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ORIGINAL PAPER

Different Effect of High Fat Diet and Physical Exercise in the Hippocampal Signaling

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Abstract Obesity is an epidemic disease that may affect brain function. The present study examined the effect of high fat diet (HF) and physical exercise on peripheral tissue and hippocampal signaling. CF-1 mice ($n = 4$, per cage) were divided into groups receiving high fat (HF) or control (CD) diets for 5 months, with or without voluntary exercise. Serum triacylglycerol, total cholesterol, HDLc, liver triacylglycerol and glycogen concentrations were evaluated ($n = 6$). Also, the phosphorylation state of the AKT → ERK 1/2 → CREB pathway (AKT, pAKTser473, ERK 1/2, pERK 1/2, CREB and pCREB, $n = 4$–6) was analyzed in the hippocampus. HF diet caused an increase in AKT phosphorylation at ser473 ($P < 0.05$), while exercise increased the phosphorylation of ERK 1/2 ($P < 0.05$) and CREB ($P < 0.05$). As expected, exercise reversed some of the harmful effects of HF, i.e., increased liver deposition of fat ($P < 0.05$) and fat gain in the abdominal region ($P < 0.05$). In conclusion, the effects of exercise and HF diet on brain signaling appear to affect the hippocampal AKT → ERK 1/2 → CREB pathway in independent ways: HF intake caused increased phosphorylation of AKTser473, while exercise increased ERK 1/2 → CREB signaling. The physiological relevance of these findings in brain function remains to be elucidated.

Keywords High fat diet · Physical exercise · Hippocampal signaling · Obesity

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Introduction

Obesity is a problem in all westernized countries and more recently in several less industrialized countries such as India and Brazil. Both environmental and genetic factors may contribute to excessive body weight gain and its comorbidities [1]. Obesity associated with abdominal fat deposits is a major risk factor for insulin resistance, type II diabetes, and the 'metabolic syndrome'. Thus, lifestyle interventions aiming to control body weight gain have been proposed to prevent obesity [2]. Physical exercise, a low-cost lifestyle intervention, is known to increase insulin sensitivity, decrease body weight, and improve serum lipid profile [3]. Moreover, there is accumulating evidence that physical exercise has beneficial effects on brain function through incompletely understood mechanisms [4]. Hormones and neurotrophins, as well as proteins involved in intracellular signaling pathways, are putative brain targets modulated by the effects of exercise and diet [5–7].

A classical signaling pathway that is proposed to be regulated by hormones and neurotrophins is the serine/threonine protein kinase B (also knows as AKT) pathway. AKT-mediated intracellular signaling is a key regulator of

cell cycle, apoptosis and cellular responses to insulin in the brain [8]. Through its phosphorylation, AKT can be activated and translocated to the nucleus to regulate gene transcription. Moreover, AKT interacts with other kinases, including mitogen-activated protein kinase (MAPK) family members such as the extracellular signal-regulated kinases (ERK1/ERK2) and cyclic-AMP response element binding protein (CREB) [9]. Recent observations indicate that ERK-associated signaling plays an essential role in cell differentiation and proliferation, and also in long-term synaptic plasticity [10]. Activated ERK translocates to the nucleus and regulates gene transcription via phosphorylation of different transcription factors, including CREB, which are involved in long-term plasticity events within the brain [11]. Both experimental and clinical studies have reported that a high-fat diet may impair overall brain function, whereas exercise has opposite effect [4]. In rat models, a high-fat diet has been found to impair performance on a variety of tasks requiring both the hippocampus and frontal cortex, an effect which has been shown to be associated with the level of saturated fatty acids on diet [11]. Moreover, epidemiological studies have shown that a high intake of saturated fat and cholesterol can contribute to cognitive decline [12]. Albeit the mechanisms by which high-fat diets alter cognitive function are not completely understood, hyperglycemia and glucose intolerance caused by such diet may be a contributing factor. Albeit the increasing rates of obesity and insulin resistance have stimulated interest in the effects of diet composition on peripheral systems, comparatively little work has been done to examine its effects on the brain. Thus, it seems relevant to determine whether a combination of high fat diet and physical exercise would influence and/or interact with specific aspects of brain signaling.

In this sense, this study evaluated hippocampal AKT → ERK 1/2 → CREB signaling, a pathway recognized to be regulated by diet and exercise, which is also putatively involved in several cellular functions including regulation of cell growth, apoptosis, survival and nutrient metabolism [8].

Experimental procedure

Animals and diet

CF-1 male mice, 2 months old, were maintained in a 12-h light/dark cycle at 22–24°C. Mice were maintained during 5 months after being assigned to one of four groups ($n = 4$ per cage): control diet/sedentary (CDS); control diet/exercise (CDE); high fat diet/sedentary (HFS); high fat diet/exercise (HFE). Animals engaged in voluntary physical activity had free access to running wheel throughout the treatment as previously reported [13]. The animals progressively increased the distance ran per day during the first month, reaching a plateau of about 3000 m/day after 30 days and maintaining it until the end of the study. To avoid social isolation we did not maintain animals in individual housing.

Diets containing a standard vitamin and mineral mix with all essential nutrients were provided *ad libitum*. The HF diet contained 60% energy from saturated and unsaturated fat (45% lard and 15% soybean oil), 15% energy from starch and 25% from protein (soybean protein), while the CD contained 15% of energy from saturated fat and unsaturated fat (soybean oil), 60% energy from starch and 25% from protein (soybean protein).

Glucose tolerance test (GTT)

An intraperitoneal injection of glucose (2 mg/g body weight) was performed in 6 h fasted mice after 5 months of treatment (at 2:00 P.M). Blood glucose was monitored with glucose oxidase method (Labtest, MG, Brazil) at 0, 30, 60 and 120 min after glucose injection. Blood was collected from tail.

Blood biochemical evaluation

Two weeks after, to prevent the effect of stress caused by GTT, animals were sacrificed by decapitation. Blood was collected and centrifuged at 2500*g*/10 min. Serum was stored at −20°C until assays were performed. The serum triacylglycerol (TAG), total cholesterol, HDL cholesterol was measured using commercial kits (Labtest, MG, Brazil). The reactions were performed using Labmax equipment (Labtest, MG, Brazil).

Liver measurements and fat pad weights

Liver was dissected and between 90–110 mg of liver were homogenized 1:10 with saline 0.9%; 20 µl of the homogenate were used to determine liver TAG. Liver glycogen was determined by the colorimetric method described by Krisman [14]. Fat tissues from retroperitoneal and epididymal regions were dissected and weighted as previously described [15].

Hippocampal preparation and Western blot analysis

Hippocampus was rapidly dissected and stored at −70°C. Hippocampal homogenates were prepared in buffer (NaF

50 mM, Tris 20 mM, sucrose 0.32 mM, ortovanadate 1 mM, EDTA mM, EGTA 1 mM, phenylmethylsulfonyl fluoride 1 mM) and centrifuged. Supernatants were collected and total protein was measured by a Bradford protein assay. For Western blot analysis, samples containing 30 μg of protein were separated by electrophoresis on a 10% polyacrilamide gel and electrotransfered to PVDF membranes. Non-specific binding sites were blocked with in Tween–Tris buffered saline (TTBS, 100 mM Tris–HCl, pH 7.5, containing 0.9% NaCl and 0.1% Tween-20) containing 5% albumin for 2 h. Membranes were then incubated overnight at 4°C with polyclonal antibodies against AKT, pAKT ser473 (Cell Signalling, 1:1000), CREB, pCREb (Upstate, 1:2000) or ERK 1/2, pERK 1/2 (Upstate, 1:500). After rinsing three times for 10 min each with TTBS, membranes were incubated with secondary antibodies (1:15000 dilution, anti-rabbit, Santa Cruz) during 2 h at room temperature. After rinsing four times for 10 min each with TTBS, membranes were incubated with peroxidase-conjugated for 5 min at room temperature, then displayed on autoradiographic film by chemiluminescence. Signals were digitally scanned and quantified using Opitquant version 02.00 (Packard Instrument Company). This procedure was repeated by at least three times.

Statistical analysis

Results are presented as means ± SEM. Differences between all groups was analyzed by using analysis of variance (ANOVA) with a post-hoc Duncan test. For analyzing differences between treatments we used a two-way ANOVA. To analyze GTT results we used repeated-measures ANOVA. Values were considered statistically different when P values were below 0.05.

Results

Body weight, fat pads weight, serum lipids and liver measurements

Body weight, fat pad weight, serum and liver biochemical analysis are demonstrated in Table 1. The HFS group presented increased fat pad weight in epididymal and retroperitoneal regions as compared to other groups ($P < 0.05$) but no increment in total body weight was observed. The HFS group also showed a high liver TAG concentration compared to other groups ($P < 0.05$). CDE animals showed an increase in serum TAG compared to the HFE group ($P < 0.05$). Body weight, fat pads and liver glycogen were not statistically different when the HFE and CD groups were compared. The HFS group had a lower hepatic glycogen concentration than other groups ($P < 0.05$). Total cholesterol and HDLc were not altered by diet or exercise.

Glucose tolerance test

The results of GTT are presented in Fig. 1a. At 30 min there was an apparent increase in serum glucose levels in both HFS and HFE groups (19% and 50% respectively); however, this effect did not reach statistical significance. At 60 min, glucose levels in the HFS group remained high compared to all other groups ($P < 0.05$, 37%). At 60 min, glucose levels in the HFE group were equal to the CD group.

Immunocontent of AKT and pAKT in hippocampus

Total AKT immunocontent was not different among groups, but HF groups enhanced the phosphorylation of AKTser473 when compared to CD groups (Fig. 1b, $F(13.3) = 25.184$ $P < 0.05$).

Immunocontent of ERK 1/2 and pERK 1/2, CREB and pCREB in hippocampus

Considering that pAKTser473 was modified by diet, we also evaluated other proteins involved in this signaling pathway. Total immunocontent of ERK1/2 (Fig. 2a) and CREB (Fig. 2b) did not change with diet, but exercise increased phosphorylation status of pERK1/2 (F (5.1) = 10.981 $P < 0.03$, Fig. 2a) and pCREB (F (4.8) = 5.593 $P < 0.05$, Fig. 2b).

Discussion

In this study, we evaluated the effects of both high-fat diet and exercise on metabolic parameters and on the hippocampal AKT → ERK 1/2 → CREB signaling pathway in mice. Research has recently been spurred by the finding that an association exists between the accumulation of TAG in tissues other than adipocytes and the development of insulin resistance [16]. In our work, exercise prevented fat deposition and TAG accumulation in the liver of HF treated mice. Similarly, Gauthier et al. [17] also showed that HF triggered the accumulation of lipid as macrovesicles in liver; but in this study when an exercise training program was pursued at the same time as the HF diet, the

Table 1 Body weight, adiposity, liver and blood measurements in mice after 5 months of treatments

	Body weight (g)	Fat pad retroperitoneal	Fat pad epididymal	Triacylglycerol serum mg%	Liver triacylglycerol mg%	Liver glycogen mg%	Cholesterol mg/dl	HDL cholesterol mg%
CDS	49.7 ± 2.6	0.9 ± 0.2	2.5 ± 0.4	170.0 ± 19[a,b]	3.0 ± 0.4	2.3 ± 0.3	129.2 ± 11.3	41.7 ± 4.1
CDE	45.8 ± 1.6	0.8 ± 0.1	1.7 ± 0.3	188.0 ± 20[a]	2.6 ± 0.2	2.6 ± 0.2	133.8 ± 8.9	45.6 ± 2.8
HFS	50.3 ± 1.7	2.3 ± 0.1[a]	3.6 ± 0.3[a]	137.8 ± 12[a,b]	4.9 ± 0.4[a]	1.3 ± 0.3[a]	138.5 ± 8.0	43.0 ± 3.8
HFE	47.0 ± 2.1	1.1 ± 0.2	2.4 ± 0.5	114.4 ± 22[b]	3.8 ± 0.7	2.2 ± 0.5	144.2 ± 14.9	49.2 ± 2.4

Values are mean ± SEM. Means in a column with distinct letter or different letter differ than other groups with distinct letter or without letter ($P \leq 0.05$). CDS (control diet sedentary, $n = 6$); CDE (control diet exercise $n = 6$); HFS (high fat diet sedentary $n = 6$); HFE (high fat diet exercise $n = 6$)

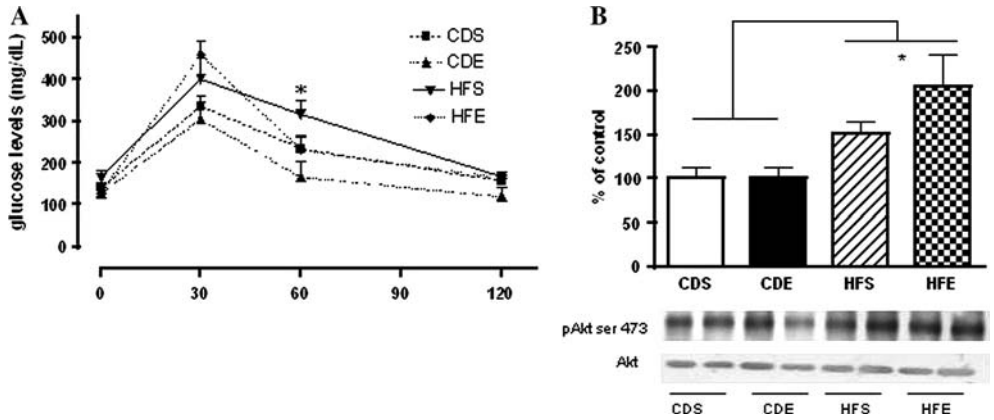

Fig. 1 Glucose tolerance test, AKT and pAKTser473 immunocontent. (**a**) 6 h-fasted mice received an intraperitoneal injection of glucose (2 mg/g of body wt). Blood samples were taken at the times 0, 30, 60 and 120 min from the tail vein of the same animal. Repeated-measures analysis of variance (ANOVA) was used to evaluate statistical significance. Results are expressed as means ± SEM. Serum glucose levels were higher in the HFS group at 60 min ($P = 0.05$) compared to all other groups. (**b**) Representative immunoblots of hippocampal pAKTser473 and AKT with their respective histograms and densitometric analyses. Results are expressed as mean ± SEM. Two-way ANOVA was used to analyze statistical significance. HF diet (sedentary and exercise) increased pAKTser473 immunocontent ($P < 0.05$). CDS (control diet sedentary, $n = 4$); CDE (control diet exercise $n = 4$); HFS (high fat diet sedentary $n = 4$); HFE (high fat diet exercise $n = 4$)

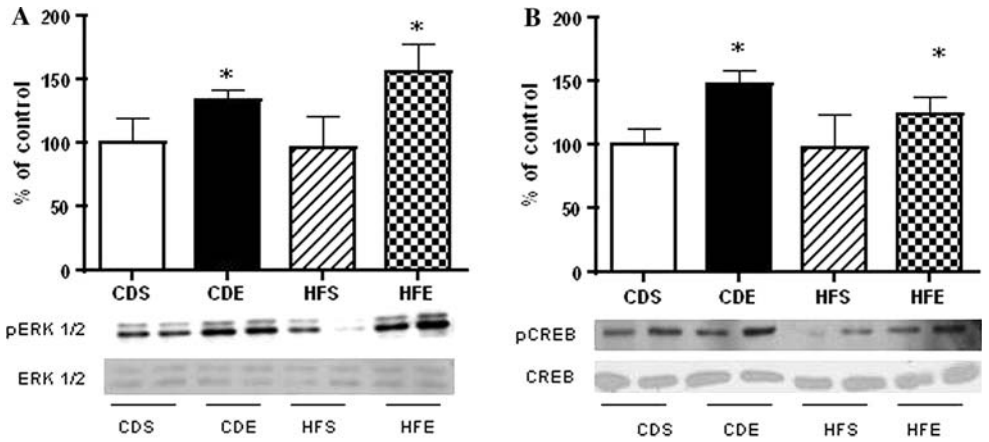

Fig. 2 Determination of ERK 1/2, pERK 1/2, CREB and pCREB immunocontent. Representative immunoblots of hippocampal ERK 1/2, pERK 1/2, CREB and pCREB with their respective histograms and densitometric analyses. Results are expressed as mean ± SEM. Two-way ANOVA was used to analyze statistical significance. (**a**) Exercise increased pERK 1/2 ($P = 0.03$). (**b**) Exercise increased pCREB ($P = 0.05$). * indicates difference in exercise groups in relation to sedentary groups. CDS (control diet sedentary, $n = 6$); CDE (control diet exercise $n = 6$); HFS (high fat diet sedentary $n = 6$); HFE (high fat diet exercise $n = 6$)

induction of hepatic steatosis was completely suppressed. Our results are somewhat convergent with the effect of exercise in reducing liver lipid accumulation induced by HF diet.

The HFS group had altered glucose levels in GTT at 60 min ($P < 0.05$). Moreover, increased AKTser473 phosphorylation was observed in hippocampal tissue from the HFS and HFE groups. It has been extensively reported that cerebral signaling may be influenced by insulin and glucose levels [8, 18–21]. Accordingly, Clodfelder-Miller et al. [22] demonstrated that physiological variations in serum glucose and insulin levels affected brain AKT phosphorylation/dephosphorylation status in the hippocampus. Moreover, streptozotocin-induced hyperglycemia caused persistent AKT hyperphosphorylation, which was postulated to be detrimental for brain function [22]. Here, we speculate that although not statistically significant, the increased glucose levels observed at 30 min in HFS (19%) and HFE (50%) groups could account in some degree for the increased phosphorylation of AKTser473. However, considering that the GTT did not present a clear pattern of glucose intolerance, it is difficult to attribute a causal effect of glucose and/or insulin levels on AKT phosphorylation.

Conversely, Mielke et al. [23] demonstrated that in C57BL/6 mice, consuming a large proportion of calories from saturated fat had limited effect on learning and memory, and also on pAKT. These results suggest that changes in brain function do not appear to be strictly related to changes in peripheral glucose tolerance and neural insulin sensitivity**.** Exercise training did not affect GTT response in CDE group when compared to CDS group, probably because this parameter is already well regulated in sedentary animals (i.e. they may not yet present pathophysiological alterations that can be further optimized by exercise).

The preliminary postulation that the effect of exercise and HF diet might affect the AKT → ERK 1/2 → CREB by similar means was not confirmed by this study. ERK 1/2 is activated in response to many different stimuli including growth factors and hormones and transduces extracellular stimuli into diverse intracellular responses. Moreover, the activation of ERK 1/2 may cause phosphorylation of CREB [9], which is consistently associated with the positive effect of exercise on brain function, at least in part, by promoting increased neurotrophic factor expression and synthesis [4].

In conclusion, the effects of exercise and HF diet on brain signaling affected the AKT–> ERK 1/2 –> CREB in different ways. HF intake caused increase phosphorylation of hippocampal AKTser473 and exercise increased hippocampal ERK 1/2 → CREB signaling. The physiological relevance of these findings for the brain function remains to be elucidated.

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