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Original Contribution

INFLUENCE OF AGING AND LONG-TERM CALORIC RESTRICTION ON OXYGEN RADICAL GENERATION AND OXIDATIVE DNA DAMAGE IN RAT LIVER MITOCHONDRIA

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Abstract—The effect of long-term caloric restriction and aging on the rates of mitochondrial H_2O_2 production and oxygen consumption as well as on oxidative damage to nuclear (nDNA) and mitochondrial DNA (mtDNA) was studied in rat liver tissue. Long-term caloric restriction significantly decreased H_2O_2 production of rat liver mitochondria (47% reduction) and significantly reduced oxidative damage to mtDNA (46% reduction) with no changes in nDNA. The decrease in ROS production was located at complex I because it only took place with complex I-linked substrates (pyruvate/malate) but not with complex II-linked substrates (succinate). The mechanism responsible for that decrease in ROS production was not a decrease in mitochondrial oxygen consumption because it did not change after long-term restriction. Instead, the caloric restricted mitochondria released less ROS per unit electron flow, due to a decrease in the reduction degree of the complex I generator. On the other hand, increased ROS production with aging in state 3 was observed in succinate-supplemented mitochondria because old control animals were unable to suppress H_2O_2 production during the energy transition from state 4 to state 3. The levels of 8-oxodG in mtDNA increased with age in old animals and this increase was abolished by caloric restriction. These results support the idea that caloric restriction reduces the aging rate at least in part by decreasing the rate of mitochondrial ROS production and so, the rate of oxidative attack to biological macromolecules like mtDNA. © 2002 Elsevier Science Inc.

Keywords—ROS production, Oxidative stress, 8-OxodG, Free radicals

INTRODUCTION

Several findings suggest that reactive oxygen species (ROS) of mitochondrial origin are involved in the aging process [1,2]. The negative correlation between mitochondrial ROS generation rate and maximum life span (MLSP) [3] and more recently, the observation that the steady-state level of oxidative damage to mitochondrial DNA also negatively correlates with MLSP [4], support the mitochondrial free radical theory of aging [5]. Comparative studies show that long-lived species have low rates of mitochondrial ROS production that could explain their low levels of mitochondrial DNA oxidative damage and so, their slow aging rates [3]. On the other hand, caloric restriction continues to be the only experimental procedure capable of decreasing the aging rate [6]. It is of main interest to study if the caloric restriction life-extension mechanism works through a decreased oxidative stress. Only one previous investigation has studied the effect of caloric restriction on the rate of H_2O_2 production [7], and it did not include the liver tissue; very few authors have studied the effect of caloric restriction on DNA oxidative damage [8–11]. Most importantly, the effect of caloric restriction on mitochondrial H_2O_2 production and DNA levels of oxidative damage has never been studied simultaneously except in two previous reports from our laboratory [12,13].

H_2O_2 production studies have been mainly performed in heart tissue but the liver is interesting, as it plays a central role in mammalian metabolism and is very susceptible to diet variations. The effect of aging on mitochondrial H_2O_2 production has also been mainly studied in the heart tissue and the available data do not agree on whether mitochondrial ROS generation increases or not with aging [14–18].

The present work was designed to find out if long-

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term caloric restriction decreases the rate of ROS production of liver mitochondria as well as to localize the ROS generator site in the respiratory chain and the mechanism responsible for such a decrease. There is only one previous localization report from our laboratory, but using immature rats [12], and the mechanism responsible for the caloric restriction effect has only been described in the heart tissue [13] or after short-term caloric restriction in the liver [12]. The marker of oxidative DNA damage 8-oxo-7,8-dihydro-2′deoxyguanosine (8-oxodG) was also measured in order to ascertain whether putative decreases in ROS production are associated with decreases in oxidative damage to mtDNA and nDNA. With the present design we have also analyzed the controversial effect of aging on mitochondrial ROS production.

MATERIALS AND METHODS

Animals and treatments

Male Wistar rats were caged individually and maintained at 22 ± 2°C, 12:12 (light:dark) cycle and 50 ± 10% relative humidity. Control animals were fed ad libitum and restricted animals daily received 60% of control animals food intake (40% energy restriction). Dietary restriction started at 12 months of age and continued for 12 more months until sacrifice; at that time these animals were 24 months old, old restricted animals (OR). Old control animals (OC) were also 24 months by the time of sacrifice. An additional group of ad libitum feed animals was maintained in parallel under the same conditions as OC animals during the last months of the dietary restriction experiment. These animals had 11 months of age at the time of sacrifice, were classified as AC (adult controls), and were used for aging-related comparisons between AC and OC animals. Animals were sacrificed by decapitation and liver samples were stored at −80°C for later 8-oxodG analyses. For liver mitochondrial H_2O_2 production determinations, fresh tissue samples were processed directly.

Mitochondria isolation

After decapitation, liver samples were immediately processed to obtain functional mitochondria. Livers were rinsed and fat was removed before homogenization in 60 ml of isolation buffer (210 mM mannitol, 70 mM sucrose, 5 mM hepes, 1 mM EDTA, pH 7.35). The nuclei and cell debris were removed by centrifugation at 1000 × *g* for 10 min. Supernatants were then centrifuged at 10,000 × *g* for 10 min. and supernatants and fat were eliminated. Pellets were resuspended in 40 ml isolation buffer without EDTA and centrifuged at 1000 × *g* for 5 min. Liver mitochondria were obtained after centrifugation of supernatants at 10,000 × *g* for 10 min. The mitochondrial pellets were resuspended in 1 ml of isolation buffer without EDTA. All the above procedures were performed at 5°C. Mitochondrial protein was measured by the Biuret method [19]. Final mitochondrial suspensions were maintained over ice and immediately used for oxygen consumption and H_2O_2 production measurements.

Mitochondrial oxygen consumption

Liver mitochondria oxygen consumption was measured at 37°C in a water-thermostatized incubation chamber with a computer-controlled Clark-type electrode (Oxygraph, Hansatech Instruments Ltd., UK) in 0.5 ml of incubation buffer (145 mM KCl, 30 mM Hepes, 5 mM KH_2PO_4, 3 mM $MgCl_2$, 0.1 mM EGTA, 0.1% BSA, pH 7.4) with 2.5 mM pyruvate/2.5 mM malate or 5 mM succinate (plus 2 μM rotenone) as substrates, in the absence (State 4) and in the presence (State 3) of 500 μM ADP. Mitochondrial respiratory control index (state 3/state 4) with succinate as substrate was 3.2 ± 0.1 (AC), 3.2 ± 0.2 (OC) and 3.6 ± 0.2 (OR).

Mitochondrial oxygen free radical production

The rate of mitochondrial H_2O_2 production was measured following the linear increase in fluorescence (excitation at 312 nm, emission at 420 nm) due to oxidation of homovanillic acid by H_2O_2 in the presence of horseradish peroxidase [20,21]. Reaction conditions were 0.25 mg of mitochondrial protein per ml, 6 U/ml of horseradish peroxidase, 0.1 mM homovanillic acid, 50 U/ml of SOD, and 2.5 mM pyruvate/2.5 malate, or 5 mM succinate (+ 2 μM rotenone) as substrates added at the end to the same incubation buffer used for oxygen consumption to start the reaction, at 37°C in a total volume of 1.5 ml. Unless otherwise stated, the assays with succinate as substrate were performed in the presence of rotenone in order to avoid the backwards flow of electrons to complex I. In some experiments rotenone (2 μM), antimycin A (2 μM), and thenoyltrifluoroacetone (TTFA; 11 μM) were additionally included in the reaction mixture to assay maximum rates of complex I, complex II + III, and complex II H_2O_2 generation, respectively. Samples were incubated for 15 min at 37°C in duplicated, the reaction was stopped by transferring the samples to a cold bath and adding 0.1 M glicyne-25 mM EDTA-NaOH, pH 12 [20], and the fluorescence was read in a LS50B Perkin-Elmer fluorometer. Known amounts of H_2O_2 generated in parallel by glucose oxidase with glucose as substrate were used as standards. Since SOD added in excess converts all O_2^- produced (if any) to H_2O_2, under these

conditions the measurements represent the total (O_2^- plus H_2O_2) mitochondrial ROS production.

Mitochondrial free radical leak

Liver mitochondria H_2O_2 production and oxygen consumption were measured in parallel in the same samples under the same experimental conditions. This allowed calculation of the fraction of electrons out of sequence which reduce O_2 to oxygen radicals at the respiratory chain instead of reaching Complex IV to reduce O_2 to water. Since two electrons are needed to reduce one molecule of O_2 to H_2O_2, whereas four electrons are transferred in the reduction of one molecule of O_2 to water, the percentage of free radical leak was calculated as the rate of H_2O_2 production divided by two times the rate of O_2 consumption, the result being multiplied by 100.

Isolation and digestion of mitochondrial and nuclear DNA

Nuclear DNA was isolated, after homogenization, centrifugation at 1000 $\times$ *g* for 10 min, resuspension of nuclear pellets from liver samples and SDS treatment, by chloroform extraction, and ethanol precipitation [22]. Mitochondrial DNA free of nuclear DNA was isolated by the method of Latorre et al. [23], with some modifications [4,24]. Isolated nuclear and mitochondrial DNA were digested to deoxynucleoside level by incubation at 37°C with 5 U of nuclease P1 (in 20 μl of 20 mM sodium acetate, 10 mM $ZnCl_2$, 15% glycerol, pH 4.8) during 30 min and 1 U of alkaline phosphatase (in 20 μl of 1 M Tris-HCl, pH 8.0) for 1 h [22].

8-oxodG and dG assays in nDNA and mtDNA

8-OxodG and deoxyguanosine (dG) concentrations were measured by HPLC with online electrochemical and ultraviolet detection, respectively. For analyses, the nucleoside mixture was injected into a reverse-phase Beckman Ultrasphere ODS column (5 μm, 4.6 mm $\times$ 25 cm), eluted with 2.5% acetonitrile in 50 mM phosphate buffer pH 5. The amount of deoxynucleosides injected in the HPLC was higher than the minimum needed to avoid potential artifacts due to injection of small quantities of deoxynucleosides in the HPLC system [25]. A Waters 510 pump at 1 ml/min was used. 8-OxodG was detected with an ESA Coulochem II electrochemical coulometric detector (ESA, Inc., Bedford, MA, USA) with a 5011 analytical cell run in the oxidative mode (225 mV/20nA), and dG was detected with a Biorad model 1806 UV detector (Bio-Rad, USA) at 254 nm. For quantification, peak areas of dG standards and of three level calibration of pure 8-oxodG standards (Sigma, St. Louis, MO, USA) were analyzed during each HPLC run. Comparison of areas of 8-oxodG standards injected with and without simultaneous injection of dG standards ensured that no oxidation of dG occurred during HPLC analyses

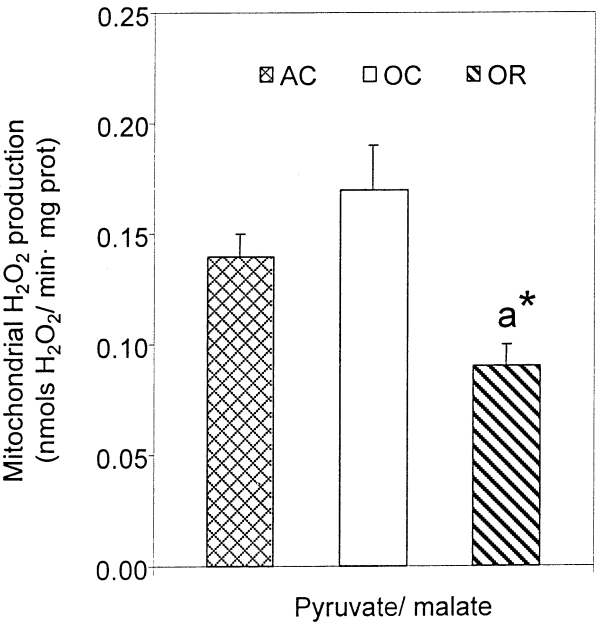

Fig. 1. Effect of aging and long-term caloric restriction on the basal rates of H_2O_2 production of rat liver mitochondria with complex I-linked substrates (pyruvate/malate). AC = adult controls (11 months); OC = old controls (24 months); OR = old restricted (24 months). Data are means ± SEM from 6–7 animals. a = significant difference between OC and OR. *$p < .05$.

Statistical analyses

Data were statistically analyzed by Student's *t*-tests. The .05 level was selected as the point of minimal statistical significance in every comparison.

RESULTS

The rate of H_2O_2 production with complex I-linked substrates (pyruvate/malate) was significantly lower in old restricted animals (47% reduction) than in old control ones (Fig. 1). No significant differences were observed between adult control and old control animals with this substrate (Fig. 1). Mitochondrial H_2O_2 production with pyruvate/malate and ADP (state 3) was undetectable in the three experimental groups. However, whereas basal rates of H_2O_2 production with pyruvate/malate were decreased by long-term restriction, this was not the case for maximum complex I H_2O_2 generation (pyruvate/

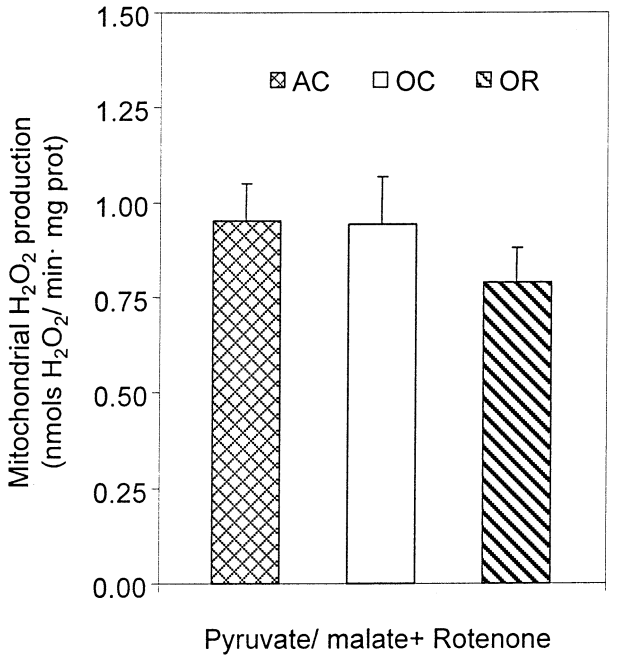

Fig. 2. Effect of aging and long-term caloric restriction on the maximum rates of H_2O_2 production of rat liver mitochondria with complex I-linked substrates (pyruvate/malate + rotenone). AC = adult controls (11 months); OC = old controls (24 months); OR = old restricted (24 months). Data are means ± SEM from 6–7 animals.

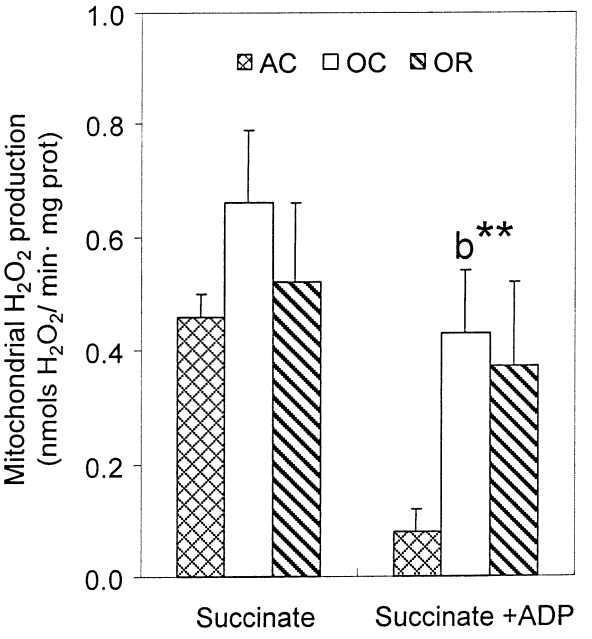

Fig. 3. Effect of aging and long-term caloric restriction on the rates of H_2O_2 production in states 4 and 3 of rat liver mitochondria with complex II-linked substrates (succinate). AC = adult controls (11 months); OC = old controls (24 months); OR = old restricted (24 months). Data are means ± SEM from 6–7 animals. b = significant difference between AC and OC. $^{**}p < .01$.

malate + rotenone) that was similar in the three groups (Fig. 2).

When a complex II-linked substrate was used (succinate) neither long-term caloric restriction nor age modified H_2O_2 production rates (Fig. 3). However, in the presence of ADP (state 3), H_2O_2 generation rate was significantly higher in old than in adult control animals (Fig. 3). Similar to what happened for basal rates with succinate, maximum complex III H_2O_2 production (succinate + antimycin A) was not changed by either long-term caloric restriction or age (Table 1) and was significantly higher than basal generation (succinate + rotenone). TTFA addition to succinate-supplemented liver mitochondria significantly decreased H_2O_2 generation in the three experimental groups (Table 1). H_2O_2 generation with succinate as substrate was significantly lower in the presence than in the absence of rotenone in the three groups (Table 1).

Long-term caloric restriction did not modify state 4 and state 3 mitochondrial oxygen consumption with either pyruvate/malate or succinate as substrates (Table 2). Old control animals showed lower state 3 mitochondrial oxygen consumption with succinate as substrate than adult controls (Table 2). The free radical leak in state 3 with succinate as substrate was significantly higher in old control animals than in adult ones (Table 3). Long-term caloric restriction significantly reduced the free radical leak with pyruvate/malate as substrate (Table 3).

Mitochondrial DNA 8-oxodG levels of long-term caloric restricted old animals were significantly lower (46% reduction) than those of old control animals (Fig. 4A). Furthermore, mt-DNA 8-oxodG levels were significantly higher in old control animals than in adult controls (Fig. 4A). Neither long-term caloric restriction nor age modified the steady-state levels of 8-oxodG in nuclear DNA (Fig. 4B).

DISCUSSION

Data from this investigation show that long-term caloric restriction decreases the H_2O_2 generation rate of rat liver mitochondria (47%) and concomitantly reduces oxidative damage to mitochondrial DNA (46%). Moreover, the decrease in mitochondrial ROS production takes place at complex I and the mechanism involved is a lower reduction degree of the complex I generator that reduces its free radical leak.

In the present study various respiratory chain inhibitors were used to localize the free radical generator on rat liver mitochondria. The rate of free radical production in the respiratory chain increases as a function of the reduction degree of the autoxidizable electron carriers [26]. When the respiratory chain is blocked with an inhibitor,

Table 1. Rate of Succinate-supported H_2O_2 Production of Rat Liver Mitochondria Under Different Conditions

	Adult control	Old control	Old restricted
Succinate	0.46 ± 0.09	0.66 ± 0.13	0.52 ± 0.14
Succinate (without Rotenone)	1.68 ± 0.48[a]*	2.47 ± 0.33[a]*	1.65 ± 0.52[a]*
Succinate + TTFA	0.11 ± 0.08[a]**	0.24 ± 0.05[a]**	0.25 ± 0.1
Succinate + AA	5.51 ± 0.5[a]***	5.67 ± 0.9[a]***	7.97 ± 1.5[a]***

Adult control animals were 11 months old; old animals, control and restricted were 24 months old. Values are Means ± SEM from 6–7 animals. TTFA = thenoyltrifluoroacetone.

[a] Significant difference in relation to succinate in the same treatment group.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

the degree of reduction of the electron carriers situated on the substrate side strongly increases, whereas those on the oxygen side become oxidized. Thus, an increase in ROS production after the addition of an inhibitor indicates that the free radical generator is located on the substrate side in relation to the inhibitor. On the other hand, a decrease in ROS production after the addition of an inhibitor means that the free radical generator is situated on the oxygen side.

Previous results from our laboratory, using immature animals, showed that rat liver mitochondria produce ROS at complex I, II, and III [12]. Studies performed in other tissues have shown that the ROS generator of heart mitochondria is located at complex I [16,27–31] and complex III [28,30–34] and in nonsynaptic brain mitochondria mainly at complex I [29,35]. In the present study, however, liver mitochondria from mature rats either adult or old generated H_2O_2 at complex I and complex III. The increase brought about by rotenone on H_2O_2 production with pyruvate/malate as substrate indicates that complex I is a free radical generator in rat liver mitochondria. Antimycin A addition to succinate-supplemented mitochondria strongly increased H_2O_2 production, indicating that complex III is also involved in ROS generation in rat liver mitochondria. In this investigation, TTFA addition to succinate-supplemented liver mitochondria from mature rats did not increase H_2O_2 production, contrary to what happened in immature rats [12], and even decreased it. So apparently, complex II H_2O_2 production disappears during development as ROS production by this complex is observed in immature rats [12] but not in adult or old mature rats (present investigation).

Long-term caloric restriction significantly decreased H_2O_2 production of rat liver mitochondria but this reduction only took place at complex I not at complex III, because the decrease is only observed with pyruvate/malate, not with succinate as substrate. With succinate (+ rotenone) the electrons flow only through the complex III generator, whereas with pyruvate/malate they flow also through complex I. Since caloric restriction decreases H_2O_2 production with pyruvate/malate but not with succinate (+ rotenone), the ROS generator responsible for the caloric restriction effect must be situated at complex I. The same was observed after just 6 weeks of caloric restriction treatment [12] and still stands after 1 year of caloric restriction, suggesting that the decrease in mitochondrial H_2O_2 production is established soon and is maintained throughout the rest of the life span. If the decrease in ROS production is responsible at least in part for the decrease in the aging rate produced by caloric restriction, it makes sense that the change is early established and maintained. Decreases in ROS production have also been found after 9 months of caloric restriction

Table 2. Effect of Aging and Long-term Caloric Restriction on Oxygen Consumption of Rat Liver Mitochondria Supplemented with Pyruvate/malate or Succinate in State 4 and 3

	Adult control		Old control		Old restricted	
	State 4	State 3	State 4	State 3	State 4	State 3
Pyr/mal	9.3 ± 1.2	33.7 ± 3.4	7.8 ± 1.2	24.0 ± 3.8	8.2 ± 0.9	25.0 ± 2.3
Succinate	27.1 ± 2.8	105.4 ± 10.3	22.1 ± 2.7	70.8 ± 8.5[a]*	21.0 ± 1.4	76.0 ± 5.2

Adult control animals were 11 months old; old animals, control and restricted were 24 months old. Values are Means ± SEM from 6–7 animals. pyr/mal = pyruvate/malate.

[a] significant difference between old control and adult control animals (aging effect).

* $p < .05$.

Table 3. Effect of Aging and Long-term Caloric Restriction on the Free Radical Leak of Rat Liver Mitochondria Supplemented with Pyruvate/malate or Succinate + ADP

	Adult control	Old control	Old restricted
Pyr/mal	0.79 ± 0.08	1.16 ± 0.18	0.61 ± 0.11[a]*
Succ + ADP	0.04 ± 0.02	0.29 ± 0.10[b]*	0.18 ± 0.12

Adult control animals were 11 months old; old animals, control and restricted were 24 months old. Values are Means ± SEM from 6–7 animals. pyr/mal = pyruvate/malate; succ = succinate.

[a] significant difference between old restricted and old control animals (restriction effect).

[b] significant difference between old control and adult control animals (aging effect).

* $p < .05$.

in brain, heart, and kidney mice mitochondria [7]. Besides, when ROS production has been comparatively analyzed in different animals it has been always found to be lower in long-lived than in short-lived species [3,29–31,35–38]. Thus, a low rate of ROS production can be involved in both the life extension effect of caloric restriction and the determination of the slow aging rate of long-lived animals. Our data also show that the decrease in ROS production of liver mitochondria after caloric restriction is time dependent. Long-term caloric restriction (12 months) produced a 47% decrease in ROS production, whereas short-term caloric restriction (6 weeks) decreased it by 23%. Previous studies have shown that the life extension effect of caloric restriction increases as a function of restriction time [39]. Moreover, long-term caloric restriction lowers the rate of mitochondrial ROS production below that present in adult control animals.

Some authors have proposed that the life-extension effect of caloric restriction involves a decrease in metabolic rate [40,41]. However, other authors have shown that total body 24 h metabolic activity does not change in caloric restricted rats [42]. Our data agrees with this last result since caloric restriction did not generally decrease mitochondrial O_2 consumption. The dietary treatment decreased the percentage of the total electron flow leading to ROS generation in complex I instead of reaching cytochrome oxidase to reduce oxygen to water (decreased the free radical leak). Thus, the mitochondria from caloric restricted rats release less ROS per unit electron flow in the respiratory chain. The mechanism responsible for this decrease in free radical leak was also studied. Using pyruvate/malate as substrate the rate of ROS production decreased in caloric restricted mitochondria; in these conditions complex I is only partially reduced. Rotenone addition to these pyruvate/malate-supplemented mitochondria leads to full reduction of complex I and in these conditions the difference in ROS production between restricted and ad libitum animals disappeared. This means that caloric restricted mitochondria have lower ROS production and lower free radical leak in the steady-state because the reduction degree of their complex I generator is lower than in control mitochondria.

Concerning the aging effect, previous reports mainly performed in heart tissue present controversial data. Sometimes authors reported increases in ROS production with age [7,14,15], whereas in other studies no changes were observed [16–18]. Thus, with the available information it cannot be concluded whether mitochondrial ROS production increases or not with aging. Nevertheless, aging is a progressive process that takes place at

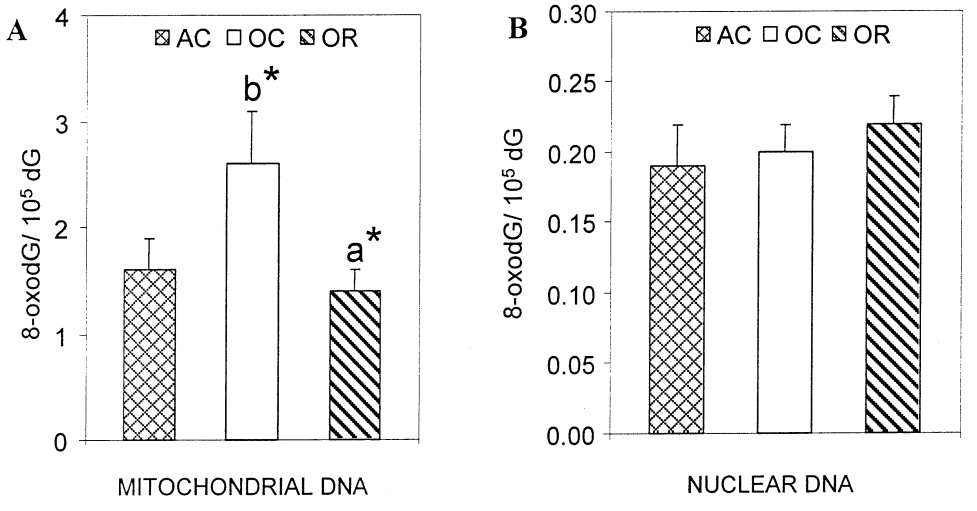

Fig. 4. Effect of aging and long-term caloric restriction on oxidative damage to rat liver mitochondrial (A) and nuclear (B) DNA. AC = adult controls (11 months); OC = old controls (24 months); OR = old restricted (24 months). Data are means ± SEM from 6–7 animals. a = significant difference between OC and OR; b = significant difference between AC and OC. *$p < .05$.

approximately similar rates at all adult ages. Thus, causes of aging (like, putatively, ROS production) must be present already in young individuals and there is no need for them to increase in old age. In agreement with these ideas and some previous works [16,17] we have not detected any increase in ROS production during aging in state 4 either using complex I- or II-linked substrates. We have only found a higher ROS production in old than in mature adult rats in state 3 and using succinate as substrate. The cause of this difference is that old control animals, differing from the mature adults, were unable to perform the normal supression of ROS production during the state 4 to state 3 energy transition [26,29].

In agreement with the reduced H_2O_2 production of the mitochondria from restricted animals, 8-oxodG values in mt-DNA also decreased after caloric restriction and no changes were observed in n-DNA. This specific decrease in mitochondrial DNA oxidative damage has also been described by us in the heart [13] and correlates with the localization of mtDNA very near to the mitochondrial free radical source. The only previous study available also found a decrease in 8-oxodG in liver mtDNA of caloric restricted rats [8]. A decrease in mitochondrial DNA mutations has also been described in rat liver after caloric restriction [43]. Nevertheless, other DNA modifications like nonoxidative type I I-compounds are not reduced by caloric restriction [44]. No changes in 8-oxodG were detected in liver nDNA, although a previous study from our laboratory using immature rats found a decrease in 8-oxodG in nDNA after short-term caloric restriction [12]. Other authors have also reported a lack of significant changes in 8-oxodG in liver and kidney after caloric restriction [11], whereas others have found decreases in 8-oxodG or 5-OH-methyluracyl in rat or mouse liver nDNA [8–11]. In any case, the mechanism responsible for these decreases does not seem to be related to the reduction of ROS production after caloric restriction because it occurred together with a 23% decrease [12] but not with a 47% decrease in H_2O_2 generation (present investigation). Thus, other possibilities like increases in 8-oxodG repair in nDNA of restricted animals could be involved. In contrast, decreases of 8-oxodG in mitochondrial DNA and decreases in mitochondrial ROS production are always linked either after short-term [12] or long-term caloric restriction (present data) in the liver, in other organs like the heart [13] and between animals with different longevities [4,45].

In agreement with the increase of H_2O_2 production in old animals in state 3 using succinate as substrate, 8-oxodG levels in mtDNA increased with aging and again this effect was specific for mtDNA, since no changes were detected in nDNA. The observed decrease in 8-oxodG in mtDNA after caloric restriction completely neutralized the increase observed with aging. However the mtDNA 8-oxodG levels of restricted animals were not below those of adult controls. In a postmitotic tissue like the heart we have detected an even greater caloric restriction effect, since both H_2O_2 production and 8-oxodG levels decreased below control values [13].

In summary, the results of this study show that a 40% caloric restriction regimen leads to a 47% decrease in the rate of mitochondrial H_2O_2 production and to a 46% decrease in oxidative damage to mitochondrial DNA. Thus a very close dose-response effect is observed between these parameters. Moreover, caloric restriction decreases aging rate by 30–50% [39,46]. The decrease in ROS production occurs in complex I and is due to a decrease in the degree of reduction of the ROS generator in the caloric restricted animals.

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ABBREVIATIONS

dG—deoxyguanosine
8-oxodG—8-oxo-7,8-dihydro-2′ deoxyguanosine
FRL—Free radical leak
MLSP—maximum life span
ROS—reactive oxygen species
TTFA—thenoyltrifluoroactetone