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Research Report

# Caloric restriction improves basal redox parameters in hippocampus and cerebral cortex of Wistar rats

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ABSTRACT

Caloric restriction (CR) has been shown to either decrease or prevent the progression of several age-related pathologies. In previous work, we demonstrated that CR modulates astrocyte functions, suggesting that CR may exert neuroglial modulation. Here, we investigated the effects of CR on hippocampal (Hc) and cortical (Cx) oxidative stress parameters of male Wistar rats. Our results showed that CR-fed rats had 17% less body weight gain after 12 weeks of treatment. CR improved locomotion performance, increased glutathione levels and decreased glutathione peroxidase activity and the production of reactive oxygen species. However, no changes were observed in lipid peroxidation, nitric oxide content and catalase activity. Single cell gel electrophoresis assay (comet assay) revealed a reduction in the extent of basal DNA damage upon CR. Our data suggest that dietary CR could induce both hippocampal and cortical modulation resulting in metabolic changes and as a consequence, significant improvement of cellular defense-associated parameters.



## 1. Introduction

Dietary caloric restriction (CR) is defined as a limitation of food intake below the *ad libitum* level without malnutrition and it is well known to extend the maximum lifespan in a wide range of different organisms.

Experiments in animal models have demonstrated that caloric restriction (CR) is able to either slow down or prevent the progression of several age-related pathologies (Gonzalez et al., 2011); for instance, cardiovascular disease (Mattson and Wan, 2005), multiple types of cancer (De Lorenzo et al., 2011; Klebanov, 2007) diabetes (Anson et al., 2003) and ischemic injury

Abbreviations: RC, caloric restriction; GS, glutamine synthetase; Hc, hippocampal; Cx, cortical; ROS, reactive oxygen species; GSH, glutathione; EPM, elevated plus-maze test; GPx, glutathione peroxidase; CAT, catalase; TBARS, thiobarbituric acid-reactive substances; NO, nitric oxide

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(Morris et al., 2011). CR may also reduce neuronal damage (Chouliaras et al., 2012) and consequently offer protection against neurodegenerative diseases (Bishop and Guarente, 2007; Gillette-Guyonnet and Vellas, 2008). Recent studies have shown that CR is sufficient and enough to induce neurogenesis in the hippocampus of adult mice (Lee et al., 2002), to enhances synaptic plasticity in the aging rat (Fontan-Lozano et al., 2008; Mladenovic Djordjevic et al., 2009), to modulates *a*-synuclein expression in the aging rat cortex and hippocampus (Mladenovic et al., 2007) and to attenuates age-related changes in mouse neuromuscular synapses (Valdez et al., 2010).

Moreover, our laboratory recently reported that CR also modulates astrocytic functions by increasing glutamate uptake and glutamine synthetase (GS) activity. This suggested that CR may exert certain neuroprotective effects against brain illness by a mechanism involving modulation of astrocytic functions (Ribeiro et al., 2009). Such results suggest that brain under CR could become somehow less sensitive to physiological aging process and better restore its functions after injury. With aging, brain undergoes neuronal loss in many areas, cognitive functions decline and it decreases in size as well as white matter integrity (Park and Reuter-Lorenz, 2009).

There is evidence that hippocampus seems to be particularly sensitive to aging and may be partly responsible for age-related cognitive decline (Jessberger and Gage, 2008). In addition, a large number of age-related changes within the hippocampus have already been documented, such as altered mitochondrial function, oxidative stress, changes in glutamate transmission and synaptic plasticity (Fontan-Lozano et al., 2008). Some studies indicated that the frontal cerebral cortex suffers a dramatic cell loss due to aging and its influence on synaptic loss was associated with significant cognitive decline (Asha Devi, 2009). Aging has a powerful effect on enhanced susceptibility to neurodegenerative diseases (Fratiglioni and Qiu, 2009).

Problems occur when production of reactive oxygen species (ROS) exceeds the cells ability to protect themselves against such molecules. Oxidative stress occurs as a result of imbalance between cellular production of ROS and the ability of the cells to defend themselves against them (Buonocore et al., 2010). Thus, it could trigger cellular damage as ROS is able to oxidize cellular components such as membrane lipids, proteins and DNA (Esposito et al., 2002). There is substantial evidence that the brain, which consumes large amounts of oxygen, has abundant lipid content but relative paucity of antioxidant enzymes, making it particularly vulnerable to oxidative damage.

As a matter of fact, oxidative damage has strongly been associated in the pathogenesis of several neurodegenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease and stroke (brain ischemia/reperfusion injury) (Hegde et al., 2011; Kamat et al., 2008). Cells possess different physiological self-defense mechanisms against free radicals-induced damage. The major ones are for instance, antioxidant scavengers such as glutathione (GSH), vitamin C (ascorbic acid), vitamin E (α-tocopherol), carotenoids, flavonoids, polyphenols, as well as antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. These antioxidant self-defense mechanisms can be upregulated in response to increased ROS or peroxide production. Although it may confer protection against ROS, they are not completely effective in preventing aging-related oxidative damage (Esposito et al., 2002; Kamat et al., 2008). Recent studies have demonstrated that age-related increases of oxidative damage in the brain is best exemplified by lipid peroxidation-derived products, protein oxidation and oxidative modifications in nuclear and mitochondrial DNA, beyond the decrease in brain and plasma antioxidants (GSH and antioxidant enzymatic activity) (Droge and Schipper, 2007; Hegde et al., 2011).

In the present study, we investigated the effects of caloric restriction on oxidative stress parameters, basal antioxidant enzymes, lipid peroxidation and DNA damage in the hippocampus and cerebral cortex of Wistar rats. Behavioral and blood biochemical parameters were also evaluated.

## 2. Results

### 2.1. *Effect of CR diet on body weight and serum biochemistry*

Sixty-day old rats were fed with laboratory chow (Table 1) *ad libitum* (control) or underwent CR for 12 weeks, and were weighted weekly. The weight gain of the experimental protocol is shown in Fig. 1. Rats submitted to caloric restriction, had a decrease of 12% ($P < 0.05$) in body weight gain in the end of the first week of the treatment. The difference in weight gain between groups was statistically significant

**Table 1 – Composition of the laboratory chow.**

| Composition | (g/kg) |
|---|---|
| Total fat | 110 |
| Sunflower oil | 5 |
| Proteins | 220 |
| Fibers | 30 |
| Ash | 60 |
| Vitamins | 20 |
| Carbohydrates | 520 |

Commercial nonpurified diet, Nuvilab-CR1 (Curitiba, PR, Brazil).

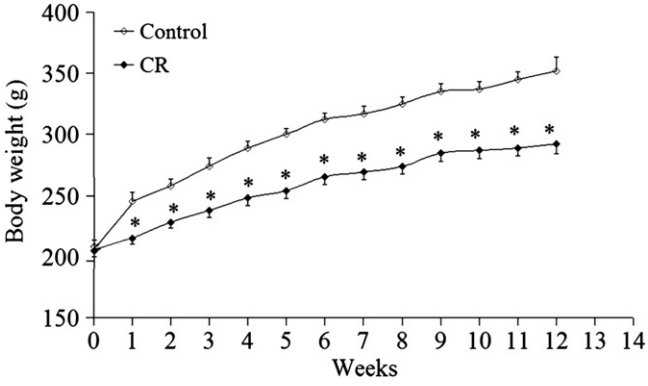


**Fig. 1 – Repeated measurement of body weight. Male Wistar rats were fed with laboratory chow *ad libitum* (control) or calorie restricted (CR) diets. The figure shows the evolution in body weight of control animals (open symbols) and CR diet-fed rats (closed symbols) throughout 12 weeks. Values are means ± S.E.M. ($n = 15$). *Significantly different from controls (Student's *t*-test, $P < 0.05$).**