The molecular basis of the effects of calorie restriction on ageing

Dianne Ford
SPARC workshop - 11 January 2007
**PI Background**

- **BSc Biochemistry**
- **PhD Biochemistry (molecular enzymology)**
- Postdoctoral research on xenobiotic metabolism then on nutrient (peptide and amino acid) transport
- **Current research focus “molecular nutrition”**
  - Zinc transport/absorption and zinc-regulated gene expression
  - Isoflavone metabolism (contribution of genetic factors to inter-individual variability)
  - Nutritional epigenomics
    - Influences of nutrition on non-sequence-related modification of DNA
    - Collaboration with Professor John Mathers, Newcastle University
Nutrition and ageing

• Importance of nutrition for health well-established
• Great potential to modulate the ageing process through nutritional strategies, or therapies that mimic the effects of effective nutritional strategies.
  - Requires an understanding of effects of nutrition on ageing at the molecular level
Background to project – calorie restriction and ageing

• Well established (based on research going back several decades) that calorie restriction (approximately 60% normal intake in rodents) increases lifespan and/or reduces incidence of ageing-related disease in model organisms including yeast, *C. elegans*, *Drosophila*, mice.

• Applies in humans?
Welcome to the Calorie Restriction (CR) Society

Our goal is to help people of all ages live longer and healthier lives simply by:

- eating fewer calories
- maintaining adequate nutrition

Since the 1930's extensive scientific research has shown that calorie restricted (CR) diets improve health and extend lifespans of nearly every species tested, including worms, spiders, rodents, dogs, cows and monkeys. We believe it is likely that people who carefully adopt a CR diet will see similar results.

The CR Society supports the efforts of people who practice CR for future longevity, current health, or other benefits; those curious about or interested in understanding the effects of the diet; and those interested in the development of related, science-based life-extension and health-enhancing technologies. Our mailing lists provide a rich forum for such topical discussions.

Calorie Restriction...the only proven life-extension method known to modern science.
Research that will change your life!

The evidence that caloric restriction extends life in animal species inspires thousands to adopt calorie restriction as a way of life. But unlike animals in research studies that have clearly defined diet parameters and protocols, humans have many ideas about how caloric restriction should be practiced.

If you are reading this, most likely you are very serious about functioning at your peak. Nothing is more precious.

But how can you know whether your limited-calorie lifestyle really slows aging? And whether or not you are practicing calorie restriction, how will you know if supplements, exercise, amount of protein intake, hormone replacements or any other of the myriad choices that are persuasively argued for - actually accelerates aging or increases risk of cancer or other serious disease? An objective method of evaluation is vital to avoid life-shortening mistakes.

That's why the Calorie Restriction Society has initiated a milestone study that will correlate human calorie restrictors' genetic expression and cell signaling indicators to clinical markers. Once these correlations are established, serious longevists will be equipped with easy-to-run clinical tests that indicate how well their regimens are working.

We've asked several renowned researchers to handle aspects of the project: Luigi Fontana, M.D., Ph.D., Stephen Spindler, Ph.D., and Shin-ichiro Imai, M.D., Ph.D.

But we can't do it without your help.

We need to raise $230,000 to make this project happen. (check status) This means we need contributions large and small. Make an investment that is priceless and learn more about slowing aging - what works and what doesn't. Please send your donation directly to the Society Treasurer,

David R. Stern
7223 S Rt 83 #142
Willowbrook, IL 60527

Or to

Bob Cavaunagh at the Calorie Restriction Society
187 Ocean Drive
Newport, NC 28570

or donate online using a Credit Card.

For other payment options, please contact The Calorie Restriction Society by phone at:

914-923-1605

or toll-free at 866-894-1812

Questions? Please be in touch with Paul McGlothlin. Want to know more about the Calorie Restriction Society? Ask me or the CR Society.
Background to project - calorie restriction and ageing

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• Applies in humans?

• Molecular basis unknown
  - Reduced metabolic rate leads to generation of fewer, DNA-damaging free radicals?

• Increased expression of the protein Sirt1 appears to be involved
  - Effects on insulin/IGF1 signalling pathway?
  - Increased stress-resistance?
    • Higher threshold for apoptosis?
  - Novel idea - effects of Sirt1 activity on epigenetic modification of DNA??
Background to project – epigenetic modification of DNA

Methylated DNA is associated with deacetylated histones

Background to project - DNA methylation and ageing

- Ageing-associated changes in DNA methylation are observed.
  - Decrease in total deoxymethylcytosine levels
  - Regional hyper- and demethylation

- Aberrant DNA methylation can result in inappropriate gene expression or gene silencing.

- Changes in DNA methylation may be causal in the ageing process.
Background to project – Sirt1 activity relevant to DNA methylation

- Recap – calorie restriction increases expression of Sirt1
- Sirt1 has histone deacetylase activity

Methylated DNA is associated with deacetylated histones

- So increased Sirt1 expression may affect histone acetylation and hence DNA methylation
Hypothesis

- The beneficial effects of calorie restriction on ageing include maintenance of DNA methylation patterns mediated through increased histone deacetylation by increased activity of the Sirt1 protein.

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  calorie restriction  ➔ increased Sirt1 expression  ➔ deacetylation of histones  ➔ increased/retained DNA methylation
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Aim

• Establish proof of principle that increased Sirt1 expression can affect DNA methylation.
Approach

• Researcher - Luisa Wakeling
• Express high levels of human Sirt1 in cultured human cells (SW480) from a “transgene” (coding sequence of the human Sirt1 gene introduced into the cells) and measure DNA methylation compared with control cells.
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Measurement of DNA methylation

Target which sequences?
LINE1 elements
C-MYC
ER
IGF2
GFP reporter gene
Future directions

• Use genome-scanning techniques (e.g. differential methylation hybridisation; ChIP on chip) to identify specific sites in the genome showing altered patterns of DNA methylation and/or altered patterns of histone acetylation in response to increased Sirt1 expression (in cells; BBSRC DTG Studentship from ICaMB) or calorie restriction (in ageing mice).

• Determine effects of such methylation patterns on target gene expression.

• Increase/knockdown expression of target genes in model organisms (C. elegans, mice?) whose expression is modulated by methylation and measure effects on ageing to identify gene targets of calorie restriction that can affect the ageing process.
Acknowledgements

• Professor John Mathers
  Newcastle University

• Luisa Wakeling
  Newcastle University