



# Effect of Carvacrol and Voluntary Exercise on Hippocampus Molecular Profile of High-Fat Dieted Male Rats

## ARTICLE INFO

### Article Type

Original Research

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### How to cite this article

Baratashvili A, Javakhishvili E, Tarkhnishvili E, Kvantidze I. Effect of Carvacrol and Voluntary Exercise on Hippocampus Molecular Profile of High-Fat Dieted Male Rats. GMJ Medicine. 2025;4(1):37-41.

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### Article History

Received: July 18, 2024  
Accepted: January 13, 2025  
ePublished: March 7, 2025

## ABSTRACT

**Aims** High-fat diet (HFD) is one risk factor in some disorders and increases oxidative stress. The use of carvacrol and voluntary exercise can be profitable. This study was thus conducted to evaluate the single and combined effects between carvacrol and voluntary exercise on gene expression in hippocampus of male rats fed with high-fat diet.

**Materials & Methods** A total number of 60 adult Wistar male rats were divided into 5 groups: 1) Healthy control, 2) HFD group, 3) VE group that received HFD plus voluntary exercise, 4) Carvacrol group received HFD plus Carvacrol and 5) VE + Carvacrol group that received HFD plus Carvacrol and voluntary exercise. Gene expression of hippocampal brain-derived neurotrophic factor (BDNF), Tropomyosin receptor kinase B (Trk-B), synapsin I and Cyclic AMP-Response Element Binding protein (CREB) were investigated.

**Findings** HFD significantly decreased expression of BDNF, Trk-B, synapsin I and CREB, but inclusion of carvacrol and the use of voluntary exercise could significantly increased gene expression of BDNF, Trk-B, synapsin I and CREB ( $p < 0.05$ ). The best responses were observed in animals fed with carvacrol in along to voluntary exercise ( $p < 0.05$ ).

**Conclusion** Carvacrol and voluntary exercise improve gene expression of BDNF, Trk-B, synapsin I and CREB in rats fed with HFD.

**Keywords** BDNF; Carvacrol; Exercise; Trk-B; Diet, High-Fat; Rats

## CITATION LINKS

[1] Nutritional modulation of cognitive function and mental ... [2] Activating transcription factor 4 regulates osteoclast ... [3] Caloric restriction leads to high marrow adiposity and ... [4] Overview of epidemiology and contribution of obesity to ... [5] Maternal obesity induced by a high fat diet causes altered ... [6] High-fat diet impairs hippocampal neurogenesis ... [7] A highfat, refined sugar diet reduces hippocampal brain-derived ... [8] A high-fat diet impairs neurogenesis: involvement of ... [9] Increased BDNF and Trk-B mRNA expression in cortical ... [10] Neurotrophins stimulate phosphorylation of synapsin I by ... [11] Synapsins as mediators of BDNF-enhanced neurotransmitter ... [12] Effects of high fat diet on Morris maze performance, oxidative stress, and ... [13] High dietary fat induces NADPH oxidase-associated oxidative stress ... [14] Effects of hypericum scabrum extract on anxiety and oxidative ... [15] The role of carvacrol as active compound of essential oils in ... [16] PPARs: Therapeutic targets for metabolic ... [17] Carvacrol Alleviated negative effects of diabetes on inflammation ... [18] Exercise, cognition, and the aging ... [19] Beneficial effects of Spirulina platensis, voluntary exercise and environmental enrichment ... [20] Exercise enhances learning and hippocampal neurogenesis ... [21] Long-term exercise is needed to enhance synaptic plasticity ... [22] Development of obesity is associated with increased calories ... [23] The role of leptin and ghrelin in the regulation of food intake ... [24] Adipose tissue leptin production and plasma leptin ... [25] Voluntary exercise and its effects on body composition depend on genetic selection ... [26] Dietary carvacrol lowers body weight gain but improves feed conversion in ... [27] Brain-derived neurotrophic factor (BDNF) and food intake regulation ... [28] BDNF regulates eating behavior and locomotor activity in ... [29] Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction ... [30] Brain-derived neurotrophic factor regulates energy balance downstream of ... [31] A de novo mutation affecting human trkb associated with severe ... [32] Molecular therapy of obesity and diabetes by a ... [33] Brain-derived neurotrophic factor plays a role as an anorexigenic ... [34] The interrelationship of metabolic syndrome and neurodegenerative diseases with focus on brain-derived ... [35] Obesity: Genes, brain, gut and ...

## Introduction

It is evident that life style and nutritional condition can have significant roles in public health, neuronal activity, memory, and learning in all the life-span of the peoples [1]. The high-fat diet (HFD) is one risk factor in some disorders including dyslipidemia and obesity [2]. There was positive correlation between body structure, adiponectin, and bone variables in animals fed with a HFD diet [3]. Obesity is significantly increasing, especially in the developed and developing countries which could increase the risk of cardiovascular disease and neurological disorders [4, 5]. The HFD can fault hippocampal long-term potentiating in the granular cells of the dentate gyrus and also change neurogenesis in the hippocampus region [6, 7]. It is shown that consumption of HFD could disrupt hippocampal neurogenesis by increased serum corticosterone. It also reduces some newly generated cells in the dentate gyrus and the level of hippocampal brain-derived neurotrophic factor (BDNF) [8]. The BDNF is one of growth factors that encourage neuronal survival and synaptic plasticity by its contact with Tropomyosin receptor kinase B (Trk-B) [9]. BDNF influences neuronal plasticity through some molecules including synapsin I and Cyclic AMP-Response Element Binding protein (CREB). Synapsin I plays a significant role in synaptogenesis and axonogenesis which affects synaptic vesicle exocytosis, and has a mediation role in modulation of BDNF for release of neurotransmitter [10, 11]. Some studies reported that oxidative stress has a significant role in the HFD-induced neurotoxicity [12-14]. Oxidative stress produces hydroxyl radicals, lipid peroxidation, and finally causes apoptotic cell death. Carvacrol is one phenol which is broadly found in some plant species [15]. It has some pharmacological properties such as anti-inflammatory [16] and antioxidant [17] that may be profitable in animals fed with HFD. On the other hand, exercise regimens are extensively used for improvement of neurological defaults [18] levels of BDNF [19], neurogenesis [20] and synaptic plasticity [21]. We believed that carvacrol and voluntary exercise, in combination form, could influence gene expression in hippocampus of rats fed with high-fat diets by influencing on BDNF and antioxidant properties. This study was thus conducted to evaluate the effects of carvacrol and voluntary exercise on gene expression in hippocampus of rats fed with HFD.

## Materials and Methods

### Animals and diets

A total number of 60 adult Wistar male rats with initial weight of  $140 \pm 5$ g and 4 to 6 Weeks of age were used. The experimental condition included temperature of  $22 \pm 2^\circ\text{C}$ , relative humidity of  $55 \pm 5\%$ , and a light diet of 12-hour light/12-hour dark cycle.

Carvacrol (Sigma-Aldrich; France) was administered in dose of 10mg/kg in oral form as recommended by previous studies [17]. Animals in the voluntary exercise group had free access to a cage that was equipped with a running wheel, as recommended by others [19]. The rats were randomly assigned to the following groups: Animals were grouped into 5 groups:

- 1- Intact animals that received a standard laboratory diet;
- 2- The HFD group received HFD (D12492) for nine weeks as suggested by previous studies [22];
- 3- VE group that received HFD plus voluntary exercise;
- 4- Carvacrol group received HFD plus Carvacrol; and
- 5- VE+ Carvacrol group that received HFD plus Carvacrol and voluntary exercise. Body weight changes were recorded in initial and end of trial.

### Preparation for molecular studies

In the end of study, the animals were killed and their brain was separated. The hippocampus were dissected out on ice, stored in liquid nitrogen, and kept in the  $-80^\circ\text{C}$  for future uses. The hippocampus were used for real time polymerase chain reaction (RT-PCR). The primers sequences were BDNF, forward (5'- GATTAGGTGGCTTCATAGGAGAC-3') and reverse (5'- AGAACAGAACAGAACAGAACAGG-3'), Trk $\beta$ , forward (5'- TATGCCGTGGTGGTGATTG-3') and reverse (5'-TGGAGATGTGGTGGAGAGG-3'), SynapsinI, forward (5'- CTCAGCAGCACAAACATACC-3') and reverse (5'-TTCTGGACACGCACATCG-3'), CREB forward (5'- CCAGAAGATGAAGCGAGTC-3') and reverse (5'-TTGATGTTGAGGCAGAAGG-3') and GAPDH forward (5'- TTCAACGGCAGTCAAGG-3') and reverse (5'- CTCAGCACCAGCATCACC-3').

### Statistical Analysis

Statistical analyses of the results were conducted by the SPSS 21 software. The data was analyzed by the one-way analysis of variance (ANOVA) and Tukey post-hoc comparison.

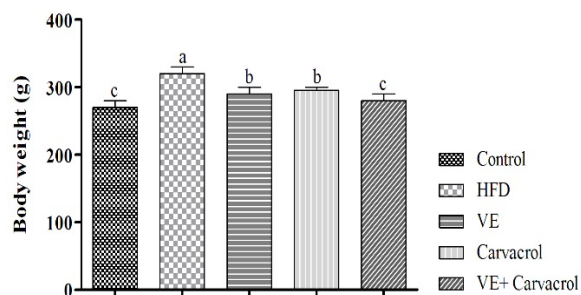
## Findings

### Body weight

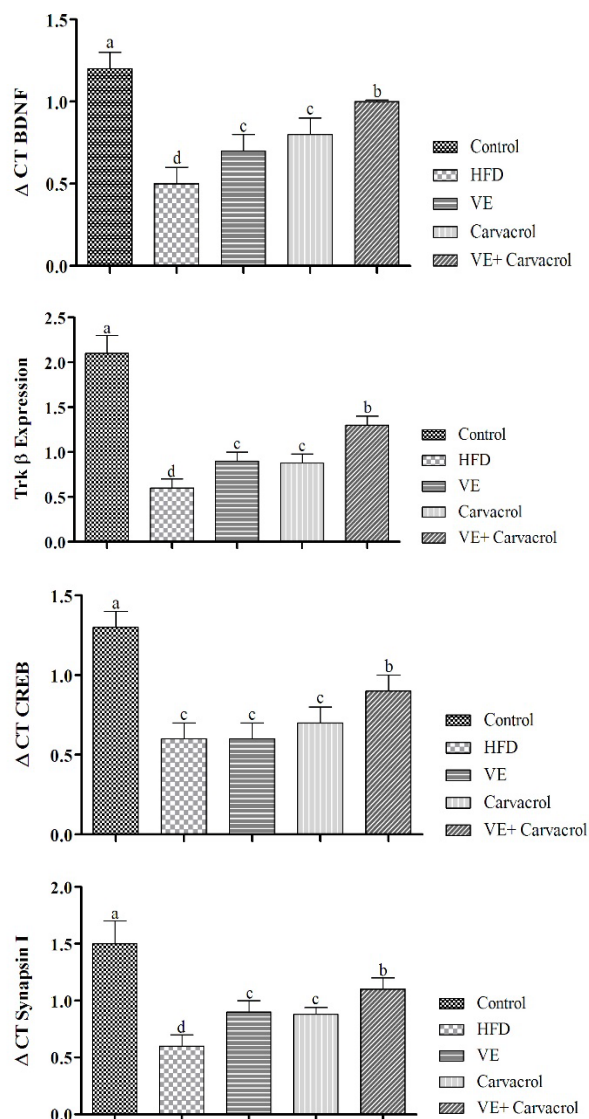
Rats fed with HFD showed higher body weight in comparison to control group ( $p < 0.05$ ). The use of carvacrol and voluntary exercise significantly decreased body weight in comparison to control group ( $p < 0.05$ ), especially in combined form ( $p < 0.05$ ; Figure 1).

### Gene expression

HFD significantly reduced expression of BDNF, Trk-B, synapsin I and CREB ( $p < 0.05$ ) in comparison to control group ( $p < 0.05$ ), but oral supplementing of carvacrol and voluntary exercise, singly and specially in combination form, could increase gene expression of BDNF, Trk-B, synapsin I and CREB ( $p < 0.05$ ; Figure 2).



**Figure 1.** Effects of carvacrol and voluntary exercise on body weight of rats fed with HFD. Superscripts (a-c) show significant difference between groups at level of 0.05



**Figure 2.** Effects of carvacrol and voluntary exercise on molecular profile in hippocampus of male rats fed with HFD

## Discussion

HFD significantly increased body weight in animals. Rats fed with HFD consume high levels of food and obtain significant energy; resulting in higher body weight. Increased body weight could be attributed to adipose tissue mass. Leptin as one adipocyte-derived hormone controls feed consumption and

energy metabolism [23]. It was reported that plasma levels of leptin increases with increasing body fat mass [24]. It is known that voluntary wheel-running activity influences body weights, but its efficiency on body composition were influenced by genetic structure [25]. On the other hand, carvacrol decreases body weight due to its role on prevention of 3-hydroxy-3-methylglutaryl coenzymeA reductase and the rate controlling enzyme of the cholesterol synthetic pathway [26]. Thus, the both have synergistic interaction on body weight and can improve body weight. It is also attributed to gene expression, as will be discussed.

HFD decreased expression of BDNF and Trk-B, but carvacrol and voluntary exercise increased expression of BDNF. It is well known that decreased hypothalamic of BDNF is involved in energy homeostasis and influences feed consumption and promotes anorectic signaling [27]. In the other words, BDNF haploinsufficiency [28, 29] or missense mutations in its receptor (Trk-B) [30, 31], are related with hyperphagia, and obesity both in human and in the animal models. Administration of BDNF in an animal model of obesity and type 2 diabetes mellitus controls normal feed consumption induces weight loss and reduces insulin resistance [32, 33]. It means that BDNF faulted in the brain induced a metabotropic faulted and caused to obesity [34, 35]. Previous studies have showed that voluntary exercise increased levels of BDNF [19]. Carvacrol improved BDNF and Trk-B levels but its mechanism is not known. It might be attributed to antioxidant properties of carvacrol that prevents oxidation of BDNF. Carvacrol and voluntary exercise increased levels of CREB and synapsin I. Synapsin I acts in synaptogenesis and axonogenesis that influences synaptic vesicle exocytosis, and acts as a mediator role production of BDNF for release of neurotransmitter [10, 11].

Mechanism of action is still unknown and needs future investigations. It can be recommended to use of the carvacrol and voluntary exercise as protective treatments in individuals fed HFD.

## Conclusion

HFD increased body weight and decreased gene expression of BDNF, Trk-B, synapsin I and CREB. This study for first time highlights synergism interaction effects between carvacrol and voluntary exercise on gene expression of BDNF, Trk-B, synapsin I and CREB in rats with HFD.

**Acknowledgements:** Compliance with ethical guidelines Approval for this study was obtained from International Center for Intelligent Research.

**Ethical Permission:** None declared by the authors.

**Conflicts of Interests:** None declared by the authors.

**Authors' Contribution:** All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.



**Funding/Support:** This study was supported by a grant from International Center for Intelligent Research (ICIR-2018-1855128).

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