**Research Article**

# Combined oral supplementation of chromium picolinate, docosahexaenoic acid, and boron enhances neuroprotection in rats fed a high-fat diet

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**Background/aim:** A novel complex of a nutritional supplement (CDB) contains chromium picolinate (CrPic), phosphatidylserine (PS), docosahexaenoic acid (DHA), and boron (B). The present study aimed to investigate the effects of CDB on the metabolic profile and memory acquisition in rats fed a high-fat diet (HFD).

**Materials and methods:** Male Wistar rats were divided into six groups and received either a regular diet or HFD supplemented with or without different levels of CDB (0, 11, or 22 mg/kg BW).

**Results:** Rats fed the HFD had greater glucose, insulin, lipid profile, and serum malondialdehyde concentrations, but lower serotonin and tryptophan in the serum and brain and lower Cr concentrations in serum, kidney, brain, and liver ($P < 0.0001$). CDB complex supplementation reversed all the effects, and the reversal effect was more pronounced with HFD for some parameters. Latency was less ($P < 0.05$) but probe was greater ($P < 0.0001$) for rats fed a regular diet. Increasing CDB complex levels in the diets resulted in a linear decrease in latency ($P < 0.0002$) but a linear increase in probe ($P < 0.0002$).

**Conclusion:** Findings of the present work indicate that the CDB complex could be considered as an alternative treatment for preventing certain metabolic diseases and improving neurological functions, such as learning and memory.

**Key words:** Chromium picolinate, docosahexaenoic acid, boron, high-fat diet

## 1. Introduction

Diet is a determining factor in maintaining metabolic, neural, and cognitive balance for humans (1). Consuming a high-fat diet (HFD) for an extended period, for example, results in obesity, metabolic disorders, and oxidative stress in humans (2,3) as well as in rats (4). Rats fed a HFD have also been reported to have decreased brain function expressed as learning and memory deficits (5,6). It has been shown that feeding a diet rich in fat decreased hippocampal neurogenesis in rats (7). Studies also indicated that HFDs significantly increased weight gain and insulin resistance, resulting in impaired glucose tolerance in rats (8), and increased triglycerides and cholesterol concentrations in other animals (9).

Chromium (Cr) is an essential trace element for mammals. Chromium picolinate (CrPic) supplementation has been reported to improve glucose utilization by neural cells (10) and to reduce hepatic and serum thiobarbituric acid reactive substance (TBARS) formation in rats (5). Kim et al. (11) found that CrPic in Cr-deficient rats leads to the

improvement of glucose intolerance, insulin sensitivity, and weight loss and also an improvement in lipid metabolism. Clinical studies suggested that oral supplementation of phosphatidylserine (PS) and docosahexaenoic acid (DHA) resulted in a little therapeutic effect in improving memory and other cognitive functions in elderly individuals with memory complaints (12), but improvement of lipid (13) and carbohydrate metabolism (14) as well as reduced reactive oxygen species in mice (15).

The dietary need for and effects of boron (B) supplementation in humans and animals have not been fully illuminated. Some studies reported the inconsistent effects of boron on insulin, energy substrates (triglycerides, glucose), and oxidative metabolism (16–18). Assessments in both animals and humans have shown that boron deprivation results in decreased brain electrical activity (19).

The aim of this study was to test whether a complex (CDB) containing CrPic, PS, DHA, and B is effective on live weight, serum glucose, insulin, total cholesterol,

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triglyceride concentrations, tissue and blood Cr content, serotonin, tryptophan, oxidative balance (malondialdehyde, MDA), as well as to evaluate the neuroprotective effect by determining learning and memory performances in rats fed a HFD. The CDB complex was greatly effective in reducing body weight, blood glucose, cholesterol, triglyceride, and oxidative stress and in improving memory in rats fed a HFD.

## 2. Materials and methods

### 2.1. Animals and diets

Male Wistar rats (n = 60, 8 weeks old) weighing 180 ± 10 g were purchased from the Veterinary Research Institute (Elazığ, Turkey). The animals were housed at a temperature of 22 ± 2 °C, humidity of 55 ± 5%, and light/dark cycle of 12/12 h throughout the experiment. All animal procedures were approved by the Institutional Animal Care and Use Committee at the Institute of Veterinary Research (Elazığ, Turkey). All procedures involving rats were conducted in strict compliance with relevant laws, the Animal Welfare Act, public health services policy, and guidelines established by the Institutional Animal Care and Use Committee of the institute. Rats were fed a regular diet (12% of calories from fat) or a HFD (42% of calories from fat). The compositions of the control and high-fat diets are given in Table 1. The CDB complex, containing

**Table 1.** Composition of diets (g/kg diet) fed to rats.

|  | Control | HFD* |
|---|---|---|
| Casein | 200.0 | 200.0 |
| Starch | 579.5 | 150.0 |
| Sucrose | 50.0 | 149.5 |
| Soybean oil | 70.0 | - |
| Beef tallow | - | 400.0 |
| Cellulose | 50.0 | 50.0 |
| Vitamin-mineral premix** | 45.0 | 45.0 |
| L-cysteine | 3.0 | 3.0 |
| Choline Bitartrate | 2.5 | 2.5 |

*: HFD: High-fat diet; **:The vitamin-mineral premix provides the following (per kg): all-*trans*-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-*rac*-a-tocopherol acetate, 12.5 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B12, 0.02 mg; folic acid, 0.55 mg; *d*-biotin, 0.1 mg; manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg.

CrPic (14 µg elemental Cr) as Chromax®, PS (4.5 mg), DHA (6 mg), and B (14 µg), was provided by Nutrition 21 (Purchase, NY, USA). The supplemental complex (total ~11 mg) was given to rats at a dose per kilogram body weight (BW) per day. The supplements were dissolved in ethanol (with the final ethanol concentration being 0.01%) before administration. Supplementation (11 mg or 22 mg/kg BW) was administered via orally inserted intragastric intubation once a day at 0900 hours. Control rats received isovolumetric intragastric intubation of drinking water containing the ethanol (concentration being 0.01%) at the same time. The experiment lasted for 12 weeks.

### 2.2. Experimental design

A total of 60 male rats were divided into 6 groups of 10 animals each in 2 × 3 factorial designs (2 diets and 3 levels of CDB complex). The experimental diets fed to rats were as follows: i) control group: rats were fed a standard diet (12% calories from fat, 3.82 kcal/g); ii) 11 mg CDB complex group: rats were fed the standard diet and received 11 mg of CDB complex; iii) 22 mg CDB complex group: rats were fed a standard diet and received 22 mg of CDB complex; iv) HFD group: rats were fed a high-fat diet (42% calories from fat, 4.70 kcal/g); v) HFD group plus 11 mg CDB complex: rats were fed a high-fat diet and received 11 mg of CDB complex; vi) HFD group plus 22 mg CDB complex: rats were fed a high-fat diet and received 22 mg of CDB complex. Diets and fresh water were offered ad libitum throughout the experiment. The diets were stored at 4 °C in a dark room.

### 2.3. Data collection and laboratory analyses

At the end of the experiment, all rats were sacrificed by cervical dislocation. Blood samples were taken and the liver, kidneys, and brain were removed and processed for biochemical and western blot analyses. Blood samples were centrifuged at 3000 × *g* for 10 min and sera were separated. Serum glucose (ACCU-Check Active, Roche Diagnostics, Mannheim, Germany) and insulin (Linco Research Inc, St. Charles, MO, USA) concentrations were measured using a commercial kit in a spectrophotometer with ELISA (EL$_x$-800, Bio-Tek Instruments Inc., Winooski, VT). Serum total cholesterol (TC) and triglyceride (TG) concentrations were also determined using an autoanalyzer (Samsung LABGEO PT10, Samsung Electronics Co., Suwon, Korea). Composite of Insulin Sensitivity Index (CISI) scores were calculated according to the following formula (20):

CISI = 10,000/square root of [(G0) (I0) (mean serum insulin during OGTT) (mean blood glucose during OGTT)].

After digestion with a mixture of concentrated $HNO_3$ (65%, Merck, Darmstadt, Germany) and $H_2O_2$ (30%, Merck) in a microwave digestion system (Berghoff, Eningen, Germany), liver, kidney, and brain samples and blood sera were analyzed for Cr content using a graphite

furnace atomic absorption spectrophotometer (AAnalyst 800, PerkinElmer Corp., Norwalk, CT, USA) as described by Dogukan et al. (21).

Serum MDA concentrations were measured by high-performance liquid chromatography (HPLC; Shimadzu, Tokyo, Japan) using a Shimadzu UV-Vis with a SPD-10 AVP detector and a CTO-10 AS VP column. The mobile phase was 30 mM $KH_2PO_4$-methanol (82.5 + 17.5, v/v %, pH: 3.6) and the flow rate was 1.2 mL/min. Column effluents were monitored at 250 nm and the injection volume was 20 µL. The serum MDA level was measured (22) using the fully automatic HPLC (Shimadzu, Kyoto, Japan).

Brain samples upon killing were rapidly removed and dissected on ice, wrapped in foil, and immediately frozen and stored at –80 °C until the serotonin concentration and binding assay could be performed. Brain samples were homogenized in 0.1 N HCl (100 µL/mg of tissue) and centrifuged for 10 min at $1500 \times g$ at 4 °C. From this extract, serotonin levels were measured using enzyme-linked immunosorbent assay kits (IBL Immuno-Biological Lab., Hamburg, Germany) according to the manufacturer's protocol. Brain insulin levels were measured as mentioned previously by Havrankova et al. (23). Serum and brain tryptophan was measured using HPLC (Shimadzu, Tokyo, Japan). Separation was obtained with a reverse-phase column and a mobile phase (flow rate: 1 mL/min) composed of 5% acetonitrile, 100 mM phosphate buffer (pH 3.6), and 1 mM ethylenediaminetetraacetic acid (EDTA). Detection was done with a Shimadzu model LC 120 (HPLC; Shimadzu, Tokyo, Japan). Excitation and emission wavelengths were 313 and 420 nm, respectively.

On five consecutive days at the end of the experiment, animals were subjected to a spatial version of the Morris water maze test to evaluate their spatial memory acquisition phase (SMAP) performance (24). In this test, time to reach the platform (latency in seconds) was measured after placing the black target platform inside the pool 1 cm above the water line as described by Baydas et al. (25). To attain the extent of the memory consolidation after learning, rats were subjected to a probe trial in which a visible platform was placed on a new location on the final day of training (26). In this test, mean percentage time spent in the target quadrant reflects the degree of memory consolidation.

### 2.4. Statistical analysis

The data were analyzed using the general linear model procedure of SAS software for a factorial design. Significant differences at 5% among treatment means were determined for all groups. When F-tests were significant, orthogonal contrasts were used to determine linear and quadratic effects of increasing CDB level.

## 3. Results

Effects of HFD and CDB complex level on body weight and various biochemical parameters are presented in Table 2. Body weights were lower for rats fed the control diet than for rats fed the HFD (276.8 vs. 306.7 g; P < 0.0001). CDB complex level did not affect BW (Table 2). Serum glucose and insulin concentrations and the ratio of glucose to insulin were all lower (P < 0.0001) for rats fed the control diet than for rats fed the HFD (Table 2). Increasing CDB complex level resulted in both decreased serum glucose concentrations and ratio of glucose to insulin but increased serum insulin concentrations (Table 2; linear, P < 0.0001). Significant dietary calorie level by CDB complex level interaction effect revealed that serum glucose concentrations decreased whereas serum insulin and the ratio of glucose to insulin concentrations increased at a lesser extent in the control rats than the HFD rats as CDB complex level increased (Table 2; P ≤ 0.05). Insulin resistance during of this study in rats was assessed by CISI score (Table 2). CISI score was greater for rats fed the control diet than for rats fed the HFD, further showing improved insulin sensitivity (P < 0.0001). The CDB complex level linearly increased the CISI score (P < 0.0001). Significant dietary calorie level by CDB complex level interaction effect revealed that CISI scores increased to a lesser extent in the control rats than the HFD rats as CDB complex level increased (Table 2; P < 0.0001).

All serum TC, TG, and FFA concentrations were lower for rats fed the control diet than for rats fed the HFD (P < 0.0001). CDB complex levels linearly decreased serum TC, TG, and FFA concentrations (P < 0.0001). Significant dietary calorie level by CDB level interaction effect revealed that serum TC, TG, and FFA concentrations decreased to a lesser extent in the control rats than the HFD rats as CDB complex level increased (Table 2; P < 0.0001).

Serum MDA concentrations were lower in rats fed the control diet than in rats fed the HFD (P < 0.0001). Supplementing CDB to the diet of rats linearly decreased both serum MDA concentrations (Table 2; P < 0.0001). Significant dietary calorie level by CDB level interaction effect revealed that serum MDA concentration decreased to a greater extent in the control rats than the HFD rats as CDB complex level increased (P < 0.0001).

Serum and brain serotonin were greater for rats fed the control diet than for rats fed the high-fat diet (P < 0.0001). CDB complex level linearly increased both serum and brain serotonin concentration (P < 0.0001). However, dietary calorie level by CDB complex level interaction effect on serum and brain serotonin was insignificant (Table 3). Serum and brain tryptophan was also greater for rats fed the control diet than for rats fed the HFD (P < 0.0001). CDB complex level linearly increased both serum and

**Table 2.** Effects of dietary calorie and CDB complex levels (0 mg/kg BW; 11 mg/kg BW; 22 mg/kg BW) on body weight and various biochemical parameters.

| Variable | Control diet | | | High-fat diet | | | SEM | Calorie | CDB level | Calorie × CDB level |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | CDB dosage, mg/kg BW | | | CDB dosage, mg/kg BW | | | | | | |
| | 0 | 11 | 22 | 0 | 11 | 22 | | | | |
| Body weight, g | 275.8 | 276.4 | 278.1 | 316.1 | 302.2 | 301.7 | 4.8 | 0.0001 | 0.33 | 0.19 |
| Glucose, mg/dL[1] | 99.4 | 92.6 | 86.8 | 129.4 | 114.9 | 107.8 | 1.2 | 0.0001 | 0.0001 | 0.001 |
| Insulin, pmol/L[1] | 37.4 | 40.2 | 42.6 | 40.2 | 45.1 | 47.1 | 0.4 | 0.0001 | 0.0001 | 0.05 |
| Glu:Ins[1] | 2.66 | 2.30 | 2.04 | 3.22 | 2.54 | 2.29 | 0.04 | 0.0001 | 0.0001 | 0.001 |
| CISI, mg/dL[1] | 2.67 | 2.75 | 2.87 | 1.56 | 1.68 | 1.73 | 0.01 | 0.0001 | 0.0001 | 0.0001 |
| TC, mg/dL[1] | 55.5 | 49.1 | 42.8 | 111.6 | 72.5 | 68.2 | 1.4 | 0.0001 | 0.0001 | 0.0001 |
| TG, mg/dL[1] | 46.5 | 36.4 | 34.5 | 104.0 | 74.0 | 63.2 | 5.6 | 0.0001 | 0.0001 | 0.04 |
| FFA, mmol/L[1] | 1.40 | 0.90 | 0.61 | 3.20 | 2.10 | 1.71 | 0.09 | 0.0001 | 0.0001 | 0.0007 |
| Serum MDA, µg/mL[1] | 0.84 | 0.65 | 0.56 | 1.71 | 1.24 | 0.91 | 0.04 | 0.0001 | 0.0001 | 0.0001 |

CDB: Nutritional supplement contains chromium picolinate (CrPic), phosphatidylserine (PS), docosahexaenoic acid (DHA), and boron; (B). 0: Rats were fed a standard diet and did not receive CDB; 11: rats received 11 mg of CDB complex; 22: rats received 22 mg of CDB complex; CISI = Composite of Insulin Sensitivity Index; TC = total cholesterol; TG = triglycerides; FFA = free fatty acids; MDA = malondialdehyde.
Data are expressed as mean ± SE of 10 rats from each group.
[1]Linear CDB level effect, P < 0.0001.

**Table 3.** Effects of dietary calorie and CDB complex levels (0 mg/kg BW; 11 mg/kg BW; 22 mg/kg BW) on serum and brain serotonin and tryptophan levels.

| Variable | Control diet | | | High-fat diet | | | SEM | Calorie | CDB level | Calorie × CDB level |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | CDB dosage, mg/kg BW | | | CDB dosage, mg/kg BW | | | | | | |
| | 0 | 11 | 22 | 0 | 11 | 22 | | | | |
| Serum serotonin, µg/g[1] | 222.4 | 232.2 | 237.4 | 200.6 | 211.9 | 217.7 | 1.4 | 0.0001 | 0.0001 | 0.76 |
| Brain serotonin, µg/g[1] | 629.6 | 644.5 | 655.2 | 559.4 | 582.0 | 594.5 | 2.6 | 0.0001 | 0.0001 | 0.66 |
| Serum tryptophan, µg/g[1] | 24.6 | 25.8 | 27.2 | 20.7 | 22.0 | 23.2 | 0.2 | 0.0001 | 0.0001 | 0.88 |
| Brain tryptophan, µg/g[1] | 7.60 | 8.39 | 8.95 | 4.90 | 5.99 | 6.66 | 0.14 | 0.0001 | 0.0001 | 0.34 |

CDB: Nutritional supplement contains chromium picolinate (CrPic), phosphatidylserine (PS), docosahexaenoic acid (DHA), and boron; (B). 0: Rats were fed a standard diet and did not receive CDB; 11: rats received 11 mg of CDB complex; 22: rats received 22 mg of CDB complex.
Data are expressed as mean ± SE of 10 rats from each group.
[1]Linear CDB level effect, P < 0.0001.

brain tryptophan concentrations (P < 0.0001). However, dietary calorie level by CDB level interaction effect on both serum and brain tryptophan was insignificant (Table 3).

Serum, liver, kidney, and brain Cr concentrations were greater for rats fed the control diet than for rats fed the HFD (P < 0.0001). CDB complex level resulted in a linear increase in serum but quadratic increases in liver, kidney, and brain Cr concentrations (P < 0.0001). Dietary calorie level by CDB complex level interaction effect on serum Cr was insignificant. However, dietary calorie level by CDB complex level interaction effects revealed that liver Cr level linearly increased in rats fed the HFD diet, whereas it increased quadratically in rats fed the control diet (P < 0.006). Elevations in kidney and brain Cr levels in the

control rats were more notable than in the HFD group as dietary CDB complex level increased (P ≤ 0.02) (Table 4).

The spatial memory acquisition responses upon treatments are given in Table 5. Latency was lower but probe was greater for rats fed the control diet than for rats fed the HFD (P < 0.05, P < 0.0001, respectively). Increasing CDB complex levels resulted in a linear decrease in latency (P < 0.0001) but a linear increase in probe (P < 0.0002). However, dietary calorie level by CDB complex level interaction effect on latency and probe was insignificant. The decrease in latency over time (41.2 on day 1 to 8.9 on day 5; P < 0.0001; Figure) was independent of dietary calorie level and CDB level.

## 4. Discussion

The consumption of a HFD resulted in greater weight gain compared with the control diet. Due to its high-calorie content, feeding a HFD was expected to promote live weight gain. Chung et al. (27) found that Sprague-Dawley rats fed a HFD (3.85 kcal/kg energy) compared with rats fed a standard diet (4.65 kcal/kg energy) for 4 weeks had an increase in weight gain. Sahin et al. (28) also found that rats fed a HFD for 12 weeks had greater body weights compared with rats fed a regular diet. Results similar to those of the present work in rodents fed a HFD in an ad libitum model have also been reported (29). Although the presence of the CDB complex in the diet changed several parameters related to energy (carbohydrate and lipid) metabolism reported in the present work, the inclusion of the CDB complex in the diet unexpectedly did not have any influences on the body weight of rats.

Recommended dietary fat intake serves a number of essential body functions; however, HFDs often lead to excessive energy intake and are positively associated

**Table 4.** Effects of dietary calorie and CDB complex levels (0 mg/kg BW; 11 mg/kg BW; 22 mg/kg BW) on the serum and tissue chromium concentrations.

| Variable | Control diet | | | High-fat diet | | | SEM | Calorie | CDB level | Calorie × CDB level |
|---|---|---|---|---|---|---|---|---|---|---|
| | CDB dosage, mg/kg BW | | | CDB dosage, mg/kg BW | | | | | | |
| | 0 | 11 | 22 | 0 | 11 | 22 | | | | |
| Serum Cr, μg/g[1] | 0.016 | 0.027 | 0.027 | 0.012 | 0.021 | 0.021 | 0.001 | 0.0001 | 0.0001 | 0.17 |
| Liver Cr, mg/kg[2] | 0.021 | 0.030 | 0.028 | 0.016 | 0.020 | 0.021 | 0.001 | 0.0001 | 0.0001 | 0.006 |
| Kidney Cr, mg/kg[2] | 0.023 | 0.030 | 0.030 | 0.017 | 0.021 | 0.021 | 0.001 | 0.0001 | 0.0001 | 0.02 |
| Brain Cr, ng/g[2] | 17.0 | 25.6 | 25.4 | 11.2 | 14.9 | 14.8 | 0.4 | 0.0001 | 0.0001 | 0.0001 |

CDB: Nutritional supplement contains chromium picolinate (CrPic), phosphatidylserine (PS), docosahexaenoic acid (DHA), and boron; (B). 0: Rats were fed a standard diet and did not receive CDB; 11: rats received 11 mg of CDB complex; 22: rats received 22 mg of CDB complex.
Data are expressed as mean ± SE of 10 rats from each group.
[1]Linear CDB level effect, P < 0.0001.
[2]Quadratic AMF level effect, P < 0.0001.

**Table 5.** Effects of dietary calorie and CDB complex levels (0 mg/kg BW; 11 mg/kg BW; 22 mg/kg BW) on latency and probe.

| Variable | Control Diet | | | HFD | | | SEM | Calorie | CDB level | Calorie × CDB level |
|---|---|---|---|---|---|---|---|---|---|---|
| | CDB dosage, mg/kg BW | | | CDB dosage, mg/kg BW | | | | | | |
| | 0 | 11 | 22 | 0 | 11 | 22 | | | | |
| Latency[1,2] | 26.6 | 15.5 | 13.4 | 32.0 | 19.6 | 16.5 | 2.4 | 0.05 | 0.0001 | 0.90 |
| Probe[2] | 29.8 | 35.2 | 37.2 | 18.8 | 26.2 | 27.5 | 16 | 0.0001 | 0.0002 | 0.82 |

CDB: Nutritional supplement contains chromium picolinate (CrPic), phosphatidylserine (PS), docosahexaenoic acid (DHA), and boron; (B). 0: Rats were fed a standard diet and did not receive CDB; 11: rats received 11 mg of CDB complex; 22: rats received 22 mg of CDB complex.
Data are expressed as mean ± SE of 10 rats from each group.
[1]Time effect, P < 0.0001.
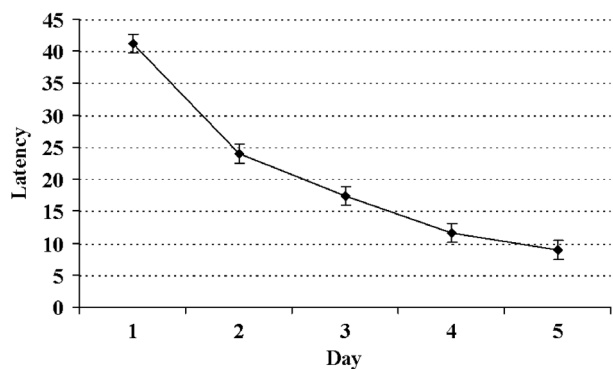[2]Linear CDB level effect, P < 0.0001.

**Figure.** Latency to find the hidden platform from the first day to the fifth day in the Morris water maze task in rats. Time effect was P < 0.0001.

with impairments in blood glucose regulation, lipid metabolism (30), and cognitive function (1). As expected, the HFD of the present work negatively influenced glucose metabolism by elevating blood glucose, insulin, and glucose:insulin ratios while decreasing CISI score, and also lipid metabolism by increasing TC, TG, and FFA concentrations. The presence of the CDB complex with a dose response ameliorated the negative effects of feeding the HFD, in particular when measured as glucose and lipid metabolism parameters. The effect of the CDB complex might have been due mostly to the Cr content of the combination. Sahin et al. (5) reported that feeding a HFD to diabetic rats increased serum glucose, total cholesterol, triglycerides, and FFA levels, but supplementary CrPic in the diet lowered these values. Pattar et al. (31) also indicated a novel effect of CrPic on cholesterol homeostasis and a blood glucose-lowering effect by altering the plasma membrane composition of cholesterol in fat and muscle cells. The positive (regulatory) effects of CrPic on serum lipid and carbohydrate values in rats have also been shown by other investigators (32,33). Supplementation of CrPic was reported to enhance insulin sensitivity and glucose disappearance and to improve lipids (total and HDL cholesterol) in obese male hyperinsulinemic rats (34). In addition, Krol and Kreipcio (35) reported that another chromium complex (chromium propionate) fed to diabetic rats for 5 weeks resulted in significant antidiabetic (insulin-sensitizing and hypolipidemic) potential and also reduced serum levels of triacylglycerols and total and LDL cholesterols. Padmavathi et al. (36) found that chronic maternal Cr restriction increased body adiposity in rat offspring and that might predispose them to obesity and associated diseases in later life.

The findings of glucose and lipid metabolism parameters could have been not only due to Cr picolinate but also DHA, PS, and B effects as well. Feeding Sprague-Dawley rats a DHA-rich diet for 9 weeks resulted in a decrease in blood triacylglycerols and total cholesterol concentrations and the intake of DHA is important for brain phospholipid DHA enrichment and brain NOS activity (37). Phosphatidylserine, a phospholipid component of cell membranes, functions in memory and cognition and has important roles in sports nutrition including hormone balance for athletes (38). Previous studies reported that physiologic amounts of boron in rats and cockerels may help reduce the amount of insulin required to maintain plasma glucose (39,40). All mentioned works (37–41) are in agreement with those of present results.

Serum and kidney, liver, and brain Cr concentrations decreased with feeding of the HFD diet, but the presence of the CDB complex containing Cr increased the Cr concentrations of these tissues. These results were expected as Cr supplements, by nature, accumulate in tissues for extended periods (42). The present work also showed that feeding a HFD depresses tissue Cr concentrations. Similar results were reported in type 2 diabetic mice (43). On the other hand, it was shown that poststroke hyperglycemia was accompanied by decreased tissue and circulating levels of chromium and increased urinary excretion level of chromium. However, daily chromium supplementation increased tissue chromium levels, attenuated brain infarction, improved hyperglycemia, and decreased plasma levels of glucagon and corticosterone in stroke rats (44). In another study, it was also reported that supplementation with chromium-containing milk powder for 3 weeks elevated serum chromium level and improved insulin resistance in obese mice (45).

Oxidative stress marker measured as MDA was greater in rats fed a HFD compared with rats fed a regular diet. Supplementing the CDB complex to both the high-fat and regular diet reduced the MDA concentrations. This is practically more important in the case of HFD feeding. In accord with the findings of the present work, Preuss et al. (46) found a decrease in hepatic TBARS formation in rats supplemented with CrPic or chromium nicotinate. A relation between free radical production and high lipid intake leads to oxidative stress, which is obvious from the increased lipid peroxidation levels in the serum and liver of rats fed the HFD in the present work. It has been reported that postprandial oxidative stress is caused by feeding an energy-dense, fatty meal and serum, liver, and heart MDA levels are increased by oxidative stress in HFD-fed rats (47,48). Although the known pathogenic mechanism of obesity with multiple etiologies remains obscure, experimental evidence indicates that ROS play an important role in the pathogenesis of obesity (49,50). Lupachyk et al. (51) also found that hypertriglyceridemia and/or increased nonesterified fatty acid concentrations cause prediabetic neuropathy through oxidative-

nitrosative stress. The results of the present work could have been not only due to Cr but also other components of the CDB complex. Similar to results of the present work, dietary supplementation of DHA and PS reduced ROS in the brain tissues of normal mice by 57% (15). Pan et al. (52) also reported that daily administration of DHA increased antioxidant enzyme activities and decreased MDA production in the brain.

Since serotonin is biochemically synthesized from tryptophan, serotonin and tryptophan were evaluated together in the present work. Serotonin and tryptophan levels were both lower in rats fed with HFD, and supplementing the CDB complex increased both serum and brain concentrations. Increases in serum and brain serotonin concentrations were paralleled to increases in serum and brain tryptophan concentrations. Similar to results of the present work, the synthesis of brain serotonin was reported to directly depend on the plasma concentration of its precursor, tryptophan (53). Serotonin is known for its feed intake depression effects and thus is an important factor in preventing obesity (54). Although not measured in the present work, feed intake could have been depressed, resulting in lower body weights for rats. In addition, serotonin reuptake transporter-deficient mice have been reported to exhibit hyperinsulinemia prior to the development of obesity. Feeding HFD to rats of the present work resulted in decreases in serotonin concentrations, in agreement with the work of Kirac et al. (55), who found that feeding a HFD to rats for 4 weeks differentially affected the stress response of striatal dopaminergic and serotonergic neurons in the brain, influencing motor activity performance.

Studies of the effects of CrPic, PS, DHA, and boron on tryptophan and brain serotonin concentration in both rats and humans with HFDs are limited. However, an animal study suggested that CrPic supplementation may alter 5-HT neuroendocrine function via increasing peripheral and central tryptophan availability and elevating brain serotonin synthesis from tryptophan (56). Khanam and Pillai (57) also found that CrPic increases serotonergic transmission in the brain of rats. Additionally, Dubey et al. (58) reported that CrPic increased serotonin concentration in the brain.

DHA is a major lipid component of the central nervous system, accounting for 30%–50% of the total fatty acid content of the human brain (59). Phosphatidylserine enhances the availability of acetylcholine and thus is proven useful in clinical trials of patients with cognitive impairment (60). Deficits in brain function have most probably been linked to dietary DHA and PS deficiencies. Overall, supplementing the CDB complex could have collectively contributed to the results of the present work.

Latency in the present work was greater in rats fed the HFD, indicating that feeding a high-fat diet impairs hippocampal-dependent memory in rats. However, even in the HFD, supplementing the CDB complex to the diets of the rats improved cognitive performance by reducing latency to find the platform. That means that supplementing the CDB complex to the diets supported rats in quickly learning to swim to the correct location with decreasing escape latencies and more direct swim paths. Although gradually declining escape latency was expected over time, CDB complex supplementation hastens the process. In an opposite way in accord with the latency results, probe was lower with the HFD. This result implies that feeding the HFD impairs retention performance in a spatial water maze probe test. In addition, supplementing the CDB complex to both HFD and regular diets resulted in increases in probe. This result implies that the rats supplemented with CDB complex, even those on the HFD, swam to the target quadrant of the pool and repeatedly across the former location of the platform until starting to search elsewhere. This spatial bias constitutes evidence for spatial memory. Similar to the results of the present work, Sahin et al. (28) also found that rats fed a HFD for 12 weeks had a 27% reduction in mean percentage time spent in the target quadrant and a 38% increase in spatial memory acquisition phase compared with rats fed a regular diet. In the same work (28), supplementing Cr (CrGly) to rats fed a HFD alleviated memory acquisition. Learning and memory are related to metabolic diseases. Similar to results of the present work, individuals with diabetes have been reported to perform less well on measures of learning and memory when compared with nondiabetics (61,62). Those cognitive deficits could be prevented by insulin treatment in type 1 diabetes mellitus (63). As proven with the results of the present work, antidiabetic effects of the CDB complex might have a role in reversing such deficits. The results of latency and probe could have been not only due to Cr but also the other ingredients of the CDB complex supplemented. Similarly, in a previous study, it was reported that feeding with DHA and/or PS supplementation not only significantly improved escape latency of animals but also improved the oxidative parameters in the brain, enhanced glutathione peroxidase activity, and reduced nitric monoxide levels in the livers of rats (64). Additionally, Liu et al. (65) indicated that DHA and PS extended the escape latency, reversed the oxidative parameters observed in the brain, and enhanced SOD activity in the liver of PTZ-induced epileptic rats. Chung et al. (66) in a work with rats reported that DHA is critical for the development and maintenance of learning memory performance. In another study, Chang et al. (67) showed that DHA exhibited neuroprotective and antiinflammatory effects against ischemic brain injury and these effects were accompanied by decreased oxidative stress and JNK/AP-1 signaling as well as enhanced

Nrf2/HO-1 expression. Hegsted et al. (68) stated that rats consuming a low-boron diet could have a negative influence on brain functions. A work in humans indicated that inadequate dietary boron resulted in lower frequency activity in electroencephalograms, as well as less favorable performance in cognitive and motor tests (69).

Findings of the present work indicated that the CDB complex with the main ingredient as CrPic along with phosphatidylserine, docosahexaenoic acid, and boron plays a crucial health-benefiting role in glucose and lipid metabolism. Cr is likely to have a proportionally greater role in these effects. The benefits of the product in most of the carbohydrate and lipid metabolism parameters are more pronounced in rats fed the HFD. In addition, the CDB complex was effective in reducing oxidative stress and improving neurological functions, such as learning and memory errors. The CDB complex could be an alternative treatment for preventing metabolic diseases and for promoting neuroprotection memories in individuals having metabolic diseases such as diabetes. However, further research should be conducted for a complete understanding of the product, including dose and side effects.

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