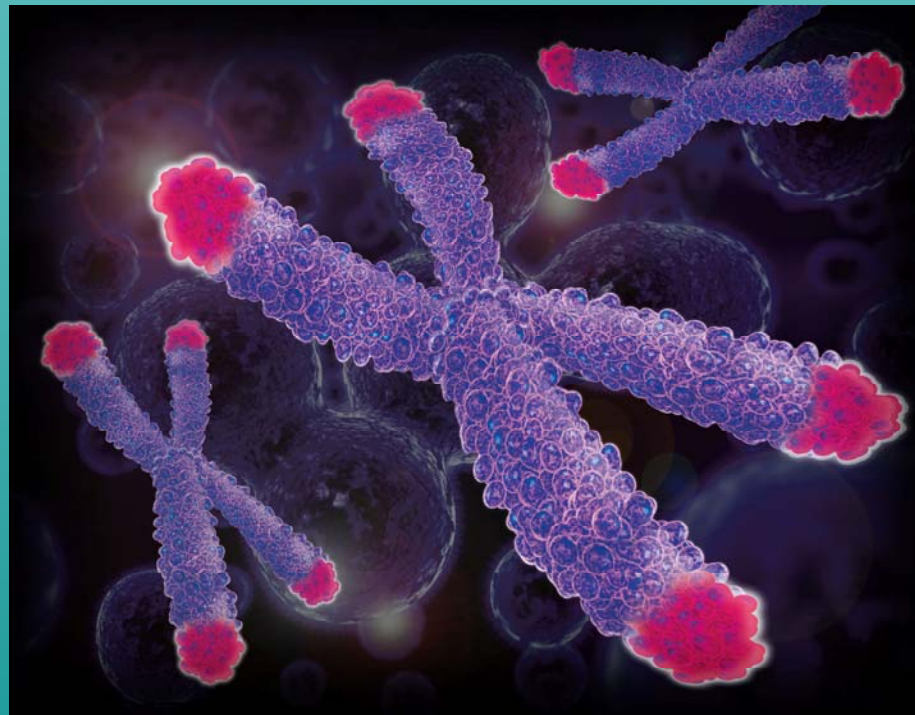


# Telomere Testing: A New Tool for Age Management



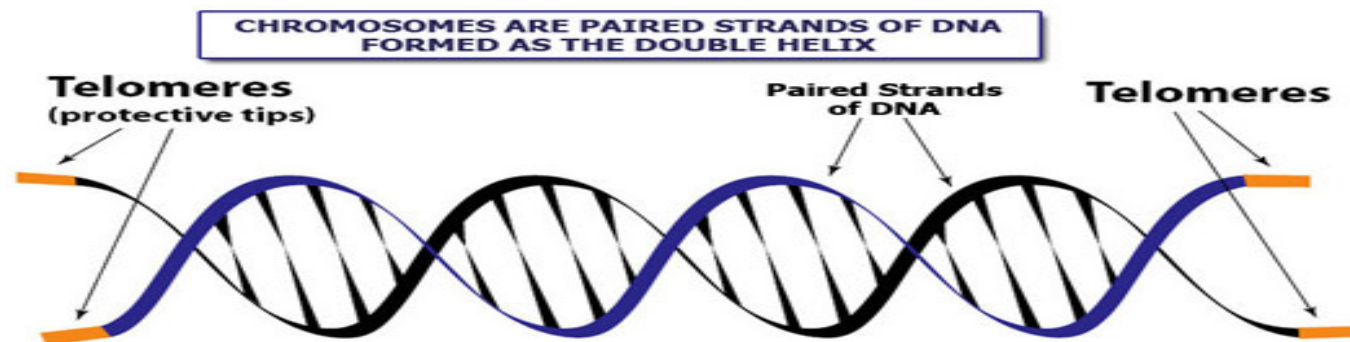
Presented by Dr. Mark C. Houston



SPECTRACELL LABORATORIES  
ADVANCED CLINICAL TESTING

## WHAT IS A TELOMERE?

- A telomere is a region of highly repetitive DNA at the end of a linear chromosome that functions as a disposable buffer.
- Every time linear eukaryotic chromosomes are replicated during late S-phase, the DNA polymerase complex is incapable of replicating all the way to the end of the chromosome.
- If it were not for telomeres, this would quickly result in the loss of vital genetic information, which is needed to sustain a cell's activities.
- Every time a cell with linear genes divides, it will lose a small piece of one of its strands of DNA.
- This process has been referred to by James Watson and Alexei Olovnikov as the “end replication problem” (1971). It is believed that telomeres have a function in the aging process.



# TELOMERE SHORTENING



It is estimated that human telomeres lose about 100 base pairs from their telomeric DNA at each mitosis.

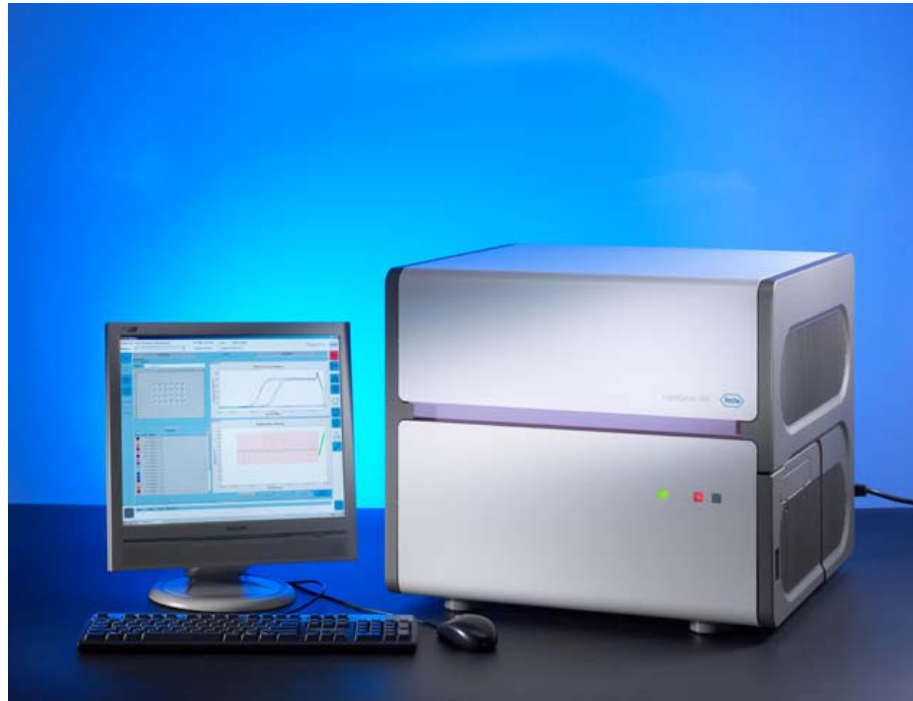
This represents about 16 TTAGGG repeats. At this rate, after 125 mitotic divisions, the telomeres would be completely gone.

This is why normal somatic cells are limited in the number of mitotic divisions before they die out.



# Quantitative Real Time PCR Assaying of Telomere Content

*Telomere  
Testing*



Roche LC480 Light Cycler



## What is PCR?

The purpose of PCR (Polymerase Chain Reaction) is to make a huge number of copies of a particular DNA segment from a small amount of starting (template) DNA, either for sequencing or visualization.

There are three major steps in a PCR run (denaturation, annealing and extension), which are repeated for 30-40 cycles. This is done on a device called a PCR thermocycler, which heats and cools the tubes with the reaction mixture in a very short time.

Real time, quantitative PCR utilizes a fluorescent reader in the PCR machine to measure the amount of DNA being amplified.

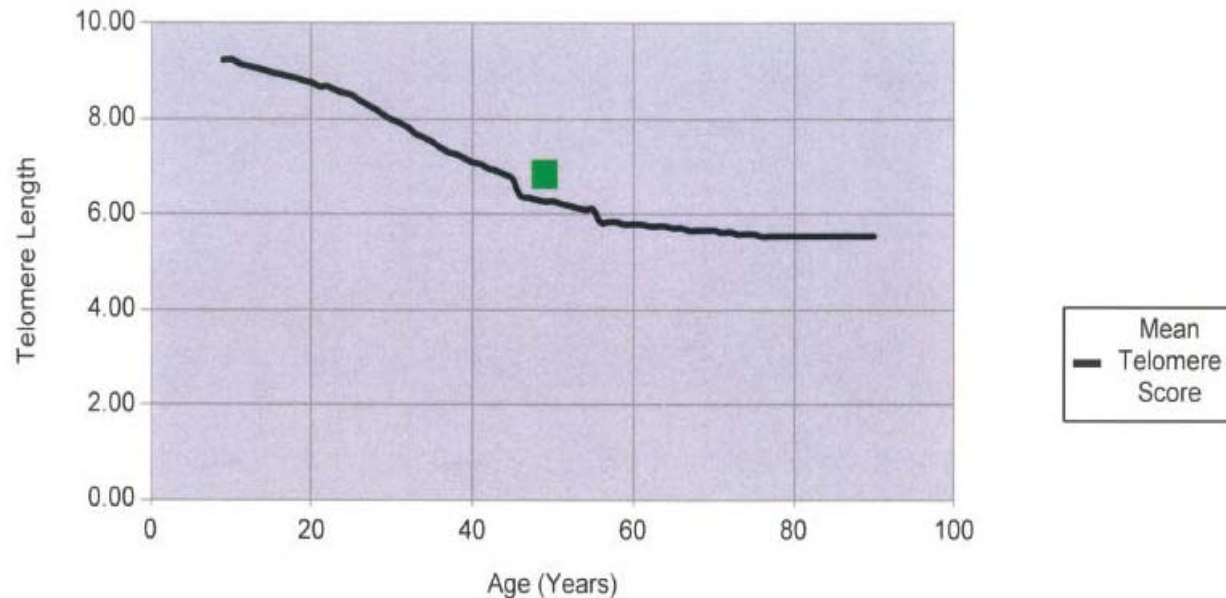
Using an internal size and quantity standard, it is possible to infer the size and quantity of the DNA sequence being amplified (ie., the length of telomere DNA). (Fenech, 2008)



**Patient Telomere Score: 6.85**  
**Percentile: 74**

# Telomere Testing

Telomere Test Results



The above graph depicts the patient's telomere score compared to the average telomere score for each age group within a random sample population of approximately 800 individuals.

A patient telomere score below the line (red box) represents a below average telomere score, and a patient score above the line (green box) represents an above average telomere score. If the patient's age was not provided, a horizontal line representing the patient's telomere score across all age groups will be shown.



# What Do the Results Mean to the Patient and the Doctor?

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Age adjusted telomere length is the best method to date to determine accurately a person's biological age using structural analysis of chromosomal damage in the telomere. Serial evaluation of telomere length will determine how rapidly one ages relative to a normal population. Therapies directed at slowing the loss of telomere length will slow aging and age-related diseases.



# FACTORS OF TELOMERE SHORTENING




1. Aging
2. CHD / MI
3. Hypertension
4. DM / IR
5. Obesity
6. Smoking
7. Arterial stress and hemodynamic stress
8. Increased pulse pressure
9. Carotid atherosclerosis
10. Aortic stenosis
11. Vascular dementia
12. Oxidative stress



# FACTORS OF TELOMERE SHORTENING (continued)

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- 
13. A-II ( $\uparrow$  AT<sub>1</sub>R stimulation) ( $\downarrow$  AT<sub>2</sub>R stimulation)
  14. Homocysteine
  15. OxLDL (PI3K / AKT path)
  16. Decreased NO
  17. GTPase – RAC-I  $\rightarrow$  ceramide  $\rightarrow$  mtROS
  18. Reduced mitochondrial cytochrome C oxidase
  19. ADMA
  20. Lack of ASA ( $\downarrow$  ADMA  $\rightarrow$   $\uparrow$  NO)
  21. Lack of arginine
  22. Mitochondrial DNA damage
  23. Lack of estrogen



## Why Telomere Length Matters

Associations with telomere length have been reported for essential hypertension, diabetes, insulin resistance, obesity, atherosclerosis, vascular dementia, and mortality due to heart disease.

Significant or borderline inverse associations were found between Telomere length and diabetes, glucose, insulin, diastolic blood pressure, carotid intima-media thickness, and interleukin-6. Associations with body size and C-reactive protein were modified by gender and age, occurring only in men and in participants aged 73 years or younger.

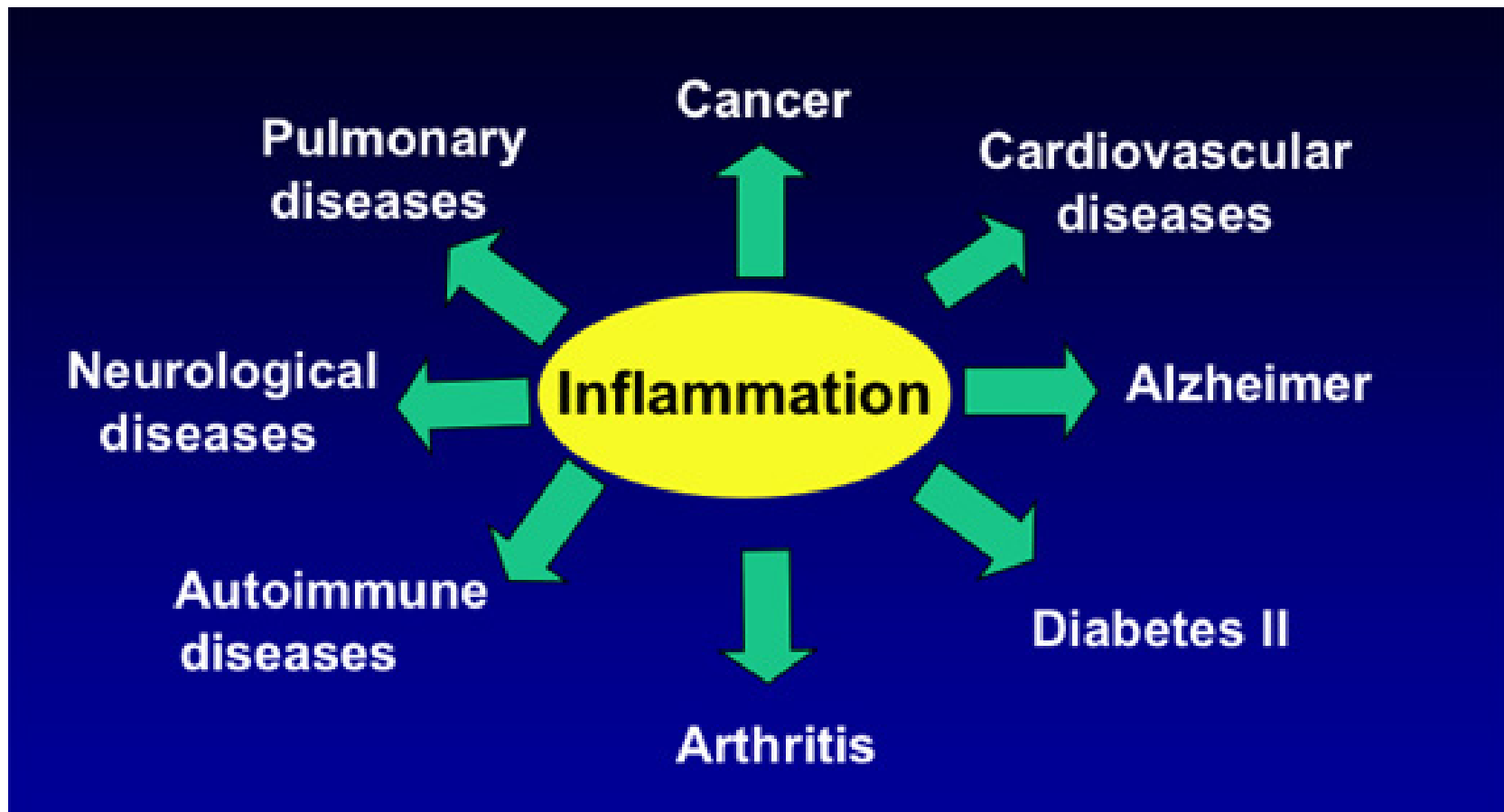
**In participants under 73, each shortened kilobase of Telomere corresponded with a threefold increased risk of myocardial infarction** (hazard ratio = 3.08, 95% confidence interval: 1.22, 7.73) **and stroke** (hazard ratio = 3.22, 95% confidence interval: 1.29, 8.02).

**These results support the hypotheses that telomere attrition may be related to diseases of aging through mechanisms involving oxidative stress, inflammation, and progression to CVD.**



Telomere attrition is associated with inflammation and high mortality in prevalent hemodialysis patients

# Telomere Testing



Reduced telomere length was associated with increased mortality, independently of age, gender and inflammation (likelihood ratio 41.6,  $P < 0.0001$ ),



# TELOMERES AND VASCULAR AGING, *Telomere Testing* CHD AND AGING

Focal replicative senescence and telomere shortening of endothelial cells play a critical role in CHD. T / C ratio smaller in CHD patients by 50% ( $p < 0.0001$ ). 3.18x greater incidence of CHD.

Short telomeres are associated with increase carotid IMT, atherosclerosis, CHD, CVD, ASCVD, increased pulse pressure and arterial stiffness in patients with hypertension ( $p < 0.01$ , difference of 220 bp), but also independent of BP levels. Also with total mortality and infectious diseases.


**Normal WBC telomere attrition rates are 25 – 38 bp per year and is indirectly related to antioxidant capacity.**

*Hypertension 2004; 43:182*  
*Arterioscler Thromb Vasc Biol 2004; 24:546*



# Leukocyte Aging is Predictive of Vascular Aging

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Blood leukocyte telomere DNA content predicts vascular telomere DNA content in humans with and without vascular disease  
**(Eur. Heart Journal, 2008)**

**The telomere:genomic DNA content was significantly reduced in wall biopsies of abdominal aortic aneurysms (AAA) vs. normal aorta, and this difference remained after adjusting for age and gender. There were strong correlations between leucocyte and vascular telomere content when the AAA and control groups were analysed either separately or grouped irrespective of the presence of vascular disease ( $r = 0.62$ ,  $P < 0.001$ ).**

**Shortened telomeres may also be an independent risk factor for CVD (Front Biosci 2008)**



# What are the Nutritional Implications on Telomere Length and Repair?


*Telomere  
Testing*

- An inflammatory diet or one that increases oxidative stress will shorten telomeres faster. This would include refined carbohydrates, fast foods, processed foods, sodas, artificial sweeteners, trans fats and saturated fats.
- A diet with a large amount and variety of antioxidants that improves oxidative defense and reduces oxidative stress will slow telomere shortening.
- Consumption of 10 servings of fresh and relatively uncooked fruits and vegetables, mixed fiber, monounsaturated fats, omega 3 fatty acids, cold water fish, and high quality vegetable proteins will preserve telomere length.
- In addition caloric restriction is advised to reduce total daily caloric intake by about 25% combined with an exercise program that increases caloric restriction by about 15 %.
- Fasting for 12 hours each night at least 4 days per week is recommended.



# The Following Will Slow Telomere Shortening

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- 
- Exercise
  - Statins
  - ACEI
  - ARB
  - Bioidentical Hormone Replacement
  - Sleep
  - Stop Tobacco
  - A good antioxidant defense program: Most antioxidants will help improve CHD risk factors such as BP, cholesterol, DM, HC
  - Weight Loss
  - Stress Reduction
  - Optimal Paleolithic Diet
  - Avoid trans fats, SFA, sodas, refined CHO etc.
  - Resveratrol
  - Possibly Omega 3 FA and MUFA




# What Lifestyle Modifications are Likely to be Helpful ?

- One should achieve ideal body weight and body composition with low body fat (less than 22 % for women and less than 16 % for men).
- Decreasing visceral fat is very important.
- Regular aerobic and resistance exercise for at least one hour per day.
- Sleeping for at least 8 hours per night
- Stress reduction
- Discontinuation of all tobacco products
- Bio-identical hormone replacement therapy will increase telomere length.



# Nutritional Supplements that will Slow Telomere Shortening

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Oxidative stress will shorten telomere length, and cause aging in cellular tissue. Antioxidant supplements can potentially reduce oxidative stress very effectively, which will ultimately improve oxidative defenses, mitochondrial function, reduce inflammation and slow vascular aging. Targeted supplementation is key, as antioxidants work synergistically and must be balanced to work most effectively and avoid inducing a prooxidant effect. Increasing antioxidant capacity at the cellular level is critical to maintaining telomere length.

Recent evidence suggests that a high quality and balanced multivitamin will also help maintain telomere length. Specifically studies have linked longer telomeres with levels of vitamin E, vitamin C, vitamin D, omega-3 fatty acids and the antioxidant resveratrol. In addition, homocysteine levels have been inversely associated with telomere length, suggesting that reducing homocysteine levels via folate and vitamin B supplementation will positively affect telomere length. Similarly, conditions such as cardiovascular disease, insulin resistance, diabetes, hypertension, atherosclerosis and even dementia affect telomere length. Correcting subclinical nutritional deficiencies that can cause such disease is crucial for telomere maintenance.



# Pharmacologic Treatments to Slow Telomere Aging

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- 
- Angiotensin converting enzyme inhibitors (ACEI)
  - Angiotensin receptor blockers (ARB)
  - Renin Inhibitors
  - Statins
  - Possibly Calcium channel blockers
  - Possibly Serum aldosterone receptor antagonists
  - Possibly metformin
  - Aspirin
  - Bioidentical Hormone Replacement Therapy



# Control all known Coronary Heart Disease Risk Factors to Optimal Levels

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- LDL cholesterol to about 70 mg % and decrease LDL particle number and increase LDL particle size
- Reduce oxidized LDL
- Increase HDL to over 40 mg% in men and over 50 mg% in women and increase HDL 2 subfraction.
- Reduce inflammatory HDL and increase protective HDL
- Reduce fasting blood glucose to less than 90 mg % and 2 hour post prandial or 2 hour GTT to less than 110 mg %.
- Keep Hemoglobin A1C to about 5.0% and keep insulin levels low
- Reduce blood pressure to about 120/ 80 mm Hg
- Reduce homocysteine to about 5 ug/ml
- Reduce HS-CRP to less than 1.0
- Ideal body weight and composition
- Stop smoking
- Treat insulin resistance and metabolic syndrome



# Telomerase: A Treatment?



## Telomerase

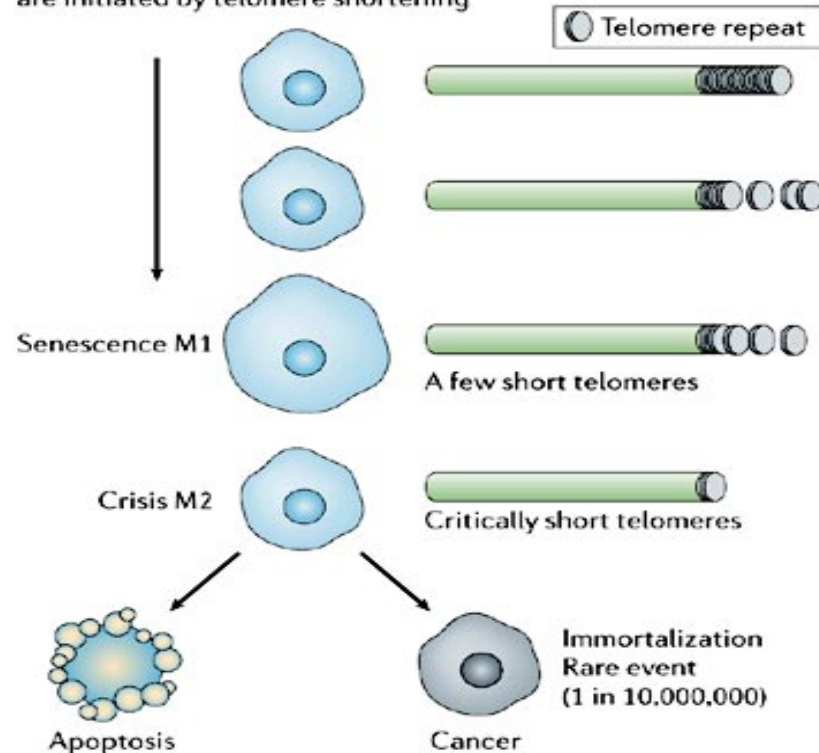
- Enzyme that repairs the telomere
- Decreased activity with aging
- Decreased activity with oxidative stress and inflammation
- In VSMC and ED cells it is activated by mitogenic stimuli via PKC dependent path. Activity decreased with aging due to decreased TERT.

*Circ Res 2007; 100:15-26*



## The Trouble With Telomerase...

M1 (senescence) and M2 (crisis) pathways are initiated by telomere shortening



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Nature Reviews | Drug Discovery

As telomeres shorten, they enter into a state called senescence, or 'telomere crisis', and stop dividing. This effects good cells as well as (most) bad ones...



# Overall the Recommendations to Maintain Telomere Length Are...

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To Involve reducing all known coronary risk factors, inflammation, oxidative stress, ADMA levels and angiotensin II levels or its action.

At the same time therapy should increase nitric oxide levels and nitric oxide bioavailability, increase arginine, increase endothelial progenitor cells, improve mitochondrial function, increase oxidative defenses In addition one should optimize hormone levels, exercise, sleep, nutrition and nutritional supplements.

Fasting and caloric restriction should be part of the regimen as well.



# When should Retesting be Considered

*Telomere  
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**Testing should be done once per year to evaluate the rate of aging and make adjustments in nutrition, nutritional supplements, weight management, exercise and other lifestyle modifications known to alter telomere length.**



## Other Relevant Tests



### MicroNutrient Testing

SpectraCell's MicroNutrient Test offers a unique means to scientifically assess the intracellular requirements of micronutrients that play an important role in overall health and wellness of your patients. Our tests measure the biochemical function of vitamins, minerals, amino acids and antioxidants, providing a powerful clinical assessment tool for your practice.



An advanced cholesterol testing technology which accurately measures both the size/density and number of lipoprotein particles.





*Thank you for attending our presentation.*

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