Alteration of Mitochondrial Bioenergetics and Apoptotic Signaling with Aging in Mice Kidney: The Effects of Calorie Restriction and Dietary Fat

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Introduction

An important mechanism to maintain cell homeostasis is apoptosis, a programmed or regulated form of cell death that controls the accumulation of defective cells. An imbalance of this process may result in the onset of several diseases, particularly those associated with aging.

Calorie restriction (CR), a reduction in the amount of dietary energy intake without malnutrition, has been proven to increase longevity and influence healthy aging by improving tissue performance and delaying the onset of age-related diseases. The underlying mechanisms of these effects likely involve decreased oxidative stress and optimization of mitochondrial bioenergetics in a tissue specific manner.

Most studies conducted to understand the effects of diet on apoptosis in relation to aging are centered on CR, which is the only treatment that has been shown to increase half-life and longevity in addition to slowing the incidence of spontaneous tumor formation in a wide variety of animals (Figure 1).

Today, the free radical theory of aging is among the most accepted theories to explain the positive effects of CR on health. CR has been demonstrated to decrease reactive oxygen species (ROS) generation and oxidative injury. Therefore, special attention has been paid to the role of the mitochondria on these phenomena due to its synthesis of ROS. Alongside CR, additional factors must also be considered such as the type of response to different apoptotic stimuli on distinct tissues and the main fat source of the diet, which directly affects the lipid composition of the cell membranes.

Methods

Levels of proteins linked to apoptosis, APAF-1 and VDAC-1 were determined by Polyacrylamide gel electrophoresis and western blot immunodetection. Samples were denatured by heating in SDS-dithiothreitol loading buffer, separated by SDS-PAGE (12.5% acrylamide) and then blotted onto nitrocellulose sheets. Subsequently, blots were stained with Ponceau S for visualization of protein lanes. To obtain digital images, photographic films and Ponceau S-stained blots were scanned in a GS-800 calibrated densitometer (Bio-Rad).

Quantification of the specified proteins was carried out using Quantity One software (Bio-Rad).

In order to determine the total amount of a ubiquinone in samples, the ubiquinone was converted into the corresponding reduced form by treatment with sodium borohydride. Chromatographic separation was monitored with a Coulochem II electrochemical detector fitted with a Model 5010 analytical cell. Quinones were detected from 1 to 3 (Figure 1).

Levels of coenzyme Q were determined by liquid chromatography and reversed-phase HPLC separation.

Results

Upon observation of the distribution of APAF-1 in the cytosol, no significant changes were noted due to either CR or the dietary fat source in the control groups of mice fed under CR (Figure 2).

Subsequently, blots were stained with Ponceau S for visualization of protein lanes.

In order to determine the total amount of a ubiquinone in samples, the ubiquinone was converted into the corresponding reduced form by treatment with sodium borohydride.

Concentration were then calculated by integration of peak areas and comparison with external standards (Sigma).

No significant changes were noted due to either CR or the dietary fat source in the control groups of mice fed under CR (Figure 5).

Upon observation of the distribution of VDAC-1 in the mitochondria, no significant changes were noted due to either CR or the dietary fat source in the control groups of mice fed under CR (Figure 1).

However, differences were observed in the distribution of APAF-1 in dietary fat source in mice fed under CR. This suggests that apoptosis signaling is decreased in CR diets in which lard is used as the primary fat source (Figure 3).

Conclusion

Results indicate that the distribution of APAF-1 in the cytosol demonstrates no significant changes in dietary fat source in the control groups of mice fed under CR with the exception of those in which lard is the primary source of dietary fat.

These findings suggest that apoptosis signaling is decreased in calorie-restricted diets in which lard is used as the primary fat source.

The distribution of VDAC-1 in mitochondria demonstrates no significant effects in dietary fat source in the control groups of mice fed under CR.

However, taking into account previous research conducted by our group, a trend demonstrating an increase in lard in diets under CR, which is potentially due to a tissue-specific effect.

Lastly, results of the levels of coenzyme Q suggest an increase with age in animals.

The results of this study suggest that CR and the predominant fat source in calorie-restricted diets induce effects on kidney apoptosis in relation to aging.

Objectives

- Investigate the effects of calorie restriction (CR) and those changes induced by the predominant fat source (lard, soybean oil, fish oil) in calorie-restricted diets on kidney apoptosis in relation to aging in mice fed experimental diets for 6 or 18 months.
- Measure markers related to apoptotic signaling, apoptotic protease activating factor 1 (APAF-1) and voltage-dependent anion channel 1 (VDAC-1), for the different experimental conditions by following a Western blot methodology.
- Due to the role of ubiquinone (coenzyme Q, CoQ) in energy metabolism, alterations in CoQ biosynthesis may modify CoQ levels in tissues. Thus, determine the levels of coenzyme Q (CoQ10) by reversed-phase HPLC separation; also, measure mitochondrial cellular respiration via citrate synthase mitochondrial marker enzyme.
- Investigate the effects of CR and those changes induced by the predominant fat source in the mitochondrial bioenergetics of kidney muscle from mice following one month of calorie restriction.
- Investigate the effects of lard, soybean oil, or fish oil in calorie-restricted diets on mitochondrial bioenergetics of the kidney from mice fed under CR.

Summary

Results indicate that the distribution of APAF-1 in the cytosol demonstrates no significant changes in dietary fat source in the control groups of mice fed under CR with the exception of those in which lard is the primary source of dietary fat.

These findings suggest that apoptosis signaling is decreased in calorie-restricted diets in which lard is used as the primary fat source.

The distribution of VDAC-1 in mitochondria demonstrates no significant effects in dietary fat source in the control groups of mice fed under CR.

However, taking into account previous research conducted by our group, a trend demonstrating an increase in lard in diets under CR, which is potentially due to a tissue-specific effect.

Lastly, results of the levels of coenzyme Q suggest an increase with age in animals.

The results of this study suggest that CR and the predominant fat source in calorie-restricted diets induce effects on kidney apoptosis in relation to aging.

References


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