2007). For example, KD has been shown to improve memory and reduce mid-life mortality in aging mice, and to mitigate cognitive decline in models of Alzheimer's disease. The cognitive benefits of KD observed here occurred even though aged rats were not genetically or chemically predisposed to neurodegeneration, suggesting that KD's effects extend to normal age-related cognitive decline. Mechanistically, the improved performance may be attributable to multiple factors induced by KD: elevated ketone bodies (like BHB) which can serve as efficient fuel for neurons, reduced systemic and central inflammation, and increased levels of neurotrophic factors that support synaptic plasticity. Our KD rats indeed exhibited higher hippocampal and cortical BDNF levels relative to controls. BDNF is crucial for learning and memory, and higher brain BDNF in KD groups likely contributed to their enhanced cognitive function. This aligns with prior studies linking KD or caloric restriction to increased BDNF expression and improved synaptic function in aged animals (Frohlich et al. 2016; Xu et al. 2010). It is noteworthy that KD's cognitive enhancement persisted even when gut bacteria were depleted (KDAB group), implying that ketone metabolism and its neural effects can partially compensate for a lack of normal microbiota-derived influences.

The antibiotic-only group (AB) provides a contrast, highlighting the importance of the gut-brain axis in aging. A week of broad-spectrum antibiotics caused notable deficits in spatial memory: AB-treated rats spent less time searching in the correct target quadrant in the MWM probe trial, suggesting poorer memory retention compared to controls. This outcome is in line with literature showing that disrupting the gut microbiome with antibiotics can negatively impact cognitive function and neuroplasticity. Antibiotic-treated adolescent mice, for instance, exhibit exacerbated memory impairments later in life (Mosaferi et al. 2021). In our study, AB rats did not show a significant deficit in the NORT, indicating object recognition memory might be less sensitive to short-term microbiota disturbance than spatial memory, or that our antibiotic duration (1 week) was insufficient to produce effects in that domain. However, the trend across tasks and biological measures suggests that an intact gut microbiome supports optimal brain function in aging, whereas its disruption can be detrimental.

Neuroinflammatory changes in AB group were somewhat paradoxical. We observed that AB rats had slightly lower levels of certain pro-inflammatory cytokines (TNF-α) in the brain relative to controls. One might expect microbiota depletion to reduce microglial activation due to fewer microbial stimuli (e.g., lipopolysaccharide). Indeed, studies have found that germ-free or antibiotic-treated mice can show blunted innate immune activation in some contexts (Godbout et al. 2005). Our AB group's modest TNF-α reduction could reflect this phenomenon. However, the same AB rats did not exhibit any increase in the anti-inflammatory cytokine IL-10 nor did they show improvements in BDNF. Thus, the net effect of antibiotics alone was an unfavorable one for the aging brain: cognitive ability declined (despite slightly lowered TNF-α), potentially because the absence of beneficial gut microbes and microbial metabolites (such as short-chain fatty acids and vitamins) led to deficits in other neuroprotective pathways. It has been reported that antibiotic-induced dysbiosis can impair hippocampal neurogenesis (Ma et al. 2018; Vogt et al. 2017). Additionally, while certain inflammatory markers might decrease, others could increase, or compensatory mechanisms might fail. For example, we saw that IL-1β in the hippocampus tended to be highest in AB group, and BDNF was lowest. These changes suggest a loss of the homeostatic, tonic signals from the microbiome that normally help maintain a balanced neuroimmune environment.

Notably, KDAB group largely avoided the specific memory impairments observed in the AB group and retained most of the neurochemical benefits of KD. KDAB rats performed nearly as well as, and in some measures even better than, KD rats in the behavioral tests. KDAB rats performed nearly as well as (and in some measures better than) KD rats in the behavioral tests. Furthermore, KDAB animals had low brain TNF-α and IL-1β levels and high IL-10 and BDNF, very similar to the KD profile. This indicates that ketosis and its downstream effects can counteract or overshadow the absence of the microbiota. One possible explanation is that ketone bodies themselves exert anti-inflammatory and neuroprotective effects. BHB also provides an alternative energy substrate that may improve neuronal survival and function during aging. Additionally, KD has been shown to alter the gut environment in ways that might mitigate some negative consequences of antibiotics; for example, ketogenic diets can increase certain beneficial bacterial metabolites (even if overall diversity is reduced) and strengthen the intestinal barrier. In our experiment, although we did not directly analyze the gut microbiota, the preserved cognitive function in KDAB rats suggests that KD could have protected the brain by maintaining metabolic and anti-inflammatory support in the absence of microbes. Our cytokine data reveal an interesting pattern: KD reliably decreased pro-inflammatory mediators (TNF-α, IL-1β) and increased the anti-inflammatory IL-10 in the aged brain. Chronic, low-grade inflammation (sometimes termed "inflammaging") is a hallmark of aging and is implicated in cognitive decline and neurodegenerative disease progression (Li et al. 2023; Nomura et al. 2024). The results imply that KD can partially reverse aspects of inflammaging in the brain. It is worth noting that even though antibiotics alone slightly reduced TNF-α, they did not raise IL-10; in fact, only the KD (with or without AB) led to substantial IL-10 elevation. IL-10 is known to support cognitive function by restraining excessive microglial activation, so its increase in KD groups likely contributed to the improved outcomes. The rise in IL-10 might be driven by KD-induced changes in gut bacteria (KD is reported to promote certain beneficial bacterial taxa capable of stimulating anti-inflammatory responses) or by ketone-mediated modulation of immune cell metabolism.

BDNF levels were significantly increased by KD in our aged rats. BDNF is crucial for synaptic plasticity, learning, and memory, and tends to decline with age (Allan et al. 2024; Hong et al. 2023; Komulainen et al. 2008). Enhanced BDNF in KD groups provides a plausible molecular explanation for their superior cognitive performance. These findings align with existing research indicating that KD can increase BDNF gene expression and protein content in the hippocampus, thereby improving memory in both aging and neurological disease models (Allan et al. 2024; Jiang et al. 2017). Interestingly, in the hippocampus, KDAB did not produce as robust a BDNF increase as KD alone, even though KDAB's cognitive performance was excellent. It is possible that the antibiotic treatment prevented a full BDNF upregulation in HC despite ketosis—perhaps due to elimination of certain microbiota that contribute to BDNF regulation (for example, through gut production of short-chain fatty acids or vitamins like B vitamins that support neuronal health). Still, KDAB hippocampal BDNF was intermediate between KD and control, and in the PFC both KD and KDAB strongly elevated BDNF. Thus, KD's capacity to increase BDNF largely persists during microbiome disruption, but region-specific nuances may exist.

One limitation of this study is that we did not directly assess the gut microbiome changes induced by the diets or antibiotic treatment. Although we infer from known literature that our antibiotic regimen caused significant depletion of gut bacteria, we lack microbiological data confirming the extent of dysbiosis or identifying which microbial populations were affected. Similarly, while KD is known to shift gut microbiota composition, we did not measure whether KDAB rats had a different microbial profile than AB rats (for example, KD might promote some residual microbiota even in the presence of antibiotics). Future studies incorporating fecal microbiota sequencing would be valuable to correlate specific microbial changes with the observed cognitive and biochemical outcomes. Another limitation is the relatively short duration of antibiotic exposure (1 week); a longer antibiotic treatment might produce more pronounced cognitive deficits or neuropathological changes, which could further clarify the interplay between microbiota absence and KD. Additionally, our behavioral tests focused on memory; it would be informative to examine other cognitive domains (such as executive function or motor coordination) to see if KD provides broad neurological benefits in aging.

To expand on our findings, future studies should investigate whether restoration of the microbiota (e.g., via fecal transplantation) can reverse antibiotic-induced cognitive impairments. Analyzing microbial metabolites and markers of inflammation or oxidative stress could reveal how KD and antibiotics differentially affect brain biochemistry. Our data suggest that KD may help protect brain health by creating a metabolic environment that compensates for microbial loss—this could potentially be tested with ketone or SCFA supplementation. Clinically, KD could be investigated as a strategy to improve cognitive function and reduce inflammation, particularly in older individuals exposed to antibiotics or affected by gut dysbiosis.

Conclusion

Our findings suggest that a ketogenic diet can enhance cognitive performance and reduce neuroinflammation in aged rats, even under conditions of microbiota disruption. KD-fed animals exhibited elevated levels of neuroprotective markers such as BDNF and IL-10, alongside reductions in proinflammatory cytokines. While antibiotic treatment alone did not significantly improve cognitive or neurochemical measures, KD appeared to retain its beneficial effects even when combined with microbiota depletion. These results highlight the potential of ketogenic strategies to support brain health in aging, especially when implemented alongside interventions that preserve gut microbial integrity.

Author Contribution R.I. and O.S.: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. E.C.A.: Writing – review & editing, Methodology, Investigation, Formal analysis. F.H. and M.A.: Project administration, Methodology, Investigation, Writing – review & editing, Data curation.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval All protocols were reviewed and approved by Animal Care Committee of Dokuz Eylul University (approval number: 35/2023). The experimental study team took care of the rats and conducted all procedures in alignment with the guidelines provided in the "Guide for the Care and Use of Laboratory Animals."

Consent to Participate Not applicable.

Consent for Publication All authors consent to the publication of this manuscript.

Competing interests The authors declare no competing interests.

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