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Intestinal morphology adjustments caused by dietary restriction improves the nutritional status during the aging process of rats

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ABSTRACT

During the aging process, the body's systems change structurally and loss of function can occur. Ingesting a smaller amount of food has been considered a plausible proposal for increased longevity with the quality of life. However, the effects of dietary restriction (DR) during aging are still poorly understood, especially for organs of the digestive system. This study aimed to describe the body weight, oxidative status and possible morphological changes of the intestinal wall of rats submitted to DR during the aging process (7 to 18 months old). Twelve 7-month-old male Wistar rats fed *ad libitum* since birth were assigned to two groups: control group (CG, n = 6) fed *ad libitum* from 7 to 18 months old; and dietary restriction group (DRG, n = 6) fed 50% of the amount of chow consumed by the CG from 7 to 18 months old. The body weight, feed and water intake were monitored throughout the experiment. Blood, periepididymal adipose tissue (PAT) and retroperitoneal adipose tissue (RAT), and the small intestine were collected at 18 months old. The blood was collected to evaluate its components and oxidative status. Sections from the duodenum and ileum were stained with HE, PAS and AB pH 2.5 for morphometric analyses of the intestinal wall components, and to count intraepithelial lymphocytes (IELs), goblet cells and cells in mitosis in the epithelium. DR rats showed a reduction in weight, naso-anal length, PAT, RAT and intestinal length; however, they consumed more water. Blood parameters indicate that the DR rats remained well nourished. In addition, they showed lower lipid peroxidation. Hypertrophy of the duodenal mucosa and atrophy of the ileal mucosa were observed. The number of goblet cells and IELs was reduced, but the mitotic index remained unaltered in both duodenum and ileum. In conclusion, 50% dietary restriction for rats from 7 to 18 months old contributed to improving their nutritional parameters but, to achieve this, adjustments were required in the structure of the body weight and morphology of the small intestine.

1. Introduction

According to a UN report, the elderly population will increase in number more than any other segments of the population by 2050 (UNO, 2009, 2011). Given the current and prospective global demographics, aging has become the subject of research aimed at deciphering the mechanisms involved in this process, together with the consequences for the structure and functioning of the organs that impact the quality of life of the aging adult population.

From conception to death, the biological organism goes through several phases during a continuous aging process that suffers multifactorial pressures, including intrinsic and extrinsic interferences, always seeking the optimal balance between gains and losses in order to prevent morbidity and mortality (Drozdowski and Thomson, 2006).

Biological organisms in the process of aging present gradual decline to the loss of function in several organs. This can occur due to gene action and/or natural aggressions (Oliveira and Schoffen, 2010). Excessive production of reactive oxygen species (ROS) and nitrogen (RNS) and reduction in the cellular level of antioxidants are among the natural aggressions. Together, free radicals are considered one of the main factors related to aging (Hewitt et al., 2012; Eckmann et al., 2013; Walsh et al., 2013).

It is known that the aging process is associated with a significant number of morphological and functional abnormalities of the gastrointestinal tract, including: the reduced amplitude and frequency of gastric emptying and peristaltic movements, which cause slow intestinal transit (Parker and Chapman, 2004; Schoffen and Natali, 2007); impairment of digestion and nutrient absorption (Schoffen and Natali, 2007) due to lower production of digestive enzymes (Parker and Chapman, 2004; Steegenga et al., 2012); changes in the composition of the intestinal microbiota as a consequence of eating habits (Steegenga et al., 2012; Britton and Mclaughlin, 2013); atrophy of the intestinal mucosa, leading

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to reduction in its activity (Grattagliano et al., 2004); an increase in the cell cycle (Thrasher and Greulich, 1965; Cirilo et al., 2013); reduction in the apoptotic index of epithelial cells (Holt et al., 1998; Xiao et al., 2001); and (6) neurodegeneration in the enteric nervous system (Drozdowski and Thomson, 2006; Schoffen and Natali, 2007; Cirilo et al., 2013; Mello et al., 2013; Rayner and Horowitz, 2013).

Among the factors that can interfere in the aging process, food deserves attention, because ingested, digested and absorbed food will determine conditions that are adequate for supporting life in elderly individuals. There are a range of dietary options being studied for elderly individuals and dietary restriction (DR) is one of the main ones recommended (Holt et al., 1998; Grattagliano et al., 2004; Cencic and Chingwaru, 2010; Reimer et al., 2010; Bauer et al., 2011; Arslan-Ergul et al., 2013; Cirilo et al., 2013; Walsh et al., 2013). Studies performed in the 1930s (McCay et al., 1935) reported for the first time that DR promoted an increase of the maximum life span of experimental animals, such as rats and mice. Above all, DR should not cause malnutrition and should therefore be monitored frequently to ensure no nutritional loss for the organism under assessment.

During the aging process, it is common to observe reduction in the production of digestive enzymes (Parker and Chapman, 2004; Steegenga et al., 2012), which can lead to lower concentrations of monosaccharides, fatty acids and amino acids in the intestinal lumen. For this reason, as a compensatory mechanism, it is necessary to enhance the process of absorption of these nutrients so that nutritional intake can continue to occur according to cellular demands. This could be related to the fact that elderly individuals usually show longer intestinal villi and slower intestinal transit (Martin et al., 1998). Thus, offering a smaller amount of food during the aging process may seem contradictory, although many studies have highlighted the beneficial effect of this practice to the health of the aging adults (Holt et al., 1998; Reimer et al., 2010; Arslan-Ergul et al., 2013; Cirilo et al., 2013). However, few studies have evaluated the effectiveness of these models in relation to structural changes caused by DR on the intestine during the aging process (Schoffen and Natali, 2007; Cirilo et al., 2013).

The current scenario is thus composed of important aspects that are interrelated, namely: the present and prospective increase in the elderly population; disruption of the body due to aging; the relevance of the intestine structure for proper digestion and absorption of nutrients; and the need to assess dietary restriction as a practice that contributes to increased longevity. Thus, the purpose of this study was to describe the body weight, oxidative status and possible morphological changes of the intestinal wall of rats submitted to dietary restriction during the aging process (7 to 18 months old).

2. Methods

All the procedures were previously approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (Universidade Estadual de Maringá, UEM), Paraná, Brazil.

2.1. Animals and treatment

This study used 12 7-month-old male Wistar rats (*Rattus norvegicus*) fed *ad libitum* since birth. They were housed in polypropylene cages with a 12-h light/12-h dark cycle and a temperature of 22 ± 2 °C. The rats were distributed into two groups: the control group (CG, n = 6) fed a standard NUVILAB-NUVITAL (Curitiba, PR, Brazil) rodent chow *ad libitum* from 7 to 18 months old; the dietary restriction group (DRG, n = 6) fed 50% of the average amount of chow consumed daily by the CG from 7 to 18 months old. Rat body weight, feed and water intake were monitored throughout the experiment. The weight of the rats was checked every two weeks. Feed intake was monitored for one week per month of the experiment in order to adjust the amount of feed supplied to the DRG. Water consumption was monitored for one week every three months.

2.2. Euthanasia and tissue collection

At 18 months old, the mitotic fuse blocker vincristine sulfate (0.5 mg/kg) was administered through the penile vein for later analysis of cell proliferation. Two hours later, the rats were anesthetized intraperitoneally with sodium thiopental (Thionembutal 40 mg/kg) (São Paulo, SP, Brazil), and the naso-anal length was measured to determine the Lee index [body weight (g) / naso-anal length (cm) × 10^3]. Blood was collected by cardiac puncture to determine biochemical analysis of the energetic profile and total protein and its fractions and to evaluate the oxidative status of the rats.

Laparotomy was performed to remove the small intestine. Its length and its circumference at the pyloric sphincter and ileal papilla were measured. The periepididymal (PAT) and retroperitoneal adipose tissues (RAT) were weighed.

2.3. Biochemical analysis of blood components

Blood was collected to analyze the levels of total proteins, albumin, globulins, triacylglycerols, total cholesterol, and glucose. Blood samples were centrifuged at 3000 rotations/min for 15 min using Analisa kits (Gold Analisa Diagnostica, Minas Gerais, Brazil).

2.4. Evaluation of oxidative status

Blood samples were placed in tubes that contained 3 mmol/L ethylenediaminetetraacetic acid as an anticoagulant and centrifuged at 1000× *g* for 10 min. The plasma was isolated and frozen at −80 °C for future analysis of lipid peroxidation. The residual sediment consisting of erythrocytes was submitted to two rinsing cycles by resuspension and recentrifugation of the sediment at 1000× *g* using 0.9% NaCl. The resulting cell suspension was hemolyzed with ten volumes of cold deionized water, centrifuged at 4000× *g* for 10 min, and the hemolysate supernatant was used to determine the level of reduced glutathione (GSH) and the activity of catalase (CAT) and glutathione peroxidase (GPx). All the procedures were performed at temperatures lower than 4 °C.

Lipid peroxidation levels in plasma were determined using the thiobarbituric acid-reactive substances (TBARS) method (Buege and Aust, 1978). This colorimetric method quantifies low-mass molecules, particularly malondialdehyde, that react with thiobarbituric acid in an acidic medium, forming a complex absorbed at 532 nm. The lipid peroxide concentration was determined using a 1.56 × 10^5 M/cm molar extinction coefficient, and the values are expressed as nanomoles of TBARS per milliliter of plasma.

Lipid peroxidation levels in erythrocytes were determined using the TBARS method (Jain et al., 1989). Aliquots of the washed erythrocyte suspension were resuspended with phosphate buffered saline (PBS, 8.1 g/L NaCl, 2.302 g/L $Na_2HPO_4^{-2}$, and 0.194 g/L $NaH_2PO_4^{-}$, pH 7.4) and deproteinized with 30% trichloroacetic acid. After sitting in an ice bath for 2 h, the material was centrifuged at 2000× *g* for 10 min and the TBARS levels were determined in the clear supernatant, similar to plasma. The values are expressed as nanomoles of TBARS per gram of hemoglobin. Hemoglobin content was determined using the cyanmethemoglobin method.

The levels of reduced glutathione (GSH) in erythrocytes were determined by spectrofluorimetry (Hissin and Hilf, 1976). The activity of the enzyme glutathione peroxidase (GPx) in erythrocytes was determined by the decrease in absorbance due to NADPH-dependent decomposition of H_2O_2 at 340 nm at 25 °C (Paglia and Valentine, 1967; Tappel, 1978). The activity of catalase (CAT) in erythrocytes was assessed by enzymatic decomposition of H_2O_2 measured by spectrophotometry at 240 nm (Aebi, 1974).