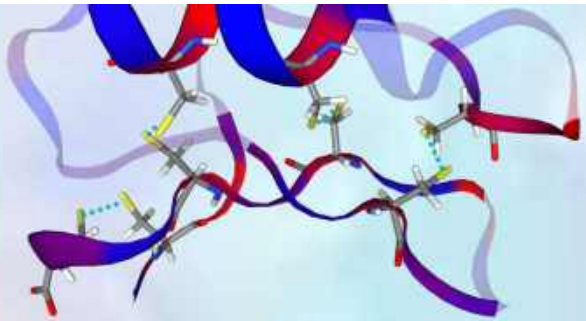


The Future of Peptides and Small Molecule Therapeutics

James LaValle, RPh, CCN, DHM, DHPh, MT

- Founder and CEO, Metabolic Code Enterprises
- Academic Co Chair A4M
- Chair Steering Committee International Peptide Society
- Precision /LaValle Metabolix

This material is provided for educational and informational purposes only to licensed health care professionals. This information is obtained from sources believed to be reliable, but its accuracy cannot be guaranteed. Dietary Supplements are very powerful and can occasionally cause dangerous allergic reactions in a small percentage of the population. Licensed health care professionals should rely on sound professional judgment when recommending herbs and natural medicines to specific individuals. Individual use of herbs and natural medicines should be supervised by an appropriate health care professional. The use of any specific product should always be in accordance with the manufacturer's directions.

Background

- Past Problems with using Peptides
  - High costs
  - Limited availability
  - Short half-lives
  - Lack of oral bioavailability
  - Side effects
  - Poor patient compliance with injections
  - Regulatory environment
  - Sub-quality products readily available on internet

Bruno BJ, Miller GD, Lim CS. Basics and recent advances in peptide and protein drug delivery. Ther Deliv. 2013;4(!1):1443-1467.

Background

- What Changed in the past decade?
  - Genomics, Metabolomics, Proteomics
  - Recombinant technology and genetic engineering
  - Interest by Pharma AND by compounding pharmacies
- Improved bioavailability
- Decreased side effects
- Improved efficacy and safety

Bruno BJ, Miller GD, Lim CS. Basics and recent advances in peptide and protein drug delivery. Ther Deliv. 2013;4(!1):1443-1467.

Peptides Now

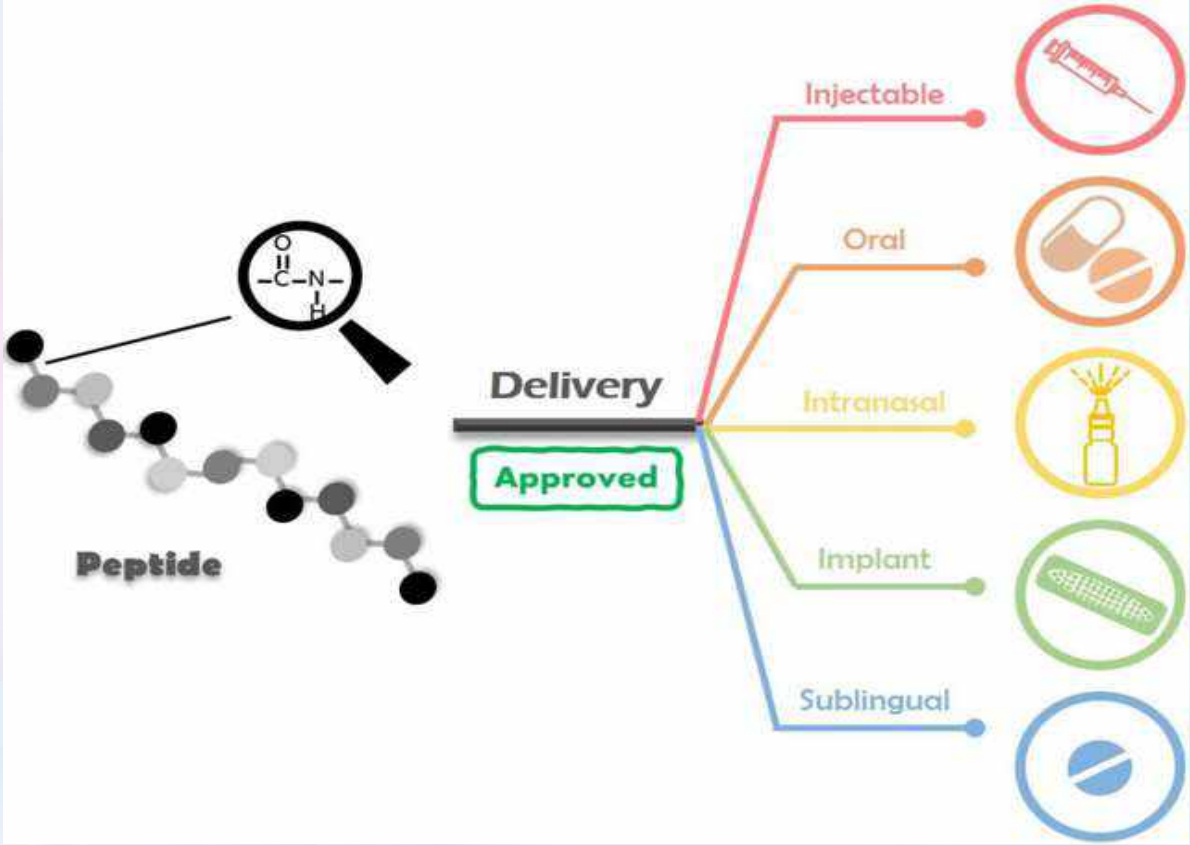
- Specialty compounding pharmacies are sequencing peptides more frequently in-house
  - SAFE
  - Q/A testing – HPLC purification, mass spectrophotometry
  - Rx’s for peptide therapies now a reality
  - Tailor made to practitioner's specs and patients’ individual needs

Recent Advances

- Novel strategies
  - Allow for the modulation of pharmacokinetic properties
  - Target specificity through amino acid or backbone modification
  - Incorporation of non-natural amino acids - polymers
  - Conjugation of moieties that extend half-life or improve solubility – 30% of peptides in clinical trials
  - Improved cell penetration
  - Nanocarrier technology
  - Liposomal technology
  - Novel formulation strategies reduce injection frequency
  - Improved stability
  - Transdermal
  - Class B receptor targets

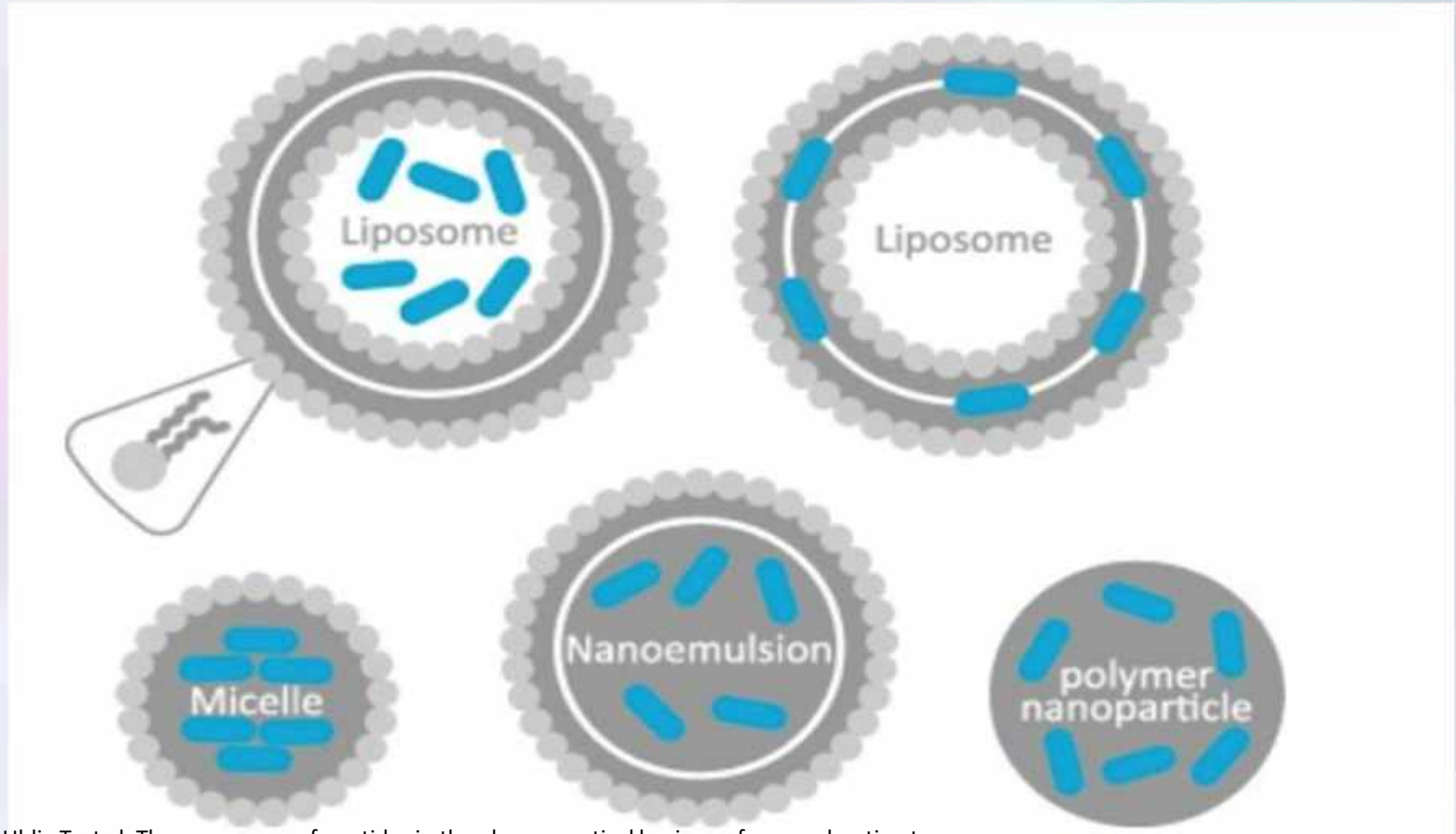
Galdiero S, et al. Peptide-based drugs and drug delivery systems. Molecules. 2017; 22(12):2185.
Herrero EP, Alonoso MJ, Csaba N. Polymer-based oral peptide nanomedicines. Ther Deliv. 2012;3(5):657-68.

Peptide Therapeutics



Zhang Y, et al. Just How Prevalent are Peptide Therapeutic Products? A Critical Review. Int J Pharm. 2020;587:119491.

Modern Peptide and Small Molecule Formulations



Uhlig T, et al. The emergence of peptides in the pharmaceutical business: from exploration to exploitation. EuPa Open Proteonimics. 2014;4:58-69.

Peptide Legal Status

- In 2022 the FDA decided to place some commonly used peptides in the DO NOT USE category
  - Bulk Drug Compounding list 503A
  - Category 2 peptides – FDA says these raise significant safety risks for patients
  - The industry is working with the FDA to take many of these peptides off the list

https://www.fda.gov/media/94155/download

Peptides placed in Bulk Drug Substances 503A Category 2

- AOD-9604
- BPC-157 - injectable
- Cathelicidin LL-37
- Cesium Chloride
- CJC-1295
- Dihexa Acetate
- Domperidone
- Emideltide (DSIP)
- Epitalon
- Germanium Sesquioxide
- GHK-Cu – injectable
- Ibutamoren Mesylate
- Ipamorelin Acetate
- Kisspeptin-10
- KPV
- Melanotan II
- Mechano Growth Factor, Pegylated (PEG-MGF)
- MOTs-C
- Quinacrine Hydrochloride for intrauterine administration
- Selank acetate (TP-7)
- Semax (heptapeptide)
- Thymosin-Alpha 1 (Ta1)
- Thymosin Beta-4, Fragment (LKKTETQ)

https://www.fda.gov/media/94155/download

Small Molecule Therapeutics

- Small molecule therapeutics are **organic compounds with a low molecular weight (< 900 daltons) that affect molecular pathways by targeting important proteins**
- Compounds obtained from natural sources
- Small molecule therapeutics are **a dominant class of pharmaceuticals**

Southey MWY, Brunavs M. Introduction to small molecule drug discovery and preclinical development. Front Drug Disc. 2023;3:.

Small Molecule Therapeutics

- Currently, approximately ½ of the clinical drugs currently on the market are derived from natural products and their derivatives

Southey MWY, Brunavs M. Introduction to small molecule drug discovery and preclinical development. Front Drug Disc. 2023;3:.

Small Molecule Characteristics

- Small molecular weight (0.1-1 kDa)
- Readily cross cell membranes to reach their target
- Act on physiological processes
- Can be administered orally

Beck H, et al. Small molecules and their impact in drug discovery: A perspective on the occasion of the 125th anniversary of the Bayer Chemical Research Laboratory. Drug Disc Today . 2022;27(6):1560-74.

Small Molecule Mechanisms

- Enzyme inhibition
  - Ex: Statins – inhibition of HMG-CoA reductase enzyme
- Receptor agonist/antagonist
  - Albuterol B2 agonist
  - Morphine – opiate agonist
- Ion channel modulation
  - Calcium channel blockers

Southey MWY, Brunavs M. Introduction to small molecule drug discovery and preclinical development. Front Drug Disc. 2023;3:.

Examples

- Acetylsalicylic acid (Aspirin) – 1st small molecule in 1899 (Bayer Pharma)
- Insulin
- Daridorexant (Quviviq®) – used for insomnia – orexin receptor antagonism
- Ciprofloxacin – antibiotic; enzyme inhibition
- Atorvastatin – HMG-CoA reductase enzyme inhibiiton

Beck H, et al. Small molecules and their impact in drug discovery: A perspective on the occasion of the 125th anniversary of the Bayer Chemical Research Laboratory. Drug Disc Today . 2022;27(6):1560-74.

Small Molecule Dietary Supplement Examples

- Rg3 ginsenoside – from *Panax ginseng* root
- Rb1 ginsenoside – from *Panax notoginseng* root
- Berberine – from several plants including Chinese *Coptis sp* and Goldenseal; antifungal (GUT) and blood glucose regulation
- Salidroside - phytocompound from *Rhodiola sp.;* improves oxygen uptake/VO2 max, improves energy and endurance
- EGCG – green tea antioxidant phytocompound
- Glutathione – antioxidant molecule

Beck H, et al. Small molecules and their impact in drug discovery: A perspective on the occasion of the 125th anniversary of the Bayer Chemical Research Laboratory. Drug Disc Today . 2022;27(6):1560-74.

Small Molecule Characteristics

- Advantages include:
  - Oral administration
  - Selectivity
  - Potency
  - Ease of synthesis

Beck H, et al. Small molecules and their impact in drug discovery: A perspective on the occasion of the 125th anniversary of the Bayer Chemical Research Laboratory. Drug Disc Today . 2022;27(6):1560-74.

Small Molecule Therapeutics - GUT

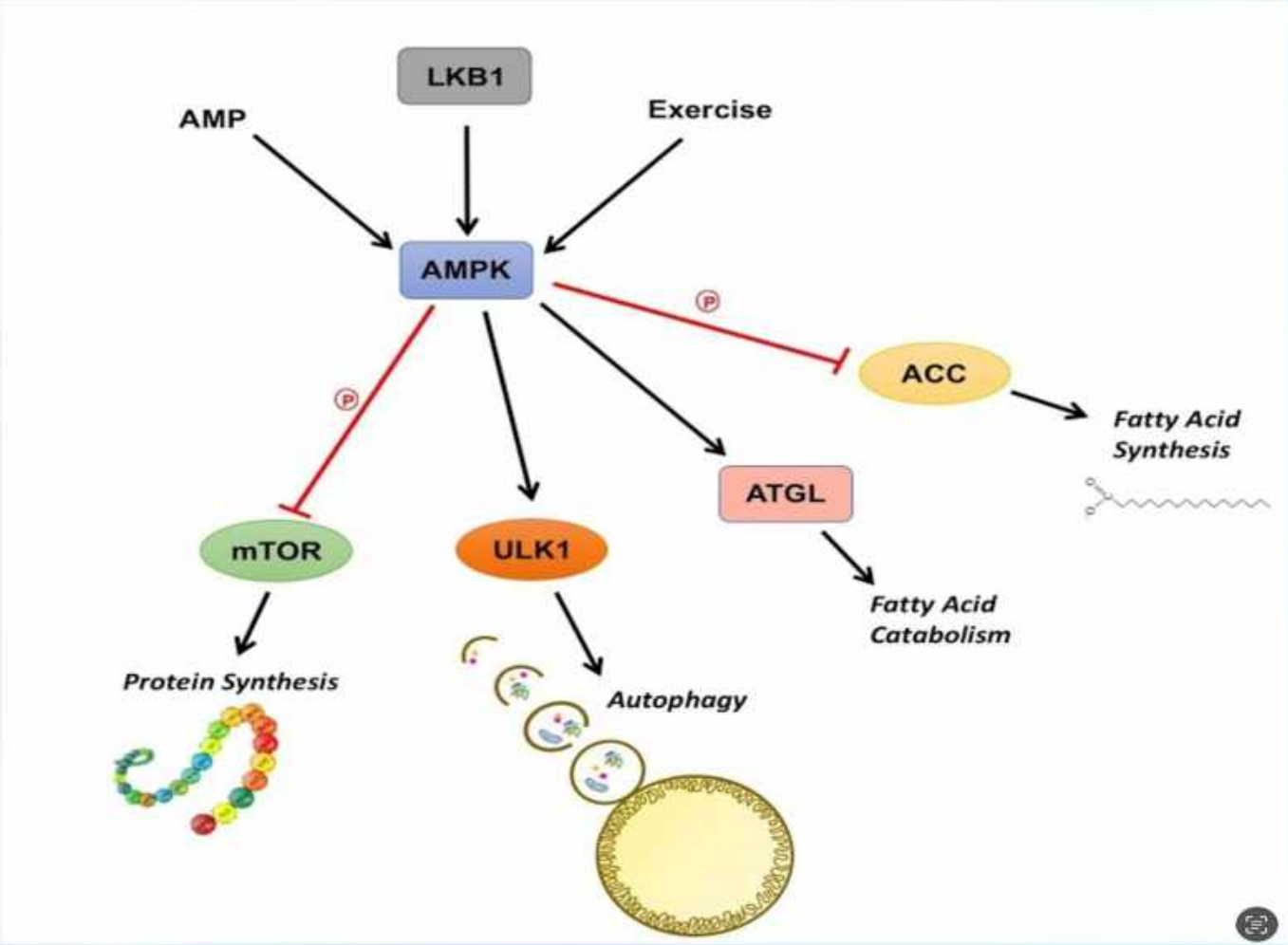
- Small-molecule drugs and biologics that alter the microbiome or its interaction with host tissues are being investigated to support GUT microbiome therapeutics

Cully M. Microbioime therapeutics go small molecule. Nat Rev Drug Discovery. 2019;18(8):569-72.

OS-01

- Small molecule AMPK agonist (activator) aka ATX-304
- Increase fatty acid metabolism and lipolysis in adipose tissue and liver
- Increase energy expenditure and cause significant weight loss effects in diet-induced obese mice while increasing food intake
- Increase glucose uptake in target tissues such as skeletal and heart muscle to restore cellular energy balance
- Improve cardiovascular performance, increasing stroke volume and exercise endurance in aged mice

Steinberg GR, Hardie DG. New insights into activation and function of the AMPK. Nature Rev Mol Cell Biol. 2023;24:255-72.



Steinberg GR, Hardie DG. New insights into activation and function of the AMPK. Nature Rev Mol Cell Biol. 2023;24:255-72.

OS-01

Potential Uses:

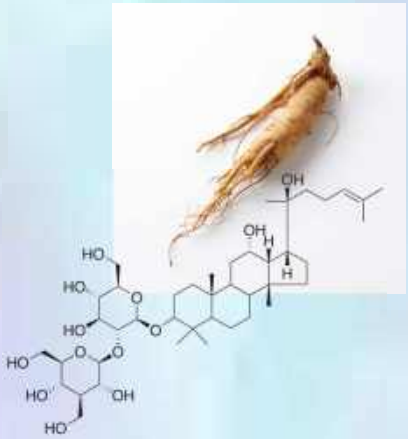
- Metabolic support – alternative to GLP-1s
  - Glucose homeostasis - Type 2 diabetes support
  - Weight management support
- Improved autophagy
- Cardiovascular support
- Improved microvascular perfusion in skeletal muscle
- Renal protection
- Decreased oxidative stress
- Pain/inflammation

Steinberg GR, Hardie DG. New insights into activation and function of the AMPK. Nature Rev Mol Cell Biol. 2023;24:255-72.

OS-01

Dosage: 100mg orally daily for 4-6 weeks

Rg3 Ginsenoside



- Ginsenosides are bio-active saponins found in Korean red ginseng (*Panax ginseng,* C.A. Meyer) root
- About 150 ginseng saponins are known
- > 90% are classified as Rb1, Rb2, Rc, Rd, Re, Rg1, and Rg3
- Rg3 ginsenoside is formed after steaming the roots of *Panax ginseng*
- Rg3 makes up approx. 0.1% of total ginsenoside

Lee H, et al. Relationship between ginsenoside Rg3 and metabolic syndrome. Front Pharmacol. 2020;11:130.

Rg3 Ginsenoside

- 20(R) Rg3 isomer
  - (R) isomer reported improved absorption over (S) isomer
  - Improved blood brain barrier penetration
  - Improved outcomes

Bae SH.0, et al. Pharmacokinetics and tissue distribution of ginsenoside Rh2 and Rg3 epimers after oral administration of BST204, a purified ginseng dry extract, in rats Xenobiotica.2014;44(12):1099-1107.

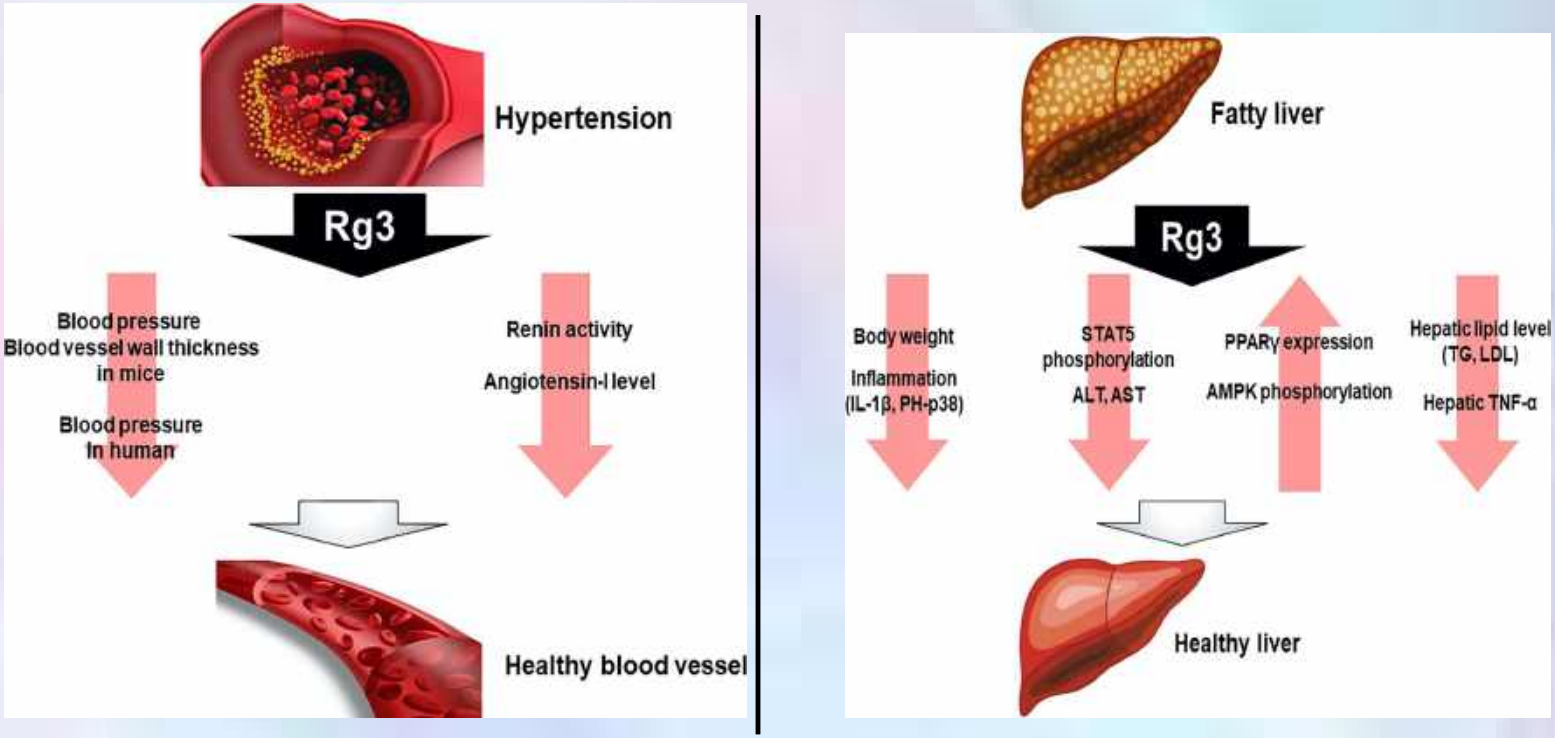
Rg3 Ginsenoside

Rg3 Physiological Properties (lab and human studies):

- Antioxidant/ Anti-inflammatory
- Neuroprotective
- Antiaging
- Anticancer
- Metabolic syndrome support
- Vascular protective
- Focus/energy supportive

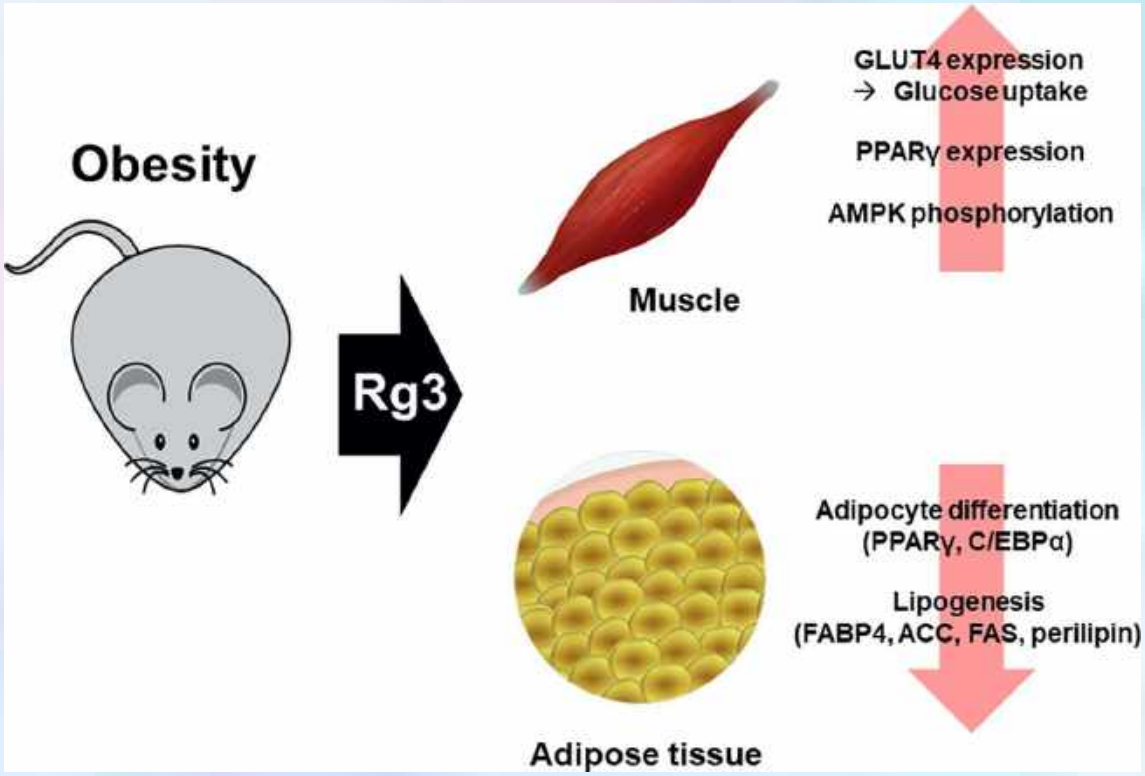
Lee H, et al. Relationship between ginsenoside Rg3 and metabolic syndrome. Front Pharmacol. 2020;11:130.

Effects of Rg3 on HTN and NAFLD



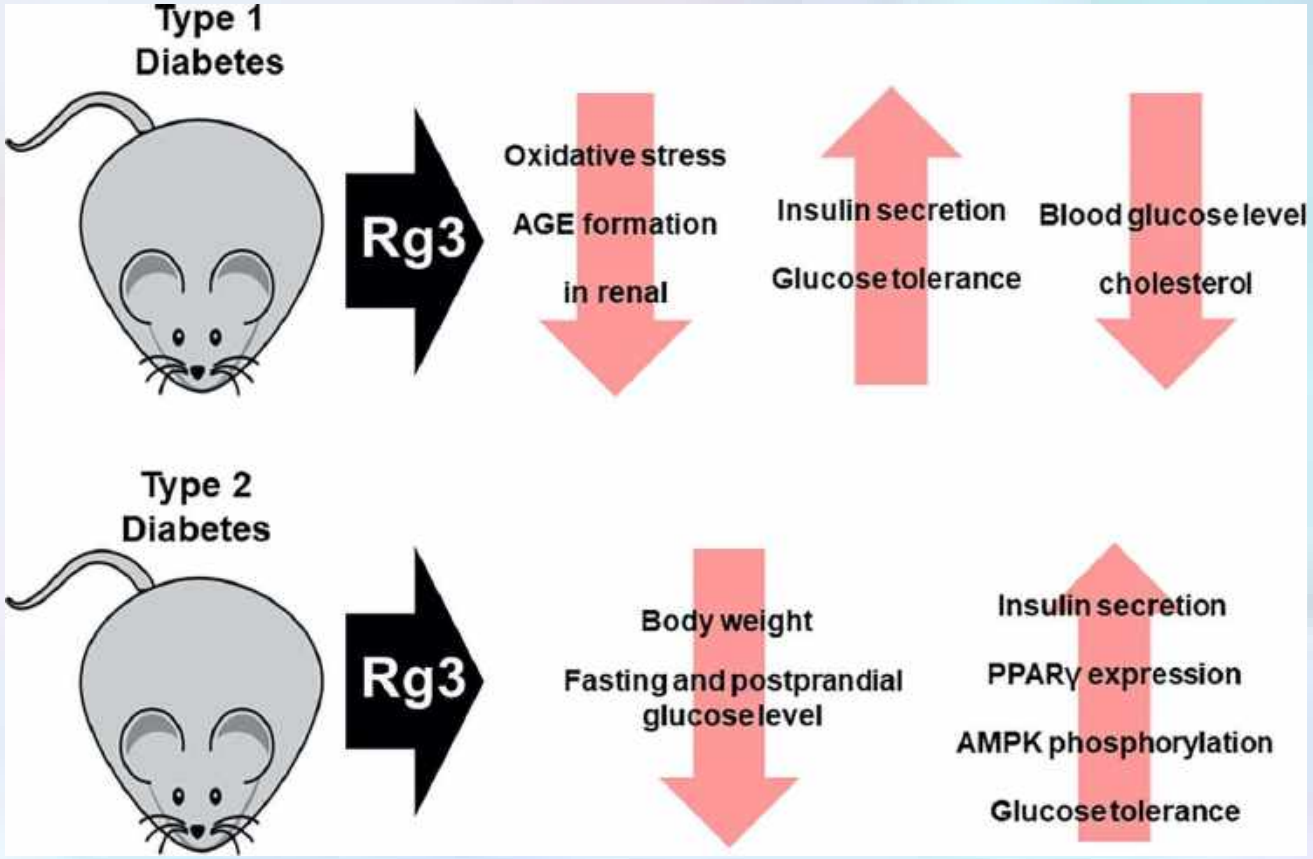
Lee H, et al. Relationship between ginsenoside Rg3 and metabolic syndrome. Front Pharmacol. 2020;11:130.

Effects of Rg3 on Obesity



Lee H, et al. Relationship between ginsenoside Rg3 and metabolic syndrome. Front Pharmacol. 2020;11:130.

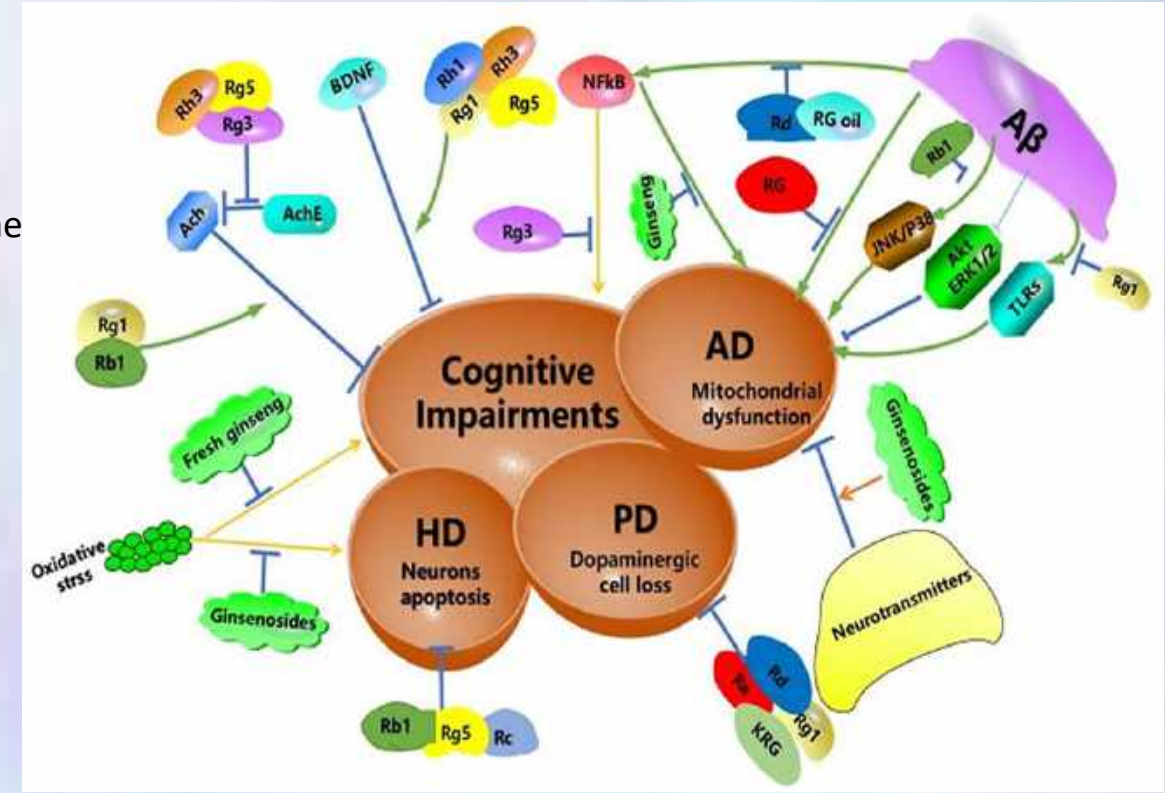
Effects of Rg3 on Glucose/Insulin Regulation



Lee H, et al. Relationship between ginsenoside Rg3 and metabolic syndrome. Front Pharmacol. 2020;11:130.

Effects of Ginsenosides on Neuroprotection

- Ginsenosides very neuroprotective
- Rg3 reported to :
  - Modulate acetylcholine
  - Decrease microglial inflammation
  - Decrease glutamate excitotoxicity
- Potentially useful in:
  - Cognitive impairments
  - Alzheimer's disease
  - Parkinson's disease
  - TBI/stroke
  - Huntington's Disease



Lee H, et al. Relationship between ginsenoside Rg3 and metabolic syndrome. Front Pharmacol. 2020;11:130.

Rg3 NeuroPharmacology

- Neuroprotective
- Decreases oxidative stress-induced inflammation
- Improves neuroinflammatory outcomes

*Joo SS et al.Prevention of inflammation-mediated neurotoxicity by Rg3 and its role in microglial activation. Biol Pharm Bull. 2008 Jul;31(7):1392-6.
*Bao HY, Zhang J, Yeo SJ, et al. Memory enhancing and neuroprotective effects of selected ginsenosides. Arch Pharm Res. 2005 Mar;28(3):335-42.

Rg3 NeuroPharmacology

- Helps attenuate microglial activation
- Decreases neuroinflammation
  - COX-2 inhibition
  - Inhibition of matrix metalloproteinase-9 (MMP-9)
  - Improves NO and ROS (reactive oxygen species) levels
  - Decreased inflammatory mediators - TNF-alpha, IL-1Beta
- Antinociceptive in lab animals – decreases pain

*Joo SS et al.Prevention of inflammation-mediated neurotoxicity by Rg3 and its role in microglial activation. Biol Pharm Bull. 2008 Jul;31(7):1392-6.
*Bao HY, Zhang J, Yeo SJ, et al. Memory enhancing and neuroprotective effects of selected ginsenosides. Arch Pharm Res. 2005 Mar;28(3):335-42.

Rg3 NeuroPharmacology

- Decreases excitotoxicity
- Attenuates NMDA (glutamate) receptor-mediated currents
- Decreases NMDA-induced neurotoxicity
- Inhibits L-type Ca(2+) channels
  - Counters increased levels seen in microglial activation

*Joo SS et al.Prevention of inflammation-mediated neurotoxicity by Rg3 and its role in microglial activation. Biol Pharm Bull. 2008 Jul;31(7):1392-6.
*Bao HY, Zhang J, Yeo SJ, et al. Memory enhancing and neuroprotective effects of selected ginsenosides. Arch Pharm Res. 2005 Mar;28(3):335-42.

Rb1 Ginsenoside

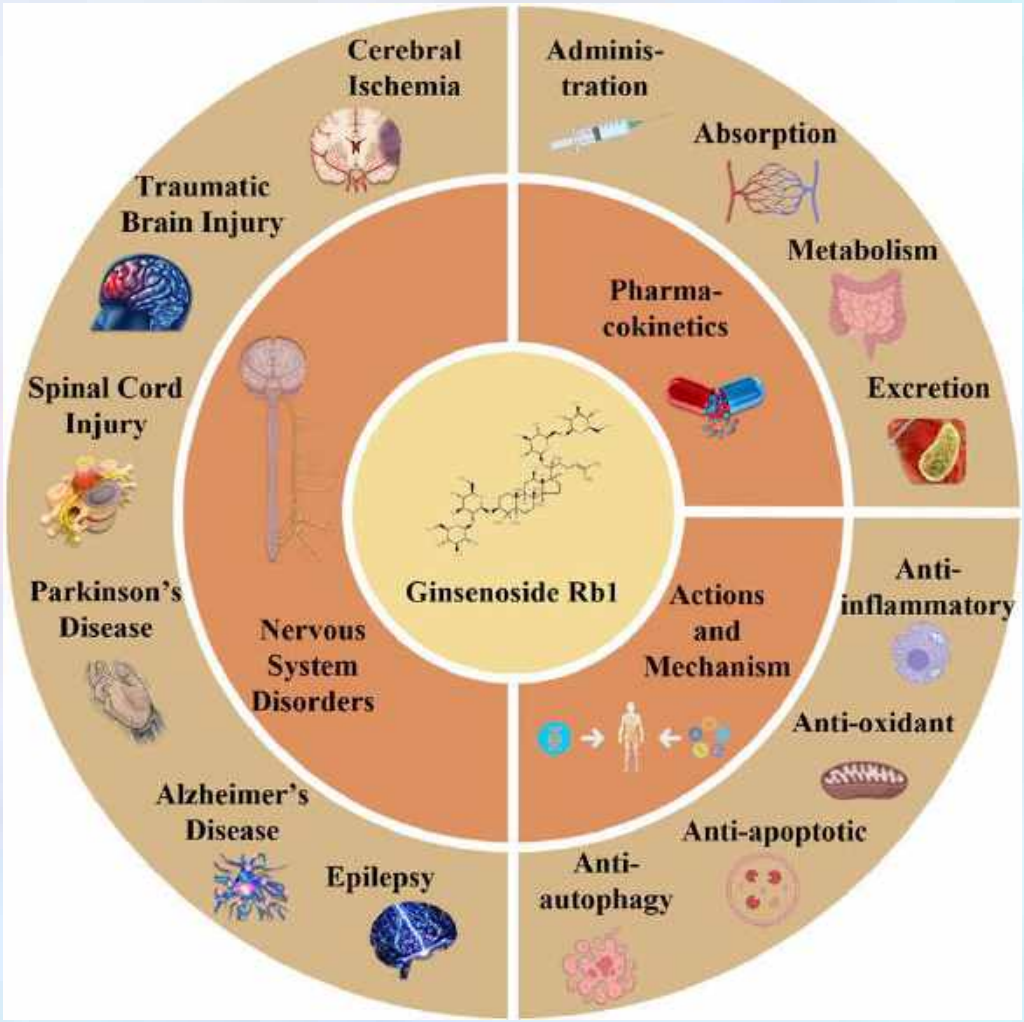
- Rb1 is a triterpene saponin (ginsenoside), produced upon steaming the root of *Panax notoginseng*
- Rb1 is reported to play a role as:
  - Neuroprotective agent – used in ischemic stroke and is reported to:
    - Attenuate brain water content
    - Promote neurogenesis
    - Antioxidant/anti-inflammatory in the brain
    - Decreases microglial activation
    - Improve cerebral circulation

*Zhang L, et al. **Ginsenoside Rb1 induces a pro-neurogenic microglial phenotype via PPARγ activation in male mice exposed to chronic mild stress. J Neuroinflam. 2021;18:171.**

Rb1 Ginsenoside –Cont.

- Anti-obesity agent
- Antioxidant/Anti-inflammatory agent
- Neuroprotective
  - Antidepressive agent – modulates amino-acidergic and monoaminergic receptors and neurotransmitters
- Apoptosis inhibitor

*Zhang L, et al. **Ginsenoside Rb1 induces a pro-neurogenic microglial phenotype via PPARγ activation in male mice exposed to chronic mild stress. J Neuroinflam. 2021;18:171.**



*Zhang L, et al. **Ginsenoside Rb1 induces a pro-neurogenic microglial phenotype via PPARγ activation in male mice exposed to chronic mild stress. J Neuroinflam. 2021;18:171.**

Rb1 Ginsenoside – Cont.

- Rb1 only available as an oral liposomal supplement in the US or as a research chemical
- Best to use synergistically with Rg3 ginsenoside for optimal therapeutic results
- 6mg Rb1 with 6mg Rg3 (R) as oral liposomal
- Chew/swallow 1 LPT, two times daily

*Zhang L, et al. **Ginsenoside Rb1 induces a pro-neurogenic microglial phenotype via PPARγ activation in male mice exposed to chronic mild stress. J Neuroinflam. 2021;18:171.**

- Innovation out of Taiwan
- Fermentation process allows for chains of peptides to be synthesized 100% accuracy
- Each peptide has a pepsin break added between the following peptide.
- Stomach acid breaks the chain which is initially a protein into peptide fragments

Formulation examples

Sleep
1. Delta Sleep Inducing Peptide (DSIP)
2. GHRH sermorelin
3. Galanin
4. Newropeptide Y
5. Vasopressin
6. Oxytocin
7. Vasoactive intestinal peptide (VIP)
8. Ghrelin
9. Melanin-concentrating hormone (MCH)
10. Epitalon
11. Soybean protein-derived peptides (SBPs)
12. Casein peptides
13. Selank
14. Semax
15. Anti-allergy peptide
16. Vilon

Allergy TGD peptide
Longevity Epitalon Vilon

Glutathione

- Tripeptide composed of glutamic acid, cysteine, glycine
- Found in high concentrations in liver tissue
- Endogenous and exogenous antioxidant
- Plays crucial role in liver detoxification and antioxidant systems
- Most important thiol reducing agent involved in modulation of hepatic redox processes

Honda Y, et al. BMC Gastroenterol. 2017;17:96.

Glutathione

- Also supports immunity
  - Modulates immune cells to function more effectively i.e. lymphocytes
  - Regulates production of pro-inflammatory cytokines
  - Supports NK cell and macrophage function
- S-Acetyl-L-glutathione – more bioactive, efficient absorption
- Acetyl glutathione oral liposome
  - 15mg/ LPT
  - Chew/swallow 1 BID

Honda Y, et al. BMC Gastroenterol. 2017;17:96.

Berberine

- Berberine
  - Antibacterial/antifungal
  - Useful in GUT microbiome imbalances
  - Decreases intestinal epithelial tight junction damage by pro-inflammatory cytokine release
  - Inhibits Candida from adhering to HT-29 epithelial cells
  - Inhibits SAP (secreted aspartyl proteinase) activity by over 70%
  - SAP marker of yeast conversion to aggressive form

Ye Y, et al. Efficacy and Safety of Berberine Alone for Several Metabolic Disorders: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Front Pharmacol. 2021;12:653887.

Berberine

- Berberine T2D/IR
  - Supports glucose and insulin regulation
  - Improves insulin sensitivity/production
  - Improves NO production
  - Slows Carb absorption in GUT
  - Induces GLP-1 secretion via activation of bitter taste receptor pathways
  - Stimulates GLP-1 via decreased mitochondrial oxidative stress
  - Improves microbiome which improves GLP-1 activation
  - 250-500mg QD std. 97% for Candida, combine with Cat's Claw (Uncaria tomentosa) vine and grapefruit seed extract
  - Oral liposomal tab – next page

Ye Y, et al. Efficacy and Safety of Berberine Alone for Several Metabolic Disorders: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Front Pharmacol. 2021;12:653887.

Berberine

- Oral liposomal tab
- Improved bioavailability and stability over liquid liposomals
- Targeted for blood glucose/insulin regulation
- Contains liposomal:
  - Berberine 97% 50mg / liposomal
  - Bitter melon 10% charantins 50mg
  - Chromium 800mcg
  - PQQ 10mg
  - P5P 5mg
  - Vanadium 500mcg

Klotho

- Alpha-Klotho is a multifunctional protein encoded by the KL gene
- “Longevity factor”
- Powerful regulator of aging processes
- Essential components of endocrine fibroblast growth factor (FGF) receptor complexes
  - FGF21 is a starvation hormone that induces stress responses by activating the sympathetic nervous system and the hypothalamus–pituitary–adrenal axis
  - FGF19 is a satiety hormone that promotes metabolic responses to feeding
  - FGF23 is a phosphaturic hormone
    - increased FGF23 levels in patients with early-stage chronic kidney disease or elderly individuals is indicative of excess phosphate intake relative to the residual nephron number

Kuro-o M. The klotho proteins in health and disease. Nat Rev Nephr. 2019;15:27-44.

Klotho

- Plays a crucial role in:
  - Anti-aging/longevity – mutations of klotho reported in animal studies to shorten lifespan (up to 80%) while overexpression extends lifespan (+30 % of longer life)
  - Oxidative stress and inflammation
  - Phosphate and calcium homeostasis
  - Fibroblast growth factor signaling
  - Regulation of cellular proliferation, differentiation and survival

Kuro-o M. The klotho proteins in health and disease. Nat Rev Nephr. 2019;15:27-44.

Klotho

- Mutations of klotho gene linked to :
  - Chronic kidney disease (CKD)
  - Diabetes
  - Cancers
  - Arteriosclerosis
  - Osteoporosis
  - Sarcopenia
  - Skin atrophy
  - Alzheimer’s disease
  - Parkinson’s disease

Kuro-o M. The klotho proteins in health and disease. Nat Rev Nephr. 2019;15:27-44.

Klotho Gel

- Used in skin health
- A clinical study reported significant improvements in skin elasticity, hydration, and firmness within weeks after using klotho gel topically
  - 80% improvement in skin hydration
  - 75% reduction in wrinkle depth
  - 70% increase in skin brightness
  - 65% reduction in pigmentation

Humble G, Manalo A. Discovery of aging gene leads to formulation of the most effective anti-aging cosmeceutical: Klotho Skin. https://cdn.shopify.com/s/files/1/0529/2500/6000/files/Klotho_White_Paper_for_End-User.pdf?v=1626437785

Klotho Gel

- Compounded for use in sarcopenia
- Treatment with topical recombinant **Klotho protein significantly improved:**
  - Mitochondrial function
  - Myogenesis
  - Muscle regeneration in the injured aged muscle

Ahrens HE, et al. Klotho expression is a prerequisite for proper muscle stem cell function and regeneration of skeletal muscle. Skelet Muscle. 2018;8:20.

Peptide Bioregulators

- Dr. Vladimir Khavinson of Russian Health Ministry pioneered bioregulator peptides in 1970s
- Developed for use in Soviet Athletes and Military uses
- Ultra-short peptides (2-7 amino acids in general)
- Helps body restore homeostatic function
- Primal signaling molecules controlling gene expression and protein synthesis
- Interact with cellular DNA
- “Less is more”

Khavinson VK, et al. Peptide regulation of gene expression: a systematic review. Molecules. 2021;26:7053.

Using peptide bioregulators is reported to improve the quality of life, prolong active longevity and restore organs at the cellular level, improving their functionality.

Khavinson VK, et al. Peptide regulation of gene expression: a systematic review. Molecules. 2021;26:7053.

How are Bioregulators Taken?

- Start with encapsulated oral **synthetic** bioregulators (called cytogens)
- Dose = 1 cap (20mg) 2 times daily (on an empty stomach) x 30 days.
- If more intensive treatments are needed, 2 caps 2 times daily on an empty stomach can be used.
- If nausea or GI upset occurs, take with small amounts of food.
- Move to natural bioregulators (cytomaxes) on follow-up therapies
- Bioregulators can be “stacked” – use more than 1 at a time therapeutically
  - Ex. Use heart, blood vessel and kidney bioregulators simultaneously
- Oral capsules, sublingual liquid and SubQ injectable bioregulators are available

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- If more intensive treatments are needed, 2 caps 2 times daily on an empty stomach can be used.
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- Move to natural bioregulators (cytomaxes) on follow-up therapies

Bioregulators on the Market Include:

- Adrenal
- Bladder
- Blood Vessels
- Bone Marrow
- Cartilage
- CNS/Brain/pineal
- Heart
- Kidney
- Liver
- Lungs
- Muscle
- Ovary
- Pancreas
- Parathyroid gland
- Pineal gland
- Prostate
- Retina/Eyes
- GUT/Digestive system
- Testes
- Thymus/Immune
- Thyroid