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ORIGINAL PAPER

Evaluation of the effect of caloric restriction on serum BDNF in overweight and obese subjects: preliminary evidences

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Abstract Brain-derived neurotrophic factor (BDNF) has emerged as a new element related with insulin resistance and obesity. *Objective* To evaluate the effect of a 3-month reduced-calorie diet (RCD) on serum BDNF concentrations in overweight and obese subjects. *Subjects* Seventeen healthy overweight and obese subjects of both sexes (24–48 years, BMI 34.6 ± 1.1 kg/m^2). *Methods* Anthropometry, oral glucose tolerance test (OGTT), lipid levels, and serum BDNF were measured at baseline and at the end of the third month**.** Reduced-calorie diet was defined as a 25% reduction in energy intake composed of: 55% carbohydrates, 20% proteins, and 25% fat (less than 10% saturated fat and over 10% nonsaturated fat). Refined sugar was not allowed. *Results* There was a significant decrease in BMI, waist circumference, body fat percentage, fasting glucose, post-OGTT glucose levels, area under the curve of glucose, and HOMA2-IR after 3 months of RCD. Serum BDNF showed a significant increase (3.97 ± 0.87 to 6.75 ± ! 1.62 ng/ml, $P = 0.02$). Final serum BDNF correlated negatively with weight ($r = -0.51$, $P = 0.03$), and basal post-OGTT insulin correlated positively with final serum BDNF ($r = 0.48$, $P = 0.04$). *Conclusions* Serum BDNF increases in insulin-resistant overweight and obese subjects after three months on a RCD. This observation could indicate that BDNF may be modulated in humans through diet composition.

Keywords Serum BDNF · Reduced-calorie diet · Obesity · Overweight

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Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, and tropomyosin-related kinase B (TrkB), its high-affinity specific receptor, are expressed in the hypothalamus [1, 2], specifically in the nuclei associated with satiety and locomotor function. Some authors have demonstrated that hypothalamic BDNF is regulated by the melanocortin-4 receptor (MC4-R), and could be an important signaling effector that controls energy balance and eating behavior [3].

On the other hand, heterozygous mice for BDNF or TrkB deficiency have locomotor hyperactivity and hyperphagia, with almost 50% displaying an increase in body fat as well as obesity [4–6]. Central infusion of BDNF in the lateral ventricle induces weight loss, and direct infusion in the hypothalamus decreases feeding [5]. In addition, in genetically obese, insulin- or leptin-resistant animal models, peripheral injection of BDNF decreases weight and appetite as well as improving glucose, cholesterol, and nonesterified free fatty acid levels [7–11].

In obese humans, some investigators have reported lower levels of serum or plasma BDNF compared to normal-weight subjects [12, 13], while others have found lower plasma BDNF without differences in serum BDNF [14]. In recent reports, it has been shown that circulating

BDNF levels are related to fasting glucose levels and HOMA-IR in type-2 diabetic patients [15, 16].

Experimentally, it has been demonstrated that diet can modulate the expression of BDNF at a central level in rats. A diet high in fat or refined sugar can reduce expression of hippocampal BDNF [17]. Other authors have described that a high fat diet can induce hyperphagia, obesity, and a decreased satiety in BDNF mutant animals [6]. In contrast, caloric restriction can increase brain BDNF expression [18–22].

To date, there are no reports in the literature regarding the influence of dietary restriction on circulating BDNF levels in obese humans.

To test the hypothesis that serum BDNF increases after a period of dietary caloric restriction associated to weight loss, and to an improvement in some metabolic parameters, we studied the following: changes in serum BDNF concentrations, body weight, body fat percentage, glycemia, insulin levels, and lipid levels, in a sample of nondiabetic overweight and obese subjects, before and after a 3-month period on a RCD.

Results

Basal and final parameters are shown in Table 1. At the end of the third month on a RCD, there was a significant decrease in BMI, body fat percentage, and waist circumference. Weight loss was 7.3 ± 1.1% of initial weight (−6.9 ± 1.1 kg). The following metabolic parameters showed a significant decrease: fasting glucose, post-oral glucose tolerance test (OGTT) glucose levels, and the area under the curve of glucose ($AUC_{Glucose}$). Also, there was a significant decrease in HOMA2-IR (2.3 ± 0.2 to 1.97 ± 0.15, $P = 0.03$), but no significant changes were observed in insulin levels. Analysis of the lipid profile showed a significant decrease in LDL and HDL cholesterol with a nonsignificant increase in triglyceride levels.

As shown in Fig. 1, serum BDNF levels show a significant increase from baseline to the end of the study period (3.97 ± 0.87 to 6.75 ± 1.62 ng/ml, $P = 0.02$). Final serum BDNF levels correlated negatively with final weight ($r = -0.51$, $P = 0.03$), but no relation was found between basal BDNF and initial weight (Fig. 2). Post-OGTT insulin levels at time 0 correlated positively with final serum BDNF ($r = 0.48$, $P = 0.04$), but not with basal BDNF

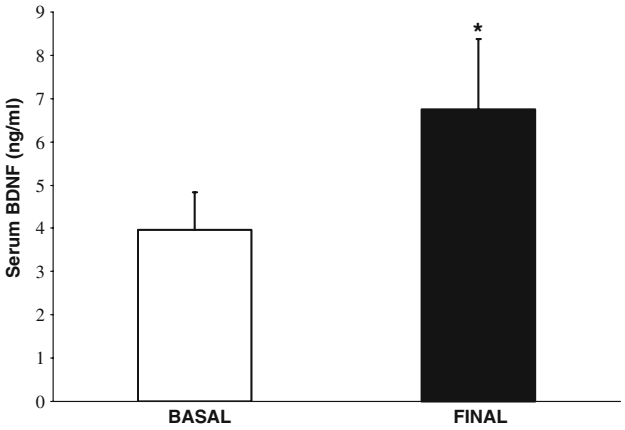

Fig. 1 Basal and final mean (±SE) serum BDNF concentration in 17 overweight and obese patients subjected to calorie-restricted diet. $P = 0.02$

Table 1 Clinical and biochemical parameters in overweight and obese subjects before and after 3 months on a calorie-restricted diet

	Baseline	Final	*P*
n	17 (9F/8M)		
Age (years)	33.5 ± 2		
BMI (kg/m^2)	34.6 ± 1.1	32 ± 1.2	0.001
Waist (cm)	115 ± 2.7	108.9 ± 3.3	0.003
Body fat (%)	38.2 ± 0.5	34.4 ± 0.5	0.0001
Total cholesterol (mg/dl)	175.5 ± 8.3	163.6 ± 7.5	0.009
HDL (mg/dl)	48.3 ± 3.3	45.2 ± 2.6	0.03
LDL (mg/dl)	101 ± 8.6	90.9 ± 6.2	0.01
Triglycerides (mg/dl)	122.3 ± 12.8	157.2 ± 40	NS
Fasting glycemia (mg/dl)	93.8 ± 2.6	85.7 ± 1.7	0.004
Post-charge glycemia (mg/dl)	117.3 ± 4.8	107.4 ± 3.3	0.02
$AUC_{Glycemia}$ (mg/dl/min)	15620.6 ± 442.9	14642.2 ± 408.2	0.01
Fasting insulin (μUI/ml)	17.9 ± 1.6	15.7 ± 1.3	NS*
Post-charge insulin (μUI/ml)	85.07 ± 14.3	82.7 ± 10.4	NS*
$AUC_{Insulin}$ (μUI/ml/min)	10718.6 ± 1175.9	10903.2 ± 1139	NS*
HOMA2-IR	2.3 ± 0.2	1.97 ± 0.15	0.03*
Serum BDNF (ng/ml)	3.97 ± 0.87	6.75 ± 1.62	0.02*

AUC: Area under the curve. Results are presented as mean ± SE

* Logarithmical transformation for statistical analysis. NS: Not significant. Statistical significance $P < 0.05$

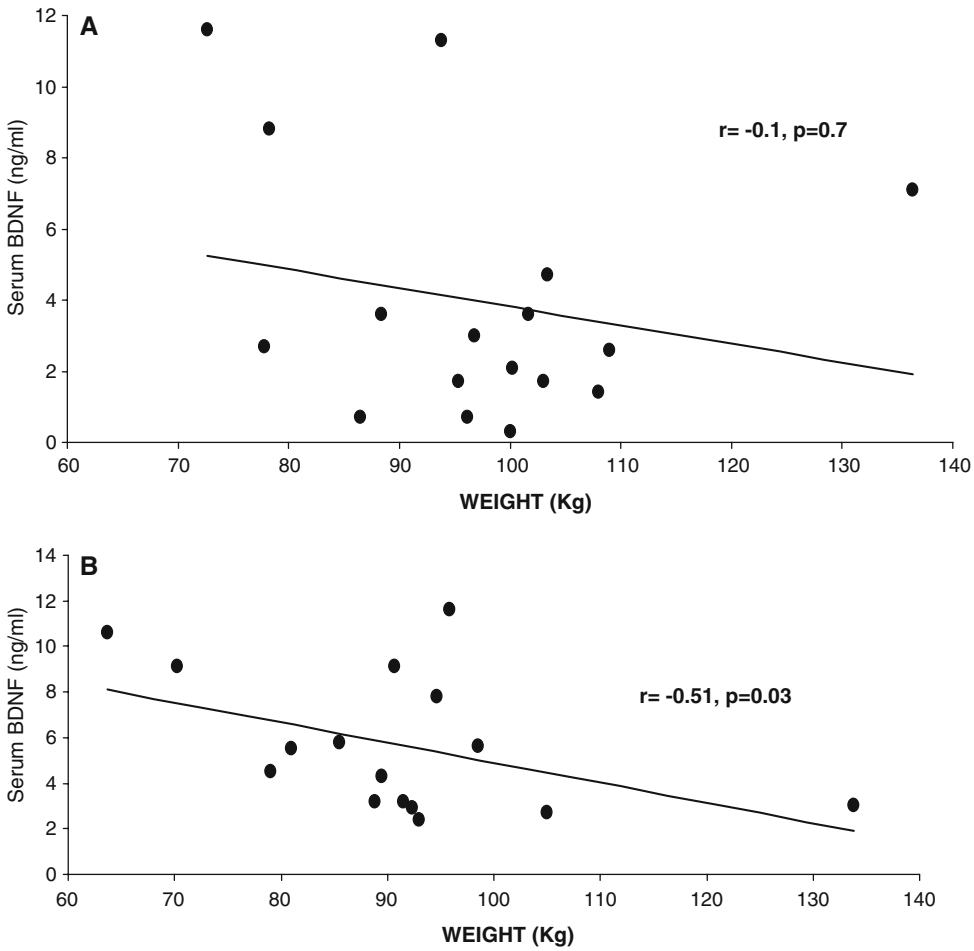

Fig. 2 Relationships between serum BDNF and weight before **a**, and after 3 months on a calorie-restricted diet **b**, in 17 overweight and obese subjects

($r = -0.21$, $P = 0.4$). No other relationships were found between BDNF and the rest of the parameters analyzed.

Other significant correlations in the basal condition were: body fat percentage correlated positively with BMI, waist circumference, and $AUC_{Insulin}$ ($r = 0.67$, $P = 0.0028$; $r = 0.57$, $P = 0.01$ and $r = 0.53$, $P = 0.02$, respectively); post-OGTT glucose levels correlated positively with LDL cholesterol and post-OGTT insulin levels ($r = 0.62$, $P = 0.0078$ and $r = 0.64$, $P = 0.005$, respectively); Post-OGTT insulin levels correlated positively with HOMA2-IR ($r = 0.52$, $P = 0.03$). At the end of the study, body fat percentage correlated only with BMI and waist circumference ($r = 0.68$, $P = 0.002$ and $r = 0.68$, $P = 0.002$); HOMA2-IR correlated positively with BMI and weight ($r = -0.5$, $P = 0.03$ and $r = 0.49$, $P = 0.04$, respectively), and weight loss correlated positively with HDL cholesterol ($r = 0.57$, $P = 0.01$).

Discussion

In this study, we observed a significant decrease in BMI, waist circumference, and body fat, and an increase in serum BDNF in overweight and obese subjects on a reduced-calorie diet. In addition, we observed an improvement in the glycemia, LDL cholesterol, and HOMA2-IR. The finding of a nonsignificant increase in triglycerides could be explained by the lipolytic effect of caloric restriction associated to an increase in triglyceride turnover [23].

Previously, other authors have demonstrated a negative correlation of plasma BDNF and weight, and a positive correlation of serum BDNF with BMI in type-2 diabetic subjects [14, 16]. Interestingly, we did not find correlations between basal serum BDNF and initial BMI or weight in these subjects. Nevertheless, after a 25% caloric restriction for a 3-month period, a negative correlation between BDNF and weight was found.

BDNF effects on weight and metabolism have been experimentally demonstrated. Peripheral injection of BDNF in mice decreases weight and appetite, and improves glucose and lipid levels [7–11]. In this context, we speculate that in overweight and obese subjects, a RCD could change serum BDNF levels contributing to metabolic improvement and weight loss.

In humans, there is no previous evidence on the influences that caloric restriction has on BDNF circulating

levels. In mice, caloric restriction can increase expression of BDNF in the hippocampus, cerebral cortex, and striatum when compared to animals fed *ad limitum* [18]. It can also reverse several abnormal phenotypes in BDNF heterozygous animals including obesity, hyperphagia, and increased locomotor activity, associated to an increase in BDNF levels in the brain [19]. In monkeys, caloric restriction increases BDNF in the caudate nucleus affected by a neurotoxin [21]. On the other hand, in animals, a diet high in fat or refined sugar can reduce expression of BDNF [17]. In our dietary prescription, refined sugar was not allowed and fat was substantially decreased with respect to basal ingestion reported by our subjects. Thus, the increase in serum BDNF observed could be the response to caloric restriction and to changes in dietary composition.

In humans, some investigators have reported lower levels of serum or plasma BDNF in obese patients compared to normal-weight subjects [12, 13]. Others have found lower plasma BDNF levels without differences in serum BDNF [14], or even higher serum BDNF levels than in controls [16, 24]. Krabbe et al. found that plasma BDNF correlates negatively with fasting glucose, suggesting that high levels of plasma glucose inhibit BDNF brain output [15]. Other authors have observed that serum BDNF directly correlated with fasting glucose and HOMA-IR in females with newly diagnosed type-2 diabetes [16]. In concordance with this observation, we found that initial post-OGTT insulin levels correlated positively with final serum BDNF. Our subjects were insulin resistant, as demonstrated by fasting insulin and HOMA2-IR. The differences observed between plasma and serum BDNF levels could be explained by the suppression of BDNF synthesis by high glucose levels, or by insulin resistance. On the other hand, BDNF is synthesized not only in neuronal and brain tissue, but also in vascular endothelial cells, smooth muscle cells, pancreatic cells, and skeletal muscle cells which synthesize BDNF and express its specific receptor TrkB [25–28]. Thus, in insulin-resistant individuals, BDNF platelet storage probably increases as a mechanism to prevent negative metabolic effects.

Our aim of obtaining a group of nontreated cases resulted in rigorous inclusion criteria, and hence a reduced study group. The small size of our population could represent a statistical limitation. The prescription of a nonstandard diet, the inability to measure BDNF synthesized only in the brain, and possibly some ethnic differences [29], could influence the results reported here. Nevertheless, they constitute a preliminary approach toward gaining a better understanding of the association between dietary restriction and this neurotrophic factor in overweight and obese individuals.

We conclude that serum BDNF can increase in overweight and obese subjects after a 3-month period on a reduced-calorie diet. This observation could indicate that BDNF can be modulated by dietary composition, and that it might be one of the factors involved in the improvement of BMI and metabolic parameters in subjects on dietary restriction.

Longitudinal clinical studies are necessary to further clarify the role of brain-derived neurotrophic factor in weight control and its modulation by diet and other environmental factors in humans.

Patients and methods

Twenty one subjects of Hispanic descent, 11 females and 10 males, age 18–48 years (34.5 ± 8.1 years), with a body mass index (BMI) over 27 kg/m^2, normal fasting glycemia, and without any of the following were recruited: chronic diseases, alcohol or drug abuse, history of depressive disorders, medications, or infection. To rule out a major depressive disorder, we applied the Beck Depression Inventory as screening method. None of the subjects had received intervention or treatment for obesity or insulin resistance within the last 2 years. Four subjects abandoned the study.

This study was carried out in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Chile Clinical Hospital. Each subject gave written consent for participation in the protocol.

Before and after 3 months on a RCD, weight, height, and waist circumference were measured. Subscapular (SS), bicipital (B), tricipital (T), and suprailiac (SI) skinfold thickness were measured with Caliper. Applying the Durnin and Womersley equation: BD (Body Density) = log (T + B + SE + SI), % Body Fat = 4.95/BD − 4.5 × 100, the percentage of body fat was calculated. This method has demonstrated a good correlation with body fat measured by densitometry [30]. After a period of 10 h of overnight fasting, a blood sample for lipid determination was taken and a 75 g OGTT was performed to determine glucose and insulin blood levels at: 0–30–60- and 120 min. The total and integrated glucose and insulin responses in the OGTT were calculated by the trapezoid method and expressed as the area under the concentration–time curve (AUC) from 0 to 120 min. To evaluate insulin resistance the homeostatic model assessment version 2 (HOMA2-IR) was calculated based on the plasma fasting glucose and insulin (HOMA calculator v2.2, http://www.dtu.ox.ac.uk/).

Glucose was measured by the enzymatic glucose oxidase method, and insulin was measured by chemoluminescent enzyme immunometric assays (Immulite, Diagnostic Products Corporation, Los Angeles, CA).

Reduced-calorie diet was defined as a 25% reduction in energy intake (25% caloric restriction) distributed as

follows: 55% of carbohydrates, 20% of proteins, and 25% of fat with less than 10% of saturated fat and over 10% of nonsaturated fat. Refined sugar was not allowed. Individual compliance to the prescribed dietary intervention was monitored by self-reported food records.

Serum BDNF was measured at the start of the study and at the end of third month. In women, the sample was taken in mid-follicular phase of the menstrual cycle. Samples were taken in the fasting state, before 10 AM. Samples were collected on ice, centrifuged immediately at 4°C, and stored at −80°C until they were processed.

BDNF was determined using a commercial ELISA kit (BDNF E_{max}® Immunoassay System, Promega, Madison, WI), according to the manufacturer's instructions. Subjects' serum dilutions from 1:10 to 1:100 were tested. The dilution 1:25 was chosen because it allowed detection of BDNF values in all the samples. BDNF concentration in serum was calculated based on a standard curve, which was linear between 7.8 and 500 pg/ml. Intra- and interassay variability was 8.8% and 18%, respectively; the lower limit of detection was 7.8 pg/ml. All samples were processed in one assay to minimize the interassay variability.

Statistical analysis

Statistical analysis was performed using the software Stata 9.0. Results are expressed as the mean ± SE. Logarithmical transformation was applied if data were not normally distributed (BDNF, insulin, HOMA2-IR). Paired Students-*t* and χ^2 tests were used to compare differences in means before and after the intervention. Pearson correlation coefficient was used to evaluate the relationship between variables. Statistical significance was defined as *P*-value < 0.05.

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