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·*Original Article*·

Age-related decrease in aromatase and estrogen receptor (ERα and ERβ) expression in rat testes: protective effect of low caloric diets*

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Abstract

Aim: To examine the effects on rat aging of caloric restriction (CR1) and undernutrition (CR2) on the body and on testicular weights, on two enzymatic antioxidants (superoxide dismutase and catalase), on lipid peroxidation and on the expression of testicular aromatase and estrogen receptors (ER). **Methods**: CR was initiated in 1-month-old rats and carried on until the age of 18 months. **Results**: In control and CR2 rats an age-related decrease of the aromatase and of ER (α and β) gene expression was observed; in parallel a diminution of testicular weights, and of the total number and motility of epididymal spermatozo was recorded. In addition, aging in control and CR2 rats was accompanied by a significant decrease in testicular superoxide dismutase, catalase activities, and an increase in lipid peroxidation level (thiobarbituric acid reactive substance), associated with alterations of spermatogenesis. Conversely, caloric restriction-treatment exerted a protective effect and all the parameters were less affected by aging. **Conclusion**: These results indicate that during aging, a low caloric diet (not undernutrition) is beneficial for spermatogenesis and likely improves the protection of the cells via an increase of the cellular antioxidant defense system in which aromatase/ER could play a role. *(Asian J Androl 2008 Mar; 10: 177–187)*

Keywords: aging; low caloric diet; rat testis; aromatase; estrogen receptors; antioxidants

1 Introduction

During aging the diminution of the blood testosterone level parallels a decline of several physiological parameters and among them, those of the reproductive function [1]. Studies using the Brown Norway rat as a model have clearly shown that aging is associated with a functional deficit of the testicular cells [2]. A decrease in the number of germ cells and, consequently, a diminution of the daily sperm production have also been reported [3]. The loss of germ cells might be related to either a decrease in the ability of the Sertoli cells to support the germ cell survival and differentiation, or to the reduced availability of the testosterone produced by Leydig cells [1], or to other unknown factors. Nutrition plays an

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important role during aging and, indeed, caloric restriction delays the appearance of age-associated physiopathological changes [1]. Therefore, caloric restriction induces some benefits on the longevity of rats [4]. As reported, most of the steroidogenic enzyme activities are decreased during aging [1]; nevertheless no information regarding the aromatase status and estrogen's role in aging is available. We have shown that rat testicular cells, including germ cells, are able to synthesize estrogens [5] and are also equipped with estrogen receptors, suggesting a putative role for these female hormones on spermatogenesis [6]. Prokai *et al.* [7] show that estrogens exert various cellular actions, including an antioxidant effect. Indeed, to protect against the adverse effects of reactive oxygen species, mammalian cells are equipped with various enzymatic and non-enzymatic antioxidant scavengers.

Therefore, the aim of the present study is: (i) to evaluate the expression of aromatase and of estrogen receptor alpha and beta (ERα and ERβ) genes in the male rat testis during aging; and (ii) to analyze the impact of low caloric diets and undernutrition on these parameters. To assess the fertility of these treated-animals, the epididymal sperm counts and the sperm motility have been evaluated. Finally, two major enzymatic antioxidants, superoxide dismutase (SOD) and catalase (CAT), as well as the lipid peroxidation (thiobarbituric acid reactive substance (TBAR) are determined to characterize some of the cellular adverse effects targeted by aging.

2 Materials and methods

2.1 Animals and treatments

Male Wistar rats, aged from 2 to 18 months, were either fed *ad libitum* (control) or submitted to diets of caloric restriction (CR1) or undernutrition (CR2), as reported elsewhere [8, 9]. The CR1 and CR2 groups received, respectively, 60% and 40% of the quantity of food given to the control rats (equivalent to 413 kCal/kg body weight/day) and the restriction was initiated in animals aged 4 weeks. All animals (six per group) were obtained through Central Pharmacy, Tunis, Tunisia; they were maintained at 24 ± 3ºC under a 12 h-controlled Light: Dark cycle, with access to water *ad libidum*. At the indicated age, the animals were weighted, killed by decapitation, and the trunk blood was collected. The serum was prepared by centrifugation (1 500 × *g*, 15 min, 4ºC) and the testes were removed, cleaned of fat and weighted; all these samples were stored at –80ºC until used. The caudal region of the epididymides was saved to collect the spermatozoa. The handling of the animals was approved by the local ethical committee for the care and use of laboratory animals.

2.2 Extraction of RNA

Total RNA from testes was extracted using the guanidium thiocyanate-phenol-chlorofom technique [10]. Testes were homogenized in 600 μL of lysis buffer (1 mol/L Tris, 4 mol/L guanidium thiocyanate, 0.5% sarcosyl and 1% β-mercaptoethanol); then 0.1 volume of 2 mol/L sodium acetate, 1 volume of phenol and 0.2 volume of isoamylic chloroform-alcohol (v/v: 49/1) were added to the preparation. After 15 min of incubation in a cold bath, the samples were centrifuged at 10 000 × *g* (4ºC, 15 min). RNA was precipitated at –80ºC by addition of 1 volume of isopropanol. After centrifugation, the pellets were washed with 75% ethanol, dried and dissolved with 50 μL of diethyl pyrocarbonate-treated water. The quality of the RNA samples was evaluated by the determination of the ratio 260 nm:280 nm and their integrity was controlled by electrophoresis on a 1.5% agarose gel. RNA was stored at –80ºC until use.

2.3 Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

Two micrograms of total RNA were reverse transcribed into cDNA in a final volume of 40 μL. RNA was incubated for 1 h at 42ºC with 200 IU Moloney murine leukemia virus reverse transcriptase, 0.5 mmol/L dNTP, 0.2 μg oligo-dT and 20 IU RNasin. Then cDNA coding for aromatase, ERα, ERβ and ribosomal protein L19 were amplified by PCR using specific primers (Table 1). The reactions were performed in a final volume of 50 μL from 4 μL of cDNA for aromatase, L19 and ERα (5 μL for ERβ), with 1.5 IU of *Taq* polymerase, 0.2 mmol/L dNTP, 1.5 mmol/L $MgCl_2$ and 25 pmoles of the forward and reverse primers. Primers were obtained from Invitrogen (Cergy Pontoise, France) and all others products used for RT-PCR were from Promega (Charbonnières, France). To quantify aromatase, ERα and ERβ transcripts, for each gene we determined the optimal number of amplification cycles to be used for the linear increase in cDNA. After an initial step of denaturation at 95ºC for 5 min, a variable number of cycles of amplification were performed: 30 s at 95ºC,

Table 1. Sequences of primers. Size of fragments amplified and number of cycles used for amplification. Genbank accession number are indicated in parenthesis. PCR, polymerase chain reaction; S, sens primer; R, reverse primer.

Gene	Primer	Sequence	Orientation and position	Size of PCR product (bp)	Number of cycle
Aromatase	5'-ARO	GCTTCTCATCGCAGAGTATCCGG	S (1555–1577)	290	33
(M33986)	3'-ARO	CAAGGGTAAATTCATTGGGCTTGG	R (1821–1844)		
ERα	5'-ERα	AATTCTGACAATCGACGCCAG	S (545–565)	345	29
(X61098)	3'-ERα	GTGCTTCAACATTCTCCCTCCTC	R (867–889)		
ERβ	5'-ERβ	GAAGCTGAACCACCCAATGT	S (1100–1120)	211	40
(U57439)	3'-ERβ	CAGTCCCACCATTAGCACCT	R (1291–1310)		
L19	5'-L19	GAAATCGCCAATGCCAACTC	S (119–138)	290	24
(NM_031103)	3'-L19	ACCTTCAGGTACAGGCTGTG	R (389–408)		

30 s at 60ºC, and 45 s at 72ºC (Table 1), followed by a final extension at 72ºC for 7 min. We have chosen L19 transcripts, which did not vary among the samples, to correct the difference in the quantities of total RNA used for reverse transcription [11]. The amplified cDNA fragments were run on a 2% agarose gel stained with ethidium bromide, visualized under ultraviolet transillumination and analyzed with National Institutes of Health (NIH) software (http://rsb.info.nih.gov/nih-image).

2.4 Epididymal sperm count

Spermatozoa were collected from an equal length of the cauda epididymis of each rat by flushing with the same volume (10 mL) of a medium containing 140 mmol/L NaCl, 0.3 mmol/L KCl, 0.8 mmol/L Na2HPO$_4$, 0.2 mmol/L KH_2PO_4 and 1.5 mmol/L D-glucose (pH 7.3). The collected samples were centrifuged at 100 × *g* for 2 min, and the pellets were re-suspended in 10 mL of fresh medium. An aliquot (100 μL) was mixed with an equal volume of 1% Trypan blue, then the number of spermatozoa and the motility were determined [12].

2.5 Steroid determinations and measurements of antioxidant enzymatic activities

After homogenisation of testes in a phosphate buffer (1 g/2 mL), steroids were extracted by diethylether according to our reported method. The estradiol level was then measured by RIA using highly specific antibodies from P.A.R.I.S (Compiègne, France). The intra-assay and inter-assay coefficients of variation were 8% and 5% for estradiol. The lipid peroxidation was determined in the homogenates from control and treated rat testes by quantification of the TBAR using the method applied by Buege and Aust [13]. The superoxide dismutase activity was assayed using the spectrophotometric method of Marklund and Marklund [14]. The activity of catalase was measured using Aebi's method [15]. The protein level was determined using the method applied by Lowry *et al*. [16].

For histological studies, pieces of testes were fixed in a Bouin's solution for 24 h, then embedded in paraffin. Sections of 5 μm thickness were stained with hematoxylin-eosin and examined under a digital camera Olympus microscope (Olympus CX41; Olympus Industrial America Inc., Orangeburg, NY, USA).

2.6 Statistical analysis

Data are presented as mean ± SEM. The determinations were performed using six animals per group and the differences were examined using one-way analysis of variance (ANOVA) followed by the Scheffe test. Significance was accepted at $P < 0.05$ (StatView, SAS Institute, Cary, NC, USA).

3 Results

3.1 Body and testicular weights

For the control rats, an increase in body weight was observed between 2 and 15 months of age, which remained stable later. In the CR1 group body weight increased but more slowly, and from 4 months of age it was significantly lower than that of the control rats. For CR2 rats, we noticed a slight increase between 2 and 12 months, then no variation was registered (Figure 1A). Compared to the controls, the body weight of CR2 rats was statistically lower whatever the age and the decrease was approximately 50% from 15 months of age. When compared to CR1 rats, the body weight of CR2 animals

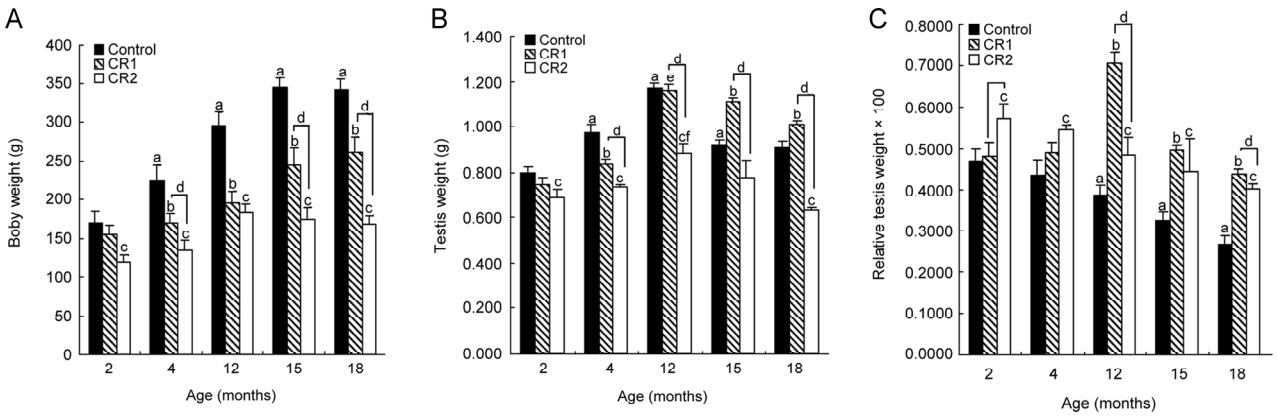

Figure 1. Body (A), testicular weights (B) and ratio testis/body weight (C) of two 18-month-old rats either fed *ad libidum* (control), or submitted to caloric restriction (CR1) or undernutrition (CR2) diet. The data are expressed as mean ± SEM (n = 6). a: control rats significantly different from 2-month-old rats; b: CR1 rats significantly different from the same age (control) rats; c: CR2 rats significantly different from the same age (control) rats; d: CR1 rats significantly different from the same age (CR2) rats; e: CR1 rats significantly different from 2-month-old CR1 rats; (f): CR2 rats significantly different from 2-month-old CR2 rats.

was also significantly lower at all ages, except at 2 and 12 months and again, the difference was greater in rats aged 15 months onwards.

In control rats, an increase in testicular weight was noticed until the age of 12 months, but in 18-month-old rats the testicular weight was identical to that of 2-month-old animals (Figure 1B). For the CR1 group, a similar pattern was observed until the age of 15 months, then the testicular weight, before diminishing slightly. At 15 and 18 months, the weight of testes in CR1 rats was significantly higher compared to that in the controls. For CR2 rats, the testicular weights at all ages studied were significantly lower than those of their age-matched controls, except at 2 months of age. Moreover, it was significantly lower compared to the CR1 animals whatever the age (except at 2 months), especially from the age of 15 months (Figure 1B).

Testicular weight in relation to body weight decreased with age in control rats, whereas in the CR1 group a significant augmentation was observed at 12 months (Figure 1C). It was also clear that relative testicular weight was significantly lower in 1-year-old CR2 rats than in CR1 animals.

3.2 Number and motility of epididymal spermatozoa

In the control CR1 and CR2 rats, an increase in the number of spermatozoa was recorded from ages 2 to 12 months; thereafter, a diminution was observed (39% at 18 months compared to 1-year-old rats). In contrast, in CR2 rats no variations were noticed between 4 and 18 months (in the older CR2 rats the amount of spermatozoa was identical to that of the controls). In 18-month-old CR1 rats, the number of spermatozoa was diminished by 24% compared to 12-month-old animals. In CR1 rats aged of 15 and 18 months, the total number of spermatozoa was significantly higher than that in the control rats (Figure 2A).

Concerning the motility of spermatozoa, between 2 and 4 months of age a sharp increase was observed in control CR1 and CR2 rats, although in 2-month-old CR2 animals the number of motile spermatozoa was very low. In 1-year-old control rats and onwards the number of motile spermatozoa was lower (36%–47%) than in 4-month-old animals, whereas in CR1 rats no changes were observed with aging. In 18-month-old CR1 rats, the number of motile spermatozoa was significantly higher (35%) than in either controls or CR2 animals. In that later group, the motility was of the same magnitude as in the controls starting from the age of 1 year (Figure 2B).

3.3 Histological changes in testes of control and treated rats

The effects of aging, caloric restriction and undernutrition were further analyzed through histological examination of spermatogenesis (Figure 3). In 18-month-old controls and CR2 rats (Figure 3B and 3D), a deple-

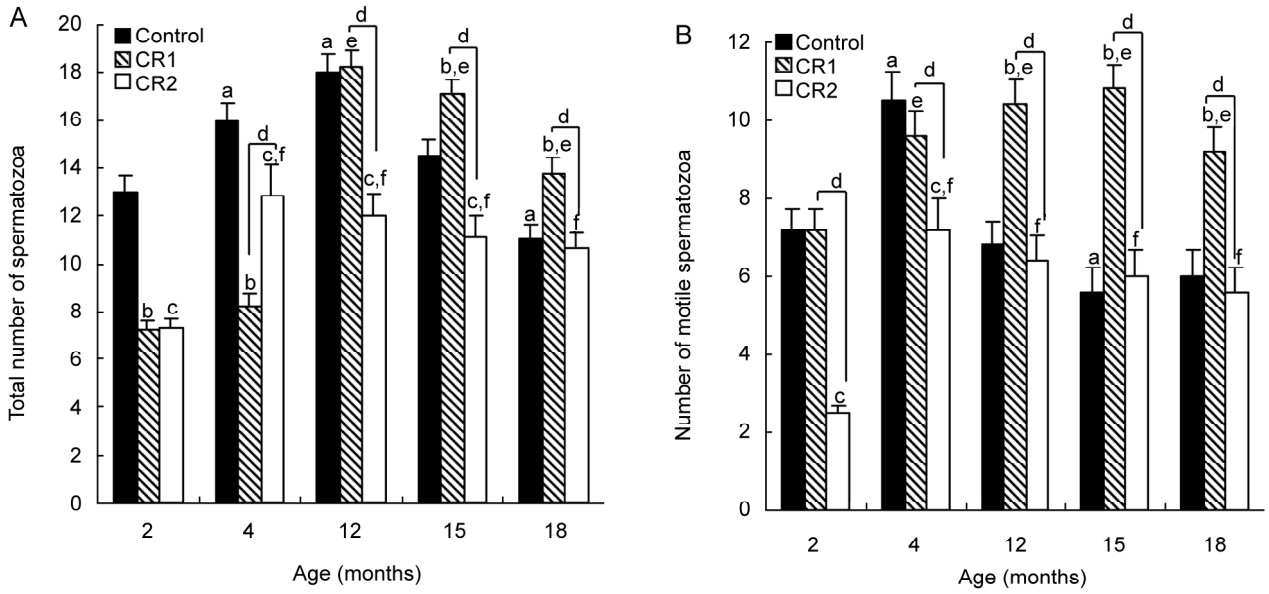

Figure 2. Total number ($\times 10^5$) of spermatozoa in cauda epididymis (A) and measurement of their motility (B) in control, caloric restriction (CR1) and undernutrition (CR2) rats aged 2–18 months. Data are expressed as mean ± SEM (n = 8). a: control rats significantly different from 2-month-old rats; b: CR1 rats significantly different from the same age (control) rats; c: CR2 rats significantly different from the same age (control) rats; d: CR1 rats significantly different from the same age (CR2) rats; e: CR1 rats significantly different from 2-month-old CR1 rats; f: CR2 rats significantly different from 2-month-old CR2 rats.

tion of germ cells was observed in comparison to 2-month- old rats (Figure 3A). In CR1 treated rats, spermatogenesis proceeded normally and was similar to that of 2-month-old rats (Figure 3C).

3.4 Aromatase expression

We performed a semi-quantitative RT-PCR to determine whether the amount of aromatase transcripts in testis was affected by aging and the caloric diets. As shown in Figure 4, an increase in the amount of aromatase transcripts was observed between 2 and 4 months in the control and CR2 groups of animals (not for CR1), followed by a sharp and significant decrease from the age of 12 months in control and CR2 animals (more than 78% and 38%, respectively, compared to 4-month-old rats). In contrast, in CR1 rats the diminutions were much lower compared with the control group (Figure 4A and 4B). From the age of 12 months onwards, the levels of aromatase transcripts were approximately twice as high in both CR1 and CR2 rats compared to the control rats. Moreover, in 12- and 18-month-old CR1 rats, the amount of aromatase transcripts was greater and significantly higher compared to CR2 rats (Figure 4B), and a significant decrease was observed only in 18-month-old rats compared to young CR1 animals.

3.5 Age-related and caloric restriction-related changes of ERα and ERβ gene expression

The values of ERα/L19 and ERβ/19 ratio are reported in Figure 5. Between 2 and 4 months of age an increase of the levels of ERα mRNAs was observed in control, CR1 and CR2 rats followed by a diminution of the amount of transcripts in older animals.

In the control group the decrease of the amount of ERα mRNA was significant at the age of one year (25%) and remained unchanged in the older rats. For CR1 animals, the decrease of ERα mRNA was significant only in 18-month-old CR1 rats (Figure 5A). Moreover in CR1 rats, the levels of ERα transcripts were higher compared to control and CR2 rats starting from the age of 12 months. In CR2 rats aged of 18 months the amount of ERα was similar to that of controls. Additionaly, in CR2 rats, ERα mRNA decreased significantly from 12-month-old rats compared to 2-month-old CR2 rats.

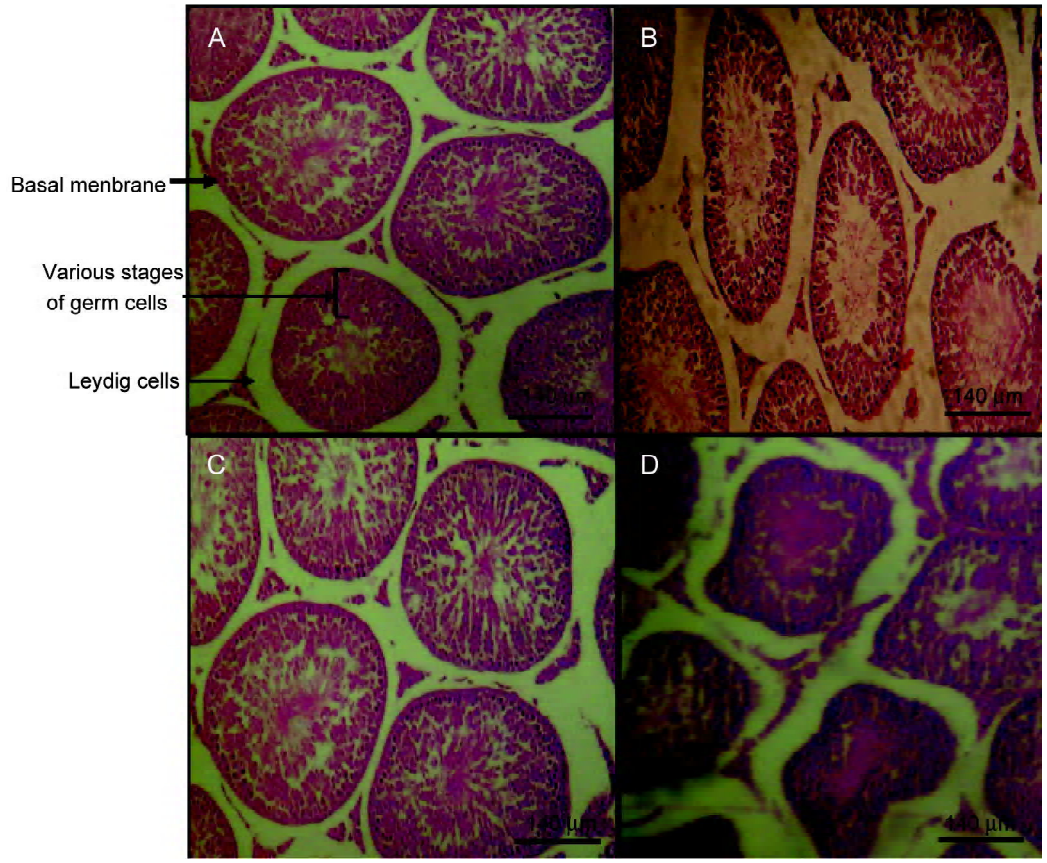

Figure 3. Effect of aging (B), caloric restriction (CR1) (C) and undernutrition (CR2) (D) on the histological morphology of rat testes (magnification × 100). Control rat aged of 12 months (A) showing a regular course of spermatogenesis. Control rat aged 18 months (B) with alterations of spermatogenesis. In CR1 testes of 18-month-old rats a positive effect was observed with well-developed germ cells (3C). In CR2-testes of 18-month-old rats damages were observed in the seminiferous tubes (D). Bars = 140 μm.

Concerning the expression of ERβ (Figure 5B) in 1-year-old CR1 rats the amount of transcripts was significantly higher compared to that in control and CR2 animals, and it remained higher than in the two other groups at 18 months of age. Whatever the age in CR1 animals there was no significant difference in the amount of ERβ transcripts.

3.6 Estradiol levels in blood and testes

Until 15 months of age, in control rats the serum estradiol level was slightly but significantly lower than in 2-month-old animals (Figure 6A). In CR1 rats, the estradiol concentrations were significantly lower compared to that of the matched controls at 2 and 4 months of age. In CR2 rats an age-related decrease of serum estradiol level was observed until 12 months of age, thereafter in older rats the levels varied slightly. From the age of 4 months onwards the blood concentrations of estradiol were significantly higher in CR1 compared to CR2 rats.

Age-related changes in testicular estradiol concentration was recorded (Figure 6B). In the three groups of rats the estradiol levels were decreased by 70% at 12 and 18 months of age compared to 4 month old animals. In 4-month-old CR1 rats the estradiol levels were not determined in that study; however in an other experimental group of rats breeded in the same conditions and used for an other protocol the testicular estradiol concentrations were 20% lower than in control rats. In CR2 rats the endogenous levels of estradiol were significantly lower from that of controls in 2 months, and from 12 months they were even higher at the age of 15 and 18 months.

3.7 SOD and CAT activities in testes of control and

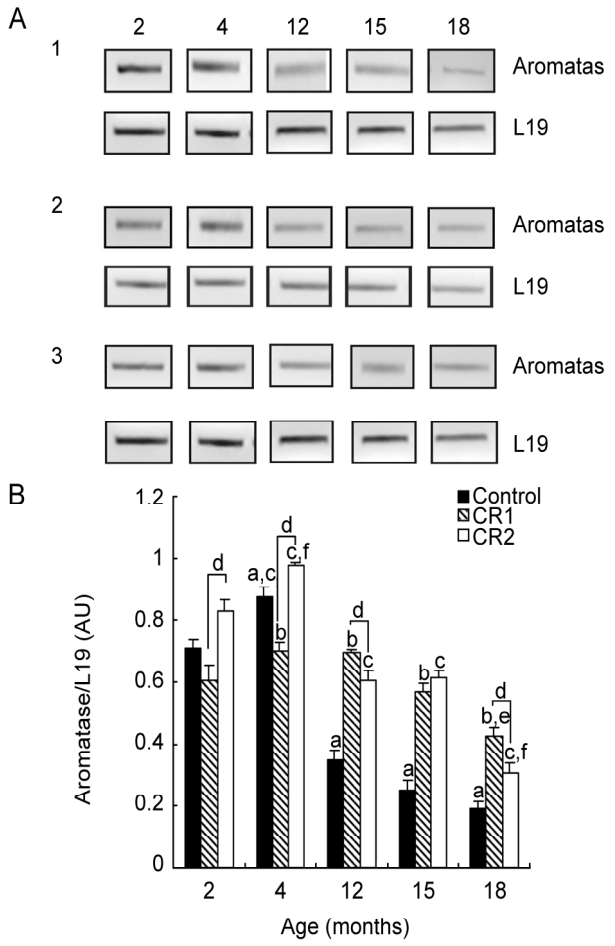

Figure 4. Effect of age on the expression of aromatase gene in testes of control, caloric restriction (CR1) and undernutrition (CR2) rats. (A): A representative signal of the amplification of aromatase or L19 gene obtained by reverse transcription polymerase chain reaction from total testis RNA of control (1), CR1 (2) and CR2 (3) rat at all ages studied (from 2 to 18 months). (B): The data are expressed as mean ± SEM (n = 6). a: control rats significantly different from 2-month-old rats; b: CR1 rats significantly different from the same age (control) rats; c: CR2 rats significantly different from the same age (control) rats; d: CR1 rats significantly different from the same age (CR2) rats; e: CR1 rats significantly different from 2-month-old CR1 rats; f: CR2 rats significantly different from 2-month-old CR2 rats. AU, arbitrary unit-statistical analyses.

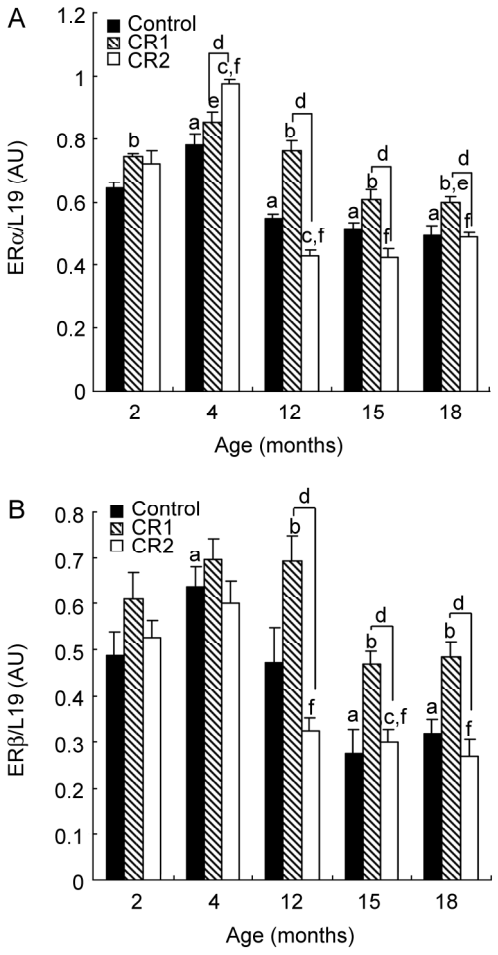

Figure 5. Expression of estrogen receptors alpha (ERα) (A) and ERβ (B) genes in testes of rats aged 2–18 months either fed *ad libitum* (control), or under low caloric diets (caloric restriction [CR1] and undernutrition [CR2]). The data are expressed as mean ± SEM (n = 6). AU, arbitrary unit-statistical analyses; a: control rats significantly different from 2-month-old rats; b: CR1 rats significantly different from the same age (C) rats; c: CR2 rats significantly different from the same age (C) rats; d: CR1 rats significantly different from the same age (CR2) rats; e: CR1 rats significantly different from 2-month-old CR1 rats; f: CR2 rats significantly different from 2-month-old CR2 rats.

treated rats (Figure 7)

In control and CR2 rats aged 15 and 18-months a sharp and significant decrease of the activity of testicular SOD and CAT was observed when compared to either 2 or 4 month old animals. In the 15-months-old CR1 rats, no such decrease of the SOD or CAT activities was observed; in oldest rats these enzyme acitivities were diminished compared to the young control animals but still remained higher than in either control or CR2 treated rats.

3.8 Thiobarbituric level in testes of control and treated rats

The testicular TBAR were significantly increased in

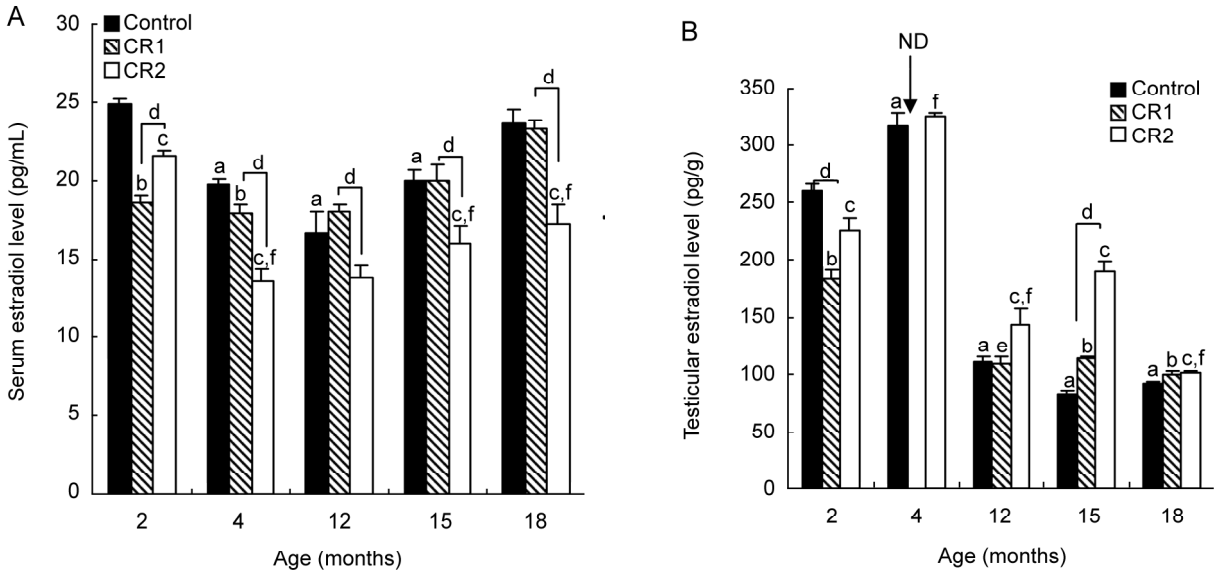

Figure 6. Serum (A) and testicular (B) estradiol concentrations in the three groups of rats aged 2–18 months. The data are expressed as mean ± SEM (n = 6). Statistical analyses as in legend of Figure 1. ND, not determined. a: control rats significantly different from 2-month-old rats; b: CR1 rats significantly different from the same age (C) rats; c: CR2 rats significantly different from the same age (C) rats; d: CR1 rats significantly different from the same age (CR2) rats; e: CR1 rats significantly different from 2-month-old CR1 rats; f: CR2 rats significantly different from 2-month-old CR2 rats.

the control and CR2 rats aged 18 months compared to the 2-month-old animals (66% and 55%, respectively). When the animals were submitted to CR1 treatment, a significant decrease in the TBAR levels by 16% and 27% was observed (Figure 8).

4 Discussion

Our results provide further evidence that aging in control and undernourished rats is accompanied by alterations of some parameters concerned with male reproductive function together with a significant decrease (of 40%) in the relative testicular weight in the oldest control rats. A loss of germ cells and, consequently, a decrease in daily sperm production was recorded during aging in control and in undernourished rats, which likely accounts for the decrease in testicular weight, as reported by Henkel *et al.* [3]. In parallel, a sharp decrease in the aromatase and estrogen receptor gene expression was observed during aging in the control rats. In addition, an age-related decline in testicular estrogen and testosterone levels [1] has also been registered. In 18-month-old controls and CR2 rats we recorded a significant diminution of the SOD and catalase activities, together with an increase in lipid peroxidation, associated with histological changes in the seminiferous tubules, and in agreement with a previous report [17]. In contrast, in CR1 animals the aging effect on aromatase and ER gene expression was much smaller than in control rats and, indeed, the CAT and SOD activities were higher. Therefore, caloric restriction without undernutrition (CR2) might exert a protective effect on the expression of both aromatase and estrogen receptor genes in rat testis, as reported by Chen *et al.* [1] for other enzymes involved in the rat Leydig cell steroidogenic pathway. Using the Brown Norway rat to study the aging effects, Chen *et al.* [1] observed a diminution in the Leydig cell capacity to synthesize testosterone and, because the aromatase expression is also diminished, a decrease in estradiol output is obvious. Wang and Stocco [18] reported that an increase in cyclooxygenase 2 is observed during aging and that the expression of StAR is dimished, which suggests that the testicular senescence affects various steps of the steroid synthesis in the Leydig cells.

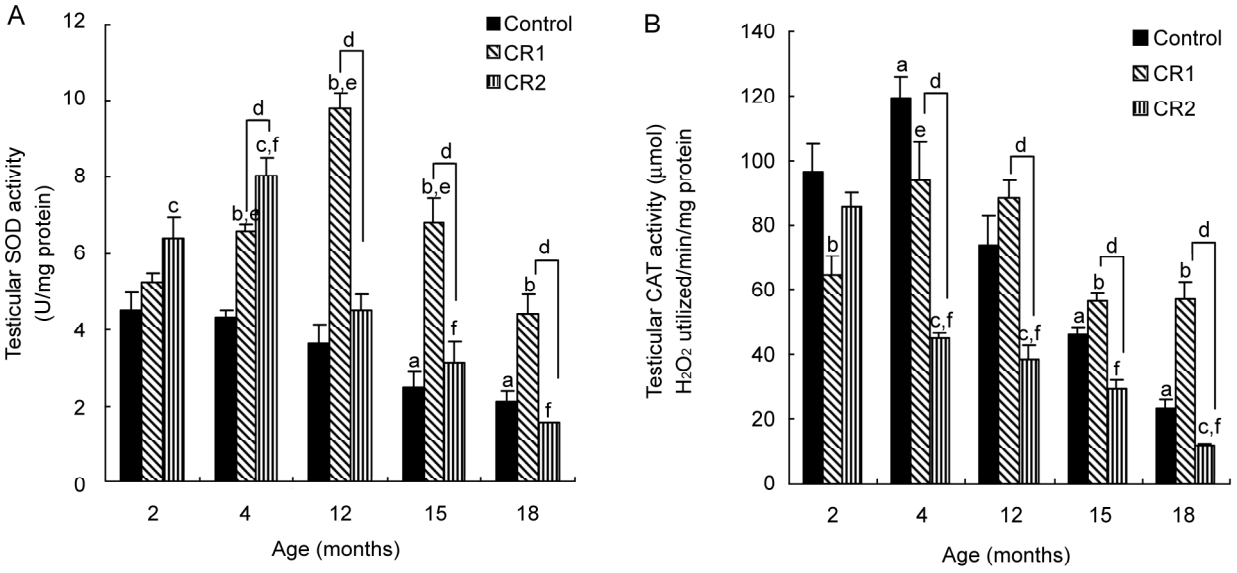

Figure 7. Testicular antioxidant enzyme activity levels superoxide dismutase (SOD) (A) and catalase (CAT) (B) in control, caloric restriction (CR1) and undernutrition (CR2) rats aged 2–18 months. Data are expressed as mean ± SEM (n = 8). a: control rats significantly different from 2-month-old rats; b: CR1 rats significantly different from the same age (control) rats; c: CR2 rats significantly different from the same age (C) rats; d: CR1 rats significantly different from the same age (CR2) rats; e: CR1 rats significantly different from 2-month-old CR1 rats; f: CR2 rats significantly different from 2-month-old CR2 rats.

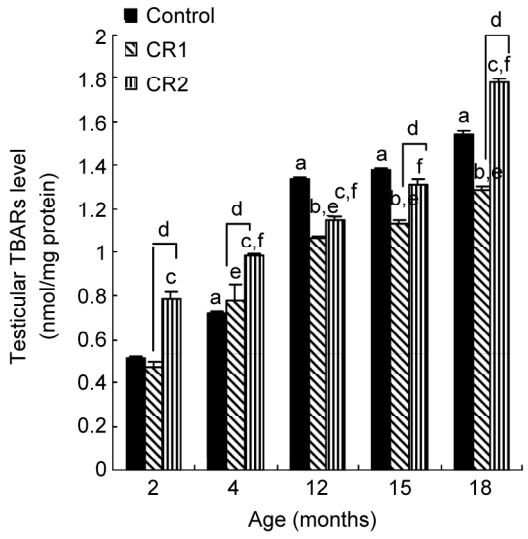

Figure 8. Testicular thiobarbituric acid reactive substance (TBAR) level in control, caloric restriction (CR1) and undernutrition (CR2) rats aged 2–18 months. Data are expressed as mean ± SEM (n = 8). a: control rats significantly different from 2-month-old rats; b: CR1 rats significantly different from the same age (C) rats; c: CR2 rats significantly different from the same age (control) rats; d: CR1 rats significantly different from the same age (CR2) rats; e: CR1 rats significantly different from 2-month-old CR1 rats; f: CR2 rats significantly different from 2-month-old CR2 rats.

In mammals, the ability of the testis to convert androgens into estrogens is well known [19]. It has been recently reported that estrogens can exert an antioxidant role by scavenging free radicals and, therefore, they might prevent any damage induced by these free radicals on cell protein and DNA contents [7]. Aromatase is present in most of the cells of rodents [19], and taking into account the widespread distribution of ER in these cells [6], the antioxidant effect of estrogens could be evoked. We have observed that the expression of aromatase and ER (mainly ERβ) in the testicular tissue of the oldest CR1 rats is higher than in control animals, especially starting from the age of 12 months, which might well suggest a protecting effect of estrogens during aging. Vina *et al.* [20] also demonstrate that estradiol as well as phytoestrogens are chemical antioxidants *in vivo* and are able to protect against aging by upregulating the expression of antioxidants and longevity-related genes, such as glutathione peroxidase (GPx) and Mn-SOD, by the mediation of ER. Indeed, Luo *et al.* [21] confirm the age-related decrease of rat Leydig cell antioxidant enzymes in terms of activities, protein levels and gene expressions.

The mechanisms concerned with the protective effect of low diet are not clearly understood, but we might

also evoke a decrease in lipid peroxidation (one of the mechanisms by which oxygen free radicals can provoke cell damage) by estrogens, which might be of benefit by delaying the apparition of cell alterations caused by the aging process. The positive effect of estrogens on sperm maturation has been clearly demonstrated in the efferent ducts and the proximal part of the rat epididymis [22]. We showed that the number and the motility of spermatozoa in aged rats under CR1 is higher than in controls, which might suggest that the beneficial effect of a low caloric diet could be in part mediated by estrogens. Indeed, a positive role of estrogenic compounds on mouse and human sperm (i.e. capacitation and loss of acrosome) has been clearly demonstrated [23]. We have also reported a positive correlation between aromatase gene expression and the motility of spermatozoa in humans [24].

Hence, a low caloric diet will help during aging to improve protection of the cells against reactive oxygen species (ROS) via an increase of the cellular antioxidant defense system in which estrogens are probably concerned, as shown by Urata *et al.* [25]. Our preliminary data (Hamden *et al.*, unpublished) supports the above hypotheses because in rats submitted to CR1 diet or treated with either estradiol or phytoestrogen, the GPx, SOD and catalase activities were similarly enhanced at the age of 18 months. Therefore, caloric restriction protects the male gonad against the adverse effects of ROS by increasing the activity of some antioxidant enzymes. Moreover, these positive effects are further supported by a low level of lipid peroxidation and estrogens might be one of the key hormones concerned in that process.

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