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Clinical Applications of Scientific Innovation

Annatto Tocotrienol & Geranylgeraniol

Ancient Phytonutrients for Modern Day Intervention

Barrie Tan, PhD

Annatto Tocotrienol & Geranylgeraniol

Ancient Phytonutrients for Modern Day Intervention

Barrie Tan, Ph.D.
American River Nutrition, President
Designs for Health, CSO

Presented at CASI, October 22nd, 2022

Disclaimers

- This presentation is intended for educational purposes only and does not replace independent professional judgment. Statements of fact and opinions expressed are solely those of Dr. Barrie Tan and derived from published studies.

- For Practitioner Use Only. This content is not medical advice. These statements have not been evaluated by the FDA. These products are not intended to diagnose, treat, cure or prevent any disease.

CASI Disclosure & Preamble

- These are based on clinical studies, not medical recommendations
- Trials (18-20) on pure T3, Delta-T3, Annatto T3 were all done with ARN's DeltaGold®; the only company in US making T3
- A new day for GG; ARN is the first (and only) in the world to make Annatto GG as GG-Gold®
- Human dosages are provided throughout; if animal studies, a 70kg human translation is shown
- Most studies were done in the last 5 years.

Roadmap

- Annatto History
- Vitamin E Background
- Tocotrienol (T3) Benefits
- Geranylgeraniol (GG) Background
- Geranylgeraniol Benefits
- Summary

My Discovery Story

1. **Palm Tocotrienol**
   - **1980s** in Malaysia
   - Visit to a palm plantation

2. **Rice Tocotrienol**
   - **1990s** in Thailand
   - Call from a Prince of Thailand

3. **Annatto Tocotrienol**
   - **2000s** in Peru
   - Search for giant marigolds

4. **Staying with Annatto T3 & GG**
   - **2010s** in USA
   - Production and clinical trials

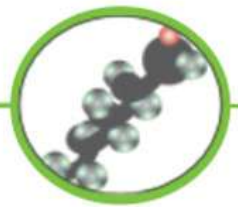
Annatto – "One Plant Wonder" from Amazonia

- Native to tropical Central & South America
- Ancient shrub of indigenous people
- Ingredient Responsibility:
  - Bixin (attractant)
  - Tocotrienol (protectant)
  - Terpenoids (repellant)

Vitamin E: A Century of Research

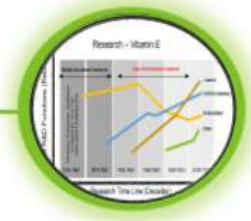
1922: Fetal reabsorption prevention

1937: Antioxidant properties

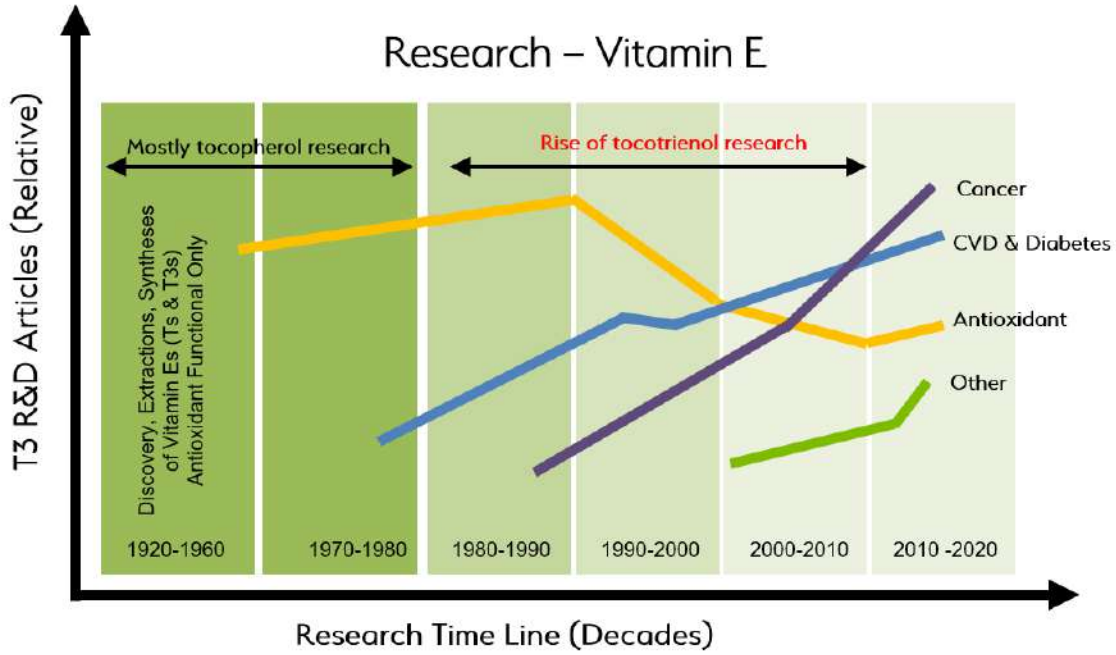


1964: Tocotrienol discovered

2003: Annatto T3 first in commerce



2022: Continued rise of T3 research

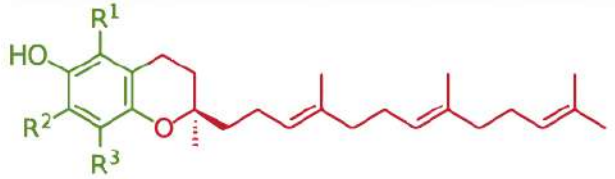


Structure – 8 Distinct Vitamers

Tocopherol (T)

- Alpha T
- Beta T
- Delta T
- Gamma T

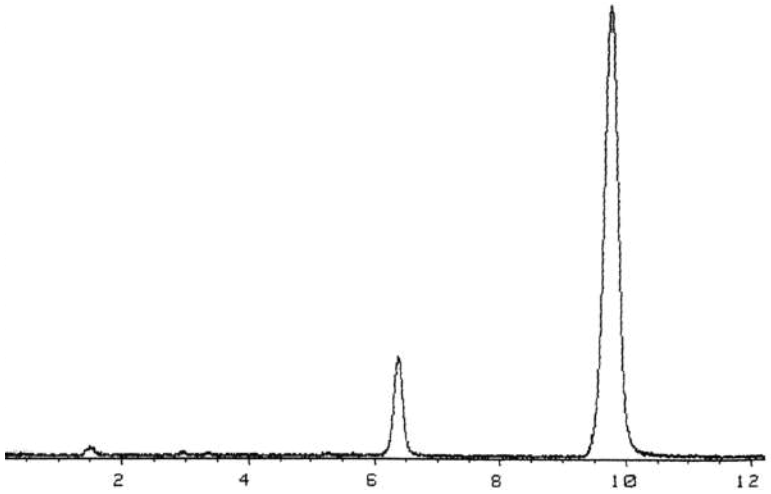
Tocotrienol (T3)

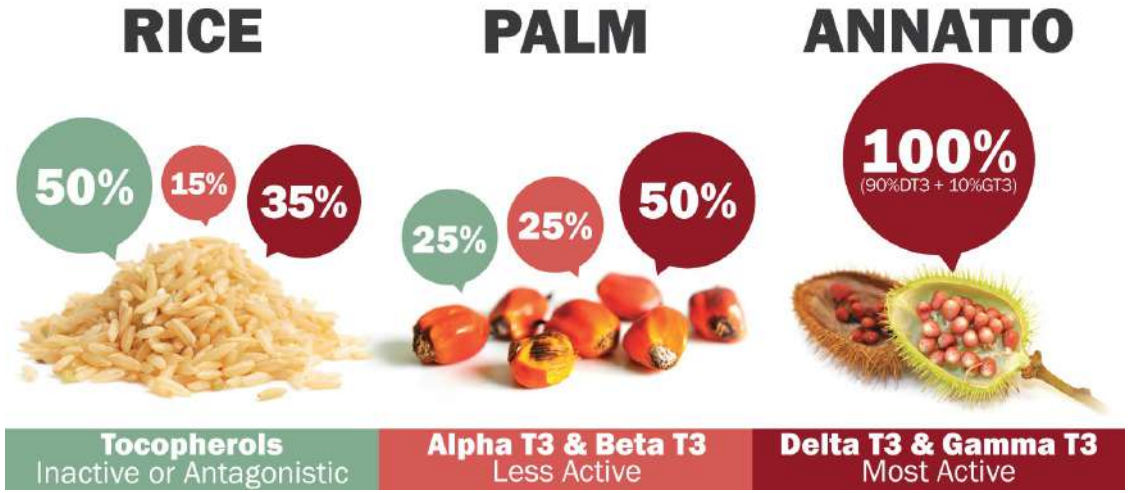


- Alpha T3
- Beta T3
- Delta T3
- Gamma T3

- Most supplements are filled with tocopherols
  - Annatto is uniquely tocotrienols

Annatto Tocotrienol





- Plant-based
- Physical process
- Made in USA

- Only 3 sources (Rice, Palm, Annatto)
- Annatto is the most potent tocotrienol

So why not Alpha-Tocopherol?

Alpha-Tocopherol Interferes with Tocotrienol

- Inhibits absorption
- Reduces adipose storage
- Compromises cholesterol and triglyceride reduction
- Attenuates cancer inhibition
- Exacerbates stroke injury

Alpha-Tocopherol Problems in Physiological Functions

- Oxidizes ‘good’ HDL and ‘bad’ LDL (50mg/d & 400mg/d)
- Increases inflammation of TNF_{α} (13–40%↑) & IL_6 (21–22%↑)
- Raises mortality and cancers (breast, prostate, lung)
- Blocks/Lowers chemo’s effectiveness (400mg/d for 1 mth)
- Increases cardiac risk in PMW (300mg/d for ~3yr)

Alpha-T puts brakes on T3 functions

Alpha-T has too many land mines

Recap

- Tocotrienol is a recent discovery
- Vitamin E = 4 tocotrienols and 4 tocopherols
- Annatto is the most potent source of tocotrienol
- Alpha-tocopherol interferes with tocotrienol
- Alpha-tocopherol is laden with land mines

Cardiovascular Disease (CVD)

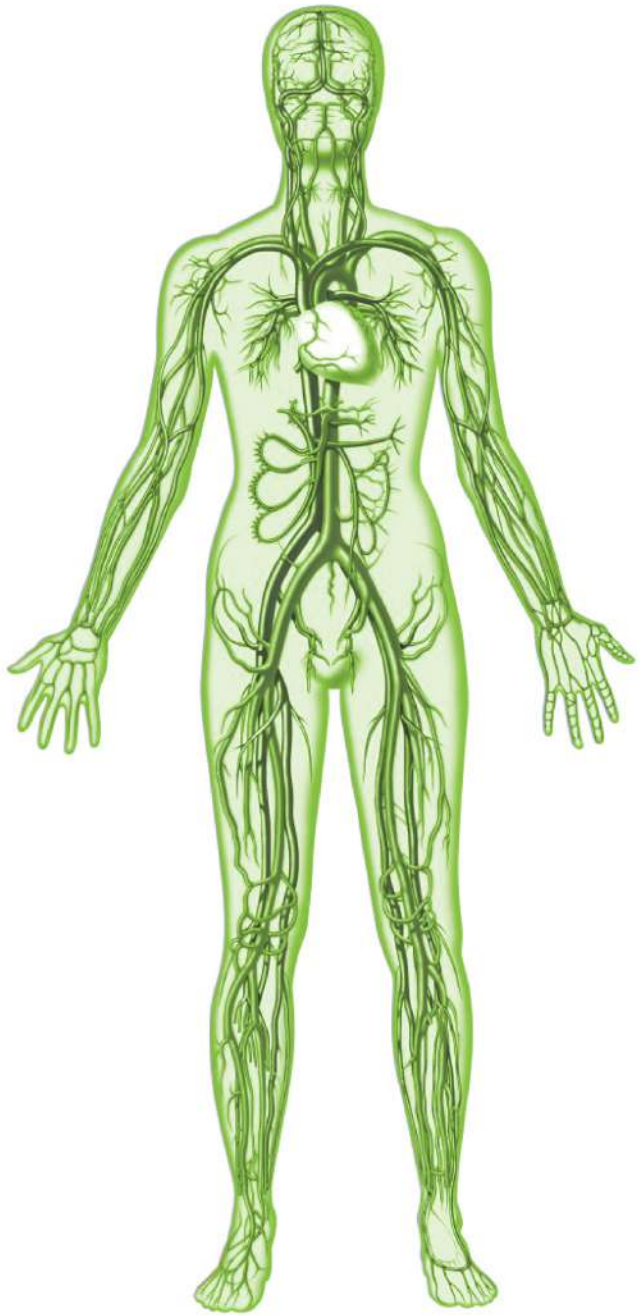
Inflammation
Lipids & Oxidation
Atherosclerosis

Annatto T3 in Preclinical Hypercholesterolemia Study

Lipid (mmol/L)	Control (2% chol. in feed)	T3 Treatment	% Change	Remarks
Cholesterol	31.5	22.8	27.6%↓	• T3 targets the down-regulation of HMGR enzyme • Neat drop because of alpha-T absence*
LDL	27.7	19.5	29.6%↓	
HDL	2.58	2.68	3.9%↑	• HDL and TG trend well with MetS control
TG	1.61	1.41	12.4%↓	
TG/HDL	0.624	0.526	15.7%↓	• Predicts small dense LDL drop

- Animal dosage translates to **135 mg/d** in a 70kg person
- Findings justify a clinical study to validate lipid findings

Shahid U, Khan DA, Noor U, Butt SH. Effect of tocotrienols on lipid profile in rabbits. JRMC. 2012;16(1):68-9.
**Hasselwander O, Kraemer K, Hoppe PP, Oberfrank U, Baldenius K, Schroeder H, et al. Effects of feeding various tocotrienol sources on plasma lipids and aortic atherosclerotic lesions in cholesterol-fed rabbits. Food Res Int'l. 2002;35:245-51.*
**Yu SG, Thomas AM, Gapor A, Tan B, Qureshi N, Qureshi AA. Dose-response impact of various tocotrienols on serum lipid parameters in 5-week-old female chickens. Lipids. 2006;41(5):453-61.*



The human body contains

30,000 miles of arteries

1.2 x the circumference of the earth

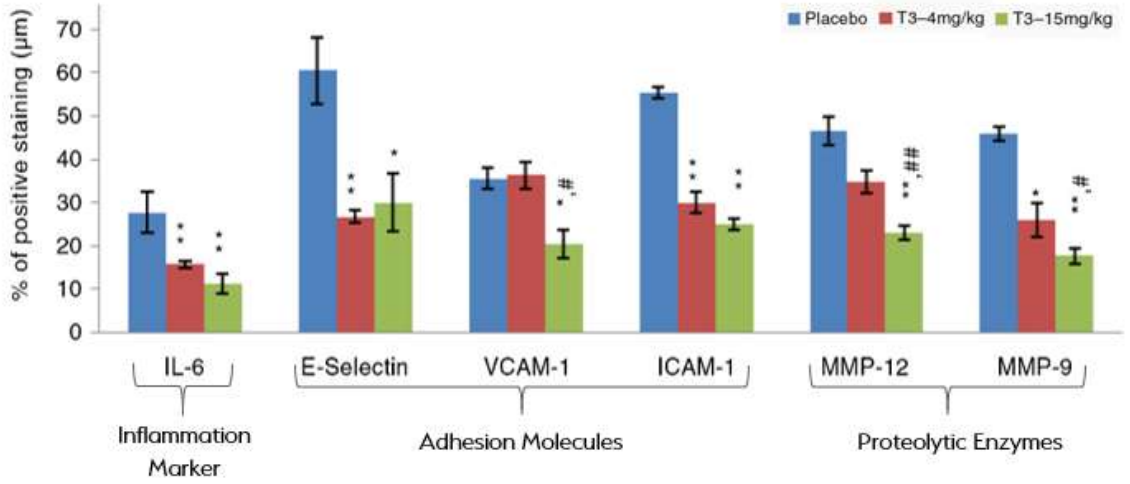
or

10 x the distance from NY to LA

Arteries carry blood cells to import oxygen & nutrients and to export carbon dioxide & wastes to

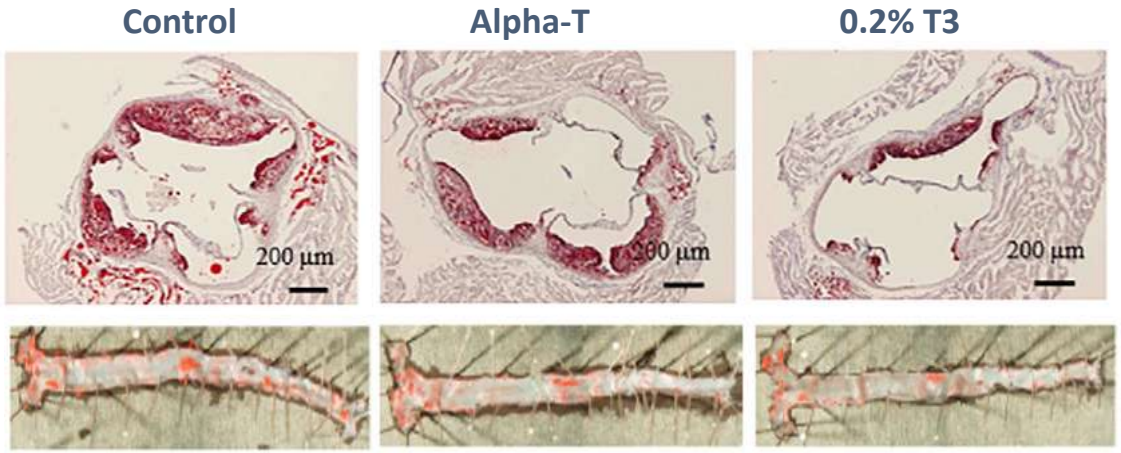
38 trillion cells

Tocotrienol Reduces Chemotaxis & Atherosclerosis



T3 Reduces "Velcro Effect" in arteries

- Inflammation Marker ↓
- Adhesion Molecules ↓
- Proteolytic Enzymes ↓



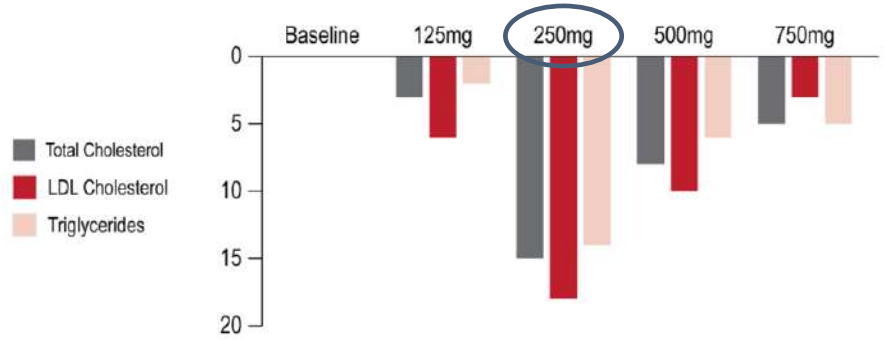
24%↓

36%↓

Translated dosage for a 70kg person: **340mg/day**

Shibata A, Kobayashi T, Asai A, Eitsuka T, Oikawa S, Miyazawa T, et al. High purity tocotrienols attenuate atherosclerotic lesion formation in apoE-KO mice. J Nutr Biochem. 2017;48:44-50. Epub 2017/08/02.
Rahman TA, Hassim NF, Zulkafli N, Muid S, Kornain NK, Nawawi H. Atheroprotective effects of pure tocotrienol supplementation in the treatment of rabbits with experimentally induced early and established atherosclerosis. Food & nutrition research. 2016;60:31525. Epub 2016/11/02.

Tocotrienol Reduces Cholesterol & Inflammation in Hypercholesterolemic Subjects

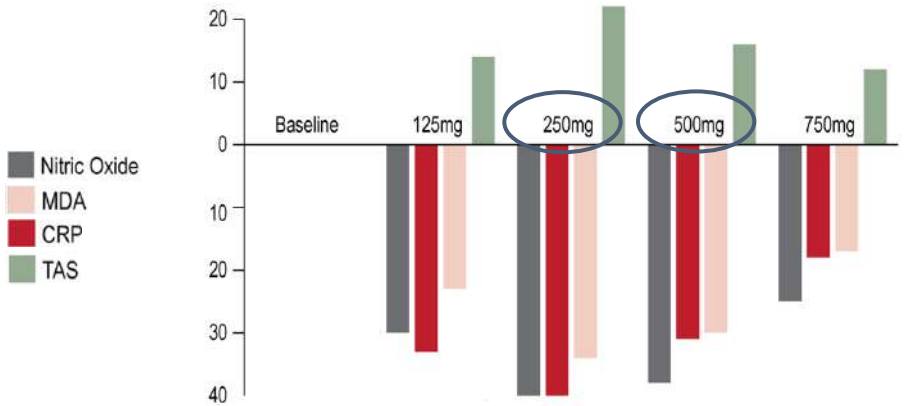


250mg/d

TC: 15%↓

LDL: 18%↓

TG: 14%↓



250mg/d & 500mg/d

CRP: 40% ↓

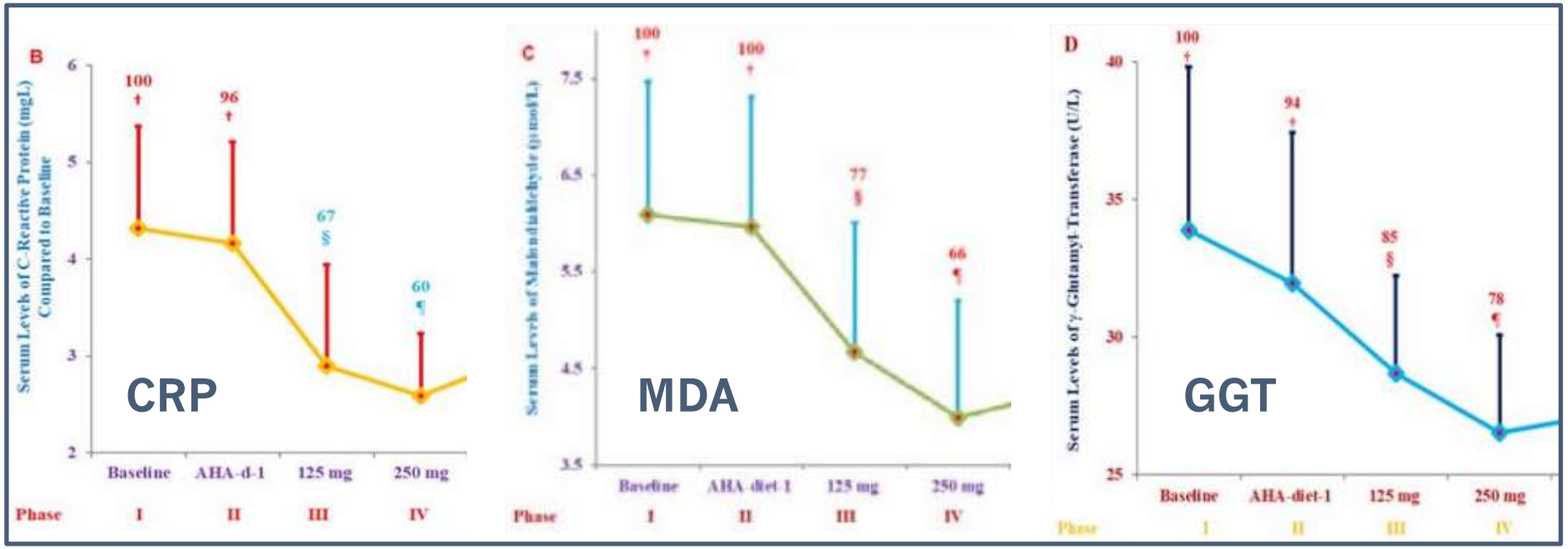
MDA 34% ↓

TAS: 22%↑

Phase duration (4 wk) and washout (2wk)

Qureshi, A.A., et al., Dose-dependent modulation of lipid parameters, cytokines, and RNA by delta-tocotrienol in hypercholesterolemic subjects restricted to AHA Step-1 diet. Brit J of Med & Med Res, 2015. 6(4): p. 351-366.
Qureshi AA, Khan DA, Mahjabeen W, Trias AM, Silswal N, Qureshi N. Impact of delta-tocotrienol on inflammatory biomarkers and oxidative stress in hypercholesterolemic subjects. Clin. Exp. Cardiology. 2015;6(4):1000367.

Tocotrienol Improves Markers in Hypercholesterolemic Subjects



- Reduces inflammation (CRP↓)
- Reduces lipid oxidation (MDA↓)
- Improves liver and heart functions (GGT↓)

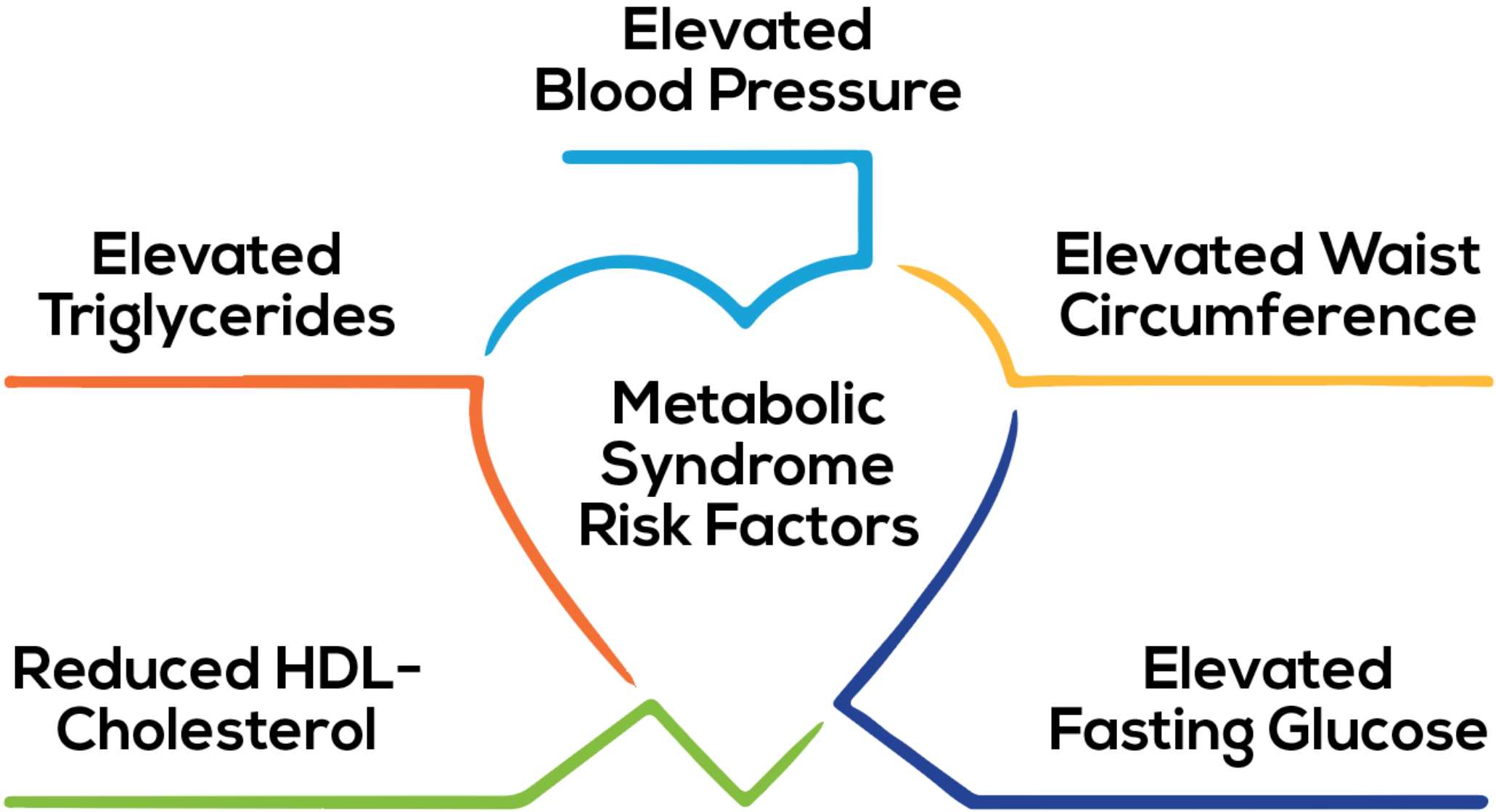
Qureshi AA. Tocotrienols: Exciting Biological and Pharmacological Properties of Tocotrienols and other Naturally Occurring Compounds, Part I. Ann Clin Case Rep. 2022; 7: 2194.

Cardiometabolic Disease (CMD)

NAFLD

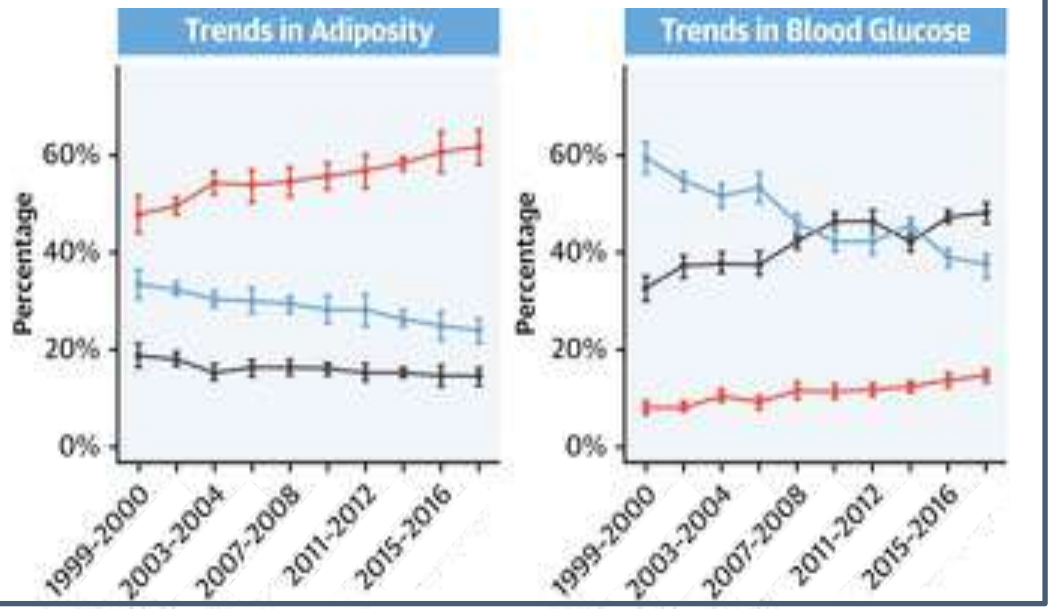
Pre-Diabetes (MetS/IR)

T2DM

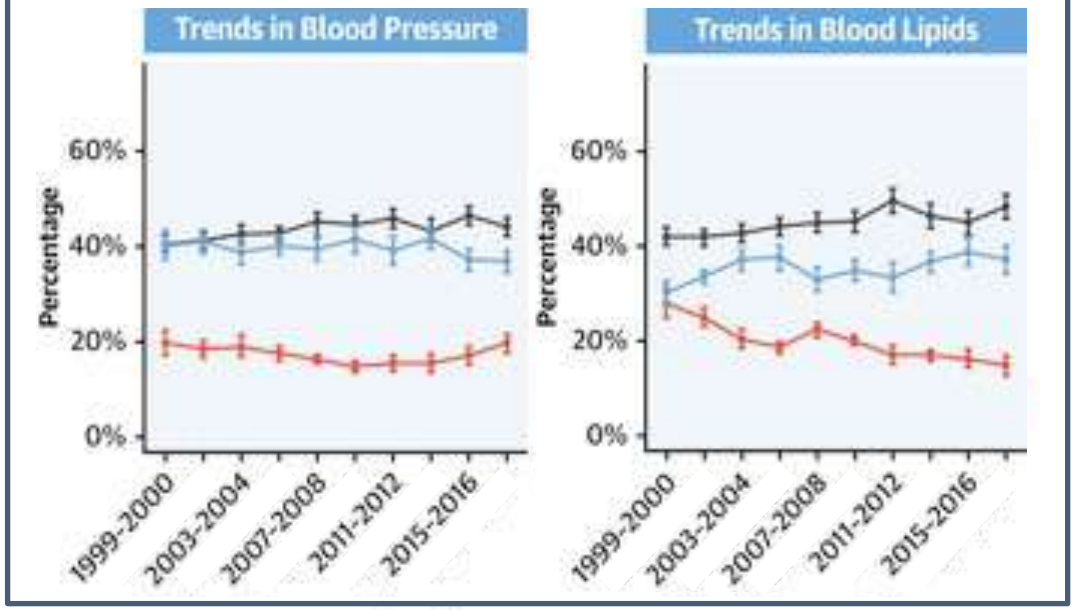


CMD – A Population **Code Blue**

Strongest Markers (BMI↑, FBG↑ or A1c↑)



Strong Markers (BP↑, Lipids↓)



- 1-in-14 (<7%) adults have optimal CM health
- MetS↑ from 36% to 47%; highest in Mexican American (52%)
- Higher in older (>65yr) than younger (20-34) adults; 78% vs 23%

Kottke TE et al (2022) Failing Cardiovascular Health. JACC 80(2) 152-154
O'Hearn M et al (2022) Trends ad Disparities in Cardiometabolic Health Among US Adults, 1999-2018. JACC 80(2)138-151
Gao Y et al (2022) Secular Trends in Risk Profiles Among Adults with Cardiovascular Disease in the United States. JACC 80(2) 126-137

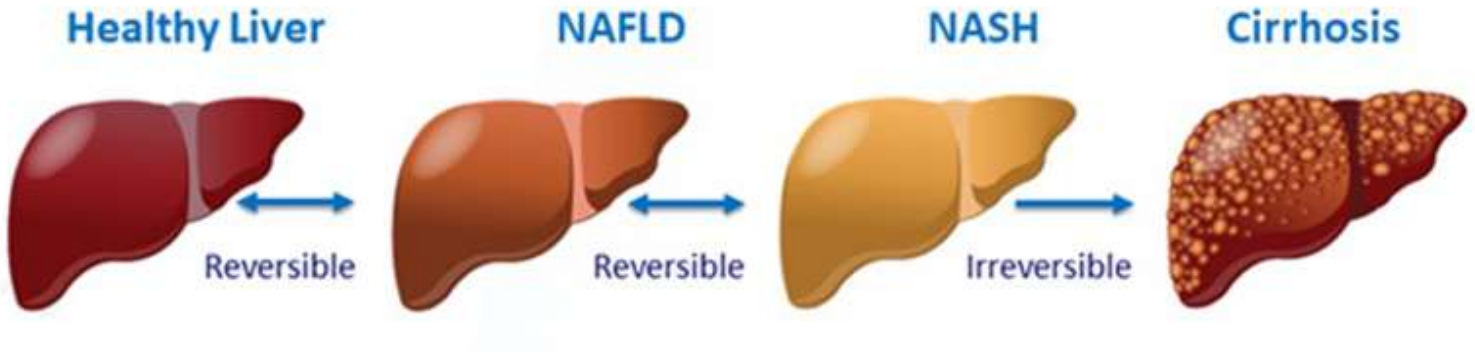
Annatto Tocotrienol and Polyphenol Combo in Healthy Elderly

Marker	Before	After	% Change	Remarks
GGT (U/L)*	26	21	**19%↓**	Ox. stress/heart failure markers dropped
CRP (mg/L)[1]	2.1	1.4	**33%↓**	Inflammation marker reduced
TAS (mmol/L)	1.45	1.61	**11%↑**	Endogenous antioxidant status improved

- Healthy men & women (60-67yo) for 4 weeks
- Combo (Tocotrienol **200mg/d**, Quercetin **300mg/d** and Resveratrol **100mg/d**)

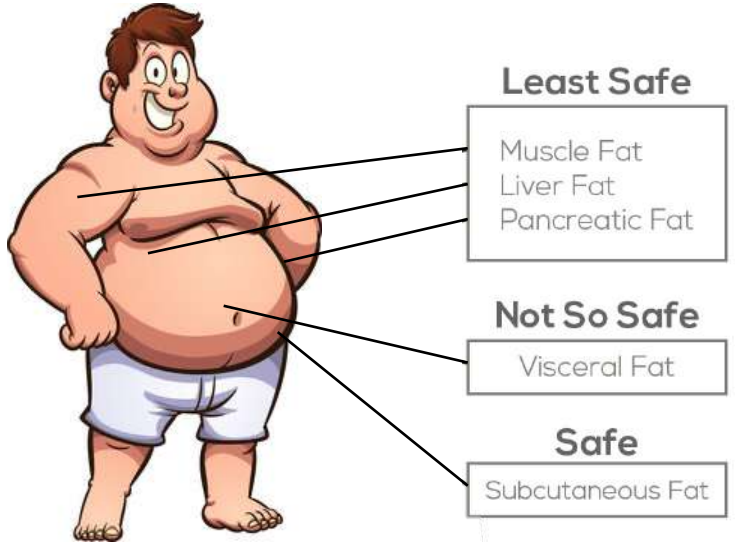
Khan DA, Mahjabeen W, Khan FA. Effects of delta-tocotrienol and polyphenol on ageing induced oxidative stress and inflammation in humans. Pakistan J Path. 2009:55-9.
**Dhingra R, Gona P, Wang TJ, Fox CS, D'Agostino RB, Sr., Vasan RS. Serum gamma-glutamyl transferase and risk of heart failure in the community. Arterioscler Thromb Vasc Biol. 2010;30(9):1855-60. Epub 2010/06/12*
**Onur S, Niklowitz P, Jacobs G, Nothlings U, Lieb W, Menke T, et al. Ubiquinol reduces gamma glutamyltransferase as a marker of oxidative stress in humans. BMC research notes. 2014;7:427. Epub 2014/07/06.*
1 Ridker PM. From C-Reactive Protein to Interleukin-6 to Interleukin-1: Moving Upstream To Identify Novel Targets for Atheroprotection. Circ Res. 2016;118(1):145-56. Epub 2016/02/04.
1 Ridker PM. Clinician's Guide to Reducing Inflammation to Reduce Atherothrombotic Risk: JACC Review Topic of the Week. Journal of the American College of Cardiology. 2018;72(25):3320-31. Epub 2018/11/13.

Global NAFLD Prevalence is 20-30%



- 90-100m Americans have NAFLD (30% of adults)
- 20% transition to non-alcoholic steatohepatitis (NASH)
- Complications: Inflammation, Fibrosis, Infections, Cancer

Livers look like those of heavy drinkers but they are not



Das, S.L., et al., *Newly diagnosed diabetes mellitus after acute pancreatitis: a systematic review and meta-analysis. Gut, 2013.*
Gukovsky, I., et al., *Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. Gastroenterology, 2013. 144(6): p. 1199-209 e4.*
Fiore, K. *Losing Fatty Liver Cuts Diabetes Risk. MedPage Today 2013.*
Le, M., Devaki, P., Ha, N., Jun, D., Te, H., Cheung, R. and Nguyen, M. (2017). Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. PLOS. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0173499.
Estes, C., Razavi, H., Loomba, R., Younossi, Z. and Sanyal, A. (2018). "Modeling the Epidemic of Nonalcoholic Fatty Liver Disease Demonstrates an Exponential Increase in Burden of Disease." Hepatology, 67(1).

Delta-Tocotrienol Improves Fatty Liver in NAFLD Patients

Three RDBPC trials in patients (80-100) on **300mg twice daily**

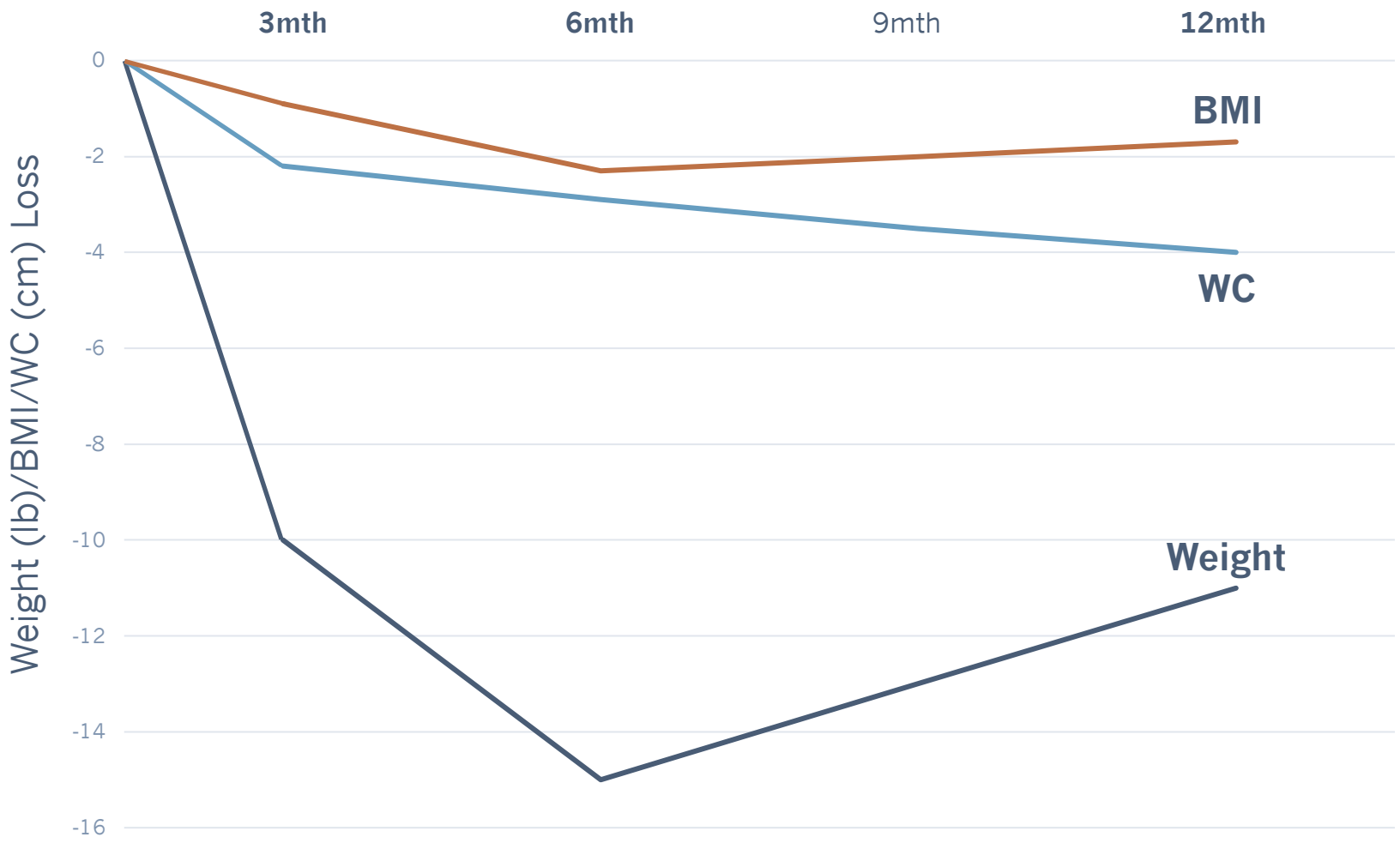
	Study Parameters	3 mth*	6 mth+	12 mth^
Patient	Weight Loss (lb)	10	15	11
	BMI (kg/m^2)	30.7 → 29.2	30.6 → 28.3	32.8 → 31.1
	Waist Circumference (cm)	100.2 → 98.0	100.4 → 97.5	106.5 → 102.5
HP & Doctors	Triglycerides	9.9%↓	13% ↓	16%↓
	ALT & AST	15% - 16%↓	18% - 21%↓	29%↓
	ALP & GGT	8% - 17%↓	17%↓	37%↓
	hsCRP	18%↓	21%↓	38%↓
	MDA	14%↓	19%↓	13%↓
Specialists	FIB-4, NFS (**fibrosis**)	------	11.4%↓	20.1%↓
	CK18 (**cirrhosis, liver cell death**)	------	18.3%↓	19.8%↓
	FLI (**steatosis**)	11.1%↓	15%↓	17%↓
	HOMA-IR homeostatic model assessment of insulin resistance	------	15%↓	24%↓

**Pervez MA, Khan DA, Ijaz A, Khan S. Effects of Delta-tocotrienol Supplementation on Liver Enzymes, Inflammation, Oxidative stress and Hepatic Steatosis in Patients with Nonalcoholic Fatty Liver Disease. The Turkish journal of gastroenterology : the official journal of Turkish Society of Gastroenterology. 2018;29(2):170-6. Epub 2018/05/12.*

+ Pervez, Muhammad Amjad, et al. "Delta-Tocotrienol Supplementation Improves Biochemical Markers of Hepatocellular Injury and Steatosis in Patients with Nonalcoholic Fatty Liver Disease: A Randomized, Placebo-Controlled Trial." Complementary Therapies in Medicine, vol. 52, 2020, p. 102494., doi:10.1016/j.ctim.2020.102494.

^ Pervez MA, Khan DA, Slobia AUR, Ijaz A. Delta-tocotrienol supplementation improves biochemical markers of hepatocellular injury and steatosis in patients with nonalcoholic fatty liver disease: A randomized, placebo-controlled trial. Complement Ther Med. 2020 Aug;52:102494.

Delta-Tocotrienol Sustains Weight Loss in NAFLD Patients



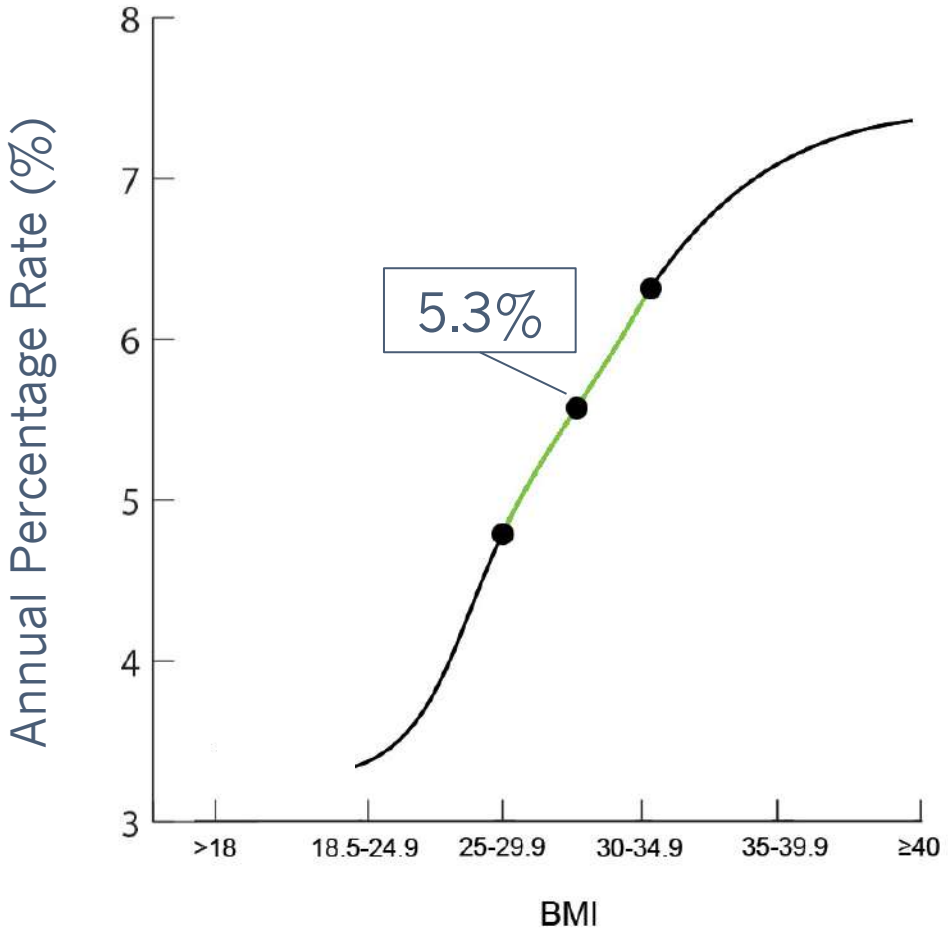
Delta-Tocotrienol Improves Glycemic Control in Prediabetics

- Clinical Trial
  - RDBPC, 3mth, DeltaGold® **300mg/day**
  - 77 prediabetic patients (FPG 100-124mg/dl; A1c 5.7-6.4%)

	T3-Supplemented	Placebo
A1C	6.04 → 5.77% (**4.5%↓**)	5.99 → 6.04%(**0.8%↑**)
FPG	4.3%↓	0.2%↓
Insulin	11.2%↓	3.0%↓
HOMA-IR	15.1%↓	3.7%↓

Suleman, F., et al., Effects of delta-tocotrienol supplementation on glycaemic control in individuals with prediabetes: A randomized controlled study. J Pak Med Assoc, 2022. 72(1): p. 4-7.

Delta-Tocotrienol Averts Pre-D-to-T2DM Conversion



- CDC says progression of A1c-confirmed PreD-to-T2DM is **5.3%** in older adults
- US has **95m** Pre-diabetics
- APR approximates BMI

Window of opportunity (green section)

Evidence of T3 in PreD-to-T2DM non-progression

Koyama, A.K., et al., Progression to Diabetes Among Older Adults With Hemoglobin A1c-Defined Prediabetes in the US. JAMA Netw Open, 2022. 5(4): p. e228158.

Non Reversion of MetS and Central Obesity

Combo (**Delta-T3 500mg/d + Resveratrol 300mg/d**) for 6mth on MetS patients

Primary Endpoints:

- SBP/DBP (5.2%/5.8%↓)
- WC ♂/♀ (3.7%/4.6%↓)
- Weight Loss (4.6% or 9.3lb↓)
- TG (16%↓)
- HDL ♂/♀ (4.0%/5.1%↑)

Secondary Endpoints:

- Inflammation (15-17%↓)
- Adiponectin (11%↑)
- VCAM (27%↓)
- MDA (7.5%↓)
- TAS (23%↑)

Conclusions of (Delta-T3 + Resveratrol) Combo:

- Reversed MetS & Central Obesity (40%) and Metabolites (55%)
- Decreased Relative Risk of reversion to MetS & Central Obesity (both by 70%)
- Improved MetS of young adults (18-43yr)

Fatima S, Khan D et al.δ-Tocotrienol in Combination with Resveratrol Improves the Cardiometabolic Risk Factors and Biomarkers in Patients with Metabolic Syndrome: A Randomized Controlled Trial .Metabolic Syndrome and Related Disorders. Epub ahead of print

Delta-Tocotrienol Helps T2DM Patients

Randomized, double-blind, placebo-controlled trials
6mth study with 60-110 patients

Study Parameters	Results (**250mg/d**)	Results (**500mg/d**)
Glucose	6.8%↓	7%↓
HbA1c	6.3%↓	8%↓
Insulin	7.6%↓	9%↓
HOMA-IR	13.1%↓	14%↓
Triglycerides	10.3%↓	8%↓
hsCRP	10.1%↓	12%↓
IL-6	15.9%↓	9%↓
TNF-alpha	13.7%↓	14%↓
MDA	8.8%↓	11%↓

Mahjabeen, W., et al. (2021). "Effects of delta-tocotrienol supplementation on Glycemic Control, oxidative stress, inflammatory biomarkers and miRNA expression in type 2 diabetes mellitus: A randomized control trial." Phytother Res.
Qureshi, A. A., et al. (2021). "A Novel Mixture of δ-Tocotrienol, Vitamin D3, Resveratrol (NS-3) Significantly Decreases Diabetes Biomarkers Including Inflammatory in People with Type 2 Diabetes." J Diab Clin Stud 5(1).
Values not adjusted against placebo

Bone Health

Many Animal Studies
One Clinical Trial

Preclinical studies conclude that annatto tocotrienols…

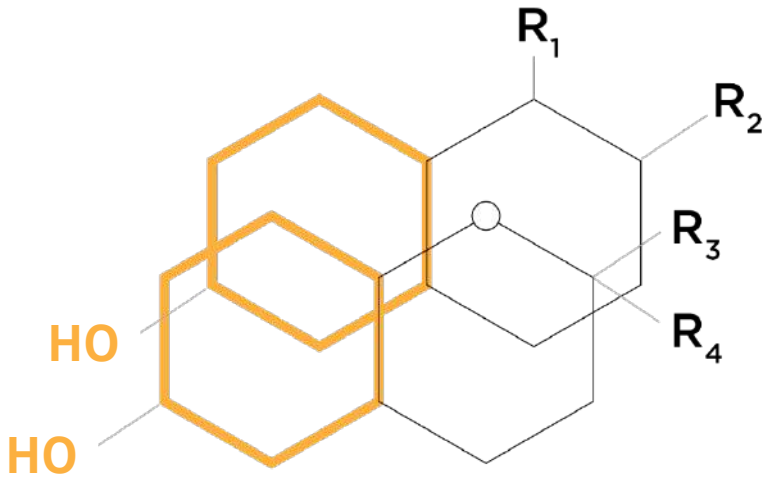
- Ovariectomized model – for women > 45 years
- Orchiectomized model – for men > 65 years
- HFHC diet (MetS & OP) model – both men & women
- Cigarette smoke & nicotine – toxicant induced
- Chronic steroid medicines – drug induced
- Fractured bone model – healing process

… promote bone formation following excessive resorption.

Chin, K.Y. and S. Ima-Nirwana, Effects of annatto-derived tocotrienol supplementation on osteoporosis induced by testosterone deficiency in rats. Clin Interv Aging, 2014. 9: p. 1247-59.
Chin, K.Y., et al., annatto tocotrienol improves indices of bone static histomorphometry in osteoporosis due to testosterone deficiency in rats. Nutrients, 2014. 6(11): p. 4974-83.
Abdul-Majeed, S., N. Mohamed, and I.N. Soelaiman, Effects of tocotrienol and lovastatin combination on osteoblast and osteoclast activity in estrogen-deficient osteoporosis. Evid Based Complement Alternat Med, 2012. 2012: p. 960742.
Ibrahim, N., et al., Targeted delivery of lovastatin and tocotrienol to fracture site promotes fracture healing in osteoporosis model: micro-computed tomography and biomechanical evaluation. PLoS One, 2014. 9(12): p. e115595.
Wong SK, Chin KY, Suhaimi FH, Ahmad F, Ima-Nirwana S. Exploring the potential of tocotrienol from Bixa orellana as a single agent targeting metabolic syndrome and bone loss. Bone. 2018;116:8-21. Epub 2018/07/11.
Shen CL, Kaur G, Wanders D, Sharma S, Tomison MD, Ramalingam L, et al. annatto-extracted tocotrienols improve glucose homeostasis and bone properties in high-fat diet-induced type 2 diabetic mice by decreasing the inflammatory response. Scientific reports. 2018;8(1):11377. Epub 2018/07/29.

Intro to Bone Health

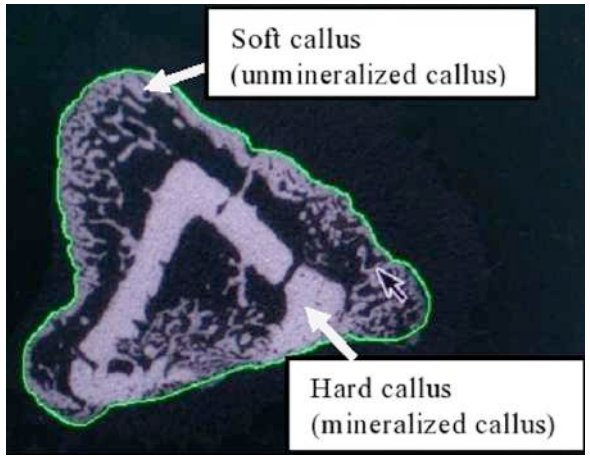
- Osteoblasts
  - Make up new bone
  - Produce collagen matrix [for structural framework]
- Osteoclasts
  - Break down old bone
  - Produce acids & enzymes [to dissolve minerals & protein]
- Menopause (45-55yo)
  - Estrogen↓ → Oxidative stress↑
- Oxidative Stress causes
  - Osteoblastic disruption → Formation↓
  - Osteoclastic differentiation → Resorption↑

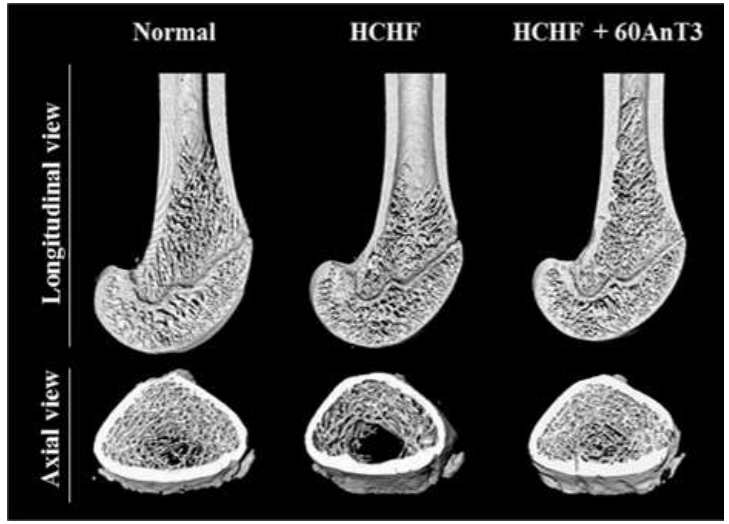


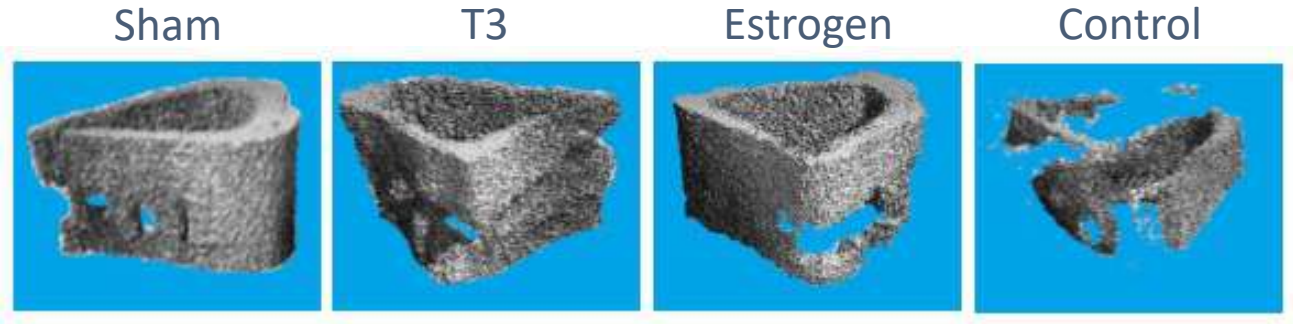
Superimposition of Estradiol & Tocotrienol Structures

Chin, K.Y. and S. Ima-Nirwana, Effects of annatto-derived tocotrienol supplementation on osteoporosis induced by testosterone deficiency in rats. Clin Interv Aging, 2014. 9: p. 1247-59.
Chin, K.Y., et al., annatto tocotrienol improves indices of bone static histomorphometry in osteoporosis due to testosterone deficiency in rats. Nutrients, 2014. 6(11): p. 4974-83.
Abdul-Majeed, S., N. Mohamed, and I.N. Soelaiman, Effects of tocotrienol and lovastatin combination on osteoblast and osteoclast activity in estrogen-deficient osteoporosis. Evid Based Complement Alternat Med, 2012. 2012: p. 960742.
Ibrahim, N., et al., Targeted delivery of lovastatin and tocotrienol to fracture site promotes fracture healing in osteoporosis model: micro-computed tomography and biomechanical evaluation. PLoS One, 2014. 9(12): p. e115595.

Tocotrienol Aids Mineralization in Fracture and MetS







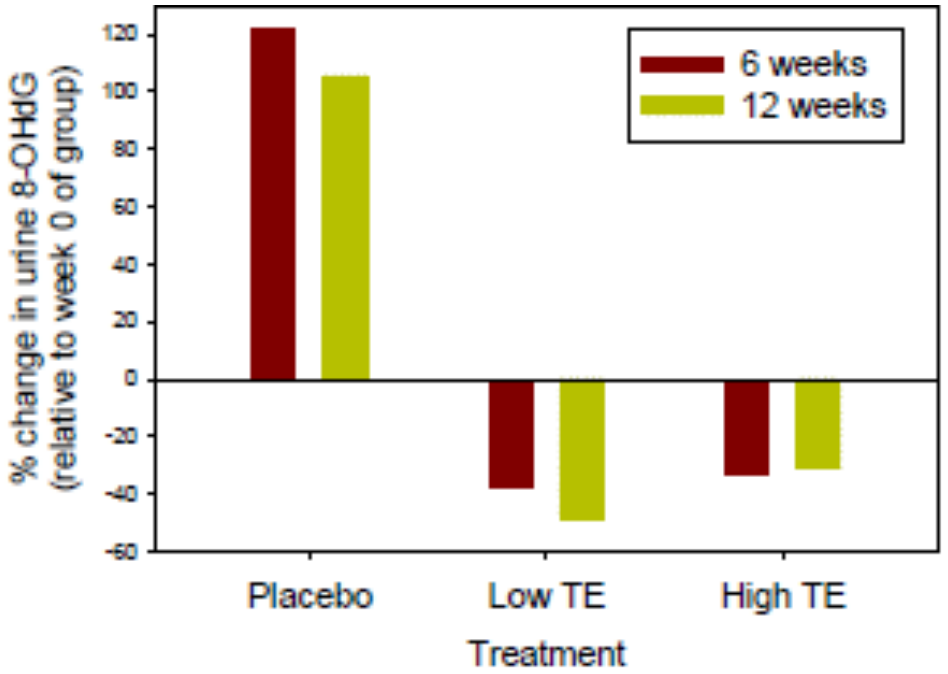
- Prevented fracture recurrence, increased callus stiffness
- Improved trabecular bone microstructure and bone strength
- Increased osteoblast surface
- Improved metabolic syndrome parameters

70kg human equivalent dose: **680mg/day**

Chin, K.Y. and S. Ima-Nirwana, Effects of annatto-derived tocotrienol supplementation on osteoporosis induced by testosterone deficiency in rats. Clin Interv Aging, 2014. 9: p. 1247-59.
Chin, K.Y., et al., annatto tocotrienol improves indices of bone static histomorphometry in osteoporosis due to testosterone deficiency in rats. Nutrients, 2014. 6(11): p. 4974-83.
Wong SK, Chin KY, Suhaimi FH, Ahmad F, Ima-Nirwana S. Exploring the potential of tocotrienol from Bixa orellana as a single agent targeting metabolic syndrome and bone loss. Bone. 2018;116:8-21. Epub 2018/07/11.
Shen CL, Kaur G, Wanders D, Sharma S, Tomison MD, Ramalingam L, et al. annatto-extracted tocotrienols improve glucose homeostasis and bone properties in high-fat diet-induced type 2 diabetic mice by decreasing the inflammatory response. Scientific reports. 2018;8(1):11377. Epub 2018/07/29.
Ibrahim, N., et al., Targeted delivery of lovastatin and tocotrienol to fracture site promotes fracture healing in osteoporosis model: micro-computed tomography and biomechanical evaluation. PLoS One, 2014. 9(12): p. e115595.

First Annatto Tocotrienol Bone Clinical Trial

- PMW in 3 treatments for 12 wks (placebo, **300mg/d** and **600mg/d**)
- Benefits to PMW at 300mg/d are:
  - Bone building (BALP/NTX) - 115%↑
  - Bone resorption (sRANKL/OPG) - 13%↓
  - Oxidative stress (8-OHdG) - 49%↓



- No adverse effects on liver and kidney function or quality of life
- PMW obesity trial for 24 wks (placebo and **300mg/d** T3)

Aryaie, Amir, et al. "Actions of Annatto-Extracted Tocotrienol Supplementation on Obese Postmenopausal Women: Study Protocol for a Double-Blinded, Placebo-Controlled, Randomised Trial." BMJ Open, vol. 10, no. 3, 2020, doi:10.1136/bmjopen-2019-034338.
Shen CL, Yang S, Tomison MD, Romero AW, Felton CK, Mo H. Tocotrienol supplementation suppressed bone resorption and oxidative stress in postmenopausal osteopenic women: a 12-week randomized double-blinded placebo-controlled trial. Osteoporos Int. 2018. Epub 2018/01/14.
Shen CL, Wang S, Yang S, Tomison MD, Abbasi M, Hao L, et al. A 12-week evaluation of annatto tocotrienol supplementation for postmenopausal women: safety, quality of life, body composition, physical activity, and nutrient intake. BMC complementary and alternative medicine. 2018;18(1):198. Epub 2018/06/30.

DeltaGold® Tocotrienol Summaries

- Annatto tocotrienol is the most potent anti-inflammatory vitamin E
- Decreases lipid oxidation and atherosclerosis in CVD
- Balances metabolites and reduces markers in CMD
- Mitigates steatosis and fibrosis in NAFLD/NASH; WC/BMI/Weight loss
- Prevents osteopenia and obesity
- Recommended Dosage (with meal)
  - Healthy: 100-300mg/day
  - Mild Chronic Condition: 300-400mg/day
  - Advanced Chronic Condition: 400-600mg/day

Geranylgeraniol

Introduction

Benefits

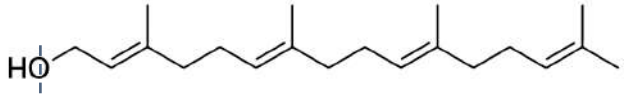
Summary

GG is a Building Block in **Plant** & **Mammal**

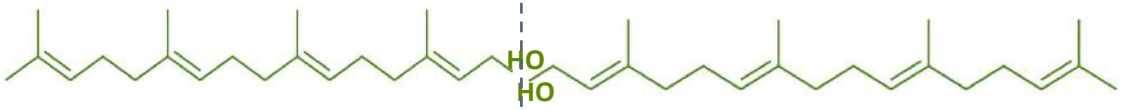
Essential:
- Growth and differentiation
- Apoptosis and survival

Endogenous:
- Plants and mammals
- For all stages of life

GG

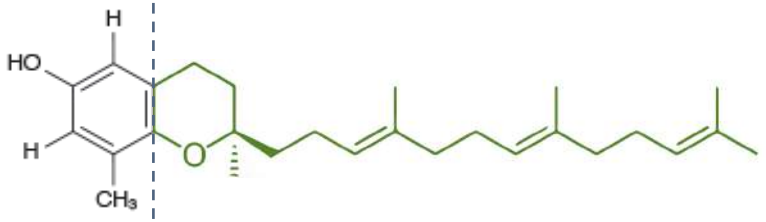


Carotenoid
[2 GG]



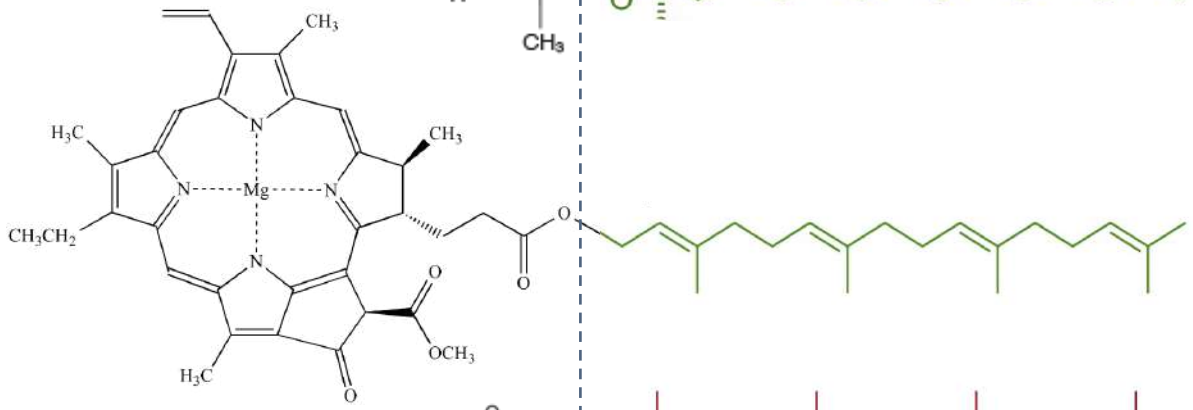
(Plant)

Tocotrienol
[1 GG]



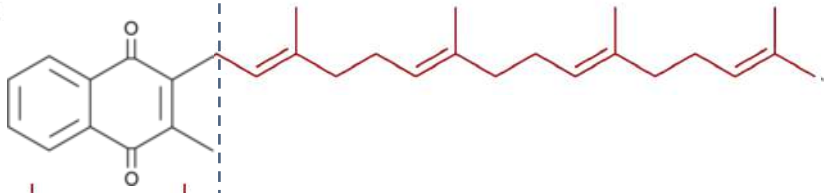
(Plant)

Chlorophyll
[1 GG]



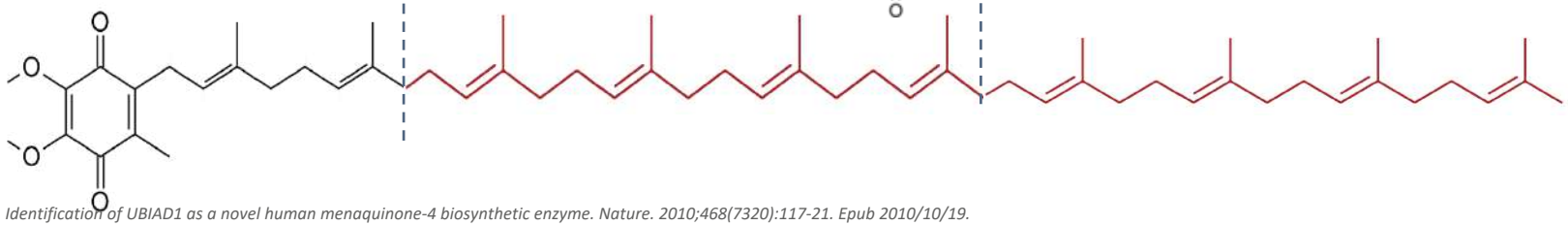
(Plant)

Vitamin K2 (MK4)
[1 GG]



(Mammal)

CoQ10
[2.5 GG]



(Mammal)

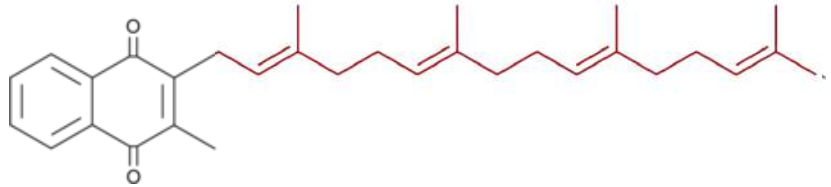
Nakagawa K, Hirota Y, Sawada N, Yuge N, Watanabe M, Uchino Y, et al. Identification of UBIAD1 as a novel human menaquinone-4 biosynthetic enzyme. Nature. 2010;468(7320):117-21. Epub 2010/10/19.
Hirota Y, Nakagawa K, Sawada N, Okuda N, Suhara Y, Uchino Y, et al. Functional characterization of the vitamin K2 biosynthetic enzyme UBIAD1. PloS one. 2015;10(4):e0125737. Epub 2015/04/16.
Huang H, Levin EJ, Liu S, Bai Y, Lockless SW, Zhou M. Structure of a membrane-embedded prenyltransferase homologous to UBIAD1. PLoS biology. 2014;12(7):e1001911. Epub 2014/07/23.

Ubiquity and Bioavailability of GG

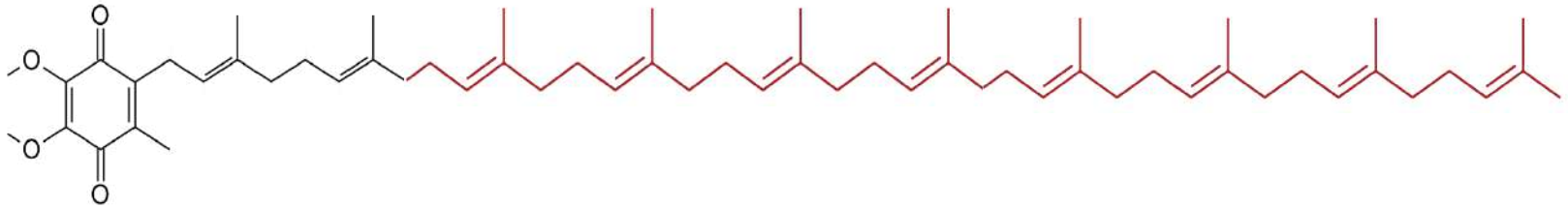
MK4 and CoQ10 are ubiquitous because GG is ubiquitous

GG

Vitamin K2 (MK4)
[1 GG]



CoQ10
[2.5 GG]

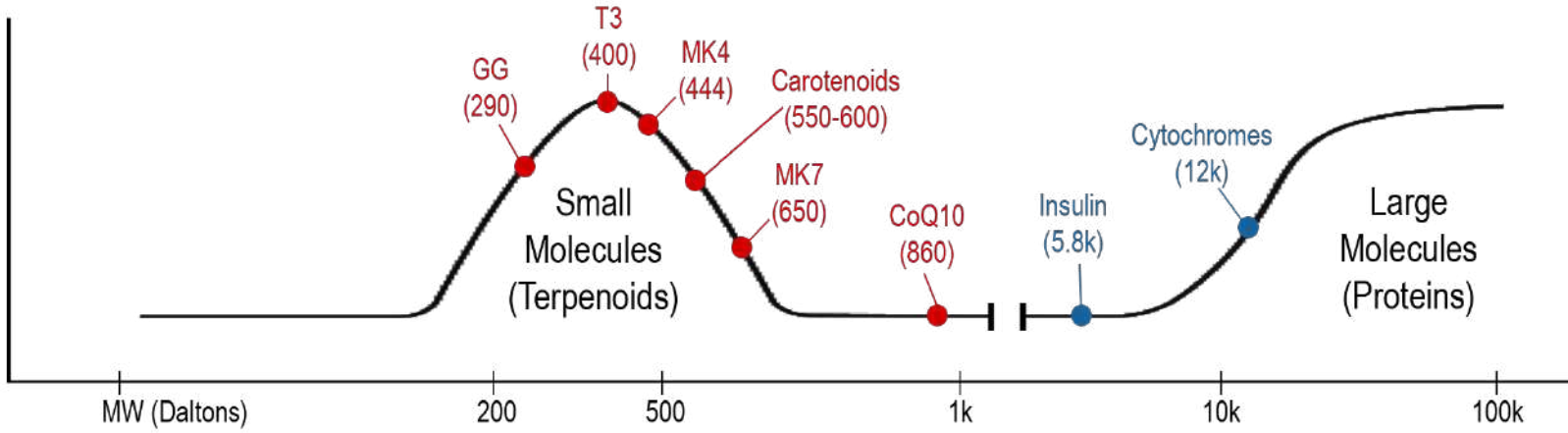


CoQ10 (MW863)

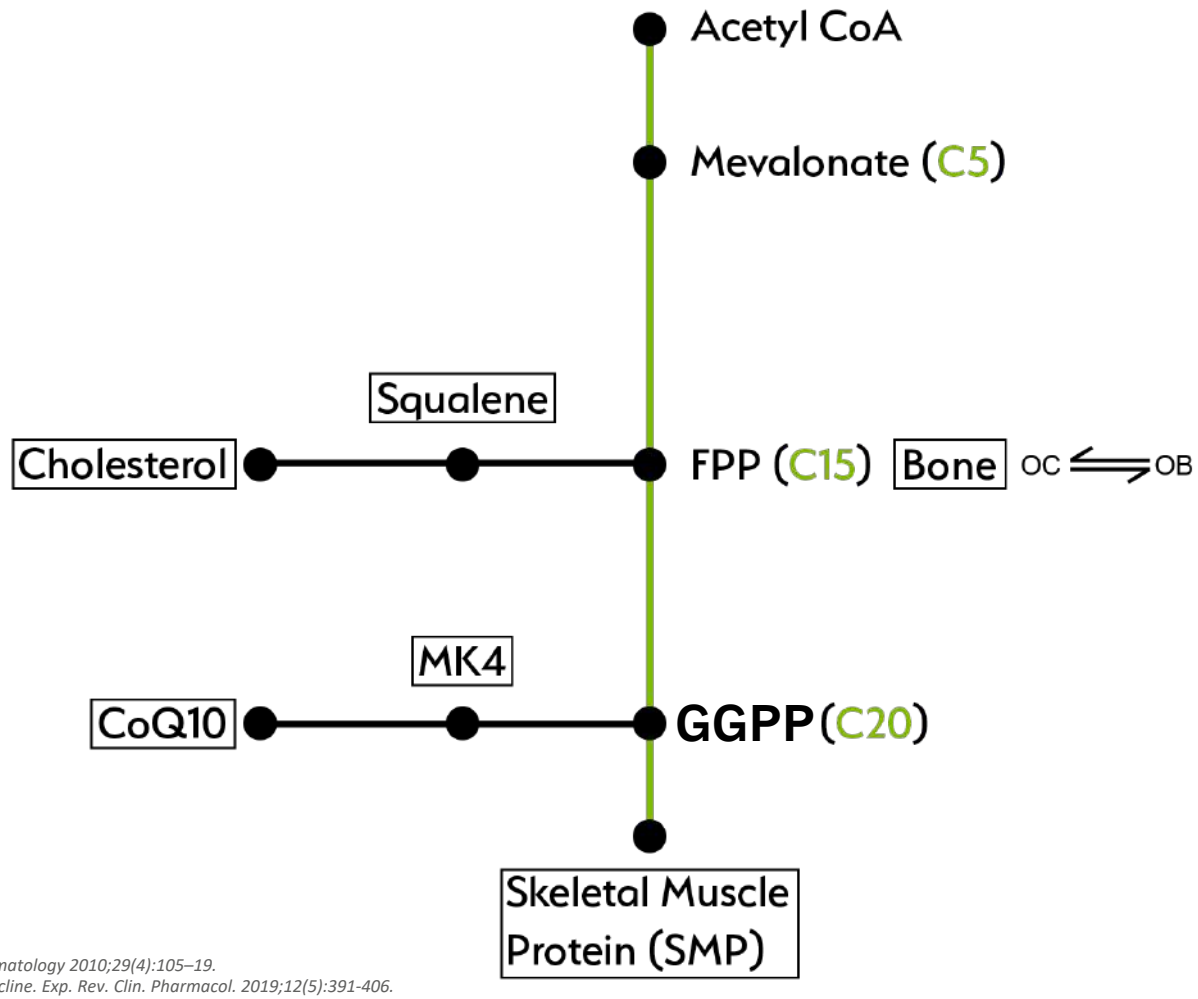
Difficult to absorb & get into membranes
Largest of the "small molecules"

GG (MW290)

Easy to absorb into cellular membranes
Smallest of the "small molecules"

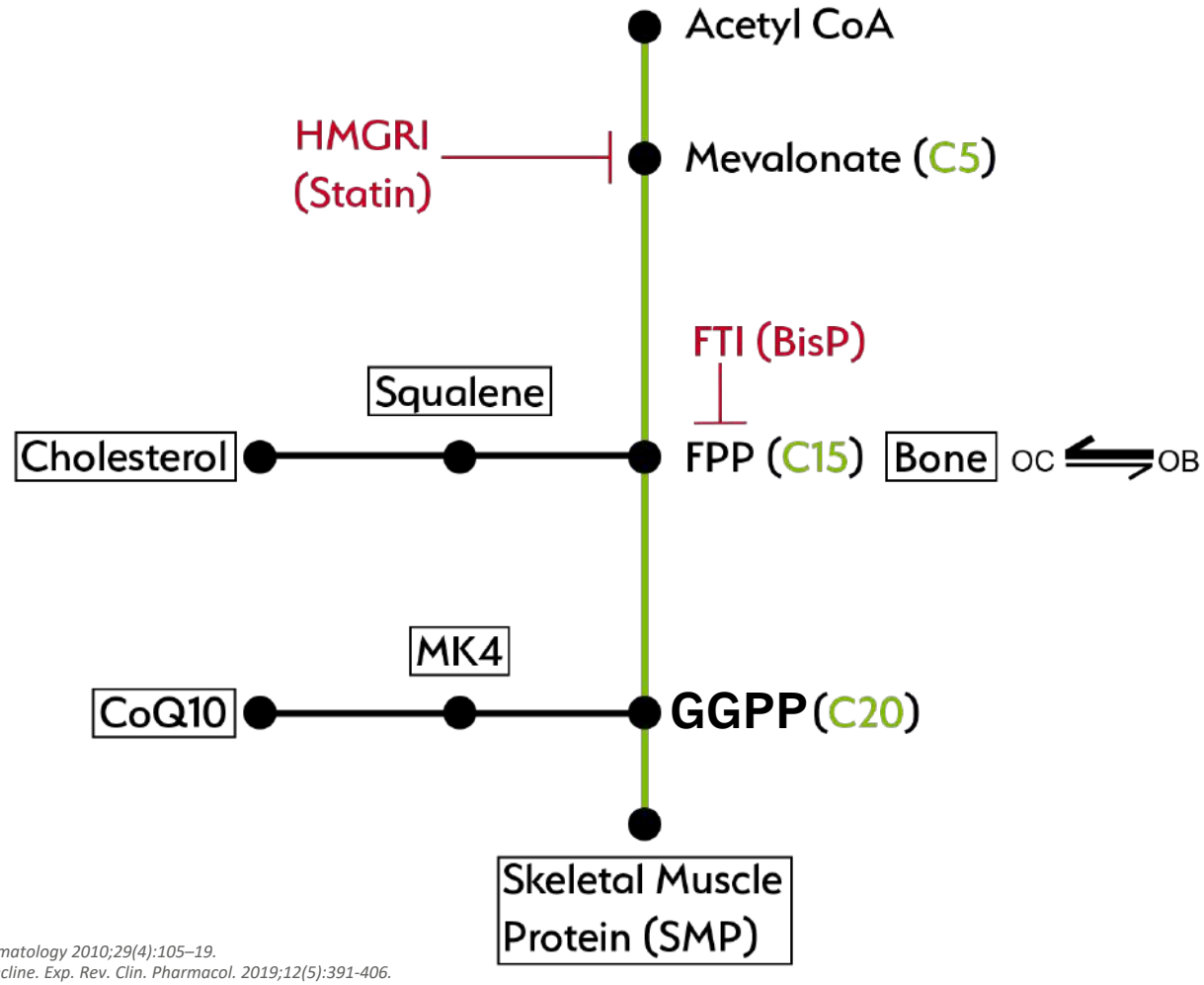


GG is Central to Mevalonate Pathway



C. Buettner et al. Statin-Induced Myopathy and its Management, Fig. 1. CML – Rheumatology 2010;29(4):105–19.
B Tan, F. Rosenfeldt, et al. Evidence and Mechanisms for Statin-induced Cognitive Decline. Exp. Rev. Clin. Pharmacol. 2019;12(5):391-406.
Hooff GP, Wood WG, Muller WE, Eckert GP. Isoprenoids, small GTPases and Alzheimer's disease. Biochim Biophys Acta. 2010;1801(8):896-905. Epub 2010/04/13.

GG is Easily Blocked in the Mevalonate Pathway

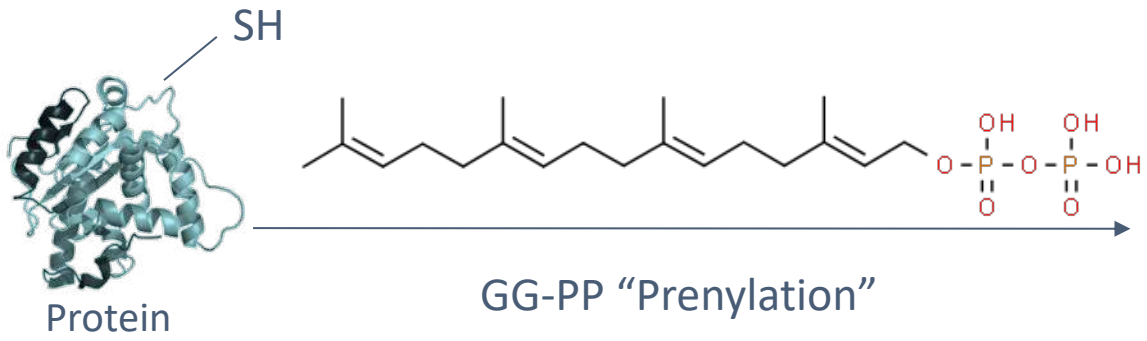


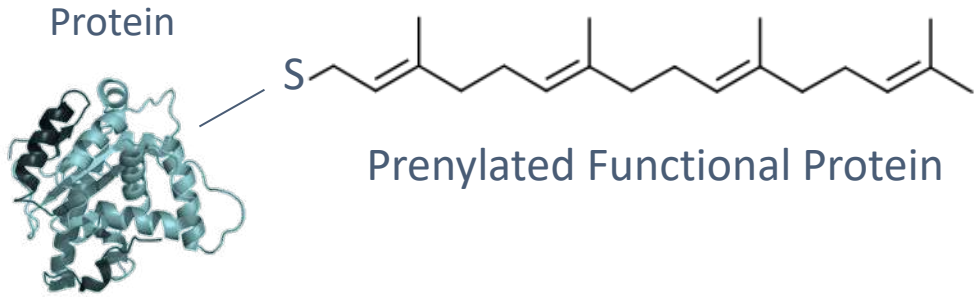
C. Buettner et al. Statin-Induced Myopathy and its Management, Fig. 1. CML – Rheumatology 2010;29(4):105–19.
B Tan, F. Rosenfeldt, et al. Evidence and Mechanisms for Statin-induced Cognitive Decline. Exp. Rev. Clin. Pharmacol. 2019;12(5):391-406.
Hooff GP, Wood WG, Muller WE, Eckert GP. Isoprenoids, small GTPases and Alzheimer's disease. Biochim Biophys Acta. 2010;1801(8):896-905. Epub 2010/04/13.

GG Benefits

Protein Synthesis
Testosterone, CoQ10 & MK4 Synthesis
Increase in Bone Strength
Mitigation of Drug Side Effects

Protein Synthesis

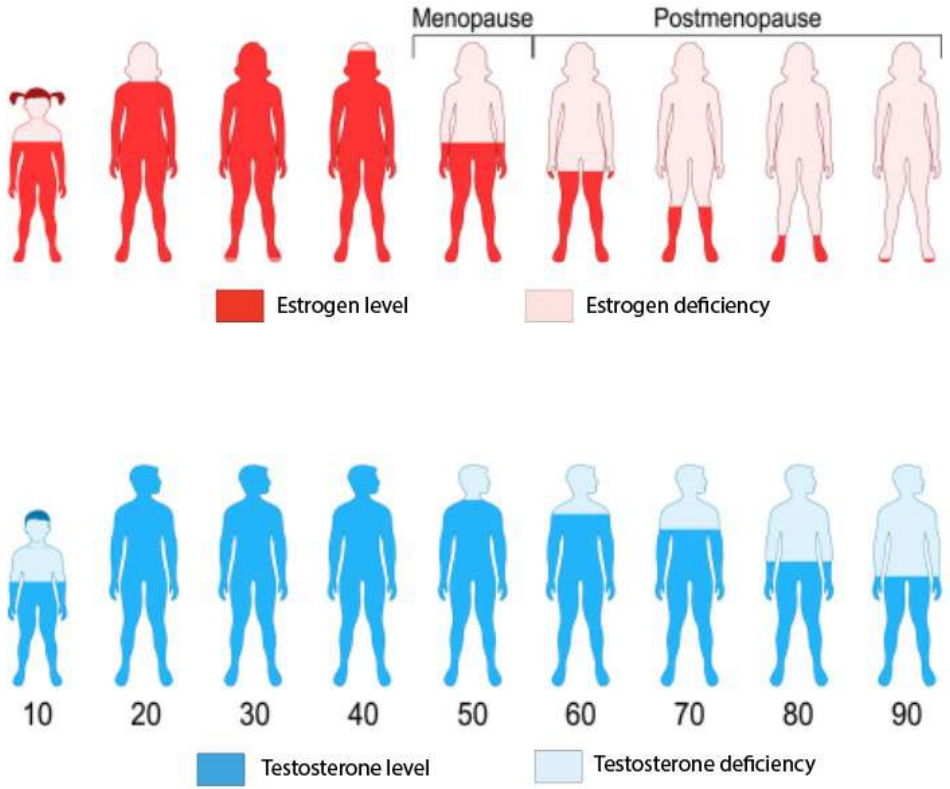




- Radiolabeled GG was found within protein fractions
- Clinical significance of protein synthesis:
  - **Myopathy** (drug-related); statins & bisphosphonates; typically >30yr old
  - **Sarcopenia** (age-related); 40yr and older

- Proteins with GG (1-2%) requirements:
  - Rho – ATP/energy production
  - Rab – Membrane/signaling protein (GG acts as anchor)
  - Rac – Regulation of cell movement (cytoskeleton & muscle)

Hormone Synthesis & GG



- Leopold Ruzicka: 1939 Nobel Prize for testosterone and GG synthesis
- GG stimulates steroid synthesis of both Testosterone and Progesterone‡
  - Mechanism of GG involvement yet unknown
  - Potentially due to GG-MK4 synthesis

**Leopold Ruzicka – Facts. NobelPrize.org. Nobel Media AB 2020. Tue. 7 Apr 2020. https://www.nobelprize.org/prizes/chemistry/1939/ruzicka/facts/*

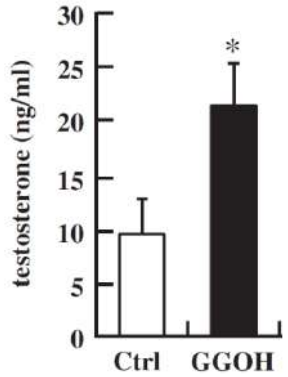
Ruzicka L, Firmenich G. Zur Kenntnis der Diterpene (37. Mitteilung). Synthese des aliphatischen Diterpenalkohols Geranyl-geraniol. Helv. 1939;22(1):392-396. (This one if Barrie wants to make reference to Ruzicka being the first to synthesize GG)

‡Ho HJ, Shirakawa H, Giriwono PE, Ito A, Komai M. A novel function of geranylgeraniol in regulating testosterone production. Bioscience, biotechnology, and biochemistry. Jun 2018;82(6):956-962.

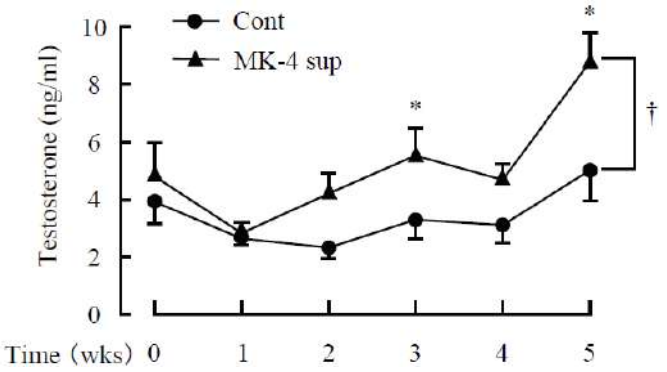
‡Ho HJ, Shirakawa H, Yoshida R, et al. Geranylgeraniol enhances testosterone production via the cAMP/protein kinase A pathway in testis-derived I-10 tumor cells. Bioscience, biotechnology, and biochemistry. 2016;80(4):791-797.

GG Stimulates Testosterone (T) Synthesis

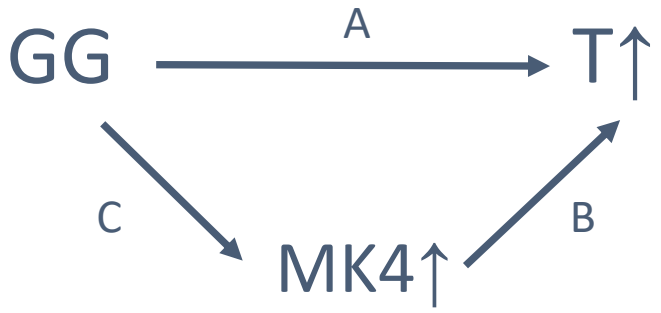
Human equivalent dose: **100mg/d**



GG supplemented diet **enhances** plasma testosterone levels in male rats



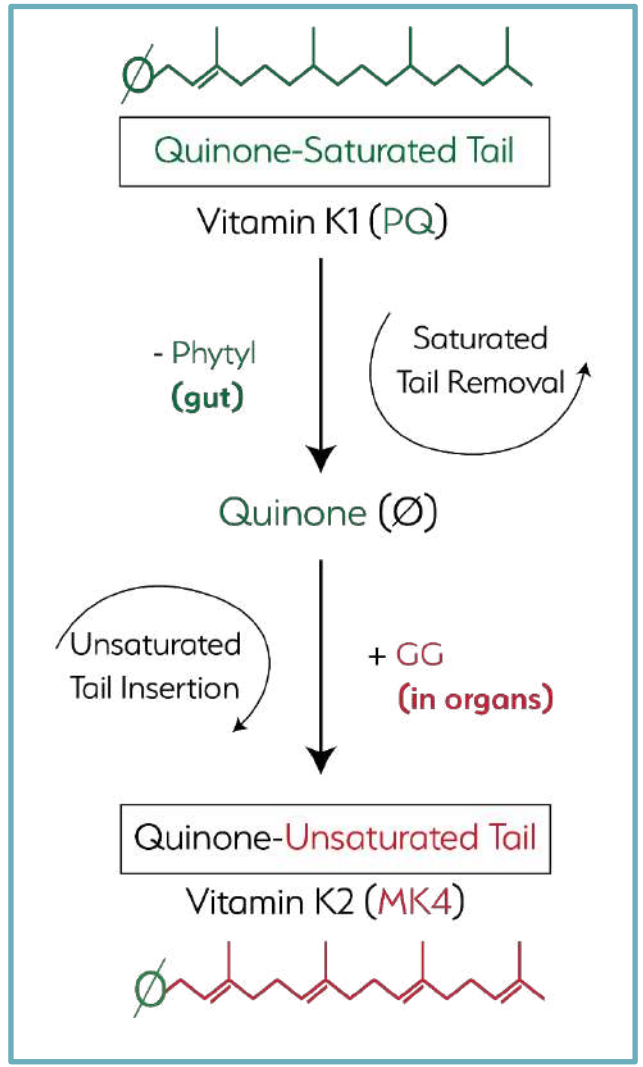
MK4 supplemented diet **enhances** testosterone production in rat testis



Ho HJ, Shirakawa H, et al. A novel function of geranylgeraniol in regulating testosterone production. Biosci Biotechnol Biochem. 2018; 82(6):956-962
Ito, A., Shirakawa, H., Takumi, N., Minegishi, Y., Ohashi, A., Howlader, Z.H., Ohsaki, Y., Sato, T., Goto, T. and Komai, M., 2011. Menaquinone-4 enhances testosterone production in rats and testis-derived tumor cells. Lipids in health and disease, 10(1), p.158.
Al Rajabi A, Booth SL, Peterson JW, Choi SW, Suttie JW, Shea MK, et al. Deuterium-labeled phylloquinone has tissue-specific conversion to menaquinone-4 among Fischer 344 male rats. J Nutr. 2012;142(5):841-5. Epub 2012/03/23.
Nakagawa, Kimie, et al. Identification of UBIAD1 as a novel human menaquinone – 4 biosynthetic enzyme. Nature. 11/4/2010; 468, 117-121.

GG Reduces Bone Resorption & Synthesizes Vitamin K2

The inhibitory effect of vitamin K_2 on bone resorption may be related to its side chain (1995) and this GG → MK4 was proven (2010-2013)



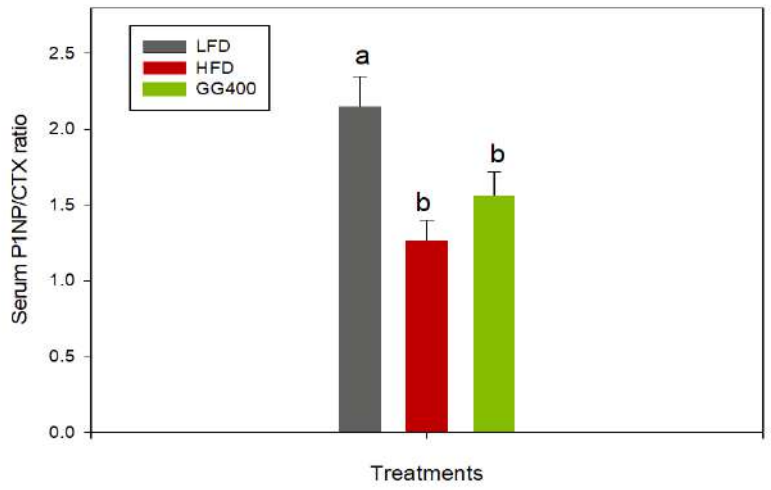
- GG → vitamin K_2; MK4 is an endogenous nutrient
- GG inhibited bone resorption similarly to vitamin K_2
- Human equivalent dose = **160mg/d**

Nakagawa K, et al. Identification of UBIAD1 as a novel human MK4 biosynthetic enzyme. Nature (Nov 4, 2010) 468, 117-121.
Hirota Y, et al. Menadione (Vitamin K3) is a catabolic product of oral phylloquinone (Vitamin K1) in the intestine and circulating precursor of tissue menaquinone4 (vitamin K2) Jbiol. Chem. (Nov 15,2013) 288, (46) 33071-33080.
Rajabi AA, et al. Deuterium-labeled phylloquinone has tissue-specific conversion menaquinone 4 among Fischer 344 male rats. J Nutr. (March 21, 2012) 142, 841-845.
Hara K, Akiyama Y, Nakamura T, Murota S, Morita I. The inhibitory effect of vitamin K2 (menatetrenone) on bone resorption may be related to its side chain. Bone. 1995;16(2):179-84. Epub 1995/02/01.
Harshman SG, Shea MK, Fu X, et al. Atorvastatin Decreases Renal Menaquinone-4 Formation in C57BL/6 Male Mice. J Nutr. Mar 1 2019;149(3):416-421.

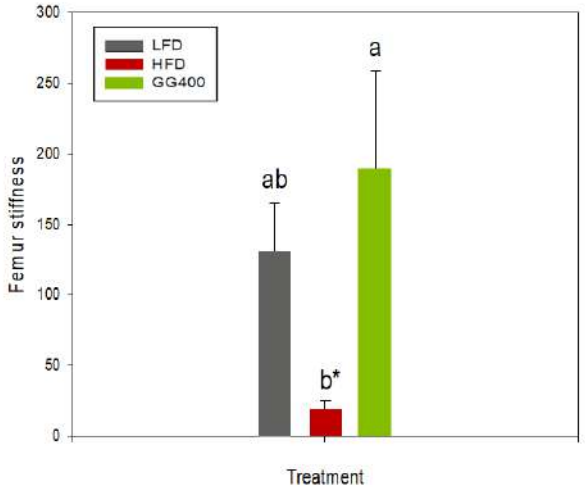
GG Improves Bone Turnover and Increases Bone Stiffness

T2DM mouse model (osteoporosis-inducing)

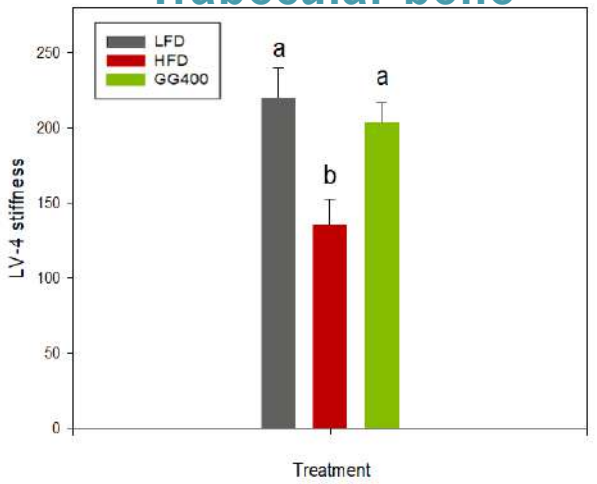
Femur bone



Femur bone



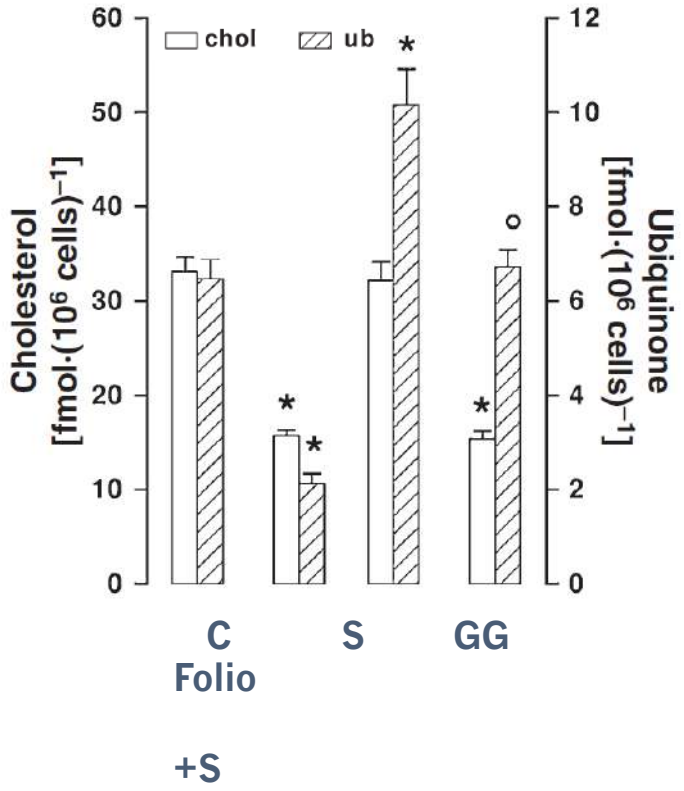
Trabecular bone



- GG protected bone microstructure and quality
- GG significantly improved bone turnover
- Human equivalent dose: **160mg/day**

Shen, C.L. et. al. 2017. Dietary geranylgeraniol improved glucose homeostasis, bone turnover biomarkers, and bone quality in C57BL/6J mice with obesity-associated type 2 diabetes mellitus. 10th International Symposium on Nutritional Aspects of Osteoporosis.

GG Supports Respiration and Synthesizes CoQ10

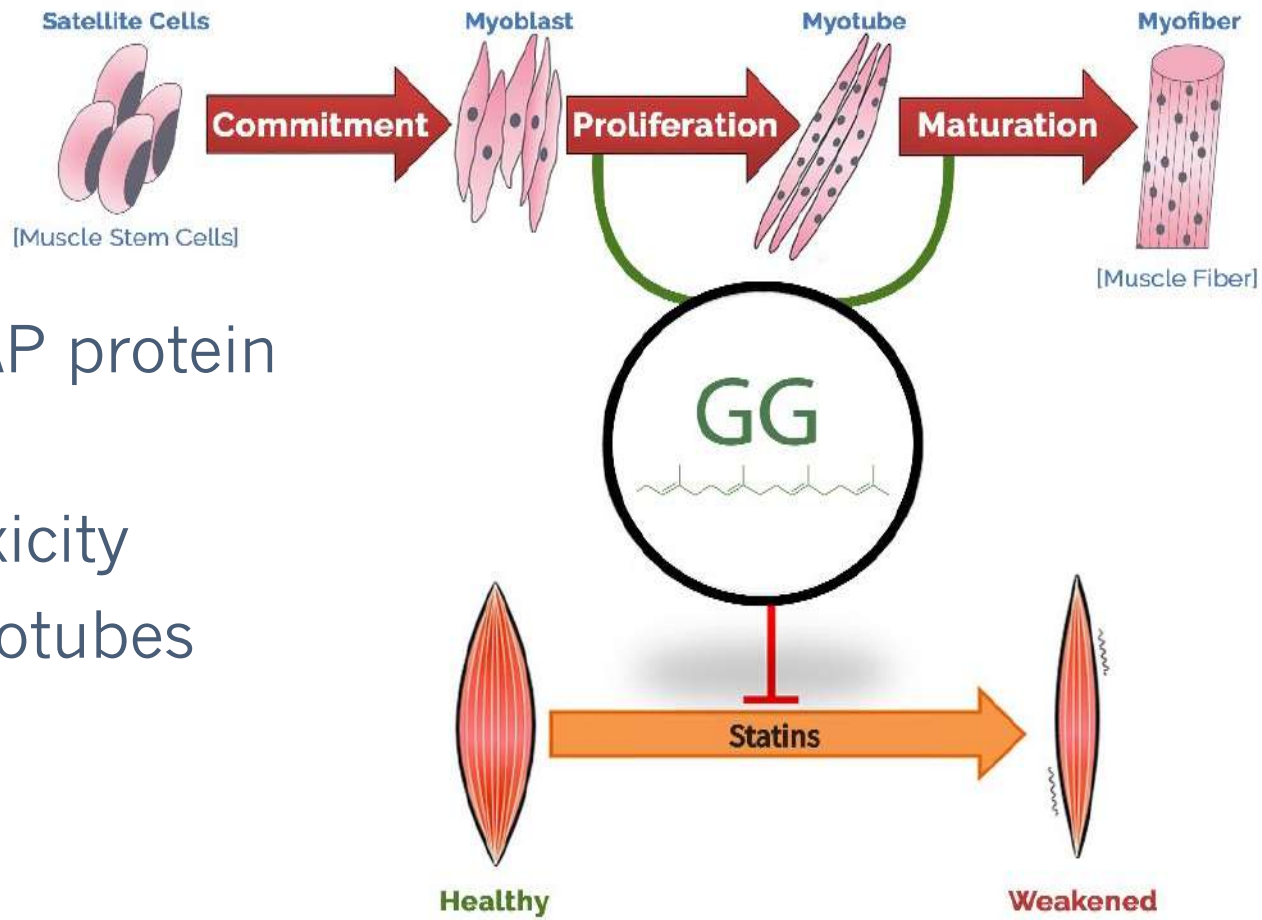


- GG reversed statins' negative impact
  - Increased mitochondrial respiration
  - Restored ubiquinone synthesis
  - Continued cholesterol reduction

GG by itself increases CoQ10 synthesis

Marcuzzi A, Piscianz E, Zweyer M, Bortul R, Loganes C, Girardelli M, et al. Geranylgeraniol and Neurological Impairment: Involvement of Apoptosis and Mitochondrial Morphology. International journal of molecular sciences. 2016;17(3):365. Epub 2016/03/16.
Campia I, Lussiana C, Pescarmona G, Ghigo D, Bosia A, Riganti C. Geranylgeraniol prevents the cytotoxic effects of mevastatin in THP-1 cells, without decreasing the beneficial effects on cholesterol synthesis. British journal of pharmacology. 2009;158(7):1777-86. Epub 2009/11/06.

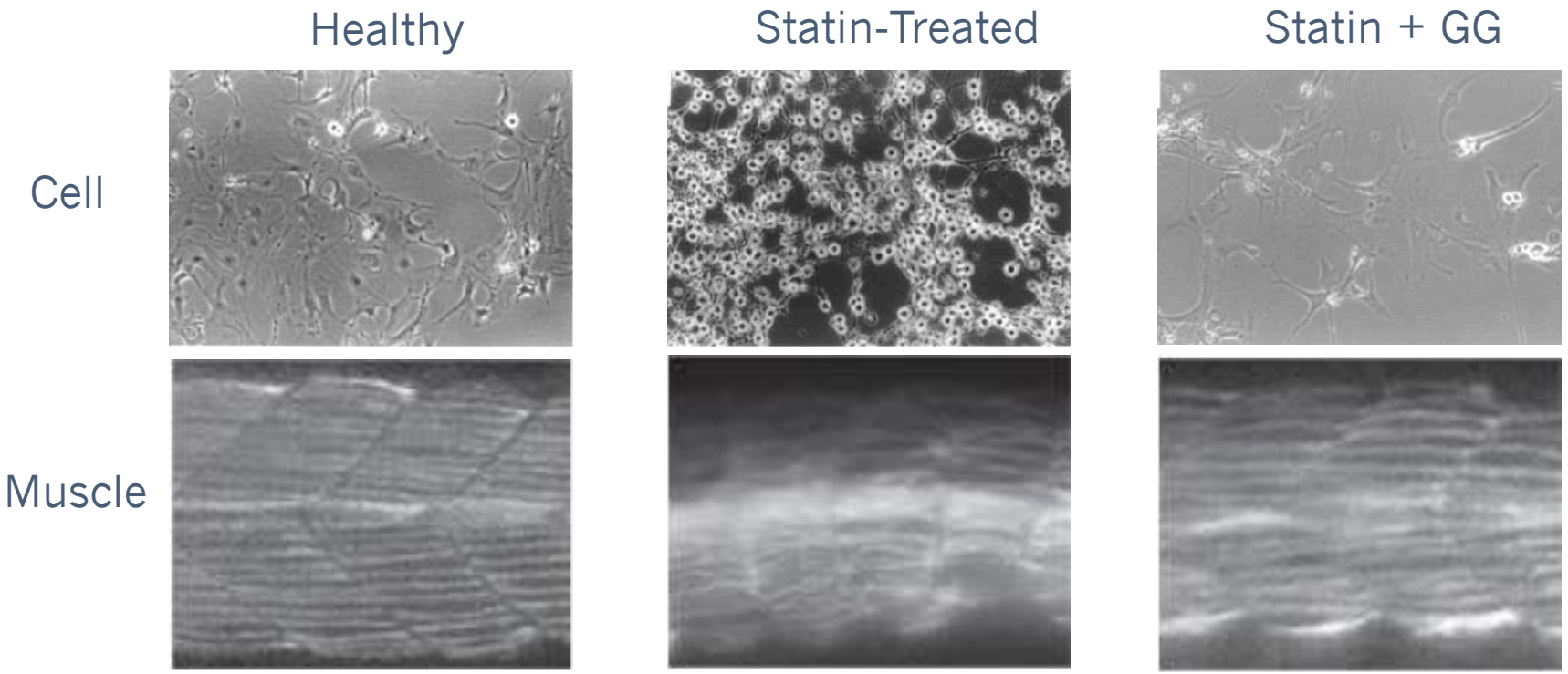
GG Prevents Statin Myotoxicity



- Statin stopped a GG-step to make RAP protein
- GG stimulated RAP1 prenylation
- GG reversed statin-associated cytotoxicity
- GG rescued detrimental effect on myotubes

Jaskiewicz A, Pajak B, Litwiniuk A, Urbanska K, Orzechowski A. Geranylgeraniol Prevents Statin-Dependent Myotoxicity in C2C12 Muscle Cells through RAP1 GTPase Prenylation and Cytoprotective Autophagy. Oxidative medicine and cellular longevity. 2018;2018:6463807.
Jaskiewicz A, Pajak B, Labieniec-Watala M, Palma C, Orzechowski A. Diverse Action of Selected Statins on Skeletal Muscle Cells-An Attempt to Explain the Protective Effect of Geranylgeraniol (GGOH) in Statin-Associated Myopathy (SAM). Journal of clinical medicine. May 16 2019;8(5).
Hsieh, Sheng-Kuo, et al. "Promotion of Myotube Differentiation and Attenuation of Muscle Atrophy in Murine C2C12 Myoblast Cells Treated with Teaghrelin." Chemico-Biological Interactions, vol. 315, 5 Jan. 2020, p. 108893., doi:10.1016/j.cbi.2019.108893.

GG Restores Cells and Muscle Fibers



- Statin destroyed cell morphology, interrupted cytoskeleton synthesis
- Statin caused visible muscle damage
- GG reduced fiber damage in myotubes

Ownby SE, Hohl RJ. Farnesol and geranylgeraniol: prevention and reversion of lovastatin-induced effects in NIH3T3 cells. Lipids. 2002;37(2):185-92.
Cao P, Hanai J, Tanksale P, Imamura S, Sukhatme VP, Lecker SH. Statin-induced muscle damage and atrogin-1 induction is the result of a geranylgeranylation defect. FASEB J. 2009;23(9):2844–2854. doi:10.1096/fj.08-128843

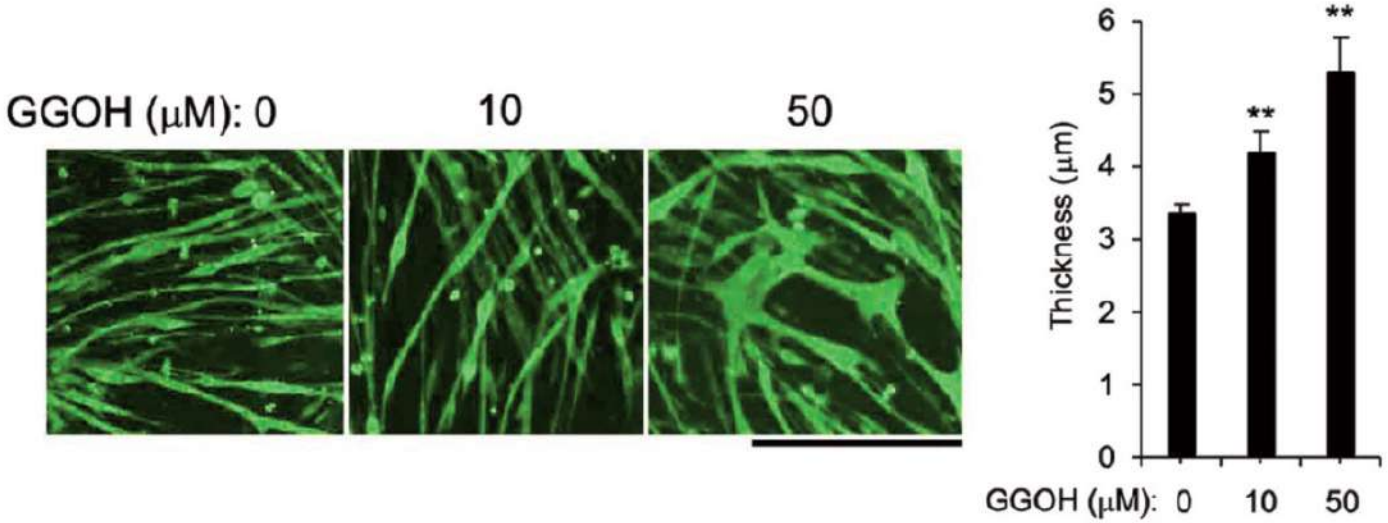
GG Increases Calf Muscle Force and Soleus Muscle

Young & Healthy Rats (Control, GG, Statin, GG+Statin)

- Increased shinbone muscle force, Reversed muscle fatigue
- Improved cardiac muscle contraction/relaxation
- Statin reduced force production, GG completely abrogated this effect
- GG increased soleus muscle (30%↑) in diabetic rats
- GG supplementation mitigated soleus muscle atrophy through
  - Increased damaged organelle clearance (mitophagy↑)
  - Improved mitochondrial quality
- 70kg person: **170mg/d** and **510mg/d** of GG

Irwin, Jordon C., et al. "Geranylgeraniol Prevents Statin-Induced Skeletal Muscle Fatigue without Causing Adverse Effects in Cardiac or Vascular Smooth Muscle Performance." Translational Research, vol. 215, 2020, pp. 17–30., doi:10.1016/j.trsl.2019.08.004.
Jiwan N et al (2022) Geranylgeraniol Supplementation Mitigates Soleus Muscle Atrophy via Changes in Mitochondrial Quality in Diabetic Rats

GG Regenerates Aging Atrophied Muscle



- GG suppressed Atrogin-1 induced muscle atrophy
- GG increased myotube thickness
- GG increased skeletal muscle fiber size

Miyawaki, Aki, Thira Rojasawasthien, Suzuro Hitomi, Y. Aoki, Mariko Urata, Asako Inoue, et al. Oral Administration of Geranylgeraniol Rescues Denervation-induced Muscle Atrophy via Suppression of Atrogin-1. In Vivo 34 2020; 2345 -2351.

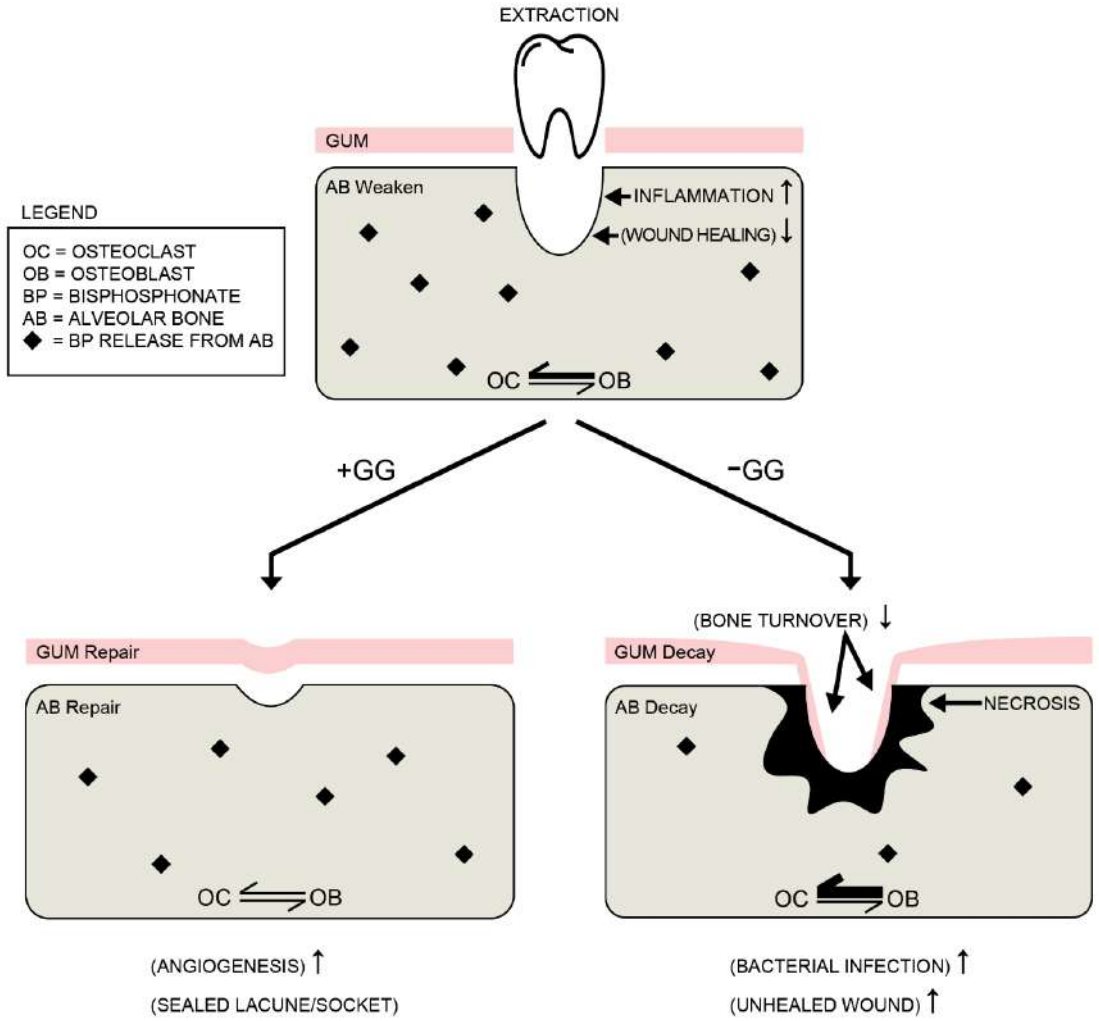
Pleiotropic Statin Benefits **Well Beyond** a Cholesterol Reducer

- 40m Statin users may reach 80m by 2030
- Statin is the 21st Century 'Aspirin'
- Statin will replace 52m acetaminophen users
- One-in-four patients have SAMS*
- 20m Statin users may benefit from GG

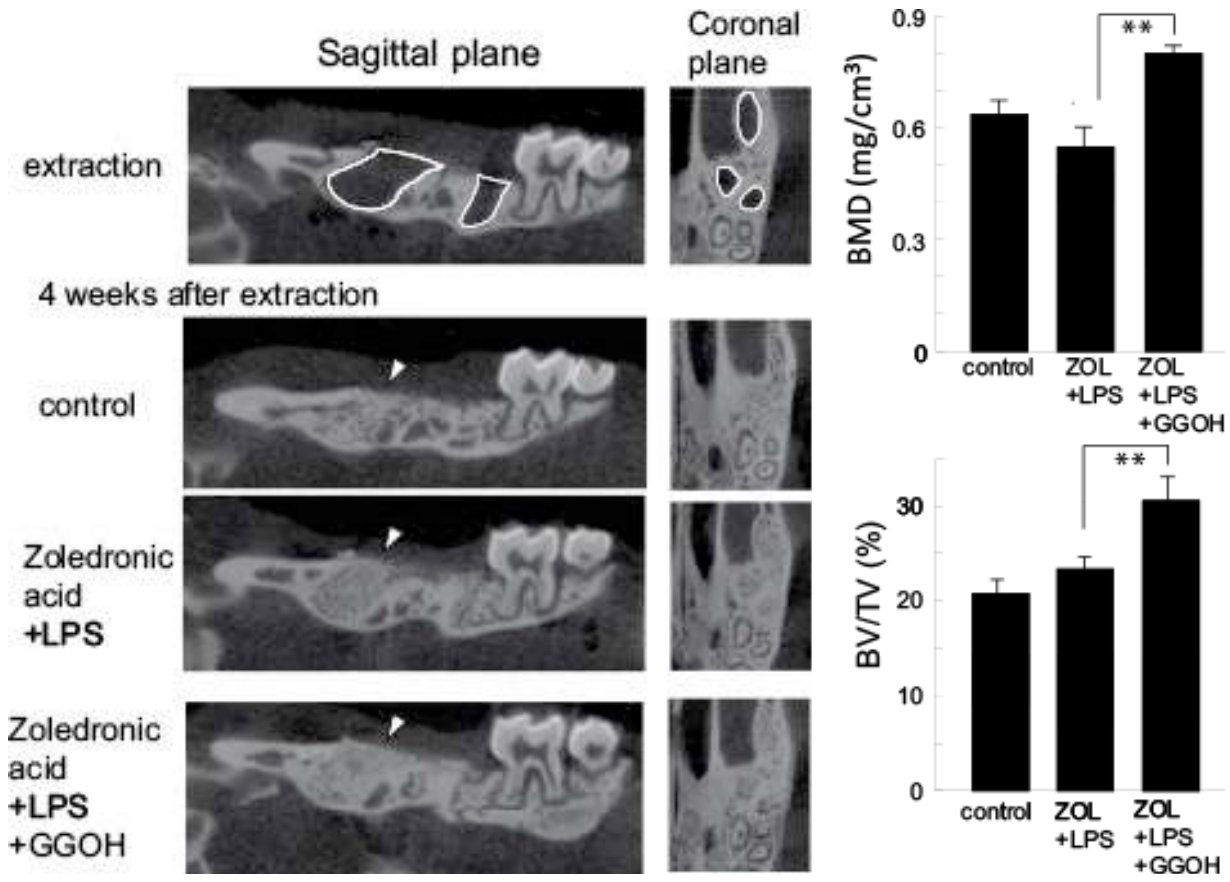
*Statin-Associated Muscle Symptoms

Orkaby, AR et al., Association of Statin Use With All-Cause and Cardiovascular Mortality in US Veterans 75 Years and Older. JAMA 324 (1) 68-78, 2020.
Eldor R, Raz I, ADA Indications for Statins in Diabetes. Diabetes Care 32, S384-391, Nov 2009.
Acetaminophen by Consumer Healthcare Product Association (CHPA) 2020.
Daniels LB, Ren J, Kumar K, Bui QM, Zhang J, Zhang X, et al. (2021) Relation of prior statin and anti-hypertensive use to severity of disease among patients hospitalized with COVID-19: Findings from the American Heart Association's COVID-19 Cardiovascular Disease Registry. PLoS ONE 16(7): e0254635.

BisP Impacts Alveolar Bone Turnover and Necrosis



GG Reduces BRONJ Damage (Systemic) [2015]



Wk0 —2wk— Wk2 —4wk— Wk6

[ZA]
[+LPS]

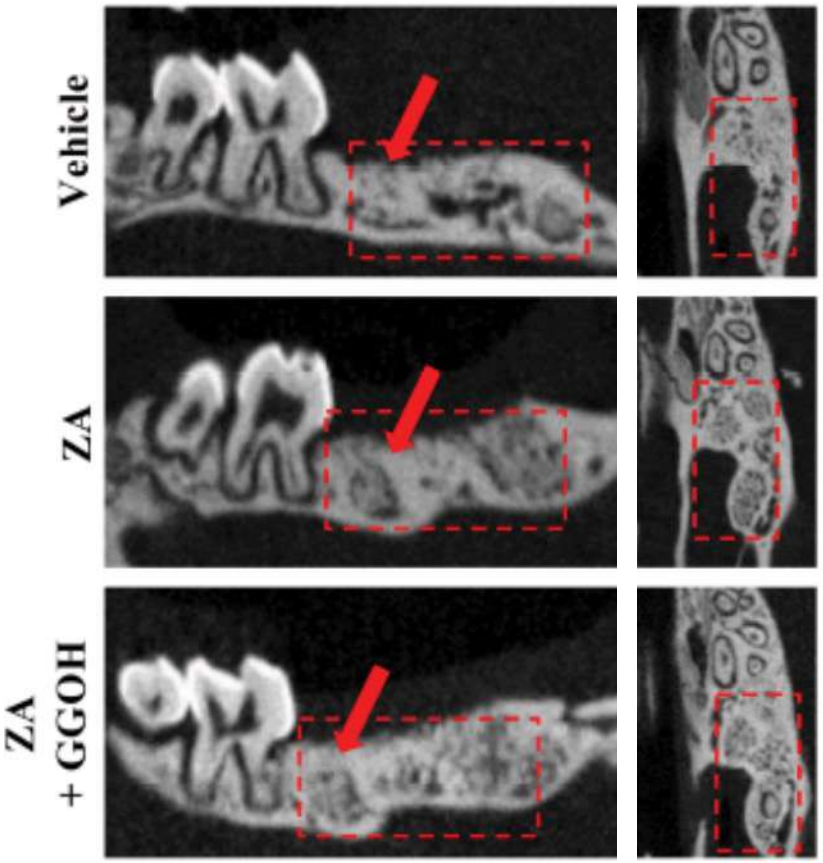
Extrn
[GG]

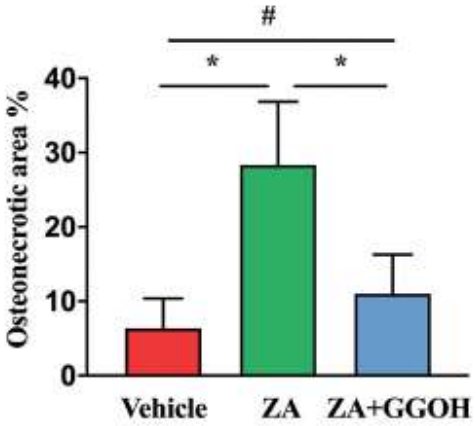
Mice: ZA & GG (twice/wk @ 3μM) IP

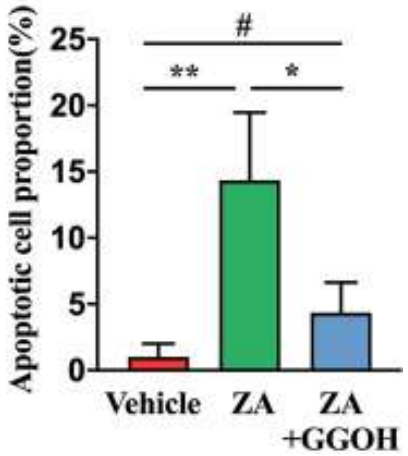
- BMD 47% ▲ and BV/TV 28% ▲
- GG improved bone in tooth sockets
- GG restored bone deposition

Nagaoka Y, Kajiya H, Ozeki S, Ikebe T, Okabe K. Mevalonates restore zoledronic acid-induced osteoclastogenesis inhibition. J Dent Res. 2015 Apr;94(4):594-601.

GG Reduces BRONJ Damage (Systemic) [2021]





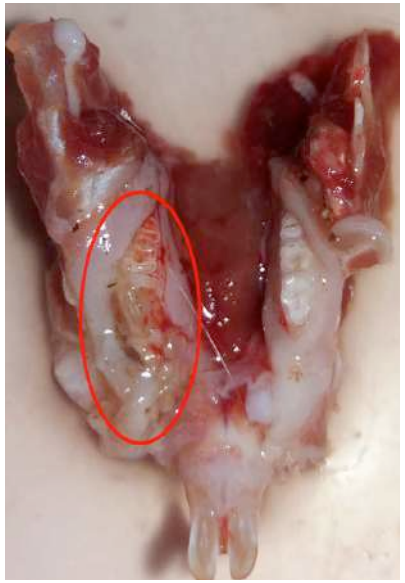


- BMD 30% ▲ and BV/TV 15% ▲
- GG prevented jaw necrosis (2.5x)
- GG prevented gum death (3.5x)

Chen, X., et al., Geranylgeraniol Restores Zoledronic Acid-Induced Efferocytosis Inhibition in Bisphosphonate-Related Osteonecrosis of the Jaw. Front Cell Dev Biol, 2021. 9: p. 770899.

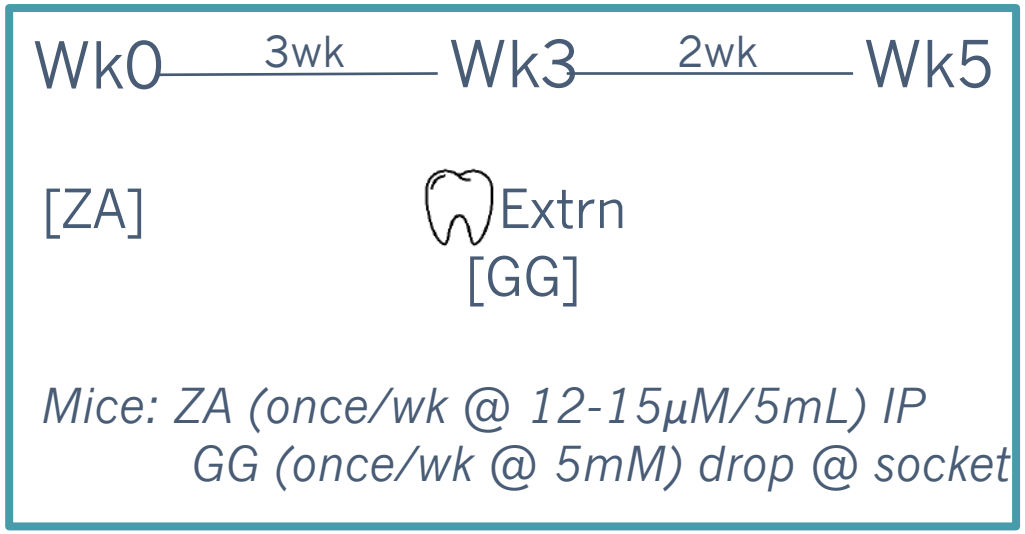
GG Reduces BRONJ Damage (Local) [2018]

Control



ZA

ZA + GG



- ZA application inflamed soft tissues, cause bone defect by 80%
- GG sealed empty lacunes (extracted sockets) by 45%
- GG improved gum (vascularity, osteoblast lining, epithelial coverage) by 67%

Zafar S, et al "Effects of zoledronic acid and geranylgeraniol on angiogenic gene expression in primary human osteoclasts." J. Oral Science 62 (1):79-83, 2020.
Koneski F, Popovic-Monevska D, Gjorgoski I, Krajoska J, Popovska M, Muratovska I, Velickovski B, Petrushevska G, Popovski V. In vivo effects of geranylgeraniol on the development of bisphosphonate-related osteonecrosis of the jaws. J Craniomaxillofac Surg. 2018 Feb;46(2):230-236.

GG-Gold® Summaries

Take Home

- Building block & endogenous nutrient
- Synthesizes CoQ10 & MK4
- Promotes cardiovascular health
- Maintains muscle and bone strength
- Decreases with age and certain meds

Recommended Dosage (with meal)

- **150mg/day** for most applications:
  - Bone and Oral health
  - CoQ10 & MK4 replenishment
  - Muscle tone and balance
- **300mg/day**:
  - Testosterone increase
  - Statin & BisP medications
  - Myopathy & Sarcopenia

Trial 1: GG and SAMS

Effect of Annatto-Derived Geranylgeraniol (GG) on Statin-Associated Myopathy

Study Outline:

- Double-blind, placebo-controlled, randomized intervention trial
- 12 week treatment with follow-ups at 3 and 6 months post-treatment
- 75 subjects
- Three groups: **150mg/day, 300mg/day**, Placebo
- Endpoints
  - GG reduces muscle-related symptoms (survey, pain inventory questionnaire)
  - GG improves muscle performance (muscle pain scoring, physical endurance/strength tests)
  - GG reduces muscle damage (creatine kinase, atrogin-1, myostatin) and mitigates inflammation (CRP, IL-6)

Virani, S.S., et al., Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. Circulation, 2021. 143(8): p. e254-e743.
Orkaby, A.R., et al., Association of Statin Use With All-Cause and Cardiovascular Mortality in US Veterans 75 Years and Older. JAMA, 2020. 324(1): p. 68-78.
Lansberg, P., et al., Nonadherence to statins: individualized intervention strategies outside the pill box. Vasc Health Risk Manag, 2018. 14: p. 91-102.
Matsubara, T., et al., Geranylgeraniol-induced Myogenic Differentiation of C2C12 Cells. In Vivo, 2018. 32(6): p. 1427-1431.
Miyawaki, A., et al., Oral Administration of Geranylgeraniol Rescues Denervation-induced Muscle Atrophy via Suppression of Atrogin-1. In Vivo, 2020. 34(5): p. 2345-2351.
Stoffel, W. and C. Martius, Zur Synthese der K-Vitamine und Ubichinone. Angew Chem, 1960. 72(17): p. 627-627.
Chen, Z., et al., Does statins promote vascular calcification in chronic kidney disease? Eur J Clin Invest, 2017. 47(2): p. 137-148.

Trial 2: GG and Sexual Health

Effect of Geranylgeraniol (GG) Supplementation on Sexual Health in Males and Females

Study Outline:

- Unique dose escalation design
- 4 weeks on **150mg/day** vs. placebo; additional 4 weeks on **300mg/day** vs. placebo
- Total 70 patients (40 male, 30 female)
- Biomarkers studied:
  - Total, bioavailable & free testosterone
  - SHBG, estrogen, progesterone
  - Renal & hepatic panels (CBC & CMP)

Okamura, K., F. Ando, and H. Shimokata, Serum total and free testosterone level of Japanese men: a population-based study. Int J Urol, 2005. 12(9): p. 810-4.
Akishita, M., et al., Low testosterone level as a predictor of cardiovascular events in Japanese men with coronary risk factors. Atherosclerosis, 2010. 210(1): p. 232-6.
Beatrice, A.M., et al., Testosterone levels and type 2 diabetes in men: current knowledge and clinical implications. Diabetes Metab Syndr Obes, 2014. 7: p. 481-6.
Huhtaniemi, I., Late-onset hypogonadism: current concepts and controversies of pathogenesis, diagnosis and treatment. Asian J Androl, 2014. 16(2): p. 192-202.
Ma, R.C. and P.C. Tong, Testosterone levels and cardiovascular disease. Heart, 2010. 96(22): p. 1787-8.
Pye, S.R., et al., Late-onset hypogonadism and mortality in aging men. J Clin Endocrinol Metab, 2014. 99(4): p. 1357-66.
Shores, M.M., et al., Low serum testosterone and mortality in male veterans. Arch Intern Med, 2006. 166(15): p. 1660-5.
Ho, H.J., et al., Geranylgeraniol enhances testosterone production via the cAMP/protein kinase A pathway in testis-derived I-10 tumor cells. Biosci Biotechnol Biochem, 2016. 80(4): p. 791-7.
Ho, H.J., et al., A novel function of geranylgeraniol in regulating testosterone production. Biosci Biotechnol Biochem, 2018. 82(6): p. 956-962.
Ito, A., et al., Menaquinone-4 enhances testosterone production in rats and testis-derived tumor cells. Lipids Health Dis, 2011. 10: p. 158.

DFH Products and Combos

- Annatto-E
- Annatto-GG
- Annatto-E GG
- CoQNol
- Resveratrol Supreme
- PerioPull
- Tri-K
- Primal Multi

Thank You!

Book Signing and Literature at Booth

Email life@american-river.com
www.americanrivernutrition.com
333 Venture Way, Hadley, MA