High-fat diets induce changes in hippocampal glutamate metabolism and neurotransmission

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GLUTAMATE (Glu) uptake is a pivotal process regulating excitatory transmission within the central nervous system, and its efficacy accounts for the time that released Glu remains in the extracellular space and, consequently, the duration of postsynaptic receptor activation. Glu uptake is carried out by specific neuronal (EAAT-3) and glial (GLT-1, GLAST) transporters, which display dynamic processes aimed at improving Glu clearance under conditions leading to increased release of Glu (27, 34). Impairment of Glu uptake due to brain injury (24, 28) or particular pathophysiological conditions, such as epileptic status (1), leads to the overstimulation of postsynaptic Glu receptors and triggers Glu-dependent excitotoxicity. In hippocampal and cortical circuits, Glu transmission accounts for learning and memory processes (5, 8), and functional consequences of Glu-evoked neurotoxicity lead to cognitive disturbances (23).

The relevance of nutritional imbalance as a source of neuropsychological disorders, including a deficient ability to perform learning and memory tasks, is supported by a number of studies suggesting that diet composition would affect limbic-mediated responses (37). Westernized societies are characterized by oversized meals that normally include high amounts of sugar and saturated fat to the detriment of satiating fiber and complex carbohydrate. These diets are integral to our lifestyle and have become a main cause of the high prevalence of obesity and type 2 diabetes. A number of studies have shown evidence that nutritional imbalance also accounts for cognitive deficits (11, 19). In particular, recent research has shown evidence that high-fat (HF) diets are deleterious for hippocampal structure and function (14, 20) and induce changes in synaptic plasticity and neurogenesis (12, 22). Moreover, previous studies carried out in our laboratory and others have demonstrated that HF diets impair hippocampal-dependent learning and memory processes (16, 35). All of this research suggests that changes triggered by HF diet within the brain could have a deleterious impact on Glu neural pathways.

Our hypothesis is that HF diet triggers significant changes in Glu transmission that are linked to the alteration of Glu uptake and metabolism. Thus, in the present study we have quantified in mouse hippocampus the influence of HF diet on 1) Glu uptake kinetics, 2) the density of Glu carriers and Glu-degrading enzymes, 3) the density of Glu receptor subunits, and 4) synaptic transmission and plasticity. Because HF diets have been shown to evoke leptin resistance within the brain (26), and leptin might theoretically account for neurochemical and functional changes in the hippocampus (17, 30), we have also analyzed the functionality of leptin receptors in this brain area.

MATERIALS AND METHODS

Animals and chow. Four-week-old C57BL/6J male mice (CRIFA, Barcelona, Spain) weighing 16–18 g were housed under a 12:12-h light-dark cycle in a temperature-controlled room (22°C), with standard food and water available ad libitum, in accordance with the European Communities Council Directive (86/609/EEC) for the Care and Use of Laboratory Animals. The experimental protocol was...
approved by the Committee on Animal Research and Ethics of the San 
Pablo-CEU University (SAF-2009-0714). After 1 wk, animals were 
divided into five groups: 1) sham (n = 12), 2) 20% saline, 3) average body weight, housed four per 
cage, and assigned to either a control (LF; D12450B, 10 kcal% fat, 70 
cal% carbohydrates, and 20 kCal% protein), 3.85 kcal/g) or HF diet 
(HF; D12451, 45 kCal% fat, 35 cal% carbohydrates, and 20 Kcal% 
protein, 4.73 kcal/g). Diets were supplied by Test Diet Limited BCM 
IPS (UK). Body weight and food intake were monitored once/wk.

Plasma leptin was measured at the end of the treatment. Assays were 
carried out after an 8-wk dietary treatment.

**Glu uptake assays.** Mice were euthanized by decapitation, and 
hippocampi were quickly dissected and sliced (400 μm thick) with a 
tissue chopper (The Mickley Laboratory). Slices were stabilized for 1 
h in oxygenated ice-cold Ringer buffer (46 mM KCl, 12 mM 
KH2PO4, 12 mM MgSO4, 1.15 mM NaCl, 250 mM NaHCO3, 25 mM 
CaCl2, and 10 mM EDTA) and then incubated for 30 min with 
creasing concentrations (0, 10, 50, 100, and 500 μM) of Glu 
(Sigma-Aldrich). After washing with ice-cold buffer (2 × 5 min), slices 
were depolarized for 1 min in 0.5 ml of 50 mM KCl. After 
derivatization with FITC, released Glu was quantified by capillary 
electrophoresis/laser-induced fluorescence detection, with a method 
based on a previous one for human serum samples (40). The analytical 
method was optimized and validated for these samples in the range of 
0.5–25 μM Glu. Quantification was carried out with a PACE 5500 
system equipped with an LIF detector. Experimental conditions were 
as follows: applied voltage, +15 kV; current, +72 μA; fused capillary, 
47 cm in length, 40 cm in effective length, 75 μm ID; assay 
buffer (BGE), 100 mM borate buffer, pH 9.75; temperature, 25°C; 
injection time, 10 s; 0.5 psi/33 mbar. LIF detection; Laser Module 488 
λ (exc), 488 nm (em); 520 nm. Samples (100 μl) were mixed with 
0.5 μM FITC solution (50 μl), derivatization buffer BGE (30 mM borate 
buffer, pH 9.75, 50 μl), and water (50 μl). Solutions were kept in 
the dark to react at room temperature for 16–24 h.

Proteins were determined by using the Bradford method (4). 
Uptake velocity was expressed in nM·mg protein−1·min−1. Km 
were expressed in μM units. Vmax and Km were calculated from the 
the Michaelis-Menten equation $V = V_{\text{max}} \times [S]/(K_M + [S])$, where $V$ 
is velocity in nM·mg protein−1·min−1 and $K_M$ is substrate concentration 
in μM by nonlinear regression analysis. Kinetic parameters were 
also calculated from the Lineweaver-Burk plot.

**Western blot assays.** Hippocampi were homogenized in ice-cold 
buffer containing 0.42 M NaCl, 20 mM HEPES (pH 7.9), 1 mM 
Na2HPO4, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 20% 
glycerol, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 20 mM sodium 
fluoride, 1 mM trisodium orthovanadate, and 2 mM phenylmethylsulfonyl 
fluoride. Tubes containing homogenates were frozen at −80°C 
and thawed at 37°C three consecutive times and then centrifuged for 
10 min at 4°C. Equivalent amounts of proteins (50 μg) present in the 
supernatant were loaded in Laemmli buffer (50 mM Tris, $p$H = 6.8, 10% 
SDS, 10% glycerol, 5% mercaptoethanol, and 2 μg/ml blue 
fluoride, 1 mM trisodium orthovanadate, and 2 mM phenylmethylsulfonyl 
fluoride. Tubes containing homogenates were frozen at −80°C 
and thawed at 37°C three consecutive times and then centrifuged for 
10 min at 4°C. Equivalent amounts of proteins (50 μg) present in the 

Perfusions were administered at 9 AM by intraperitoneal injection. After 90 min, 
fields were euthanized and hippocampi dissected and stored at −80°C 
until assay.

**Statistics.** Statistical significance was determined by one-way 
ANOVA or repeated-measures ANOVA, followed by appropriated 
post hoc tests (Student-Newman-Keuls and Mann-Whitney U-tests). 
All values are expressed as means ± SE.

**RESULTS.**

**HF diet triggers overweight and increases plasma leptin 
concentration.** Body weight increased during dietary treatment from 
20.1 ± 0.3 to 27.6 ± 0.4 g in LF animals and 34.6 ± 0.6 g 
in the HF group (*P < 0.001*). Plasma leptin concentration 
also appeared to be increased in HF-treated animals (5.4 ± 0.3 
ng/ml in LF vs. 16.0 ± 1.7 ng/ml in HF mice, *P < 0.001*). 
**HF diet improves hippocampal Glu uptake.** Figure 1 illustrates the kinetic characteristics of the Glu uptake process in 
hippocampal slices in both control and HF mice. Repeated-measures ANOVA revealed an effect of dietary treatment on 
Glu uptake velocity [$F_{(1,24)} = 87.78, P < 0.001$], which was 
independent of Glu concentration.

*Km*, calculated by nonlinear regression analysis, was 
33.85 ± 15.60 μM for LF mice vs. 12.34 ± 2.41 μM for HF 
(not significant). $V_{\text{max}}$ appeared to be almost twofold enhanced 
in samples from HF animals [152.77.2 ± 17.95 
N:Mg protein−1·min−1 in LF vs. 353.64 ± 28.58 N:Mg 
protein−1·min−1 in HF mice; 1-way ANOVA, *F* = 34.42, 
$P < 0.001$]. These kinetic parameters were also
calculated from the Lineweaver-Burk plot, and in this case both $K_m$ [27.9 ± 4.7 μM in LF vs. 13.8 ± 2.3 μM in HF mice; 1-way ANOVA $F_{(1,8)} = 7.35, P < 0.05$] and $V_{max}$ [106.2 ± 15.5 nM·mg protein$^{-1}$·min$^{-1}$ in LF vs. 366.7 ± 33.3 nM·mg protein$^{-1}$·min$^{-1}$ in HF mice; 1-way ANOVA, $F_{(1,8)} = 50.2, P < 0.001$] were significantly different between groups.

HF diet upregulates the density of Glu transporters but downregulates the glucose transporter GLUT1. The effect of HF diets on the density of Glu transporters was examined in whole hippocampus by Western blot. As illustrated in Fig. 2A, HF mice exhibited enhanced levels of both GLT-1 [31.7 ± 1%, 1-way ANOVA $F_{(1,16)} = 11.55, P < 0.01$; Fig. 2A] and GLAST [26.8 ± 4.2%, 1-way ANOVA $F_{(1,14)} = 11.78, P < 0.01$; Fig. 2B]. In contrast, the neuronal carrier EAAT-3 remained unchanged under our experimental conditions (data not shown).

The glucose transporter GLUT1 appeared to be decreased significantly in HF mice [1-way ANOVA $F_{(1,16)} = 9.48, P < 0.01$; Fig. 2C].

Basal synaptic transmission and plasticity are modified in hippocampal slices from HF mice. The preceding data suggest that Glu transmission within the CA1 area of the hippocampus would be altered in HF mice. Thus, changes in basal synaptic transmission and plasticity might be expected. Stimulus-response curves were constructed by using stimuli intensities from 0 to 120 μA (10-μA increments). As illustrated in Fig. 3A, one-way ANOVA revealed a blunting of I/O curves in HF mice [$F_{(1,104)} = 103.69, P < 0.001$ for diet]. These data show that HF diets induce a severe decrease in hippocampal synaptic efficacy of CA1 pyramidal neurons (Fig. 3A).
Moreover, we have investigated the effect of HF diets in hippocampal synaptic plasticity. Because NMDA has been shown to induce LTD (NMDA-LTD) in hippocampal slices through dephosphorylation of the GluR1 subunit of AMPA receptors, we have investigated the effect of NMDA on our experimental model. Our results show that perfusion with 15 μM NMDA for 6 min induced LTD in hippocampal slices, whereas LTD-NMDA was not induced in hippocampus from HF-treated mice (Fig. 3B). Differences between groups were maintained during the whole time course of the recording after NMDA application [LF: 82.57 ± 2.18% vs. HF: 134.45 ± 16.49%, 1-way ANOVA, F(1,6) = 5.54, P < 0.05]. Although NMDA perfusion seems to induce the potentiation of synaptic transmission in HF-treated mice, statistical differences were not found between EPSP values during baseline or after NMDA perfusion in the HF group. Nevertheless, no differences were found in other types of synaptic plasticity such as E-LTP and L-LTP (Fig. 3, C and D, respectively), indicating that HF diet affects specifically NMDA-LTD.

**HF diet downregulates the NMDA receptor subunit NR2B.** NR2A and NR2B subunits of the NMDA receptor were quantified by Western blot (Fig. 4). As illustrated in Fig. 4A, NR2B content was lower in HF than in LF samples [1-way ANOVA F(1,10) = 10.2, P < 0.01]. In contrast, the content of NR2A was not modified by the dietary treatment.

GluR1 and GluR2 subunits of the AMPA receptor were also quantified (Fig. 4B), but in this case no effect of HF was detected.

**HF diet downregulates Glu-degrading enzymes.** Expression of Glu-degrading enzymes was estimated by means of Western blotting. One-way ANOVA revealed that GS was downregulated in the hippocampus of HF mice [F(1,16) = 7.26, P < 0.05; Fig. 5A]. GABA-decarboxylase-65 (GAD-65) and -67 (GAD-67) (Fig. 5B), which are the membrane and cytosolic isoforms of GABA-decarboxylase, respectively, and are involved in GABA synthesis, also appeared to be downregulated in the hippocampus of HF animals [1-way ANOVA F(1,14) = 5.03, P < 0.05, and F(1,14) = 13.14, P < 0.01, for GAD-65 and GAD-67, respectively]. In contrast, GluDH1 remained unchanged in HF animals (Fig. 5C).

**HF diet desensitizes the Akt pathway coupled to leptin receptors.** To assess the functionality of leptin receptors within the hippocampus, we administered a single dose (1 mg/kg) of acute leptin increased STAT3 phosphorylation [2-way ANOVA, F(1,12) = 24.945, P < 0.001, for leptin treatment] independently of the type of dietary treatment. In contrast, one-way ANOVA revealed a significant effect of leptin on Akt phosphorylation [F(1,6) = 10.899, P < 0.05] only in control animals (Fig. 6B).

**DISCUSSION**

The present study shows that HF diets alter Glu metabolism and neurotransmission in mice hippocampus. Our major find-
The improvement of Glu uptake kinetics together with the upregulation of glutamate transporters does not necessarily imply that Glu uptake is actually more efficient in HF mice. In fact, the downregulation of the glucose transporter GLUT1 detected in HF mice would contribute to limit Glu uptake (36). Moreover, an eventual increase of Glu uptake should be accompanied by a parallel increase of Glu release (18). The improvement of Glu uptake kinetics together with the upregulation of the glial Glu carriers GLT-1 and GLAST. These neurochemical alterations occur concomitantly with a significant reduction of basal synaptic transmission and also with the impairment of NMDA-induced LTD, suggesting that HF diets trigger significant changes in glutamatergic transmission.

Upregulation of activity and expression of glial Glu transporters is a well-characterized event integral to physiological mechanisms involved in the homeostasis of Glu transmission (33) that can be triggered by pharmacological manipulations, leading to the increase of synaptic Glu (2, 9, 25). Taken together with the downregulation of the NMDA receptor (NMDAR) subunit NR2B detected in the present study, our data suggest that HF diets would enhance Glu release (18).

Fig. 4. A: effect of HF diet on the density of NR2A and NR2B subunits of the NMDA receptor (NMDAR). B: effect of HF diet on the density of GluR1 and GluR2 subunits of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPA) Data are expressed as %control group and are means ± SE of 6–8 animals. **P < 0.01 compared with LF controls. Student-Newman-Keuls test.

Fig. 5. A: effect of HF diet on the density of glutamine synthase (GS). B: glutamate dehydrogenase (GluDH). C: GABA-decarboxylase (GAD). Data are expressed as %control group and are means ± SE of 6–8 animals. *P < 0.05 and **P < 0.01 compared with LF controls. Student-Newman-Keuls test.
processes have been classically associated with LTP, a recent (3, 6). Despite the fact that hippocampal-dependent cognitive dependent LTP and LTD, has been proposed as the primary hippocampal glutamatergic synapses, particularly NMDAR-has been demonstrated that activity-dependent plasticity of mission in HF mice and also an impairment of NMDA-LTD. It would rather suggest an increase in Glu-degrading enzymes. Nevertheless, this possibility does not fit adequately with a more efficient uptake process that might impair Glu metabolism by triggering a downregulation as GS activity (29). In accord, it can be speculated that HF diets shown to increase GAD-65 and GAD-67 expression (7) as well nutritional facts. Interestingly, caloric restriction has been ened by a more efficient metabolism, namely by GS. In our hands, the expression of this enzyme appears to be reduced in HF mice. Finally, the downregulation of other Glu-degrading enzymes is also an intriguing issue that might be related to nervous system and to facilitate NMDA neurotransmission through the NR2B subunit (17), whereas an impaired NMDA-dependent plasticity together with the downregulation of NR2B has been detected in HF mice. Therefore, we speculate that there is a link between Akt desensitization and functional alterations of NMDAR. Accordingly, and as proposed previously by other authors (15), HF-evoked alterations at the level of NMDAR might be linked to leptin resistance. Our study shows that leptin resistance develops in the HF hippocampus for the Akt but not for the STAT3 pathway. This selective desensitization of a downstream signaling pathway coupled to leptin receptors, which has been described previously by our group in peripheral tissues (32), suggests that the integrity of the STAT3 pathway would be necessary for leptin effects on hippocampus plasticity. Furthermore, obese rodents with dysfunctional leptin receptors display impaired LTP and LTD within the hippocampus (16, 21). Thus, we speculate that leptin resistance might account for deficits in hippocampal synaptic plasticity (21). Otherwise, impaired neuronal plasticity might be related to the type of diet used in our study. Finally, HF diets have been shown to trigger type 2 diabetes, a pathological entity able to impair hippocampal function. Nevertheless, this possibility can be ruled out because under our experimental conditions insulin sensitivity appears to be preserved (35).

In summary, present results show that HF diet triggers significant changes in Glu uptake and Glu-degrading enzymes within the hippocampus, which are accompanied by a reduced synaptic efficacy and changes in NMDA-induced plasticity. Our data suggest that HF diets might have a significant impact on the cognitive function of obese individuals by promoting neurochemical changes affecting Glu metabolism.

GRANTS

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