Mitochondrial and nuclear DNA base excision repair are affected differently by caloric restriction

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SPECIFIC AIMS

Aging is associated with increasing levels of oxidative damage and mutations in mitochondrial DNA (mtDNA), and these are prevented by caloric restriction (CR). We tested the hypothesis that CR initiates a program of enhanced mtDNA repair, allowing maintenance of mitochondrial genomic integrity and function throughout the extended life span of CR mice.

PRINCIPAL FINDINGS

1. Caloric restriction lowers DNA repair activity in brain and kidney but not liver mitochondria

Most DNA repair processes present in the nucleus have not yet been detected in mitochondria, and may be absent. However, mitochondria possess a significant capacity for the repair of oxidative DNA damage via base excision repair (BER). The ability of CR and pair-fed (PF) mouse mitochondria to repair damaged DNA was assessed by measuring BER activity in mitochondrial extracts prepared from liver, brain, and kidney mitochondria. Mice were maintained on CR (60% caloric intake) and PF (100% caloric intake) diets for 14 months beginning at 8 wk of age. The BER assay measured uracil-initiated repair synthesis incorporation activity: accumulation of full-length oligonucleotide containing $^{32}$P-dCTP after removal of uracil (present at a defined position in the oligonucleotide), processing of the abasic site, incorporation of the new nucleotide ($^{32}$P-dCTP), and religation of the strand (Fig. 1a).

In liver mitochondria, BER activity was ~18% higher in CR relative to PF mice, but this difference was not statistically significant. In brain and kidney mitochondria, CR resulted in 30% reductions of BER activity ($t$ test; $P=0.06$) compared with PF controls (Fig. 1).

2. Caloric restriction induces subtle increases in uracil DNA glycosylase but decreases in AP endonuclease and polymerase γ activities in mitochondria

The first steps in the repair of uracil by the BER pathway are recognition and incision of the damaged base by uracil DNA glycosylase (UNG) and processing of the resulting abasic site by AP endonuclease. These activities were measured as percent of oligonucleotide containing the lesion of interest at a defined site that was incised by mitochondrial extracts. Both activities were measured in CR and PF mice. UNG activity showed a trend toward marginal increases (~10%) in heart, brain, and liver of CR mice, but these were not statistically significant (Fig. 2a, b). However, kidney UNG activity was ~40% higher in CR mice ($P<0.05$). AP endonuclease activity was decreased in mitochondria isolated from all tissues, but this was statistically significant ($P<0.05$) only in brain (Fig. 2c, d). The next step in the BER pathway is filling the gap in the DNA left by UDG and AP endonuclease, catalyzed in mitochondria by DNA polymerase γ. Polymerase γ activity in brain and kidney mitochondria was decreased 20–30%, in parallel with decreases in BER synthesis incorporation activity (Fig. 2e, f).

3. OGG1 activity is higher in liver mitochondria of CR mice

Specific DNA glycosylases recognize and incise different DNA lesions. OGG1 incises 7,8-dihydroxyguanine (8-oxodG), a lesion known to accumulate with age. NTH1 recognizes and incises 5-hydroxycytosine and other oxidized pyrimidines. The effect of CR on mitochondrial OGG1 and NTH1 activities was determined. OGG1 activity was increased almost 20% by CR in liver mitochondria, whereas 5-OH cytosine incision activity was ~40% greater in kidney mitochondria.

4. CR increases nuclear BER activity

BER activity and the activities of proteins catalyzing individual steps in the BER pathway were measured in nuclear extracts from liver and kidney. CR increased BER activity by 26% in liver and 42% in kidney, though this was statistically significant only in kidney ($P<0.05$). No differences in the activities of DNA glycosylases or
Figure 1. Mitochondrial BER synthesis in liver (a, b), kidney (c), and brain (d) mitochondrial extracts. a) Representative gel showing the product of uracil-initiated repair synthesis incorporation after incubation of a double-strand, uracil-containing (dsU) oligonucleotide with CR and PF liver mitochondrial extracts for 45, 60, or 90 min; b) relative repair activity at 90 min. Repair activity was quantified as $^{32}$P-dCTP signal strength, relative to PF = 100%. c) relative repair activity at 90 min. d) relative repair activity at 60 min. For all graphs, open bars = PF, filled bars = CR. Values presented are means ± SE of duplicate measurements from 4–5 animals for each experimental group *Statistically significant difference: $P < 0.05$ (brain); $P < 0.065$ (kidney).

Figure 2. DNA glycosylase and AP endonuclease activities in mitochondria from CR and PF mice. a) UNG activity: representative gel of a U-containing substrate by mitochondrial extracts. U = single-strand U oligonucleotide; C = single-strand C oligonucleotide; L = liver; B = brain; H = heart; K = kidney. b) UNG activity in CR and PF mice; c) AP endonuclease activity: representative gel showing the incision of a THF-containing substrate. d) AP endonuclease activity in CR and PF mice; e) polymerase γ gap-filling activity: representative gel showing the 39 nt product after incorporation of $^{32}$P-dCTP into an oligonucleotides containing a 1 nt gap by a kidney mitochondrial extract; f) mean gap-filling activities in kidney and brain mitochondria. *$P = 0.07$ for comparison of PF and CR kidney.
AP endonuclease were found between nuclear extracts from CR and PF mice.

**CONCLUSIONS AND SIGNIFICANCE**

Our findings indicate that a universal enhancement of mtDNA repair did not occur in CR. CR actually reduced uracil-initiated repair synthesis incorporation activities in brain and kidney whereas they were unchanged or marginally elevated in liver mitochondria. That CR did not induce greater mitochondrial BER activities was surprising, as mtDNA oxidative damage (8-oxodG) and mutation load are both decreased by CR. However, steady-state levels of mtDNA oxidative adducts, such as 8-oxodG, represent an equilibrium between the opposing effects of de novo formation and removal by BER. Therefore, lower steady-state levels of oxidative damage can be achieved by lowering only the rate of lesion formation. The rate of superoxide generation is indeed reduced, ~45–50%, in mitochondria from CR mice. Even combined with a 30% reduction in the rate of removal, steady-state levels of oxidative damage should equilibrate at lower values in CR mouse brain and kidney. This is, in fact, what was observed: mtDNA from postmitotic tissues of CR mice have 30% lower 8-oxodG levels than controls; steady-state 8-oxodG levels in liver mtDNA are 46% lower in CR rats. This is also consistent with the present data: ROS production is reduced by CR similarly in liver and muscle mitochondria. Maintenance of higher rates of removal (higher activities of BER and OGG1) will result in lower equilibrium values of lesion.

Combined with information regarding mitochondrial ROS generation and the incidence of oxidative lesions in mtDNA, our results allow a complete model of the maintenance of mitochondrial genomic stability and function in CR to be constructed (Fig. 3). CR appears to promote mitochondrial genomic stability largely by reducing mitochondrial ROS production. Our data suggest that, at least in postmitotic cells (brain, kidney), mitochondrial BER activity is regulated by mitochondrial ROS production. This effect of CR is opposite of that observed in mitotic cells (liver) and of the effect on nuclear DNA repair, indicating differential regulation of mtDNA repair in mitotic and postmitotic cells and in mitochondrial and nuclear compartments. Details of the physiological regulation of mtDNA repair remain to be elucidated in further studies.

**Figure 3.** Schematic diagram showing the sites of reactive oxygen species (ROS) metabolism modified by caloric restriction (CR). Steady-state levels of oxidative lesions (*) in mtDNA represent an equilibrium between rates of their formation by reactive oxygen species (ROS) and removal by base excision repair (BER). Unrepaired lesions are mutagenic (M) and destabilize the mitochondrial genome. Levels of oxidative lesions and mutations are lowered by CR, but this does not involve substantial and universal induction of BER. CR targets the production of oxidative lesions through substantial reductions in mitochondrial ROS production (thick arrow), but no detectable increase in mitochondrial BER (dashed arrow).