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Caloric restriction in young rats disturbs hippocampal neurogenesis and spatial learning

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ABSTRACT

It is widely known that caloric restriction (CR) has benefits on several organic systems, including the central nervous system. However, the majority of the CR studies was performed in adult animals and the information about the consequences on young populations is limited. In this study, we analyzed the effects of young-onset CR, started at 4 weeks of age, in the number of neuropeptide Y (NPY)-containing neurons and in neurogenesis of the hippocampal formation, using doublecortin (DCX) and Ki67 as markers. Knowing that CR treatment could interfere with exploratory activity, anxiety, learning and memory we have analyzed the performance of the rats in the open-field, elevated plus-maze and Morris water maze tests. Animals aged 4 weeks were randomly assigned to control or CR groups. Controls were maintained in the *ad libitum* regimen during 2 months. The adolescent CR rats were fed, during 2 months, with 60% of the amount of food consumed by controls. We have found that young-onset CR treatment did not affect the total number of NPY-immunopositive neurons in dentate hilus, CA3 and CA1 hippocampal subfields and did not change the exploratory activity and anxiety levels. Interestingly, we have found that young-onset CR might affect spatial learning process since those animals showed worse performance during the acquisition phase of Morris water maze. Furthermore, young-onset CR induced alterations of neurogenesis in the dentate subgranular layer that seems to underlie the impairment of spatial learning. Our data suggest that adolescent animals are vulnerable to CR treatment and that this diet is not suitable to be applied in this age phase.

1. Introduction

The size and frequency of meals can cause enormous effects on the health and lifespan of experimental animals (Mattson, 2005). Caloric restriction (CR), i.e. moderate caloric consumption reduction without inducing malnutrition (Fontana & Partridge, 2015; Sohal & Weindruch, 1996), is well known for its general beneficial health effects in many species, such as yeast, worms, flies, rodents and non-human primates (Fontana, Partridge, & Longo, 2010; Roth & Polotsky, 2012). Indeed, CR is recognized as an intervention capable of increasing the mean and maximal lifespan and to prevent or delay age-related diseases and cognitive decline (Anton & Leeuwenburgh, 2013; Cava & Fontana, 2013; Roth, Ingram, & Lane, 2001; Weindruch, 1996). Some of the general benefits of CR include reduction of body fat and obesity, amelioration of glucose regulation, improvement of cardiovascular diseases and diabetes mellitus, as well as reduction in the incidence of nephropathy and several types of cancer (Andrade, Lukoyanov, & Paula-Barbosa, 2002; Colman et al., 2009; Fontana et al., 2010; Mattison et al., 2012). Concerning the central nervous system (CNS), CR has been shown, in numerous experimental settings, to reduce brain atrophy associated with aging and decrease or delay age-associated cognitive decline and neurodegenerative diseases (Del Arco et al., 2011; Gillette-Guyonnet & Vellas, 2008), with improvement on learning and memory tasks (Kuhla et al., 2013). Additionally, this restriction protected neurons from metabolic events, ischemic insults (Andrade, Mesquita, Assuncão, & Pereira, 2006; Andrade et al., 2002; Bruce-Keller, Umberger, McFall, & Mattson, 1999; Duan & Mattson, 1999) and increased the resistance to epileptic seizures (Azarbar, McIntyre, & Gilby, 2010; Bough, Valiyil, Han, & Eagles, 1999; Colman et al., 2009).

However, besides all these CR beneficial effects, there are also several studies that have reported some alterations induced by CR that seem to be detrimental, including a reduction of the immune response (Adler & Bonduriansky, 2014; Kristan, 2008) and reproductive status (Adler & Bonduriansky, 2014; Leonhardt,

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Shahab, Luft, Wuttke, & Jarry, 1999). There was also a report of reduced immune responses in Rhesus monkeys initiated in CR at juvenile ages (1–2 years) (Messaoudi et al., 2008). In the brain, CR induces subtle alterations in the dendritic arborization of dentate granule cells (Andrade et al., 2002) and contributes to anxiety and depression disorders (Jahng et al., 2007). A loss of facial motoneurons after injury in CR Sprague-Dawley rats was also reported (Aperghis et al., 2003) and CR did not prevent and even increased the decline of synapses of the layer 2 of the sensorimotor cortex of old male F1 Brown Norway × Fischer 344 rats (Shi, Poe, Constance Linville, Sonntag, & Brunso-Bechtold, 2002). The different species, strains and sexes of the animals used in the experimental studies, the level of caloric restriction and duration of treatment may explain some of the detrimental effects and the reported conflicting results. Maximal beneficial effects and extension of lifespan are achieved when CR is initiated in young adult animals (Mattson, 2005). Although the effects in other age phases are not well characterized (Rizza, Veronese, & Fontana, 2014), the age of the animals at the beginning of treatment seems to be crucial for the beneficial health effects of the restriction of calories. This is based on the fact that each specific age phase has precise nutritional needs and susceptibilities (Morgane et al., 1978). In the adult phase, animals are more resistant to environmental variations and aggressions. Apparently, when animals are either young or old, they tend to be more vulnerable to the environmental changes, nutritional deprivations and external aggressions (Morgane et al., 1978). Although it was thought that CR applied to aged animals could not have effects on the extension of lifespan and health (Mattson, 2005), we have recently reported that CR applied during aging does not lead to neuronal loss in the hippocampal formation (HF) and impedes the age-associated decrease of neuropeptide Y (NPY) and somatostatin (Cardoso, Silva, Magano, Pereira, & Andrade, 2014). This was accompanied by the prevention of the age-associated reduction of acetylcholine varicosities, also avoiding the several aging-associated alterations related to cognitive functions (Cardoso et al., 2014). These recent results demonstrated that prolonged CR also induced structural and cognitive improvements in aged rats. However, the information about the CR effects in young rodents is scarce (Jahng et al., 2007; Kuhla et al., 2013). Adolescence is known to be a time of adjustment and vulnerability in humans to many psychiatric disturbances including eating disorders, addictions, major depression, anxiety and schizophrenia (Casey, Jones, & Hare, 2008; Chowdhury et al., 2014; Simon & Moghaddam, 2015). Data in the literature also suggests that the HF is not yet fully mature by puberty (Andersen & Teicher, 2004; Yildirim et al., 2008) and may be involved in the vulnerability of the adolescent brain to these disorders. Therefore, food restriction was initiated in adolescent rats at postnatal day 28. High levels of caloric restriction in rodents are known to be one of the causes of the different responses among strains regarding beneficial health effects, oxidative events, chronic stress and extension of life span (Heiderstadt, McLaughlin, Wright, Walker, & Gomez-Sanchez, 2000; Speakman & Mitchell, 2011; Stankovic et al., 2013). Nevertheless, we selected a moderate caloric restriction of 40% based on the evidence which suggests that a moderate restriction (Stankovic et al., 2013) has antioxidative properties and increases the maximal longevity in rats (Stankovic et al., 2013; Weindruch, 1996).

Taking this into consideration, we decided to analyze in the rat the effects of young-onset moderate CR treatment, starting as early as 4 weeks of age, on the HF, one of the brain regions that is more affected by nutritional deficits (Cardoso, Castro, Pereira, & Andrade, 2013; Cintra, Diaz-Cintra, Galvan, Kemper, & Morgane, 1990; Hipólito-Reis, Pereira, Andrade, & Cardoso, 2013), and essential to several cognitive functions, including spatial learning and memory (Aggleton & Brown, 2006; Cardoso, Lukoyanova, Madeira, & Lukoyanov, 2011; Eichenbaum, 1999; Morris, 1984). Moreover, knowing that NPY-positive interneurons are an important and representative population of gamma-aminobutyric acid (GABA)-ergic neurons of the HF, and their number is influenced by dietary changes (Cardoso et al., 2014), we decided to analyze if the young-onset CR will interfere with the number of the NPY-positive interneurons in the HF. Furthermore, given that CR may interfere with neurogenesis (Bondolfi, Ermini, Long, Ingram, & Jucker, 2004; Lee, Seroogy, & Mattson, 2002), we also analyzed the effects of young-onset CR on the neurogenesis of the HF dentate granule cells using the doublecortin (DCX) and Ki67 neurogenic markers. Although it is prolonged into adulthood, the focus on neurogenesis is important because it occurs mainly in early phases of life and even small alterations in the number of dentate granule cells may have profound influences on learning and memory (Murphy, Dias, & Thuret, 2014; van Praag, Kempermann, & Gage, 1999). Finally, knowing that NPY-positive interneurons are also involved in emotions and cognitive functions (Thorsell, Slawecki, El Khoury, Mathe, & Ehlers, 2006; Wettstein, Earley, & Junien, 1995) and that newly born granule cells in adult HF are very important for spatial learning and memory processes (Murphy et al., 2014), we have also evaluated the impact of young-onset CR on the behavioral performance of the animals, including spatial learning and memory, anxiety levels and locomotor activity.

2. Material and methods

2.1. Animals and diets

Male Wistar rats obtained from the colony of the Institute of Molecular and Cell Biology (Porto, Portugal) were maintained under standard laboratory conditions (20–22 °C and a 12 h light/-dark cycle) with free access to food and water. At 4 weeks of age, 20 animals weighing 159 ± 11.5 g were randomly distributed into 2 groups. At the beginning of the experimental study, two animals were housed per cage to allow daily quantification of liquid and food consumption and avoid social isolation. Rats were weighed weekly and bedding was changed at the same time minimizing stress due to handling. The control animals (n = 10) have maintained the *ad libitum* consumption of standard laboratory chow (Mucedola, Italy) containing: proteins (17%) supplemented with lysine (0.7%), methionine (0.3%) and cysteine (0.5%), carbohydrates (57%), fat (4%) and salts (7%) throughout the entire experimental period (2 months). Caloric-restricted rats were fed, during 2 months, with 60% of the amount of food consumed by control animals (Andrade et al., 2002; Cardoso et al., 2013). These CR rats (n = 10) were fed once a day at 09:00 h and food pellets were available until depletion. During the food consumption period of CR rats, given that there were 2 animals per cage, a transparent acrylic plate was placed in the cage, separating one animal on each side of the cage. After the total food consumption (1–2 h) the acrylic barrier was removed. This method was used to separate the animals only during the meal period to avoid social isolation. All diets were supplemented with diet vitamin fortification mixture (MP Biomedicals, USA). All rats had free access to water throughout the experimental period. The handling and care of the animals followed the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985) and the European Communities Council Guidelines in Animal Research (86/609/UE). All efforts were made to minimize the number of animals used and their suffering.

2.2. Behavioral procedures

Behavioral testing began when animals were aged 3 months and was conducted by experimenters blinded to the treatments.