Melatonin
Not Just for Sleep

“The Pineal Gland is the seat of the soul”...
....Rene Descartes...1700
• Melatonin levels 10-15 x higher at night than day in all organisms from bacteria to humans
• Conveys info about day length to organism
• Connects life to the cycles of the universe
  – Days
  – Seasons
  – Years
Tryptophan - Serotonin - Folate, B6


Melatonin

(N-acetyl-5-methoxytryptamine)

\[
\text{CH}_3\text{O}\]

\[
\text{C} - \text{C} - \text{N} - \text{C} - \text{CH}_3
\]

Pineal gland

Inhibition

Stimulation

Retinohypothalamic tract

Suprachiasmatic nucleus (the "biologic clock")

Superior cervical ganglion
Melatonin—\(N\)-acetyl-5-methoxytryptamine

- Secreted by pineal gland as well as extrapineal production
- Produced in darkness, suppressed by light
- Concentrated in nucleus and mitochondria
- Levels decline with aging - 10-15% per decade
- Manages circadian rhythm of inner clock
  - Controls sleep wake cycle
Correlation between age and peak levels of plasma melatonin
Low Melatonin associated with

- Alzheimer's
- CV disease
- Insulin resistance
- Cancer
- Infectious disease
- Immune dysfunction
• Scavenges Free radicals
  – Most effective FR scavenger of hydroxyl radical known
    • More than Glutathione or Vitamin E
    • Hydroxyl radical damages mitochondria
  – Protects DNA from Injury
  – Especially in Pharmacologic concentrations
  – Protects against pro-oxidation effect of Fe
• Herrera J et al. Melatonin prevents oxidative stress resulting from iron and erythropoietin administration *Am J Kidney Dis* 2001 Apr;37(4):750-7
The ultimate anti-oxidant?

• Protects lipids, proteins, DNA
• Stimulates glutathione
• Protects mitochondria
• Protects against ischemia-reperfusion injury
• Protects against ionizing radiation

Enhances Immune function

- Inhibits tumor growth
- Counteracts stress induced immunodepression
- Increases in CD4 cells, natural killer cells
- Activates cytokine system when needed
- Decreases pro-inflammatory cytokines
- Increases immune function in winter when there are more environmental stressors
- Nelson RJ Melatonin mediates seasonal changes in immune function Ann N Y Acad Sci 2000;917:404-15
Aging

• Lengthens Lifespan and Healthspan in mice

• May protect against aging through quenching free radicals or immune system enhancement

• Dilman VM, Increase in the lifespan of rats following polypeptide pineal extract. *Exp Pathol* 1979;17:539-45. 78.

• Prolongs survival mice from 23.8 to 28.1 months and preserves aspects of their youthful state

Exercise - Melatonin

- Strenuous exercise induces inflammatory reactions and high production of free radicals and subsequent muscle damage
- 50km run 2800m ramp
- Increase in TNF-α, IL-6, IL-1,
- Increase in 8-hydroxy-2'-deoxyguanosine (8-OHdG)
- Inflammation and oxidative stress limited by Melatonin before exercise
Melatonin TMD Pain

• Melatonin 5 mg
• Pain level decreased
• Independent of sleep quality

• Vidor LP et al. Analgesic and Sedative Effects of Melatonin in Temporomandibular Disorders: A Double-Blind, Randomized, Parallel-Group, Placebo-Controlled Study. *J Pain Symptom Manage.* 2012 Nov 27
Melatonin: Analgesic

- Chronobiotic, antioxidant, antihypertensive, anxiolytic and sedative
- Potent analgesic effects in a dose-dependent manner.
- Fibromyalgia, irritable bowel syndrome, migraine
- Mechanism: melatonin receptors, opioid μ-receptors, GABA-B receptors, better sleep
Melatonin and Ionizing Radiation

- Used to prevent damage induced from ionizing radiation in Fukushima disaster

Melatonin Buffers Immune

• Stimulates under basal immunosuppressive conditions
• Anti-inflammatory under exacerbated immune response
  – Inflammation or autoimmune immune conditions

Aging Brain Protection

• Melatonin added to drinking water of mice

• Melatonin prevented mitochondrial impairment associated with aging

Melatonin Protects against B amyloid

• Intra-hippocampal injection of beta amyloid in rats
• Cognitive impairment reversed with melatonin treatment

Melatonin, Mitochondria and Alzheimer’s

• Melatonin protects against beta amyloid damage to mitochondria

• Reiter, R et al. Accumulation of exogenous amyloid-beta peptide in hippocampal mitochondria causes their dysfunction: a protective role for melatonin. *Oxid Med Cell Longev.* 2012;2012:843649
Melatonin: Brain Anti-aging

• Counteracts processes that occur during aging and age-related neurodegenerative disorders
  – Oxidative stress and oxidative damage
  – Chronic and acute inflammation
  – Mitochondrial dysfunction
  – Loss of neural regeneration

• Escames, G et al. The Role of Mitochondria in Brain Aging and the Effects of Melatonin  *Current Neuropharmacology*, 2010, 8, 182-193
Melatonin, Hypoxia and Neural stem cells

- Melatonin turns on neural stem cells in setting of neonatal hypoxia

- Jie Fu et al. Melatonin Promotes proliferation and differentiation of neural stem cells subjected to hypoxia in vitro. *J Pin Res* Feb 1 2011
Insulin

“Daily melatonin administration at middle age suppressed male rat intraabdominal visceral fat and plasma insulin to youthful levels”

Wolden-Hanson T Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat Endocrinology 2000 Feb;141(2):487-97
Cancer

• Inhibits tumor growth in humans
  – Anti-mitotic activity
  – Downregulate activity of receptors
    • Decreased Estrogen binding to cells in breast cancer
  – Enhanced Immune Response
  – Free Radical scavenging
  – Anti-angiogenesis

• Improved outcome in glioblastoma, malignant melanoma, breast cancer
  – Used along with chemo, radiation
  – Protects against chemo/radiation toxicity
  – Large doses used 20-700 mg /day
Melatonin and Cancer

- 370 patient, with advanced solid CA
- Chemotherapy alone vs chemo/melatonin 20 mg orally at bedtime
- Significant tumor regression rate improvement and survival in combination group
Blindness and Breast Cancer

- 1392 Blind women
- No Light Perception vs. light perception
- ½ rate of breast cancer

Meta-analysis Cancer/Melatonin

- 10 adjunctive or sole treatment studies of melatonin and solid tumors 1992-2003
- Melatonin reduced the risk of death at 1 year (relative risk: 0.66, \( P \leq 0.56 \))
- Low adverse effects
- Low cost
- Conclusion: Great potential for melatonin and cancer therapy

Melatonin -CV

- Coronary heart disease – low melatonin levels
- Protects against ischemia – reperfusion injury
- Anti-arrhythmic
- Anti-inflammatory
- Anti-hypertensive
Melatonin and Hypertension

- Free radical scavenging and antioxidant effects
- Preservation of NO availability
- Sympatholytic effect
- Useful in nocturnal hypertension and LVH

Protective effect of melatonin and/or growth hormone against myocardial infarct in rats

Protective effect of melatonin against ventricular fibrillation & arrhythmias in rats

Melatonin and Cardiac Ischemia and Ischemic Stroke

• Melatonin has advantages over other anti-oxidants
  ▪ Reducing hypoxia
  ▪ Preventing reoxygenation-induced damage.

• Endogenous levels of melatonin in the elderly are reduced - more severe cardiac damage during an acute MI


Melatonin in ST segment elevation MI

- Patients who had developed adverse events during follow-up had significantly lower nocturnal melatonin levels than patients without events.  P < .0001
  - Cardiac Death, Repeat MI, CHF
- Vasodilator
- Free radical scavenger
- Inhibits oxidation of LDL-C
Melatonin and CRP after MI

- Inverse Correlation

MARIA Study

- IV Melatonin used as an adjunct of treatment
- Patients with acute MI undergoing primary Angioplasty
- A unicenter, prospective, randomized, double-blind, parallel-group, placebo-controlled study
- End point- decreased infarct size at 0-72 hours or clinical events in first 90 days ie death
- No published results yet

• Decreases CRP, IL-6, VCAM
• Attenuates tissue damage from reperfusion
• Decreases V Tach and V fib after reperfusion
• Low M in CHD, AMI and Sudden death
• Attenuates cellular a molecular damage from ischemia

Maria Study Update

• Phase II clinical trial
• Multicenter, randomized, controlled clinical trial
• IV and Intracoronary Melatonin in STEMI patients ongoing
Melatonin Protects Cardiac Myocyte Mitochondria

- Xu M et al. Melatonin Protection Against Lethal Myocyte Injury Induced by Doxorubicin as Reflected by Effects on Mitochondrial Membrane Potential. *J Mol Cell Cardiol* 2002 Jan;34(1):75-79
Melatonin Cardioprotection

• Both melatonin and resveratrol protect against reperfusion injury
• Melatonin reduces infarct size in mouse model

Melatonin and CV

STEMI

Opening artery:
- Thrombolysis
- PPCI

Ischemia/reperfusion injury

↑ O₂ consumption

Cardiac dysfunction:
- ↑ Free radicals
- ↓ Coronary flow
- ↓ NO availability
- ↑ Platelet aggregation

Intervention

MEL (oral or iv):
- Antioxidant
- Anti-inflammatory
- ↓ Platelet aggregability

Restore cardiac function
Melatonin and Sleep

- Exogenous melatonin improves quantity and quality of sleep
- No withdrawal or addiction

Melatonin Dose - 1/2 hr before sleep

- Try small dose 0.5 mg at first
- If no unpleasant reaction can increase dose in increments to 3-30 mg
- Time release for people who wake up in middle of night
- Sublingual lozenge or drops for people who have trouble falling asleep
- Some tolerance develops but usually levels off at 3-30 mg
- For some people, less in more and better sleep at low dose 0.3 mg
Vitamin D Pandemic

• Balanced diet or living near equator not sufficient
• Everyone who does not get lots of sun or ingests at least 2000-10,000 IU per day is at high risk for skeletal and non-skeletal consequences
• High rates of Vitamin D Deficiency:
  • USA, Mexico, Europe, Middle East, India, Asia, Australia and New Zealand
Vitamin D Deficiency - USA

• All age groups from children to elderly
• Especially African Americans
• USA: Very Low 25(OH)D = Calcidiol <20 ng/mL
  – 36% - age 18-29
  – 42% - African American women 15-49
  – 41% - Outpatients 49-83
  – 57% - Inpatients
• Europe: 28-100% of healthy adults

Vitamin D - Mexican children

- Obese and non-obese Mexican children
- Skin phototypes III-IV
- Monterey, Mexico (Latitude 25 degrees, 40’)
- 62% - insufficiency of 25-OHD (21-29 ng/ml)
- 20% - deficiency (<20 ng/ml).
- Obese significantly lower 25-OHD

Colon cancer mortality rates, males, 1970-94
All percentages reference a common baseline of 25 ng/ml as shown on the chart. %'s reflect the disease prevention % at the beginning and ending of available data. Example: Breast cancer incidence is reduced by 30% when the serum level is 34 ng/ml vs the baseline of 25 ng/ml. There is an 83% reduction in incidence when the serum level is 50 ng/ml vs the baseline of 25 ng/ml. The x's in the bars indicate 'reasonable extrapolations' from the data but are beyond existing data.

References:
Estimated Proportion of Conditions Preventable by Specified Range of Serum 25(OH)D Level

- 50% Falls, women
- 50% All fractures combined
- Multiple sclerosis: 50% - 60%
- Type 1 Diabetes: 50% - 80%

Serum 25(OH)D, ng/ml
Few foods contain vitamin D

- Fatty fish species:
  - Herring, 85g (3 oz) 1383 IU
  - Salmon, cooked, 3.5 oz 360 IU
  - Sardines, in oil, 1.75 oz, 250 IU
  - Tuna, canned in oil, 3 oz 200 IU
- One whole egg, 20 IU
- Fortified Milk 1 cup 100 IU
- Cod liver oil, 1 Tbs (15 mL) 1,360 IU
Vitamin D physiology

- Technically not a "vitamin"
- Vitamin D is in a class by itself.
- Its metabolic product, 1,25 dihydroxyvitamin D = *calcitriol*, is a *secosteroid hormone* that targets over 1000 genes
- Every cell has a vitamin D receptor that responds to 1,25 dihydroxy vitamin D
Secosteroid hormone
Vitamin D3 = Cholecalciferol
“B” Ring is “Broken”
Vitamin D → 25(OH)D → Kidney → 1,25(OH)₂D

- Skin
- Milk
- Orange Juice
- Diet
- Supplements

Regulation of Cell Growth (cancer prevention)
Regulation of Immune Function (diabetes type 1, MS, RA, autoimmune disease prevention)
Calcium, Muscle Bone Health & Regulation of Blood Pressure, Insulin Production (heart disease and diabetes prevention)
Why are old Vitamin D3 limits wrong?

- Can synthesize as much as 26,000 IU per day in the sun
- Lowest intake of Vitamin D3 associated with hypercalcemia – 40,000 IU per day for several months
- 300,000 IU per week produced hypercalcemia and 25(OH)D >400 ng/mL

VITAMIN D INTAKE & TOXICITY

- No toxicity below 30,000 IU/day
- No toxicity below 500 nmol/L (200 ng/mL)

- 15 studies of adults receiving vitamin D supplementation (means)
- 8 studies reporting toxicity (individual values)
D deficient girls can’t jump

- Higher 25 OH D3 levels:
  - Improved jump height, velocity and force

- Ward KA et al. Vitamin D status and muscle function in post-menarchal adolescent girls. *JCEM* 2009 Feb;94(2):559-63
Vitamin D and Brain

- D insufficiency > 2X dementia, Alzheimer's disease and stroke, and MRI indicators of cerebrovascular disease
- Buell, J et al. 25 Hydroxyvitamin D, dementia and cerebrovascular pathology in elders receiving home services. *Neurology* 2010;74:18-26
Annual incidence rate of Type I diabetes, children, by latitude of population centroid, reporting countries, Source: WHO data

$R^2 = 0.25$

$p < 0.0001$
NEONATAL VIT D & DIABETES*

- 10,366 northern Finnish children
- 2000 IU Vit D/d 1st year of life
- prevalence of type I diabetes assessed at age 21
- RR calculated vs. no supplementation

*Hypponen et al., Lancet 2001;358:1500–03
Testosterone

Androstenedione

E2

E1

17Beta HSD

Sulfotransferase

Sulfatase

Sulfotransferase

Sulfatase

E2, P4 sulfatase inhibitors

E2, Vit D aromatase inhibitor

Progestin (MPA) sulfatase stim
Molecular Actions of Vitamin D Contributing to Cancer Prevention

- Vitamin D or metabolites have direct inhibitory action on initiation and progression of various cancers
- Renal production of Calcitriol regulates Calcium metabolism with PTH
- Extra-renal production of Calcitriol relates to cancer risk
- Calcitriol is anti-inflammatory and turns off NFκB
- Growth Arrest of malignant cells

US Vitamin D-Sensitive Cancer Deaths

- Digestive system: 118,000
- Breast: 41,000
- Genital system: 51,000
- Urinary system: 27,000
- Lymphoma: 20,000
- Total: 257,000

46% of all cancer deaths in the US in 2007

1739 Framingham Offspring members
age: 59 yrs
follow-up: 5.4 yrs
120 individuals developed a CV event
HR calculated against 25(OH)D values > 15 ng/mL
Wang et al. Circulation 2008
Independent Association of Low Serum 25-Hydroxyvitamin D and 1.25-Dihydroxyvitamin D Levels with All-Cause and Cardiovascular Mortality

- Lowest Quartile <17.8ng/mL had a 26% increase in mortality

CHD – Vitamin D Mechanisms

- Reduces blood pressure
- Reduces risk of infection.
- Increases insulin sensitivity.
- Reduces circulating cytokines

Vitamin D and Cardiovascular Diseases

• Vitamin D insufficiency
  – Hypertension
  – Diabetes and metabolic syndrome
  – Left ventricular hypertrophy
  – Heart failure
  – Chronic vascular inflammation

## 25 (OH) Vitamin D and mortality

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All-Cause (n = 1,493)</th>
<th>Cardiovascular (n = 767)</th>
<th>Noncardiovascular (n = 726)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25-hydroxyvitamin D, nmoVL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25.0</td>
<td>2.50 (1.64–3.80)</td>
<td>3.08 (1.72–5.52)</td>
<td>2.02 (1.11–3.66)</td>
</tr>
<tr>
<td>25.0–49.9</td>
<td>1.51 (1.14–2.00)</td>
<td>1.62 (1.08–2.42)</td>
<td>1.42 (0.98–2.07)</td>
</tr>
<tr>
<td>50.0–74.9</td>
<td>1.24 (0.95–1.62)</td>
<td>1.37 (0.92–2.03)</td>
<td>1.13 (0.79–1.61)</td>
</tr>
<tr>
<td>75.0–99.9</td>
<td>1.24 (0.93–1.65)</td>
<td>1.36 (0.89–2.07)</td>
<td>1.13 (0.78–1.65)</td>
</tr>
<tr>
<td>≥100.0</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
</tbody>
</table>

- Ginde, A et al. Prospective Study of Serum 25-Hydroxyvitamin D Level, Cardiovascular Disease Mortality, and All-Cause Mortality in Older U.S. Adults *JAGS* SEPTEMBER 2009–VOL. 57, NO. 9
Vitamin D and Risk for AMI

- 18,000 men 45-75 without CV disease
- 10 year follow up
- Adjusted for confounding variables
- 25 (OH) < 15 ng/mL 2.5 x risk
- 15-30 ng/mL 2.0 x risk
- > 30 ng/mL 1.0

Vitamin D and Cardiovascular

• Check 25 (OH) Vitamin D on all cardiovascular patients
• Maintain serum level 60-80 ng/dl

Future?

Oral or IM or IV loading dose of 150,000 units for acute coronary syndromes
Infectious Diseases

- Calcitriol induces production of human cathelicidin (LL-37) a polypeptide antimicrobial
- LL-37 can fight bacterial and viral infections.

Vitamin D and infectious disease


• Rosenau MJ. Experiments to determine mode of spread of influenza *JAMA* 1919, 73:311-313
“The snot study”

- Donors: 1-3 day of disease
- Collected mucous secretions of mouth, nose, bronchi mixed together
- 1cc of the “stuff” sprayed into 10 volunteers throat and eye. No got sick.
- Recipients: Navy volunteers. None had flu the year before

- Rosenau MJ. Experiments to determine mode of spread of influenza. *JAMA* 1919, 73:311-313
Influenza and Vitamin D

- Seasonal Variation – winter
- 1,25(OH)2D acts as an immune system modulator
- Prevents excessive expression of inflammatory cytokines and increases the 'oxidative burst' potential of macrophages
- Dramatically stimulates the expression of potent anti-microbial peptides, which exist in neutrophils, monocytes, natural killer cells, and in epithelial cells lining the respiratory tract.
Prevention of Influenza A Children

- 1200 IU D3/day
- Placebo, double blind, controlled
- Relative Risk getting Influenza A
  - All treated children in study - .58
  - Not previously taking Vitamin D - .36
  - Started preschool > 3 y/o - .36