CELL BIOCHEMISTRY AND FUNCTION
Cell Biochem Funct 2009; **27**: 473–478.
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/cbf.1599

High-fat diet and hydrochlorothiazide increase oxidative stress in brain of rats

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This study evaluated the effect of possible synergic interaction between high fat diet (HF) and hydrochlorothiazide (HCTZ) on biochemical parameters of oxidative stress in brain. Rats were fed for 16 weeks with a control diet or with an HF, both supplemented with different doses of HCTZ (0.4, 1.0, and 4.0 g kg^{-1} of diet). HF associated with HCTZ caused a significant increase in lipid peroxidation and blood glucose levels. In addition, HF ingestion was associated with an increase in cerebral lipid peroxidation, vitamin C and non-protein thiol groups (NPSH) levels. There was an increase in vitamin C as well as NPSH levels in HCTZ (1.0 and 4.0 g kg^{-1} of diet) and HF plus HCTZ groups. Na^+–K^+-ATPase activity of HCTZ (4.0 g kg^{-1} of diet) and HCTZ plus HF-fed animals was significantly inhibited. Our data indicate that chronic intake of a high dose of HCTZ (4 g kg^{-1} of diet) or HF change biochemical indexes of oxidative stress in rat brain. Furthermore, high-fat diets consumption and HCTZ treatment have interactive effects on brain, showing that a long-term intake of high-fat diets can aggravate the toxicity of HCTZ. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS — high fat diet; hydrochlorothiazide; hyperglycemia; oxidative stress; neurotoxicity

ABBREVIATIONS — CT, control diet; HCTZ, hydrochlorothiazide; HF, high fat diet; NPSH, non-protein thiol groups; TBARS, thiobarbituric acid reactive substance; ROS, reactive oxygen species

INTRODUCTION

A high fat intake is considered to be an important factor in the development of insulin resistance[1,2] and oxidative stress.[3] Furthermore, results from both rodent and human studies provide evidence that chronic consumption of high-fat diets is associated with alterations in brain chemistry and structure, increased risk of cognitive decline and dementia.[4,5] One mechanism potentially linking high-fat diets to cognitive deficits is the development of insulin resistance and/or type 2 diabetes mellitus.[4] Therefore, chronic ingestion of high fat diet may have direct effects on neuronal function but at the same time can be a major contributor to other chronic diseases, including type 2 diabetes mellitus, cardiovascular disease and hypertension, all of which are considered independent risk factors for cognitive decline and dementia. Thus, it is unclear whether diet directly impacts on brain function or mediates its effects indirectly through of other chronic diseases.

Hydrochlorothiazide (HCTZ) belongs to the thiazide class of compounds used as diuretics in the treatment of hypertension, edema associated with congestive heart failure, and edema associated with hepatic cirrhosis.[6] However, its side effects include metabolic abnormalities, such as hypokalemia, hypercholesterolemia, and hyperglycemia.[7,8] Thus, a variety of studies have reported that thiazide diuretics therapy may impair glucose tolerance and decrease insulin sensitivity and thereby accelerate the development of diabetes mellitus.[7–9] However, few studies are available about the effect of HCTZ on brain.

Oxidative stress occurs in biological systems when there is an overproduction of reactive oxygen species (ROS) as well as a deficiency of enzymatic and non-enzymatic antioxidants. In other words, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of prooxidant/antioxidant reactions in living organisms.[10] In this context, brain is particularly vulnerable to oxidative damage because of the high oxygen utilization, the high content of unsaturated fatty acids (that are more liable to peroxidation), the presence of redox-active metals (Cu, Fe),[10,11] and a low reserve of antioxidant defences.[11] Interestingly, brain makes up about 2% of a person's mass but consumes 20% of their metabolic oxygen.

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Received 22 April 2009
Revised 30 June 2009
Accepted 4 July 2009

The vast majority of this energy is used by neurons.[12] In this context, it has been shown that the oxidative stress increases neuronal death, which contributes to the neuropathology associated with diabetes.[13] In this way, enhanced formation of ROS occurs in tissues during hyperglycemia[14] and these oxidant radicals contribute to increase neuronal death through protein oxidation, DNA damage, and peroxidation of membrane lipids.[15,16] Since animal models of diabetes and insulin resistance can contribute to clarify the effects of diabetes on brain functioning, the role of oxidative stress in brain damage has been extensively studied in experimental diabetes and diabetic patients.[17–19]

In the present study, the possible negative synergic interaction between high fat intake and HCTZ treatment, two risk factors for the diabetes development, was assessed by measuring biochemical parameters related to oxidative stress in brain.

MATERIALS AND METHODS

Chemicals

Casein (technical grade), comassie brilliant blue G, sodium sulfate dodecyl (SDS), ethanol, reduced glutathione, ouabain, malondialdehyde (MDA), and thiobarbituric acid (TBA) were obtained from Sigma (St. Louis, MO, USA). Mono and dibasic potassium phosphate, acetic acid, ascorbic acid, *ortho*-phosphoric acid, tris buffer (tris[hydroxymethyl]aminomethane) and trichloroacetic acid were obtained from Merck (Rio de Janeiro, Brazil). Hydrochlorothiazide, were purchased from commercial sources cornstarch, lard, bone meal, wheat bran, soybean oil,

Animals and diets

Adult male Wistar rats (2 months old), weighing 250–300 g were used for the experiments. The animals were kept on a 12 h light/12 h dark cycle, in a room with the temperature regulated to 21–25°C and humidity at roughly 56% and with free access to food and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil.

Rats were randomly divided in eight experimental groups with five animals per group and fed for 16 weeks with: (1) control diet (CT); (2) CT plus HCTZ (0.4 g kg^{-1} of diet); (3) CT plus HCTZ (1.0 g kg^{-1} of diet); (4) CT plus HCTZ (4.0 g kg^{-1} of diet); (5) high fat diet (HF); (6) HF plus HCTZ (0.4 g kg^{-1} of diet); (7) HF plus HCTZ (1.0 g kg^{-1} of diet), and (8) HF plus HCTZ (4.0 g kg^{-1} of diet). HCTZ doses were selected on the basis of a previous study where a dose of 300 mg kg^{-1} of HCTZ was found to be the NOAEL for HCTZ in rats.[6] Here we have estimated a consumption of about 25 g day^{-1} rat^{-1}, which correspond to doses of HCTZ ranging from about 30 to 300 mg HCTZ per kg of body weight. The composition of the diets is shown in Table 1. Diets were prepared weekly and stored at 4°C. HCTZ was first mixed with the vitamin and mineral mixtures. Then, this new mixture was extensively mixed with casein and sequentially with sucrose, wheat bran and the other components of the diet until a homogenous mixture was obtained. Diet (25–30 g rat^{-1}) was offered daily and the leftovers were removed and weighted to calculate the daily food consumption (food consumption varied from about 20 to 25 g rat^{-1} day^{-1}).

Table 1. Composition of the diets (g kg^{-1})

Components	High fat diet	Control diet
Sucrose	200	200
Cornstarch	—	280
Casein	180	180
Albumin	22	22
Lard	280	—
Soybean oil	20	20
Bone's flour	60	60
Wheat bran	188	188
Mineral mixture*	40	40
Vitamin mixture†	10	10

*The mineral mixture contained (g kg^{-1}): bone meal (449); NaCl (38); KCl (134.2); $MgSO_4$ (20); $ZnCl_2$ (0.4); $CuSO_4$ (0.175); $MnSO_4$ (1.2); $FeSO_4$ (2), and cornstarch (355).

†The vitamin mixture (mg or IU g^{-1}) was composed of Vitamin A, 2000 IU; Vitamin D, 200 IU; tocopherol, 10 IU; menadione, 0.5 mg; choline, 200 mg; folic acid, 0.2 mg; *p*-aminobenzoic acid, 1.0 mg; inositol, 10 mg; calcium D-panthotenate, 4.0 mg; riboflavin, 0.8 mg; thiamin-HCl, 0.5 mg; pyridoxine-HCl, 0.5 mg; niacinamide, 0.3 mg; and biotin, 0.04.

Tissue preparation

At the end of the 16-week treatment, after 12 h of fasting, the animals were decapitated under mild ether anesthesia and blood was collected by cardiac puncture in heparinized tubes for the measurement of blood glucose levels. Brain was quickly removed, rinsed with saline, weighted, placed on ice, and homogenized in 10 volumes (w/v) in cold 50 mM Tris–HCl pH 7.4. The homogenate was centrifuged at 4000*g* at 4°C for 10 min to yield low-speed supernatant fraction (S1) that was used for biochemical assays.

Blood glucose levels

Blood glucose levels were measured by using commercial Kits (Labtest, Minas Gerais, Brazil).

Lipid peroxidation (LPO) levels

Lipid peroxidation was estimated by measuring TBA reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content, according to the method of Ohkawa *et al.*,[20] in which MDA, an end product of fatty acid peroxidation, reacts with TBA to form a colored complex. In brief, samples were incubated at 100°C for 60 min in acid medium containing 0.45% SDS, 1.27 mol L^{-1} acetic acid/270 mmol L^{-1} HCl, pH 3.5 and 0.8% TBA. After centrifugation, the reaction product was determined at 532 nm using 1,1,3,3-tetramethoxypropane as standard.

DOI: 10.1002/cbf

Vitamin C levels

Cerebral vitamin C (ascorbic acid) levels were determined by the method of Jacques-Silva *et al*.[21] Proteins of brain were precipitated with 1 volume of a cold 10% trichloroacetic acid followed by centrifugation. An aliquot of 300 μl of supernatants was mixed with 2,4-dinitrophenylhydrazine (4.5 mg ml^{-1}), $CuSO_4$ (0.075 mg ml^{-1}) and trichloroacetic acid 13.3% (final volume 1 ml) and incubated for 3 h at 37°C. Then, 1 ml of H_2SO_4 65% (v/v) was added to the medium. Ascorbic acid levels were measured spectrophotometrically at 520 nm and calculated using a standard curve (1.5–4.5 μmol L^{-1} ascorbic acid freshly prepared in sulfuric acid).

Non-protein thiol groups (NPSH) levels

Non-protein thiol groups content from brain were determined as described by Ellman.[22] For the NPSH determination the samples of S1 from brain were precipitated with 200 μl of 10% trichloroacetic acid followed by centrifugation. The colorimetric assay was carried out in phosphate buffer 1 M, pH 7.4. A standard curve using glutathione was constructed in order to calculate the non-protein thiol groups in the tissues samples.

Determination of Na^+–K^+-ATPase activity

Cerebral Na^+–K^+-ATPase activity was measured spectrophotometrically by determining the organic phosphate (Pi) released according to the method of Fiske and Subbarow.[23] Na^+–K^+-ATPase activity was calculated as the difference between the total Mg^{2+} ATPase activity (without ouabain) and Mg^{2+} ATPase activity determined in the presence 0.5 mmol L^{-1} of ouabain. Both activities were determined in the presence of 125 mmol L^{-1} NaCl and 20 mmol L^{-1} KCl.

Statistical analysis

All values obtained are expressed as mean ± standard error. Data were analyzed by one-way or two-way ANOVA analyses of variance followed by Duncan's multiple range tests when appropriate. Differences between groups were considered to be significant when $p < 0.05$.

RESULTS

Organ weight

Two-way ANOVA (2 diets × 4 HCTZ doses) revealed no significant main effect of diet ($p > 0.10$) or HCTZ ($p > 0.10$) or diet versus HCTZ interaction ($p > 0.10$).

Blood glucose levels

Two-way ANOVA of blood glucose levels revealed a significant main effect of the diet [$F(1, 32) = 9.74$, $p < 0.05$] and a significant main effect of the HCTZ treatment [$F(3, 32) = 4.73$, $p < 0.05$. HCTZ and HF treatment tended to increase blood glucose; however, post-hoc comparisons indicated that a significant increase in glucose levels occurred only after simultaneous ingestion of HF and HCTZ (1.0 and 4.0 g kg^{-1}; Figure 1, $p < 0.05$).

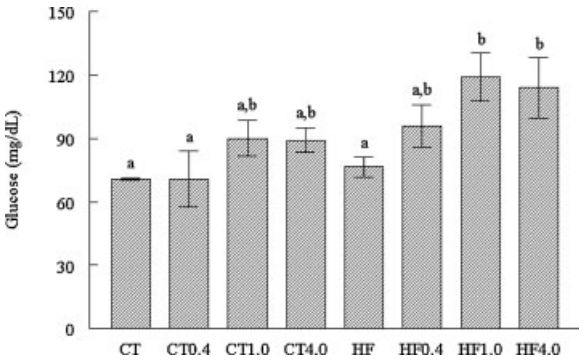

Figure 1. Effect of co-treatment with hydrochlorothiazide and control or high fat diet on glucose blood levels. Data are expressed as means ± SEM of five animals. [ab]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

Lipid peroxidation (LPO) levels

Two-way ANOVA of LPO levels revealed a significant main effect of the diet [$F(1, 32) = 15.43$, $p < 0.05$] and a significant main effect of the HCTZ treatment [$F(3, 32) = 5.56$, $p < 0.05$. HCTZ and HF treatment tended to increase LPO levels; however, post-hoc comparisons indicated that a significant increase in LPO levels occurred only after ingestion of 4.0 g kg^{-1} of HCTZ alone or after simultaneous ingestion of HF and HCTZ at 1 and 4 g kg^{-1} of HCTZ (Figure 2). Of particular importance, positive correlation was found between cerebral LPO and blood glucose levels ($r = 0.49$, $p < 0.05$).

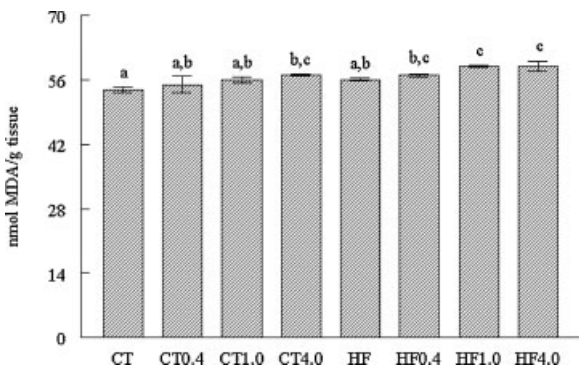

Figure 2. Thiobarbituric acid reactive substance levels in rats co-treated with hydrochlorothiazide and with control or high fat diets. Data are expressed as means ± SEM of five animals. [abc]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

DOI: 10.1002/cbf

Vitamin C levels

Two-way ANOVA of cerebral vitamin C levels revealed a significant main effect of diet [$F(1, 32) = 31.47$, $p < 0.05$] and of HCTZ treatment [$F(3, 32) = 24.72$, $p < 0.05$]. HCTZ caused a significant increase in the vitamin C levels in brain of animals. Diet × HCTZ treatment interaction was also significant [$F(3, 32) = 7.15$, $p < 0.05$]. Post-hoc comparisons revealed that the HCTZ caused a dose-dependent increase in cerebral vitamin C in rats fed with the CT, whereas only the highest dose of HCTZ caused a significant increase in vitamin C in rats fed with the high fat diet (Figure 3).

Non-protein thiol groups (NPSH) levels

Two-way ANOVA of cerebral NPSH levels revealed a significant main effect of the diet [$F(1, 32) = 21.89$, $p < 0.05$] and a significant diet versus HCTZ interaction [$F(3, 32) = 8.79$, $p < 0.05$]. Post-hoc comparisons indicated that a significant increase in NPSH levels occurred in brain from rats fed the CT only after of ingestion of 1.0 and 4.0 g kg^{-1} of HCTZ. In rats fed the HF, HCTZ caused an increase in NPSH at all doses (Figure 4).

Determination of Na^+–K^+-ATPase activity

Two-way ANOVA of cerebral Na^+–K^+-ATPase activity revealed a significant main effect of diet [$F(1, 32) = 47.10$, $p < 0.05$] and HCTZ treatment [$F(3, 32) = 13.44$, $p < 0.05$]. Post-hoc comparisons indicated that a significant decrease in Na^+–K^+-ATPase activity in rats fed the CT occurred after ingestion of 4.0 g kg^{-1} of HCTZ. In rats fed with the HF, HCTZ decreased Na^+–K^+-ATPase activity at all doses tested (Figure 5). In addition, negative correlation was found between the Na^+–K^+-ATPase activity and glucose levels ($r = -0.65$, $p < 0.05$).

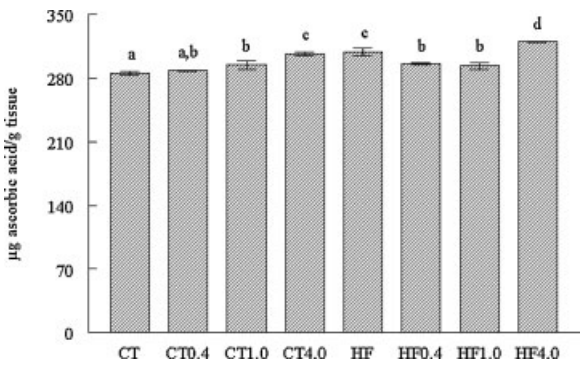

Figure 3. Vitamin C levels in rats co-treated with hydrochlorothiazide and with control or high fat diets. Data are expressed as means ± SEM of five animals. [abcd]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

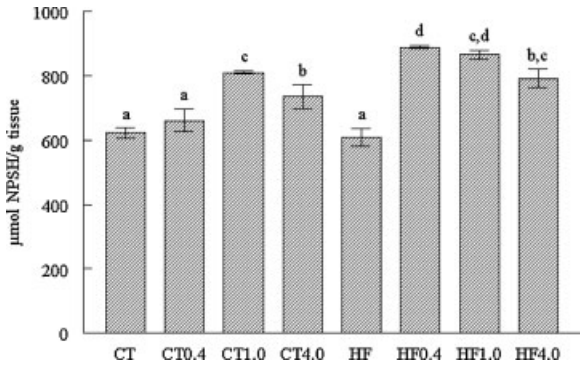

Figure 4. Non-protein thiol groups levels in rats co-treated with hydrochlorothiazide and with control or high fat diets. Data are expressed as means ± SEM of five animals. [abcd]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

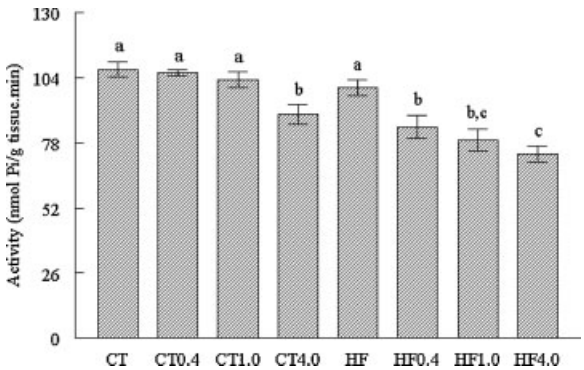

Figure 5. Effect of co-treatment with hydrochlorothiazide and with control or high fat diet on Na^+–K^+-ATPase activity. Data are expressed as means ± SEM of five animals. [abc]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

DISCUSSION

Literature data have indicated that long-term consumption of high-fat diets[3] as well as HCTZ treatment[7] are important factors for the appearance of some metabolic changes related to type 2 diabetes. In this way, results of the present study indicate that high doses of HCTZ associated with HF caused an increase in blood glucose levels, which are compatible with the development of insulin resistance. Thus, we can suggested that simultaneous ingestion of high-fat diets and the use of thiazides as diuretics for the treatment of hypertension could potentiate the increase of blood glucose levels caused by this class of drug. The mechanism by which thiazide induces an increase in glucose levels and glucose intolerance is not completely understood. However, it has been implicated a decreased insulin secretion by pancreatic β cells and decreased tissue insulin sensitivity.[24]

In this context, it is well accepted that low to medium range doses of this diuretic is effective in lowering blood pressure with minimal side effects. When the dose is

DOI: 10.1002/cbf

increased, little contribution is observed regarding the control of blood pressure, whereas side effects increase substantially.[7,8,24] In fact, previous studies have indicated a clear correlation between the dose of thiazide and an increase in fasting glucose concentrations.[25] In the same vein, hydrochlorothiazide (at an average dose of 40 mg day^{-1}) caused hyperglycemia.[26] Recent data have indicated that prolonged treatment of hypertensive patients with a low dose of HCTZ (12.5 mg day^{-1}) improves arterial elasticity, but not in patients with type 2 diabetes mellitus or impaired fasting glucose. In addition, they have demonstrated that treatment with a full dose of HCTZ (25 mg day^{-1}) can aggravate metabolic parameters and arterial stiffness.[27] The doses tested here were higher than that commonly used for the treatment of hypertension; however, they are lower than the NOAEL of HCTZ for rats[6] and can indicate that a direct extrapolation of toxic doses from rats to human is not possible.

For years researchers have reported that ROS can cause cell degeneration, especially in brain,[18,19] since it is particularly vulnerable to oxidative stress due to limited antioxidant capacity.[12] In this context, it has been shown that chronic intake of a HF[3,28] as well as the hyperglycemia condition are linked to oxidative stress generation and that increased levels of ROS are involved in the development of insulin resistance[29–32] and diabetic neuropathy.[13,18,19,33] However, data about the potential facilitating effects of HCTZ in promoting insulin resistance and oxidative stress in animal models are scarce or lacking in literature. Herein we found that chronic HF consumption was associated with an increase in LPO levels in brain tissue that was aggravated by HCTZ treatment. In line with this, literature data have indicated that hyperglycemia causes an excessive non-enzymatic glycation of protein structures, with marked inactivation of enzymes, increased lipid peroxidation, and changes in antioxidant defense systems.[30,34]

In this study, we observed a small but statistically significant increase in cerebral vitamin C levels in rats fed with high doses of HCTZ alone or in combination with HF. Similarly, NPSH levels were increased in animals treated with 1.0 and 4.0 g kg^{-1} of HCTZ and the increase was proportionally higher in rats fed the HF diet. Accordingly, an increase on the antioxidant defense systems has been observed in a variety of experimental models of pathologies possibly as a compensatory response of the tissues to the presence of oxidative insults.[35,36] However, here the significance of the levels of Vitamin C remains unclear, particularly, in view of the fact that the actual differences in Vitamin C levels across the various groups were small.

Na^+–K^+-ATPase, a sulfhydryl-containing enzyme, is embedded in the cell membrane and is responsible for the active transport of sodium and potassium ions in the nervous system. This process regulates the cellular Na^+/K^+ concentrations and hence their gradients across the plasma membrane, which are required for vital functions such as membrane co-transports, cell volume regulation and membrane excitability.[37,38] This dimeric enzyme exists in several isoforms in brain and consumes the greater part of available ATP.[39] The inactivation of Na^+–K^+-ATPase leads to partial membrane depolarization allowing excessive Ca^{2+} entry inside neurons with resultant neurotoxic events.[40] In this study, we observed a significant inhibition in Na^+/K^+-ATPase activity in brain of the animals treated with high doses of HCTZ. HCTZ-induced enzyme inhibition may be associated with an increase in oxidative stress which can accelerate Na^+–K^+-ATPase denaturation.[41] In fact, –SH groups of this enzyme are highly susceptible to oxidative stress[42] and oxidizing agents.[43] Moreover, our data have indicated an interaction between HF and HCTZ effects on Na^+–K^+-ATPase activity in brain. Simultaneous co-treatment with HCTZ and the HF diet could cause additional pro-oxidative stress in this organ with concomitant Na^+–K^+-ATPase inhibition. However, further studies are necessary to understand the mechanism(s) involved in the interactive effects of diet and HCTZ on cerebral Na^+–K^+-ATPase.

In summary, our data indicate that chronic intake of the high doses of HCTZ or HF changes the biochemical parameters related to cerebral oxidative stress. The significant positive correlation between cerebral TBARS and blood glucose and the negative correlation between Na^+–K^+-ATPase and blood glucose levels may indicate a potential role for hyperglycemia, at least in part, in neurochemical changes after exposure to HCTZ and/or a high fat diet. In short, the results of the present investigation suggested that simultaneous consumption of high fat diets and HCTZ can exacerbate oxidative stress in brain.

ACKNOWLEDGEMENTS

The financial support by FINEP Research Grant ''Rede Instituto Brasileiro de Neurociência (IBN-Net)'' # 01.06.0842-00, FAPERGS, CAPES/SAUX, VITAE Fundation and CNPq and CNPQ-INCT is gratefully acknowledged. J.B.T.R. is the recipient of CNPq fellowships.

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DOI: 10.1002/cbf