Effects of chronic caloric restriction on kidney and heart redox status and antioxidant enzyme activities in Wistar rats

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Caloric restriction (CR) has been associated with health benefits and these effects have been attributed, in part, to modulation of oxidative status by CR; however, data are still controversial. Here, we investigate the effects of seventeen weeks of chronic CR on parameters of oxidative damage/mutation of proteins and on antioxidant enzyme activities in cardiac and kidney tissues. Our results demonstrate that CR induced an increase in protein carbonylation in the heart without changing the content of sulfhydryl groups or the activities of superoxide dismutase and catalase (CAT). Moreover, CR caused an increase in CAT activity in kidney, without changing other parameters. Protein carbonylation has been associated with oxidative damage and functional impairment; however, we cannot exclude the possibility that, under our conditions, this alteration indicates a different functional meaning in the heart tissue. In addition, we reinforce the idea that CR can increase CAT activity in the kidney. [BMB Reports 2012; 45(11): 671-676]

INTRODUCTION

Caloric restriction (CR), a reduction in total calories intake without essential nutrient deficiency, has been shown to exert opposing effects in association with many diseases such as cancer, diabetes, obesity, cardiovascular and autoimmune diseases (1-3). The reduction in food intake used in different rat strains usually ranges from 30 to 50% below the level of control ad libitum (AD) (4). In humans, although there are few results available, CR provides sustained beneficial effects that may reduce insulin resistance, obesity, inflammation, oxidative stress and left ventricular diastolic dysfunction (5). At the cellular level, the basic mechanism proposed to explain the benefits of CR is that resistance to oxidative and metabolic insults occurs as a response to the cellular stress caused by reduced calorie availability to cells (1, 6). For example, CR has been shown to modulate free radical metabolism in the liver of aging rats (7).

Pro-oxidant molecules, such as reactive oxygen species (ROS), result from normal cellular metabolism and have a role in many physiological processes, such as cellular signaling (8, 9). However, in conditions of imbalance between oxidant agents and antioxidant defenses, the phenomenon of oxidative stress occurs, which is involved in cellular function decline, the pathogenesis of many diseases and the aging process (10). In this context, protein carbonylation, which occurs by oxidation of lysine, arginine, proline and threonine residues, has been used frequently as a marker for the oxidative modification of proteins, and the presence of carbonyl groups has been associated with protein, oxidative stress and some diseases (11, 12).

To protect against the deleterious effects of oxidative by-products of normal cellular metabolism, and also under conditions of oxidative stress, cells have an effective endogenous system of defense. Non-enzymatic thiols are very active organic compounds characterized by the presence of -SH (thiol) groups in their structures. Low molecular-mass thiols are critical cellular components involved in numerous physiological and pathological processes, as well as in the metabolism and homeostasis (13). The -SH groups represent the first line of non-enzymatic cellular protection against oxidative stress in cells (14). In addition, two important enzymes are essential for cellular defense against oxidative insults, superoxide dismutase (SOD) and catalase (CAT). SOD is an enzyme that converts the superoxide radical into hydrogen peroxide (H₂O₂), which is converted to 2H₂O and O₂ by CAT. Together SOD and CAT are part of the major enzymatic system to protect the cells against free radical damage (10).

Investigations about the changes of oxidative parameters induced by CR were reported from many tissues, particularly liver and brain (e.g. 6, 15). Our group has focused on brain effects (16, 17). However, the effects of CR in oxidative alterations in proteins and enzymatic activities in different tissues are still controversial.

The aim of the current study was to evaluate the effect of seventeen weeks of CR on carbonyl levels, -SH content and on...
SOD and CAT activities in heart and kidney of Wistar rats, organs that are centrally involved in the regulation of circulatory and ionic homeostasis.

RESULTS

Our results demonstrating weight gain showed that, at the end of experiment, the CR rats (368 ± 14 g) had an approximately 28% reduction in body weight gain, when compared to AD rats (450 ± 17 g, P = 0.001), as illustrated in Fig. 1. In addition, as shown in Table 1, no significant differences were observed between AD and CR rats in biochemical serum parameters, such as glucose, total protein, albumin, urea, creatinin, HDL cholesterol and triacylglycerol. However, total creatinin showed a significant 20% reduction in CR rats, when compared to AD rats.

We observed no significant change in the total content of carbonyl groups in the kidney after CR (AD = 0.027 ± 0.002; CR = 0.032 ± 0.002 nmol carbonyl/mg protein, P = 0.118), as shown in Fig. 2A. Interestingly, our results for the heart tissue (Fig. 2A) indicate that chronic CR induces a significant increase in carbonyl group content (0.009 ± 0.0005 nmol carbonyl/mg protein) when compared to carbonyl group content for the AD diet (0.006 ± 0.0007 nmol carbonyl/mg protein, P = 0.001). No difference was found in the total -SH content in the kidney tissue (AD = 3.06 ± 0.1; CR = 3.28 ± 0.1 μmol SH/mg protein, P = 0.111; Fig. 2B) and no difference was observed in the heart tissue (AD = 2.7 ± 0.1; CR = 2.7 ± 0.1 μmol SH/mg protein, P = 0.755; Fig. 2B).

As shown in Fig. 3A, no changes were observed in the SOD activity in the kidney tissue (AD = 6.5 ± 0.5; CR = 5.9 ± 0.5 U SOD/mg protein, P = 0.43), as well as in the heart tissue (AD = 4.4 ± 0.4; CR = 5.5 ± 0.6 U SOD/mg protein, P = 0.158). On the other hand, our results showed that CR increased CAT activity in the kidney (34 ± 2, U CAT/mg protein), as compared to the AD diet (23 ± 4 U CAT/mg protein, P = 0.021; Fig. 3B). Furthermore, in the heart (Fig. 3B), CAT activity was not altered by CR (6 ± 0.4 U SOD/mg protein), as compared to the AD diet (6 ± 0.4 U SOD/mg protein, P = 0.331).

DISCUSSION

For weight gain analyses, we monitored the animals’ weights weekly; after seventeen weeks, the rats submitted to the CR showed a significant 28% reduction in body weight gain, when compared to AD rats. This result is in agreement with recently data published by our research group, in which twelve weeks of CR result in a 27% reduction in body weight gain (16), suggesting a good reproducibility of this model of CR.

We performed biochemical serum analyses to assess the nutritional and the metabolic status of rats under AD or CR diets at the end of the experimental period. No significant differences were observed between AD and CR animals in serum parameters evaluated, indicating a good health state and adequate nutrition of all animals. However, CR caused a significant reduction in total cholesterol and this data is in agreement with previous results (16).

To evaluate the parameter of oxidative damage/modification of proteins, we measured the content of carbonyl groups in the kidney and heart tissues of rats submitted to AD and CR diets. Our results demonstrated that chronic CR did not alter protein carbonylation in the kidney. Furthermore, Sohal and colleagues (1994) compared AL and CR mice at nine, seventeen and twenty-three months of age and found that protein carbonyl content in the kidney and heart increased with age and was significantly reduced by CR in both tissues at each of the three ages (18). In addition, CR was found to reduce the protein carbonylation in the kidney of rats (19).

Interestingly, our results in heart tissue indicated that chronic CR induces a significant increase in carbonyl group content. This data is in agreement with those of a previous study, in which short-term CR (two months) caused a significant in-

### Table 1. Serum biochemistry

| Parameter          | AD    | CR    | P value |
|--------------------|-------|-------|---------|
| Glucose (mg/dl)    | 101 ± 6 | 109 ± 5 | 0.286   |
| Total protein (g/dl)| 6.2 ± 0.1 | 6.0 ± 0.1 | 0.103   |
| Albumin (g/dl)     | 2.7 ± 0.02 | 2.8 ± 0.03 | 0.106   |
| Urea (mg/dl)       | 61 ± 5   | 65 ± 3  | 0.503   |
| Creatinin (mg/dl)  | 0.57 ± 0.04 | 0.60 ± 0.07 | 0.237   |
| Cholesterol (mg/dl)| 80 ± 4   | 64 ± 3  | 0.009   |
| HDL (mg/dl)        | 32 ± 3   | 30 ± 2  | 0.633   |
| Triacylglycerol (mg/dl)| 50 ± 6 | 61 ± 7  | 0.270   |

Data are expressed as mean ± standard error of mean (n = 8-10 for each group). *Significantly different from AD by Student’s t-test (P = 0.01).
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Fig. 2. Effects of seventeen weeks of ad libitum (AD) or caloric restriction (CR) diets on protein carbonylation and sulfhydryl (-SH) content. (A) Carbonyl protein levels in the kidney (AD n = 9; CR n = 9) and in the heart (AD n = 7; CR n = 7). Results indicate that CR can induce oxidative modification to proteins in the heart tissue. *Significantly different from AD (P = 0.001, Student’s t-test). (B) Total -SH content in the kidney (AD n = 10; CR n = 8) and in the heart (AD n = 8; CR n = 9). The data indicate that neither the AD diet nor the chronic CR diet alter the total -SH content in the kidney or the heart. Data are mean ± S.E.M.

Fig. 3. Effects of seventeen weeks of ad libitum (AD) or caloric restriction (CR) diets on superoxide dismutase (SOD) and catalase (CAT) activities. (A) SOD activity in the kidney (AD n = 10; CR n = 10) and in the heart (AD n = 9; CR n = 9). The data indicate that neither the AD nor chronic CR diets alter the SOD activity in the kidney or the heart. *Significantly different from AD (P = 0.021, Student’s t-test). Data are mean ± S.E.M.

Protein carbonylation has been associated with oxidative damage to proteins, i.e., this parameter may reflect the proportion of proteins that have been damaged by oxidation, leading to defective function of proteins with a central role in the basic mechanisms of disease and aging (12, 22). However, protein carbonylation may also have a potential role in protein quality control and cellular deterioration (23) and also occurs in young animals and normal tissues, as well as, in pathological conditions and older animals, indicating that carbonylation may have a physiological role (23, 24).

We also measured the total -SH content in rats on AD or CR diets. No differences were found between the AD or CR diets in either the kidney or heart tissues; to our knowledge, this is the first study to measure the -SH content in the kidney of rats submitted to chronic CR. However, others results indicate that chronic CR in aged mice increases heart -SH content, when compared to aged AL mice (25).

We evaluated the activities of two key antioxidant enzymes, SOD and CAT. No changes were observed between AD and CR diets for the SOD activity in the kidney and heart tissues, suggesting that chronic CR does not alter the activity of this enzyme in either organ. However, a previous study indicated that CR reduced SOD activity at seventeen months of age in the heart and at twenty-three months in the kidney (18).

However, our results showed that CR increases the CAT activity in the kidney, indicating that a chronic reduction in caloric intake can alter the activity of this antioxidant enzyme in the kidney. This result is in accordance with those of a previous study, in which the CR diet increased the CAT in this organ at nine, seventeen and twenty-three months of age in mice (18). Furthermore, the same study indicated that CR reduced the CAT activity in the heart at nine months, but increased this activity at seventeen and twenty-three months of age. In this study, we did not find any difference between the AD and CR diets for CAT activity in the heart.

We speculate that a chronic reduction in the ingestion of calories could lead to a compensatory increase in endogenous antioxidant CAT activity in the kidney. However, it has been proposed that a chronic reduction in the ingestion of antioxidant vitamins, such as vitamin E, could lead to compensatory mechanisms in the endogenous antioxidant systems (26). We discard this possibility, taking into account that the majority of studies with 40% reduction in calorie intake provide vitamin and mineral supplementation to ensure an equivalent nutrient intake for animals on CR and AD diets (18, 25, 27). However, it has been shown a decrease in the levels of vitamin E in kidney and heart of rats submitted to 40% of chronic CR (28).

We adopted a 30% reduction in the calorie intake and serum biochemistry analyses indicated no differences in the nutritional state of the CR animals, except the reduction in total cholesterol levels in CR rats. Therefore, the increase in CAT activity observed in the kidney tissue is probably a compensatory response for the maintenance of appropriate kidney homeostasis, which can increase the $\text{H}_2\text{O}_2$ decomposition capacity in rats under CR. Meantime, it has been reported that there are no effects of CR in CAT activity in the kidney of mice.
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(26). CAT activity was also not altered by CR in the rat heart tissue in this work, in agreement with a previous study of rats under intermittent feeding at twelve and eighteen months of age, which had no significant effects on the levels and distribution of antioxidant enzymes, such as SOD and CAT, in the left ventricular wall (29).

Protein carbonylation may also occur as the consequence of other mechanisms in addition to oxidative damage (23, 24). We can speculate that this increase in carbonylation, under our experimental conditions, may have different effects to those caused by oxidative damage, since our results showed that CR caused an increase in protein carbonylation in the heart tissue without changing the content of -SH groups or SOD and CAT activities (key enzymatic defenses against oxygen toxicity).

Meantime, we may speculate that the increase in carbonyl group content in heart proteins may be a consequence of the lack of increase in CAT activity or SOD. Additionally, -SH groups are the most important non-enzymatic antioxidant defenses in cells; this content was also not altered in the heart, suggesting that the anti-oxidant protection system did not work in an efficient manner in the CR group hearts. The increase observed in kidney CAT activity probably attenuated redox imbalance and prevented protein carbonyl formation in this organ.

From the comparison of our results with those of previous studies, we speculate that the effects of chronic CR on oxidative parameters and on antioxidant enzyme activities may vary depending on tissue type, duration of CR diets, the strains and the type of damage/defense studied. For example, other factors that can explain the different effects of CR could be the nature and quantity of the specific proteins present in a particular tissue and exposed to carbonylation or loss of -SH groups (25).

In summary, our results showed that CR induces an increase in protein carbonylation in the heart and increases CAT activity in the kidney. No changes were observed in SOD activity and -SH content in either tissue types. These data contribute to the understanding of the effects of CR on the heart and kidney. However, further studies are needed to explain the underlying effects of chronic CR in the different tissues.

**MATERIALS AND METHODS**

**Animals**
Twenty male 120-day-old Wistar rats were obtained from our breeding colony and were maintained under controlled light and environmental conditions (12-h light/12-h dark cycle at a constant temperature of 22 ± 1°C) with free access to water. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications 80-23), revised 1996, and following the regulations of the local animal house authorities and all experiments were approved by the Institutional Animal Care and Use Committee (IACUC).

**Diets**
All groups received regular laboratory chow (Nuvilab-CR1, from Nuvital, Brazil) shortly before dark cycle onset. Animals were weight matched and divided into two groups: ad libitum (AD) and caloric restriction (CR). The AD animals had free access to chow, whereas the CR was progressive, being initiated at 10% restriction during the first week, and changed to 20% during the second week and to 30% in the third week, until the end of treatment (total of seventeen weeks of CR). The food intake was monitored daily and the animals were weighed weekly (17).

**Experimental procedures**
After a total time of seventeen weeks of dietary regimes, all animals were overnight-starved for 8 h and anesthetized with ketamine/xylazine (75 and 10 mg/kg, respectively, i.p) and the whole blood was obtained with an intracardiac puncture using a 0.37-mm diameter needle. The blood samples were incubated at room temperature (25°C) for 10 min and centrifuged at 3,000 rpm for 10 min; the serum was collected and immediately stored at −80°C until the day of biochemical analysis. After killing the animals by decapitation, the kidneys and heart were immediately dissected out in ice and immediately stored at −80°C until the day of analysis.

**Biochemical analyses**
Biochemical serum analyses were carried out with a multi-test analyzer (Mega; Merck, Darmstadt, Germany), using specific kits supplied by Labtest (Brazil): glucose, total protein; albumin; urea; creatinin; total cholesterol, HDL cholesterol and triacylglycerol.

**Protein carbonylation**
The oxidative damage to proteins was measured by the quantification of carbonyl groups, based on a reaction with 2,4-dinitrophenylhydrazine (30). Proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in dinitrophenylhydrazine and the absorbance read in a spectrophotometer at 370 nm. Results are expressed as nmol carbonyl/mg protein.

**Measurement of total -SH content**
An assay that serves to analyze the oxidative status of tissues was used to measure the level of reduced thiol (-SH) content in samples (31). Briefly, a 100-μg sample aliquot was diluted in PBS 10 and 10 mM 5,5-dithionitrosobis 2-nitrobenzoic acid and read in a spectrophotometer at 412 nm after a 60 min incubation at 25°C. All results are expressed as μmol SH/mg protein.

**Antioxidant enzyme activities**
The activities of two important antioxidant enzymes were analyzed; catalase (CAT) and superoxide dismutase (SOD). Catalase (EC 1.11.1.6) activity was assayed by measuring the
rate of decrease in hydrogen peroxide (H₂O₂) absorbance in a spectrophotometer at 240 nm (32). CAT activity was expressed as Units (U) CAT/mg protein.

Superoxide dismutase (E.C.1.15.1.1) activity was assessed by quantifying the inhibition of superoxide-dependent adrenaline auto-oxidation in a spectrophotometer at 480 nm (33). SOD activity was expressed as U SOD/mg protein.

**Protein content**
The total protein content was determined by the modified method of Lowry (34), using BSA as standard.

**Protein content**

**Statistical analysis**
Data are expressed as mean ± standard error mean (S.E.M.) and were considered significant. All analyses were performed using the SPSS program, Version 15.0 (SPSS, Chicago, IL).

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