INVESTIGATION OF THE EFFECTS OF TREE FRUIT SUPPLEMENTATION ON THE REPAIR OF OXIDATIVE DNA BASE DAMAGE IN MOUSE EXTRACTS

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INTRODUCTION

The DNA is a relatively unstable molecule that can be easily modified (Lindahl, 1993). Modifications of DNA bases, particularly those caused by oxidative stress, can lead to mutations and have been directly linked with some diseases such as cancer. DNA lesions introduced by oxidative stress also accumulate during the normal aging process. To counteract their deleterious effects, living organisms have developed DNA repair systems that remove the damaged base and replace it with a new, unmodified nucleotide. DNA repair is a set of enzymatic processes that work jointly to correct DNA damage. These modifications are recognized by specific proteins and then cascades of enzymatic reactions take place. The particular process and set of enzymes utilized is then dependent upon the kind of lesion that is being dealt with. Small, non-helix distorting lesions, such as oxidative modifications, are repaired by the base excision repair pathway (BER). BER is active both in the nucleus as well as in mitochondria, and thus functions to prevent accumulation of oxidative DNA damage in both genomes (Bohr et al., 2002).

Since fruits and vegetables are good sources of anti-oxidants, it has been long speculated that dietary modulation would decrease the levels of oxidative DNA damage. Very few studies have directly addressed this question and the results are heterogeneous (Moller et al., 2003;Zhu et al., 2000). On the other hand, the possibility that dietary components also modulate DNA repair activities has been poorly investigated. Recently, Collins and colleagues (Collins et al., 2003) demonstrated that consumption of kiwifruit for 3-week periods decreased endogenous levels of oxidized DNA bases and increased DNA repair efficiency. This DNA repair stimulation was not due to an increase in the repair enzyme protein levels, suggesting that kiwifruits may directly affect the activity of the proteins.

Previous results from our laboratory suggested that peach extracts could modulate the repair of oxidative lesions *in vitro*. This study was designed to address the question of whether a peach or nectarine-enriched diet modulates DNA repair and damage levels *in vivo*, using mice as our

animal models. Our interest concentrates on one particular class of DNA lesions, formamidopyrimidine (Fapy) modifications. In a previous experiment we observed that the levels of fapyadenine in liver DNA from mice that ate the fruit diet were significantly lower than in mice eating the control diet. The results obtained from these studies will further our understanding of how diet modulates human health at the molecular level and may stimulate the consumption of peaches and nectarines, if it appears that these fruits may contribute to lower oxidative DNA damage levels, and thus decrease risk for certain diseases such as cancers and age-associated degenerative diseases.

PROPOSED SPECIFIC AIMS

1. In the previous mouse-feeding experiment we observed that mice fed a diet containing 8% fruit extract (wt/wt) showed significantly lower levels of an oxidized base lesion, fapyadenine (Fig. 1), which can cause mutations and cytotoxicity. In this study we propose to investigate whether P/N extracts enhance DNA repair efficiency towards fapyadenine and the closely related fapyguanine lesion. We measure the effect of adding P/N extracts to mitochondrial and nuclear extracts on their ability to incise fapy-containing oligonucleotide substrates. We will also investigate whether fapy incision is elevated in liver extracts from mice fed a diet containing 8% P/N extract.

2. We propose to extend the mouse-feeding study to older mice. In the previous study we utilized 3 months old mice and we would like to use 18 months old mice in this follow-up study. Several of these oxidatively induced lesions accumulate with age and we postulate that older mice will be more sensitive to small variations in DNA repair capacity, as casued by the high fruit diet. Two groups of mice (24 animals each) will be fed a control diet or a diet containing 8% elegant lady peach extract (wt/wt) for 3 months. At the end of the study the animals will be sacrificed and livers and brains processed for mitochondria/nuclei isolation, DNA isolation and RNA isolation for micro-array studies.

PROGRESS REPORT:

Aim 1- One critical element of the experiments proposed under this aim is the fapy-containing oligonucleotide substrates. These substrates are proprietary, not commercially available and obtained trough collaboration with an investigator in another institution. The substrates are synthesized as short oligos, 16 bases long, which are too small for use in the in vitro incision experiments. Thus, we have to further prepare the substrates by ligating the lesion-containing oligo to another oligonucleotide in order to obtain substrates that are 30-36 bases long. We are now preparing the substrates and performing the ligation reactions. Once the final substrates are prepared we will continue with the in vitro experiments testing the direct effect of peach extracts on the incision kinetics of these lesions.

Aim 2- To address aim 2 we devised the following experimental design:

1. ***Experimental design***: 3 feed groups (control, peach-enriched and nectarine-enriched), 18 mice/group, 3 months feeding

2. ***Assays to perform***:

a. DNA repair activities in mitochondria and nuclear fractions
b. Levels of DNA damage by HPLC-EC or LC-MS
c. Expression of DNA repair/damage response genes

3. Organs to collect:

Brain, Liver, Heart, Kidney and Testis

4. Sample preparation:

a. *Brains*:

- Dissect 9 brains into regions; combine 3 regions for each sample, for a total of 3 samples. Quick freeze in liquid N_2 for isolation of mito/nuc
- Combine 3 whole brains per sample, for a total of 3 samples, for DNA isolation. Quick freeze

b. *Livers*:

- Freeze livers of 12 mice individually, to be used for mito/nuc isolation and DNA isolation
- Freeze 6 livers in RNALater for gene expression analysis

c. *Heart, Kidney and Testis*:

- Freeze organs from 6 mice individually for mito/nuc isolation
- Combine 3 each, for a total of 3 samples, for DNA isolation. Quick freeze.
- Freeze 3 organs individually in RNALater for gene expression analysis

5. Progress:

- The 3 groups were fed for 3 months and all animals sacrificed. The tissues were collected as described above and when appropriate processed into mitochondrial and nuclear extracts.
- The mitochondrial extracts were tested for nuclear contamination by Western Blot against a highly abundant nuclear protein, Lamin B2, and the respiratory chain component, Cytochrome oxidase subunit IV (COX IV), as a marker for mitochondrial content. The absence of Lamim B signal in the lanes with mitochondrial proteins shows that all mitochondrial extracts are virtually free of nuclear contamination (Figure 1). The enrichment for mitochondria was confirmed by the several fold enrichment for the mitochondrial marker COX IV. These results allowed us to proceed with the measurements of DNA repair activities in these two compartments.
- We have initiated the *in vitro* repair measurements using two prototypical DNA lesions, the common cytosine deamination product uracil, and the common oxidative lesion 8-hydroxyguanine. Incision reaction conditions were optimized for these two lesions (Figure 2 for uracil and Figure 4 for 8-hydroxyguanine) using increasing concentrations of nuclear and mitochondrial extracts from a control mouse.
- We found that uracil incision activity is similar in nuclear and mitochondrial extracts, in a protein concentration range from 0.5 – 40 µg of extract. Because the reaction reaches a saturating plateau at the higher protein concentrations, we chose 2.5 µg of extracts for the experiments comparing the different groups. We have thus far analyzed one set of animals, i.e., one mouse of each feeding group, for the uracil incision activity (Figure 3). Our preliminary results suggest that the fruit-enriched diet may modulate DNA repair

activities differently in nucleus and mitochondria. In the one set of animals analyzed we observed increased incision activity in mitochondria from the fruit-fed mice and decreased activity in the nuclear extracts from the same animals. However, since these results represent only one animal for each group, it is at this point too preliminary to draw conclusions. We are now analyzing the remaining samples in order to have statistically relevant results.

- For the 8-hydroxyguanine incision experiments we observed a slightly lower incision activity in the mitochondrial extracts when compared with nuclear extracts (Figure 4, compare panels B and D). For that reason, the amounts of extract chosen for the comparison of the feeding groups differs for the two types of extracts. We will use 20 µg of nuclear extracts and 40 µg of mitochondrial extracts. The experiments comparing the extracts from all the treated animals are now under way.

REFERENCES/LITERATURE REVIEW

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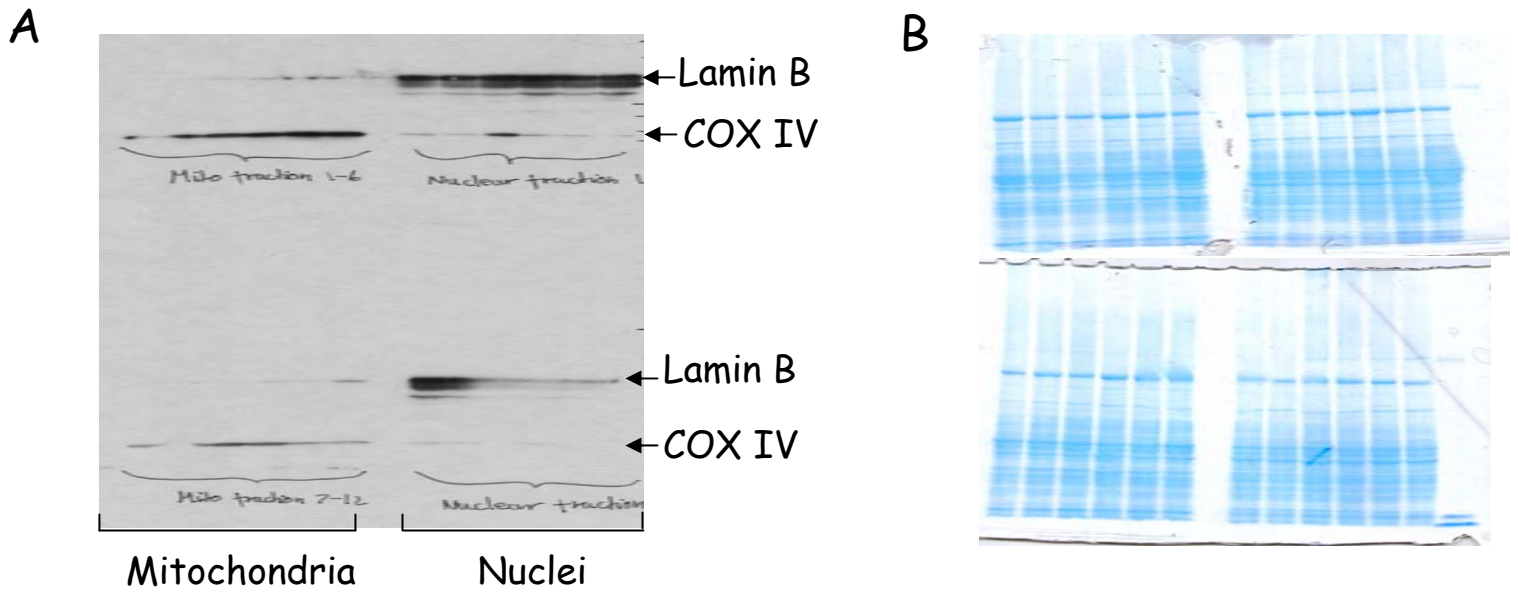
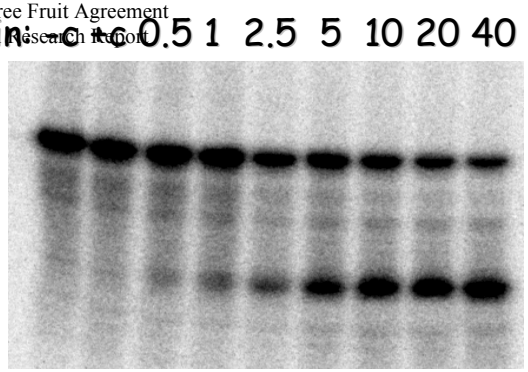
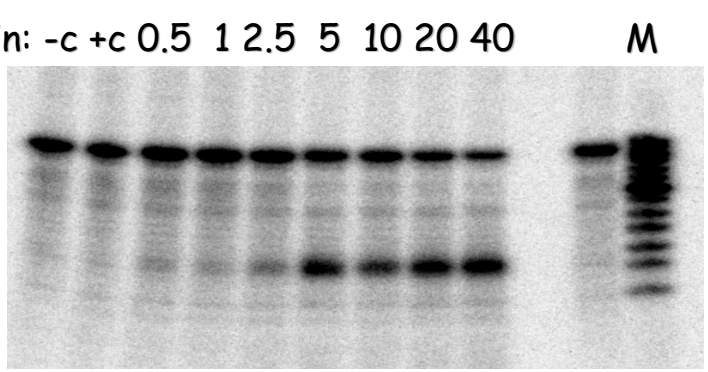


Figure 1. *Western blot detection of nuclear and mitochondrial protein markers in mouse liver*: Fifty micrograms of mitochondrial or nuclear extracts from each mouse liver were separated using a 12% denaturing polyacrylamide gel, transfer to a PVDF membrane and probed with monoclonal antibodies against Lamin B and COX IV. Panel A shows the immunoblot, as detected by ECL. Panel B shows the amido-black staining of the membranes to confirm equal loading in all lanes.

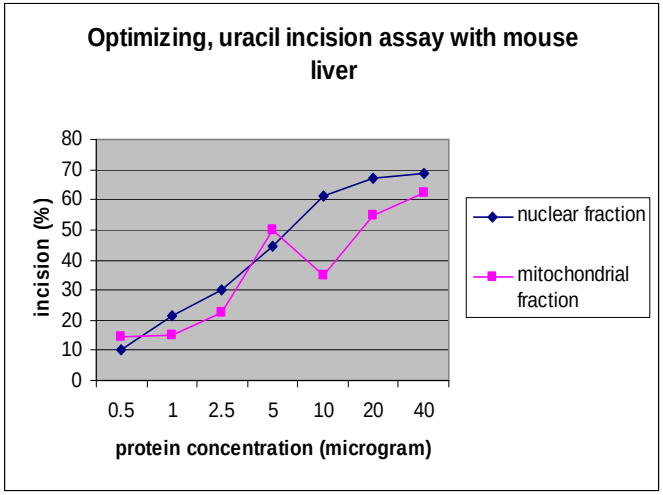
A



B



C

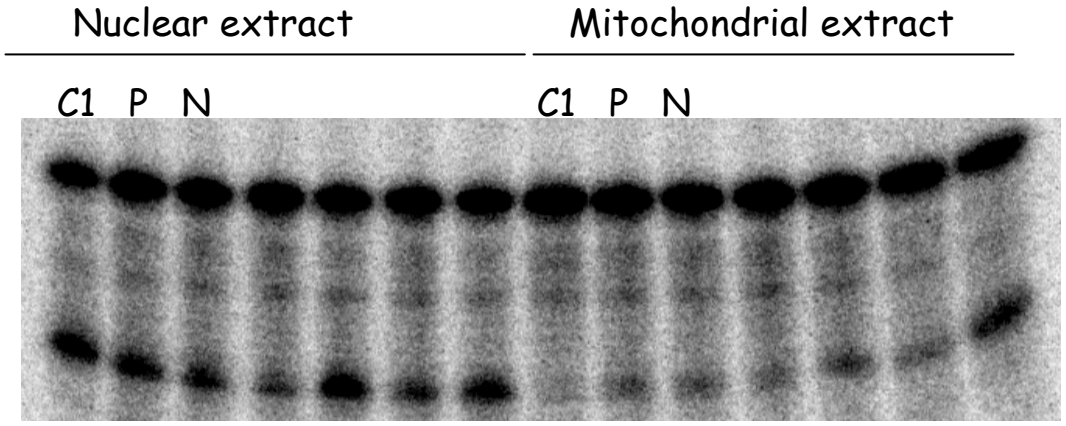


D

ATATACCGCGGUCGGCCGATCAAGCTTATT
TATATGGCGCCGGCCGGCTAGTTCGAATAA

Figure 2. *Optimization of reaction conditions for UDG incision activity with nuclear and mitochondrial extracts:* Increasing concentrations of nuclear (panel A) or mitochondrial (panel B) extracts were incubated with 100 fmoles of uracil-containing duplex oligonucleotide (D) for 30 min, at 37 °C. The reactions were resolved in a 20% polyacrylamide/7 M urea gel and exposed to Storm screens. The percent of incision was quantified using a ImageQuant software and a graph representation is presented in panel C.

A



B

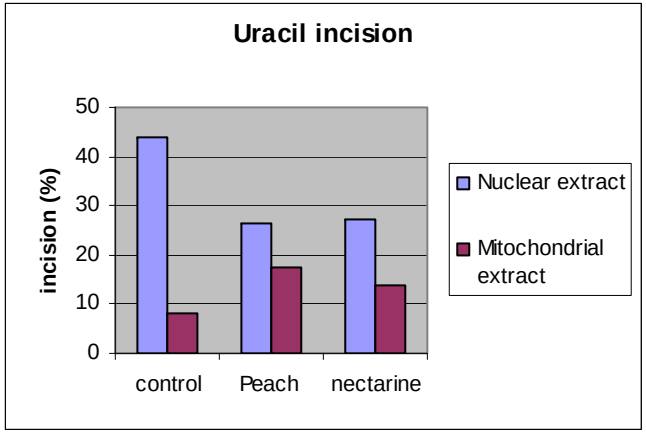
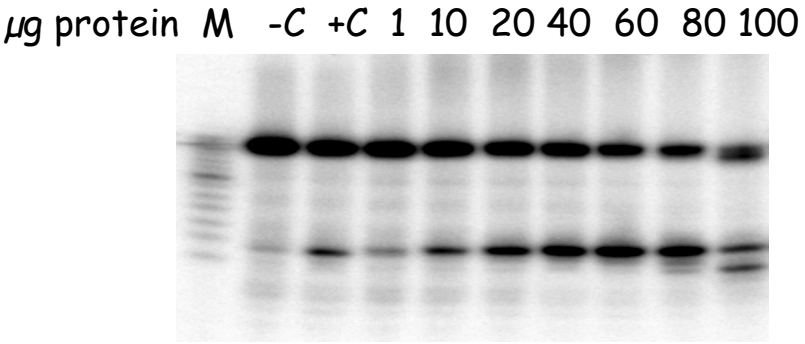
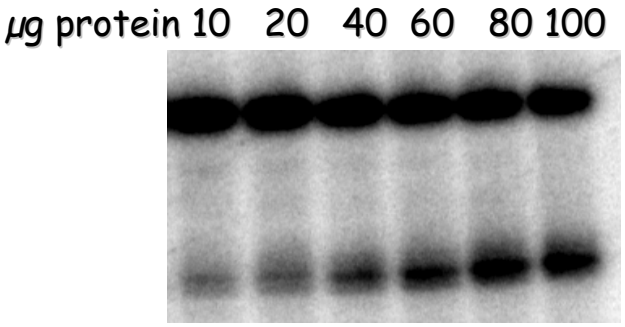


Figure 3. *UDG activity in control and fruit fed mouse liver extracts:* 2.5 µg of nuclear or mitochondrial extracts from a control (C1), a peach-fed (P) or a nectarine-fed (N) mouse were incubated with 100 fmoles of uracil-containing substrate and resolved as described earlier (A). The percentage of incision for each sample was quantified and is presented in panel B. The results presented are the average of duplicate reaction for one mouse per group.

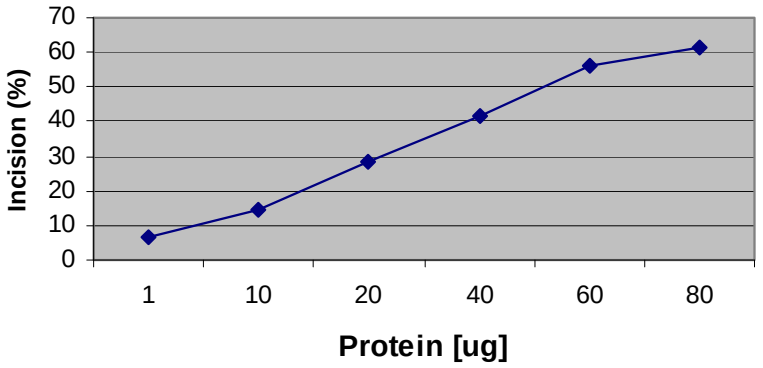
A



B



C



D

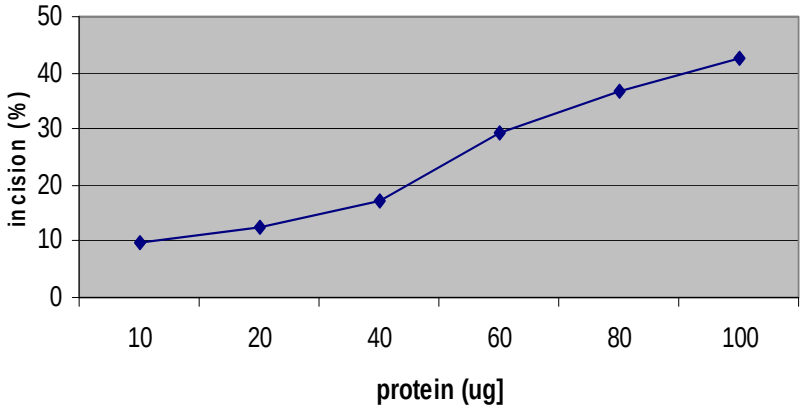


Figure 4. *Optimization of reaction conditions for OGG1 incision activity with nuclear and mitochondrial extracts:* Increasing concentrations of nuclear (panels A and C) or mitochondrial (panels B and D) extracts were incubated with 100 fmoles of 8-hudroxyguanine-containing duplex oligonucleotide (same sequence as U-substrate, but with 8-oxoG in the position 11) for 4 hr, at 32 °C. The reactions were resolved and quantified as described before