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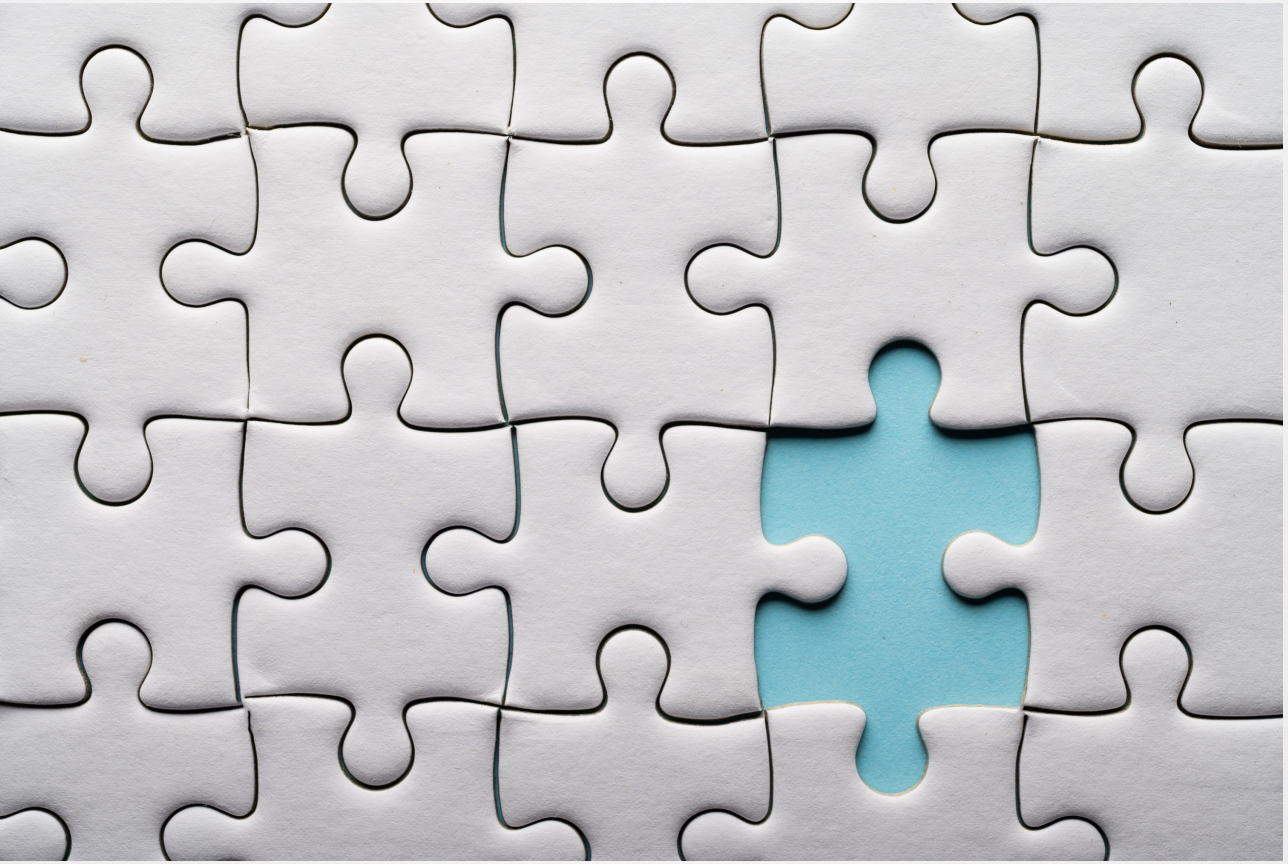
Cellular Senescence and Atherosclerosis

Abid Husain, MD
FACC, ABAARM

BOONE HEART INSTITUTE

PRACTICE IN THE CURRENT ERA



Conventional Medicine

Functional Medicine

Integrative Medicine

Regenerative Medicine

Performance Medicine

Longevity Medicine

Holistic Medicine

Naturopathic Medicine

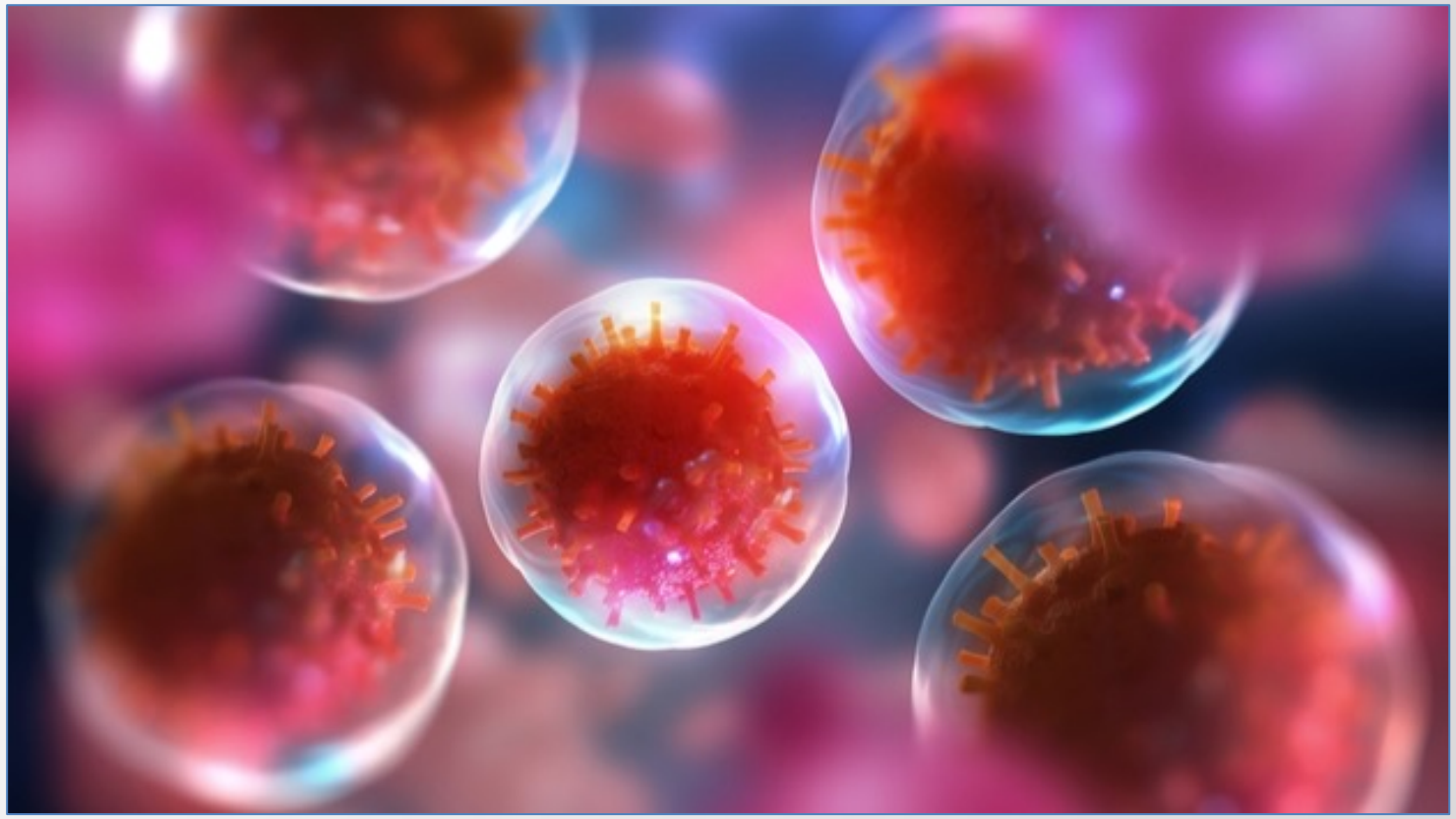
Precision Medicine

Using a compartmentalized approach will not provide the solution.

Is there a unifying principle?

The Universal Commonality is

Cellular Senescence



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WHAT IS CELLULAR SENESCENCE?

- Stable cell cycle arrest that can be triggered in normal cells. It is a highly dynamic, ***multi-step process***, during which the properties of cells continuously evolve and diversify in a context dependent manner.
- Senescence is triggered by ***developmental*** or ***stress*** signals. Depending on the cell type, intensity and nature of the stress; cells may respond by inducing repair, cell death or senescence
- Unregulated cellular senescence can lead to unregulated inflammation, compromising tissue repair and regeneration, thereby contributing toward aging. Removal of senescent cells can attenuate age-related tissue dysfunction and extend health span.
- It is a cellular program which acts as a double-edged blade, with both beneficial and detrimental effects on the health of the organism

K Ruchi, ; Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype, Front. Cell Dev. Biol., 29 March 2021

TYPES OF SENESCENCE

- replicative senescence / telomere dependent
- oncogene-induced senescence (OIS)
- unresolved DNA damage induced senescence
- programmed senescence
- stress-induced premature senescence including / non-telomeric
- epigenetically induced senescence
- mitochondrial dysfunction associated senescence
- Immuno-senescence

K Ruchi, ; Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype, Front. Cell Dev. Biol., 29 March 2021

CELLULAR SENESCENCE

- Senescence plays key physiological roles in normal development, maintaining tissue homeostasis, tissue remodeling and repair, wound healing, secretion of hormones, and limits tumor progression by ensuring that potentially dysfunctional, damaged or transformed cells do not perpetuate their genomes to the next generation
- Senescent cells have been found to accumulate exponentially with increasing chronological age in multiple tissues
- Unregulated / inappropriate senescence has deleterious effects as it can hinder tissue repair and regeneration in itself and in neighboring cells via the **SASP**

K Ruchi, ; Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype, Front. Cell Dev. Biol., 29 March 2021

Illustration by Paweł Jońca

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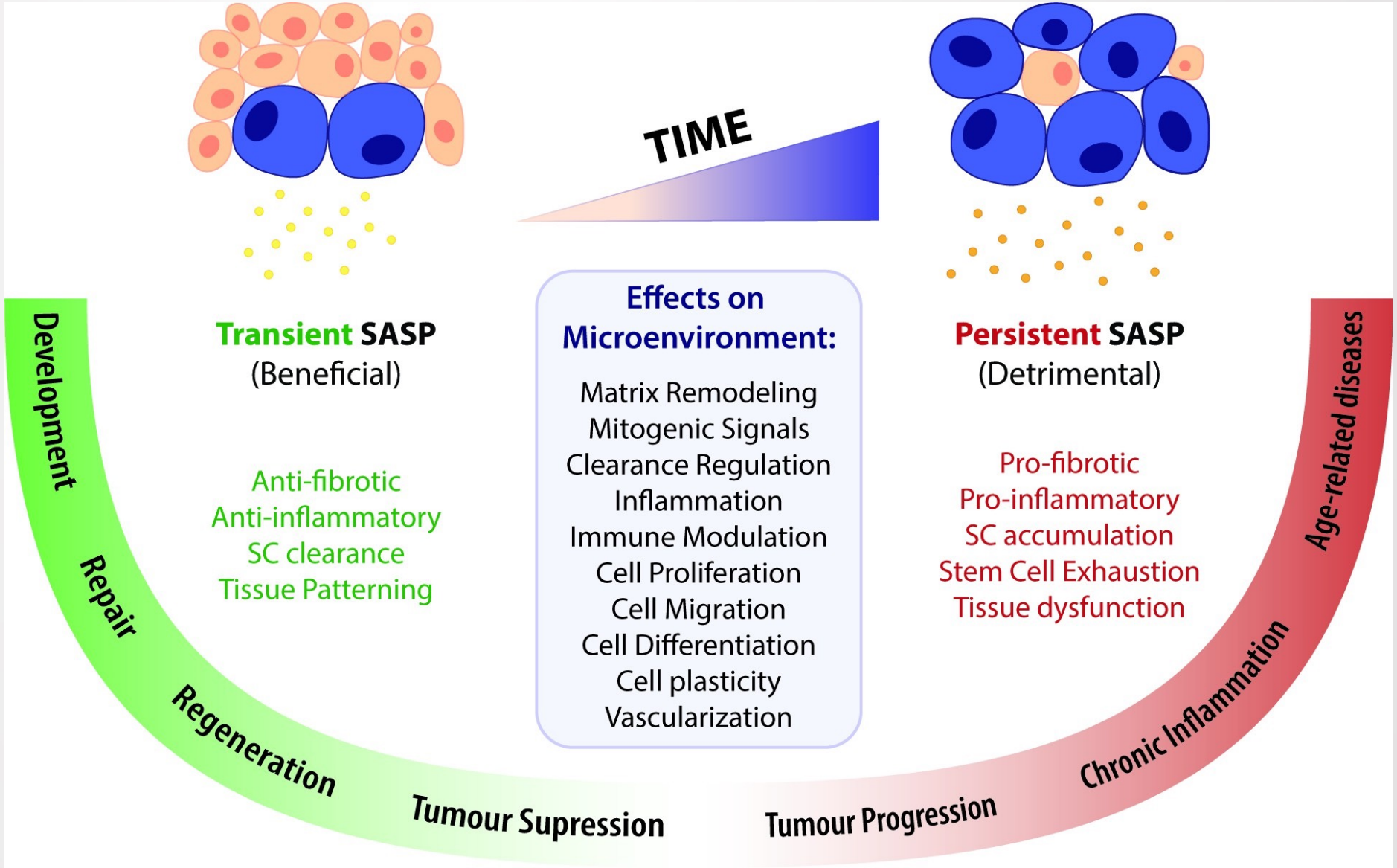
SASP- SENESCENCE ASSOC SECRETORY PHENOTYPE

- Although senescent cells are in a growth arrested state, they remain metabolically active. Senescence does not only affect the events inside the cell but has the potential to affect the surroundings and communicate with neighboring cells by secreting a complex mixture of secreted factors which can alter the behavior of nearby non-senescent cells.

- In a prolonged state of senescence, DNA and mitochondrial dysfunction occur, driving the conversion of normal senescence to phenotype that releases a plethora of signaling factors, pro-inflammatory cytokines, chemokines, growth modulators, angiogenic factors, proteases, bioactive lipids, extracellular matrix components, and matrix metalloproteinases (MMPs)

- SASP will promote inflammation in the immediate timeframe & neighboring tissue as well as negatively affecting the regional satellite (stem) cells preventing future regenerative capacities

K Ruchi, ; Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype, Front. Cell Dev. Biol., 29 March 2021



Diogo Paramos-de-Carvalho, Antonio Jacinto, Leonor Saúde (2021) The right time for senescence eLife 10:e72449

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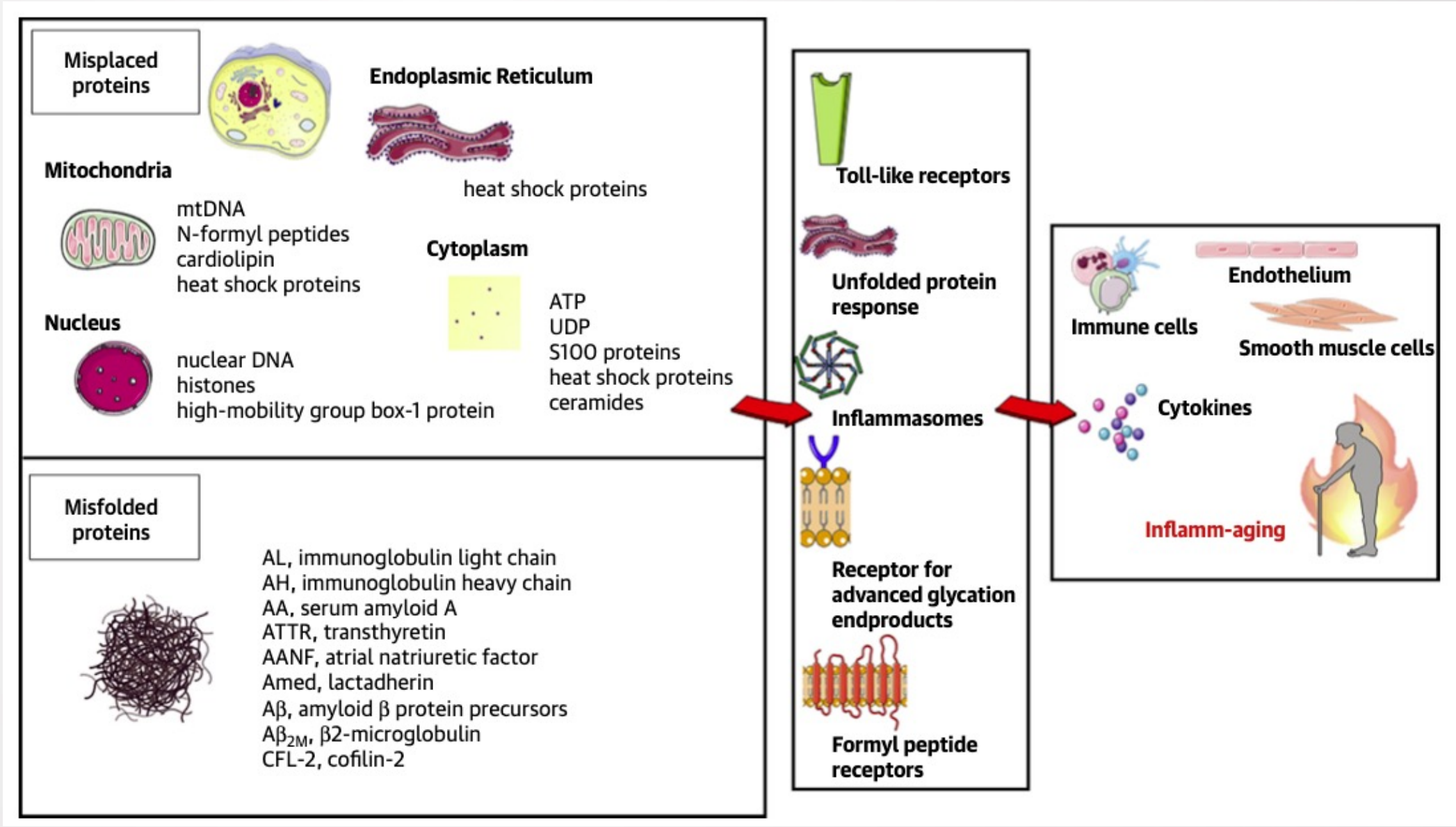
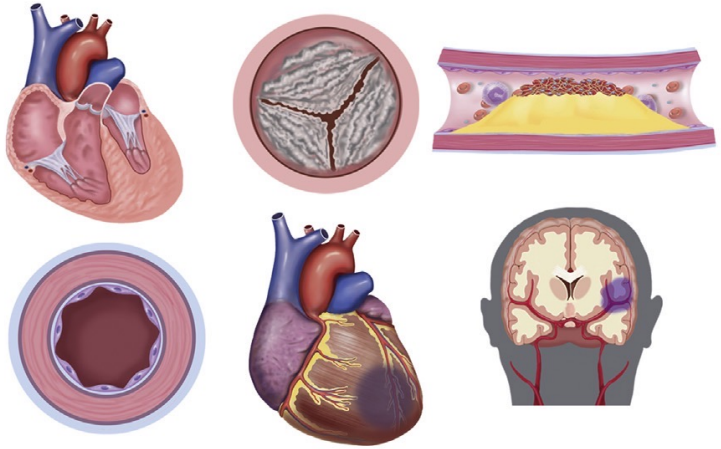


Liberale, L. et al. J Am Coll Cardiol. 2022;79(8):837–847.

Liberale, L. et al. J Am Coll Cardiol. 2022;79(8):837–847.

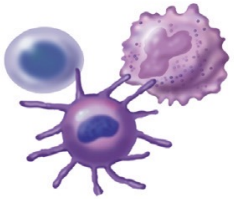
Inflamm-aging

Age-Dependent CV and CBV Afflictions

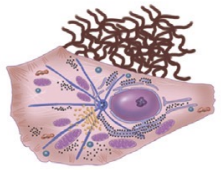


Mechanisms

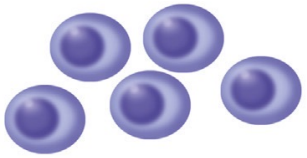
Immunosenescence



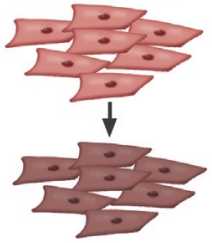
Garbaging



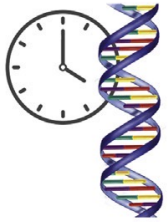
Clonal Hemopoiesis of Indetermined Potential



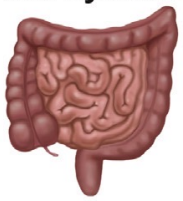
Senescence



Epigenetic Clock



Metaflammation and Dysbiosis



Potential Interventions

- Senolytics
- Pharmacological chaperones
- Proteasome activators
- Autophagy activators
- Anti-cytokines
- Epi-drugs
- Dietary interventions and supplementations
- Prebiotics and probiotics

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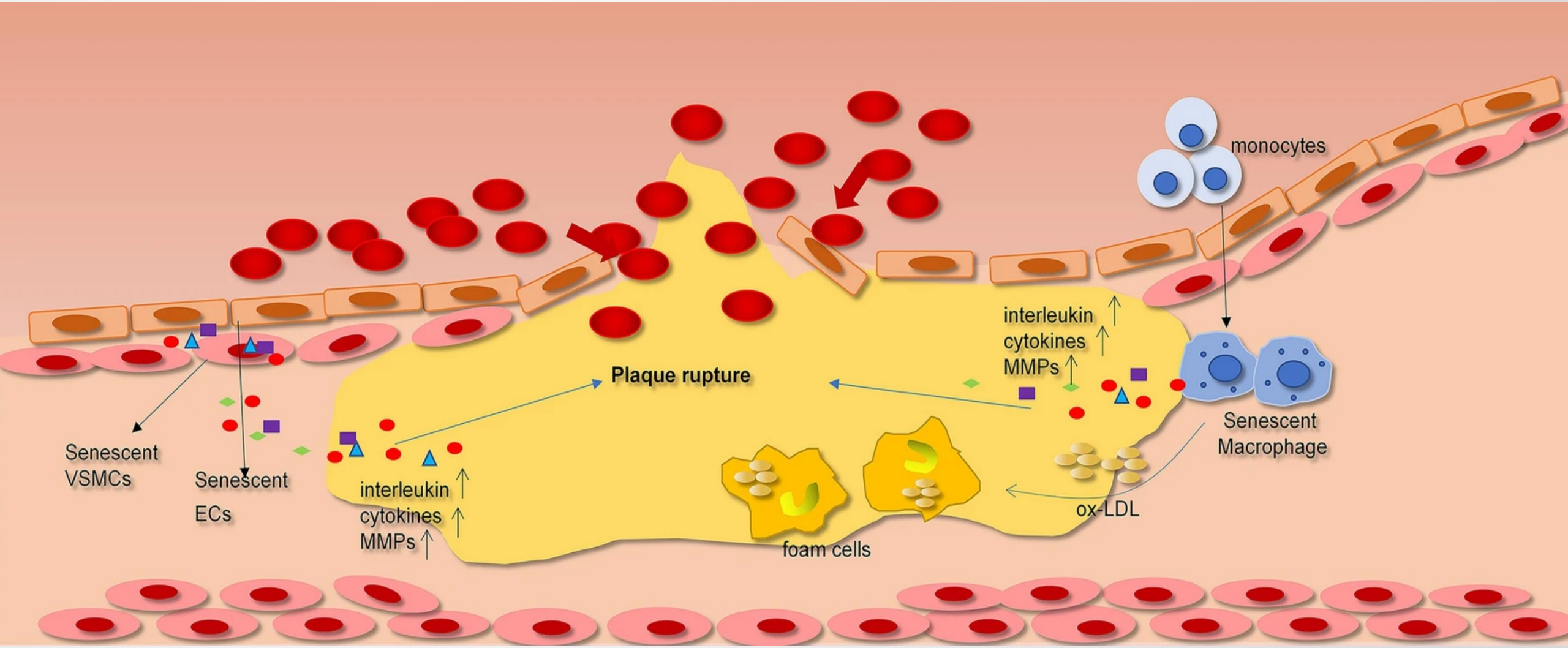
SENESCENCE & ATHEROSCLEROSIS

- Senescent cells promote features of plaque instability, including elastic fiber degradation and fibrous cap thinning, by heightening metalloprotease production.

- **Together, these results demonstrate that senescent cells are key drivers of atheroma formation, maturation and plaque rupture.**

SCIENCE, 28 Oct 2016, Vol 354, Issue 6311, pp. 472-477

Sun, Y., Wang, X., Liu, T. *et al*. The multifaceted role of the SASP in atherosclerosis: from mechanisms to therapeutic opportunities. *Cell Biosci* **12**, 74 (2022)



THE CELLULAR MEDICINE APPROACH

Health is Maintained by a Complex Interplay of Cellular Dynamics

- **Energy Production**
- **Protein Manufacturing & Repair**
- **DNA Synthesis, Maintenance & Repair**
- **Balance of Oxidative Stress**

When the cell loses efficiency in any or all of these dynamics, it will try to repair by going into a Senescent state. It is a necessary function of healing and repair. When this state is persistent, extensive and uncontrolled it leads to disease in all cell types.

THE CELLULAR MEDICINE APPROACH

We are at peak cellular efficiency and modulation of senescent cells at young adulthood.

Why?

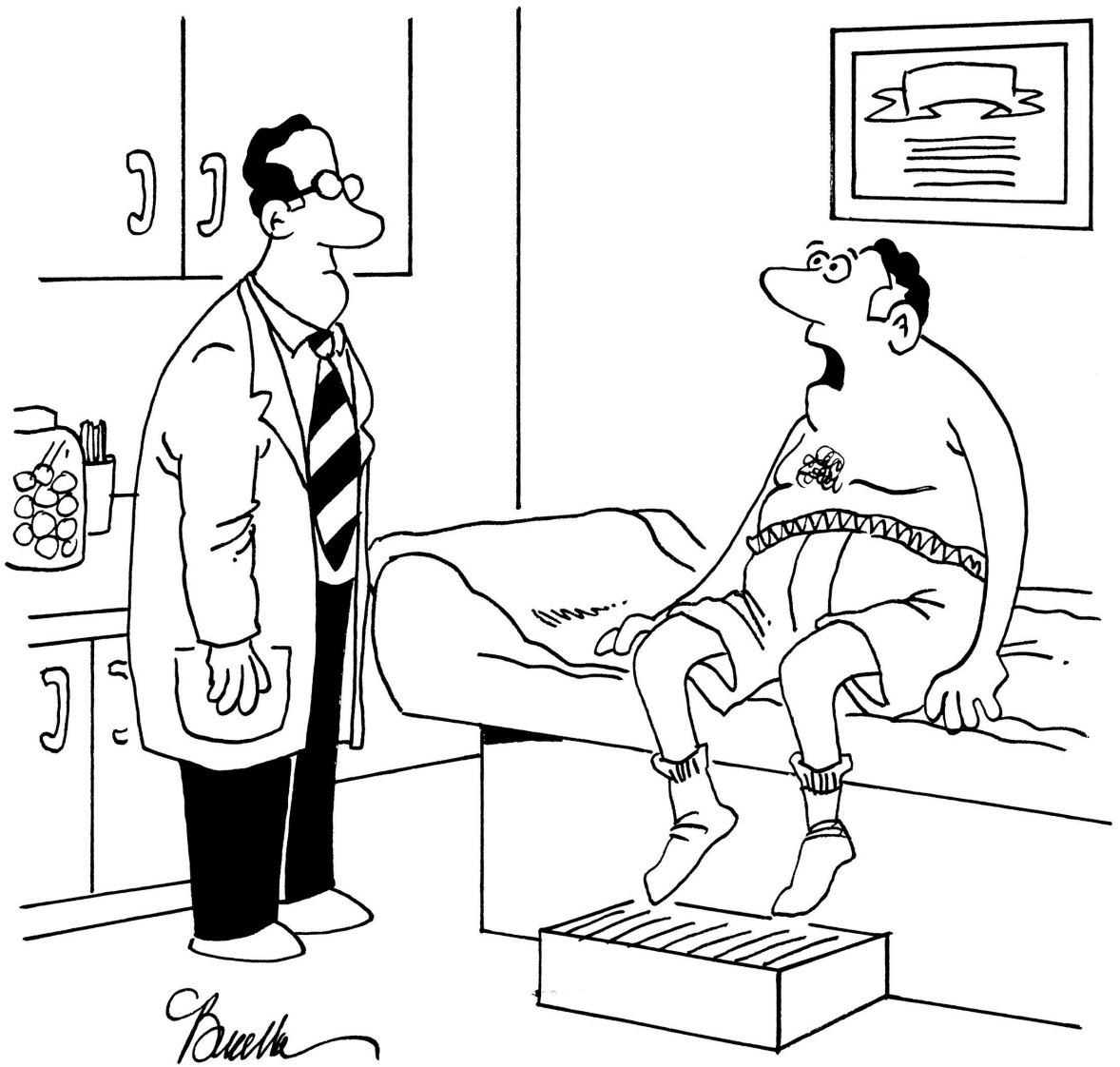
Hormones and Peptides are maintaining cellular efficiency.

These are the levels we should try to achieve with hormonal therapies. "Age appropriate" levels are not achieving cellular efficiency and treatment for cellular senescence.

THE CELLULAR MEDICINE APPROACH

Young Adulthood → **Cellular Efficiency**

Testosterone Estrogen Progesterone	Thyroid Growth Hormone Oxytocin	Melatonin Cortisol



"Low T? How's the rest of my alphabet?"

TESTOSTERONE?

Is testosterone replacement therapy safe for the cardiovascular system?

Multiple links between low testosterone and cardiovascular disease

- Lipoprotein Lipase
- Prostaglandin metabolism
- Lipid levels and Lp(a)
- Blood pressure
- PAI-1 (plasminogen activator inhibitor) and fibrinolysis
- Fibrinogen and blood viscosity
- Inflammatory/Immune status
- Glucose management

TESTOSTERONE!

- Lipoprotein Lipase - increased activity with preferential abdominal fat loss
- Prostaglandin metabolism - increased prostaglandin to thromboxane ratio
- Lipid levels and Lp(a) - reduction
- Blood pressure - arterial tone reduction
- PAI-I and fibrinolysis - increased
- Fibrinogen and blood viscosity - reduced
- Inflammatory/Immune status - decreased systemic inflammation
- Glucose management - increased Insulin sensitivity
- REDUCED ALL CAUSE MORTALITY

TESTOSTERONE!

- Despite more than 70 years of positive data on testosterone therapy there remains trepidation about its use and “controversy” about its safety.
- The two major studies citing increase in adverse outcomes were largely debunked and redacted by the authors yet their mark remains.
- The single greatest obstacle to getting a consensus is a unifying study.
  - What is being measured - total vs free vs bioavailable
  - When it is being measured - before or after their last dose
  - Target range for levels - average for age vs youthful
  - Type of replacement - topical vs injectable vs pellet vs oral vs nasal application
  - Schedule of replacement - daily vs weekly vs monthly

TESTOSTERONE

Functions as a natural modulator of Senescent Cells in multiple cell lines:

-Vascular tissue-

-Progenitor cells-

-Endothelium-

-Pancreas-

-Muscle-

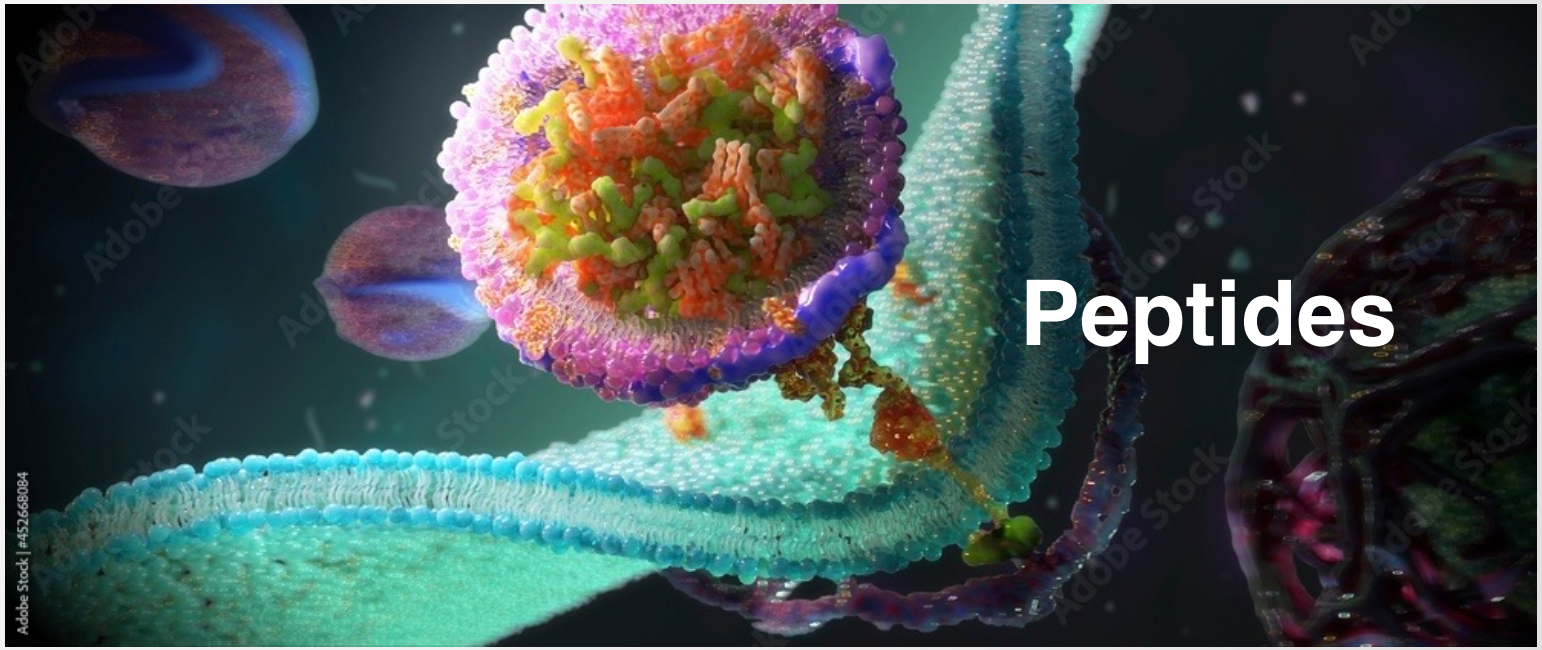
-Brain-

Chen, Yq., Zhao, J., Jin, Cw. *et al.* Testosterone delays vascular smooth muscle cell senescence and inhibits collagen synthesis via the Gas6/Axl signaling pathway. *AGE* **38**, 60 (2016)

World J Mens Health. 2021 Oct; 39(4): 724–732. Published online 2020 Nov 16.

Hormones are large protein molecules that can’t penetrate the cell or create effects without receptors or channels (i.e., a Middle Man).

There is a way that our bodies can send messages and communicate without the Middle Man:



MORE ABOUT PEPTIDES

Have been a part of standard medical care for >100 years!

Molecular messengers that preserve or improve cellular efficiency.

Peptides and Hormones are both needed to maintain optimal health.

Peptides also have the advantage of stimulating Regenerative Mechanisms.

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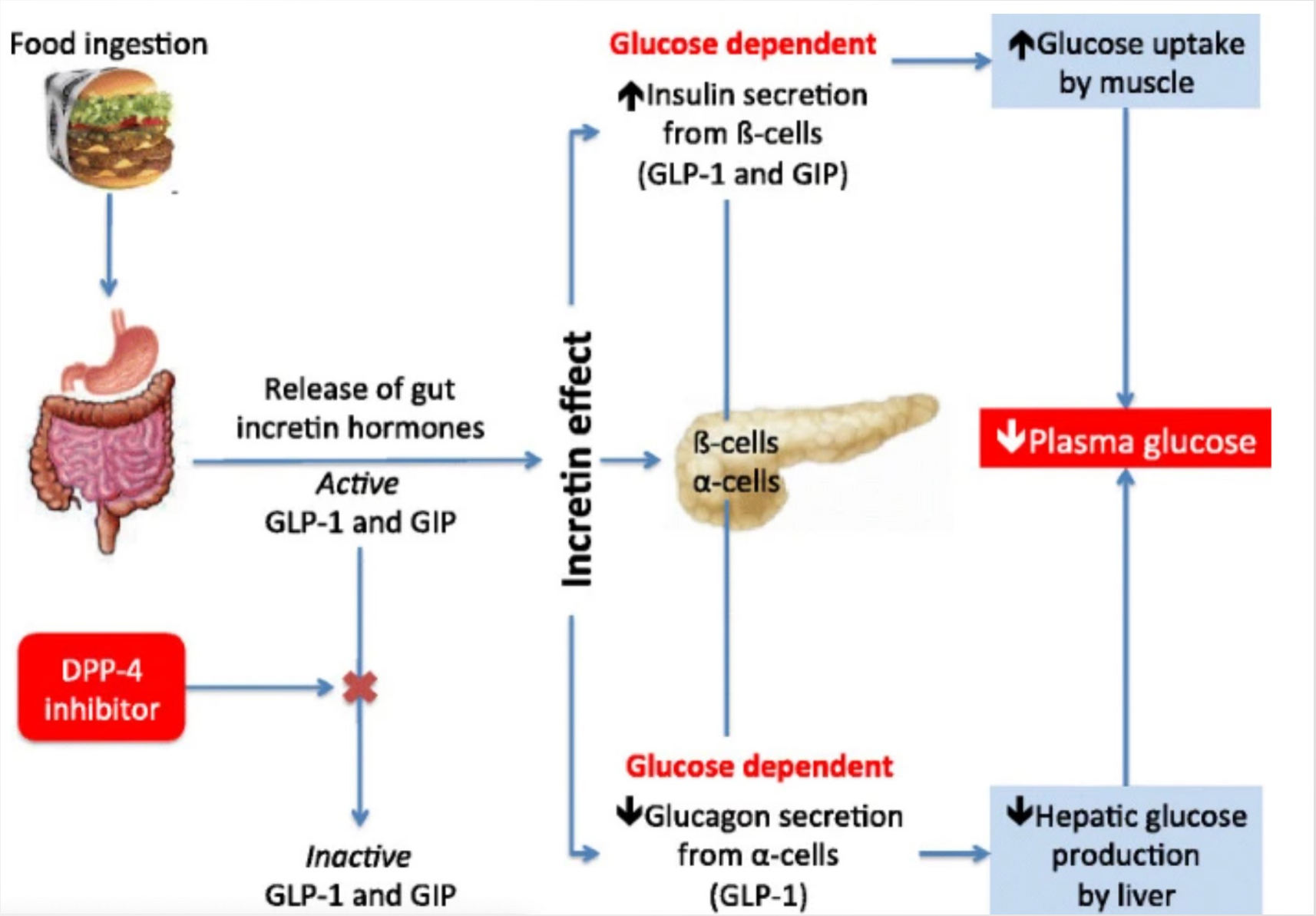
Incretin Peptides

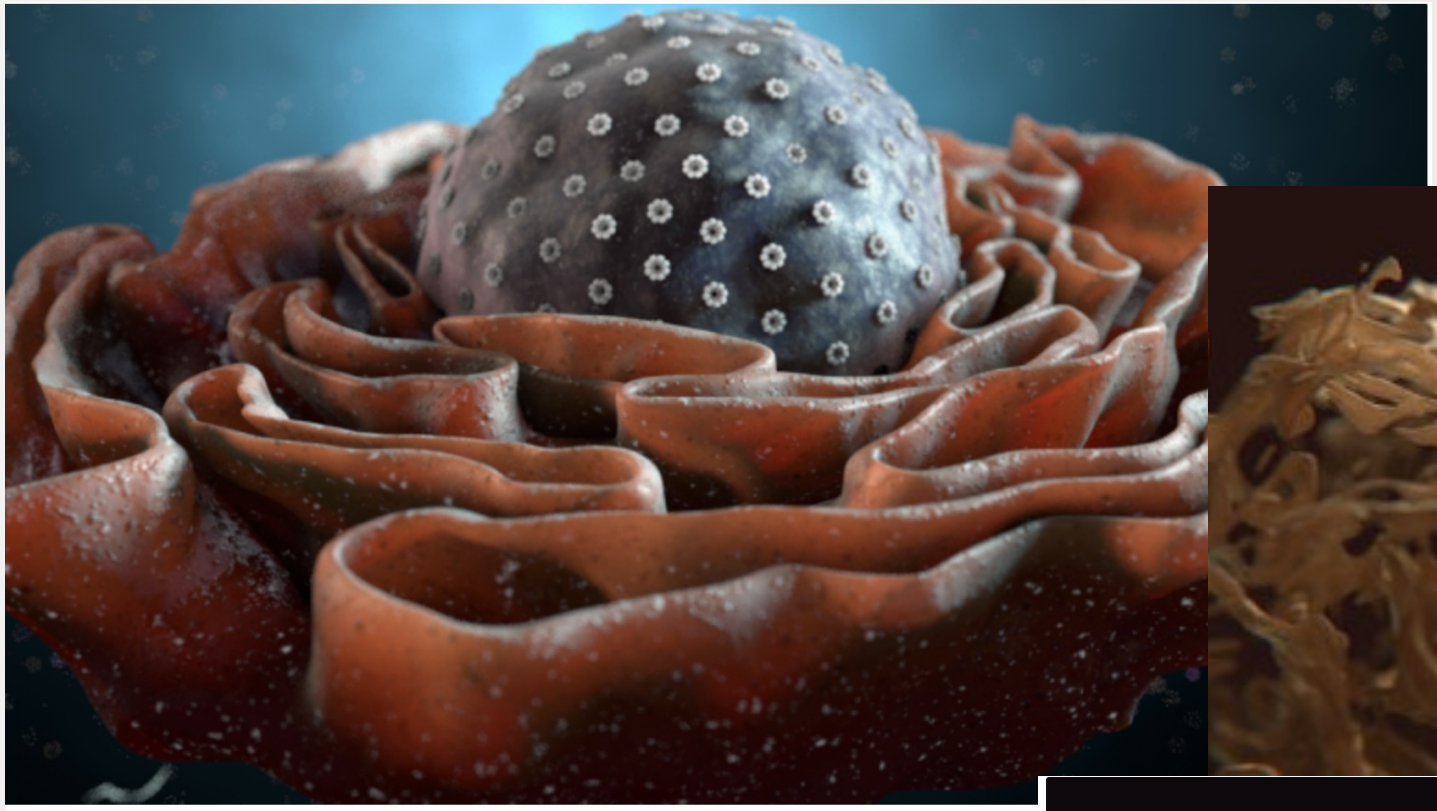
- GLP-1 & Glucose-dependent insulinotropic polypeptide (GIP)
- Short half-life of 2 minutes and rapidly degraded by dipeptylpeptidase IV enzyme (DPP-4)
- Mediates post-prandial blood glucose control and satiety
- Represents a therapeutic intervention in pancreatic beta and alpha cells
- Both GLP-1 is continuously secreted from entero-endocrine cells (EECs) at low basal levels in the fasting or inter-prandial state
- Found in all vertebrates

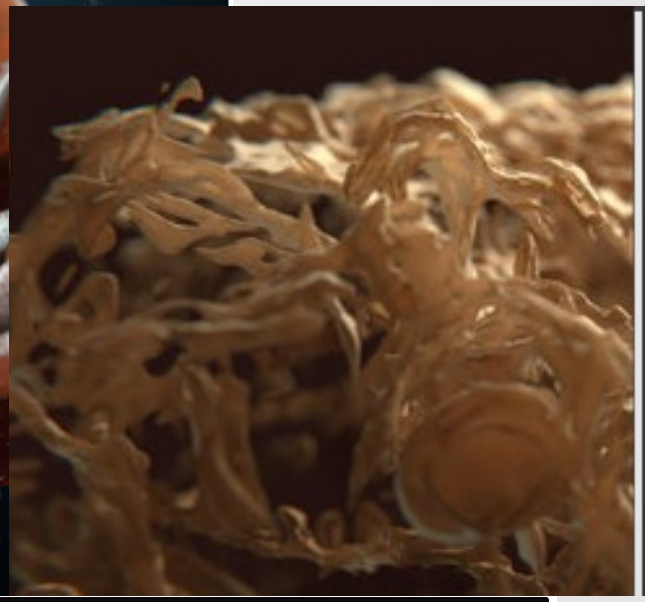


GLP-1 receptor agonist	Number in trial	Trial duration (median)	MACE absolute risk reduction versus placebo	MACE hazard ratio (95% confidence interval) versus placebo	Statistically significant
Liraglutide	9340	3.8 years	1.9%	0.87 (0.78–0.97)	Yes
Semaglutide	3297	2.1 years	2.3%	0.74 (0.58–0.95)	Yes
Exenatide once-weekly	14752	3.2 years	1.2%	0.91 (0.83–1.00)	No
Lixisenatide†	6068	2.1 years	–0.2%	1.02 (0.89–1.17)	No
Dulaglutide	9901	5.4 years	1.4%	0.88 (0.79–0.99)	Yes
Albiglutide	9493	1.6 years	2.0%	0.78 (0.68–0.90)	Yes
Oral semaglutide	3183	1.3 years	1.0%	0.79 (0.57–1.11)	No

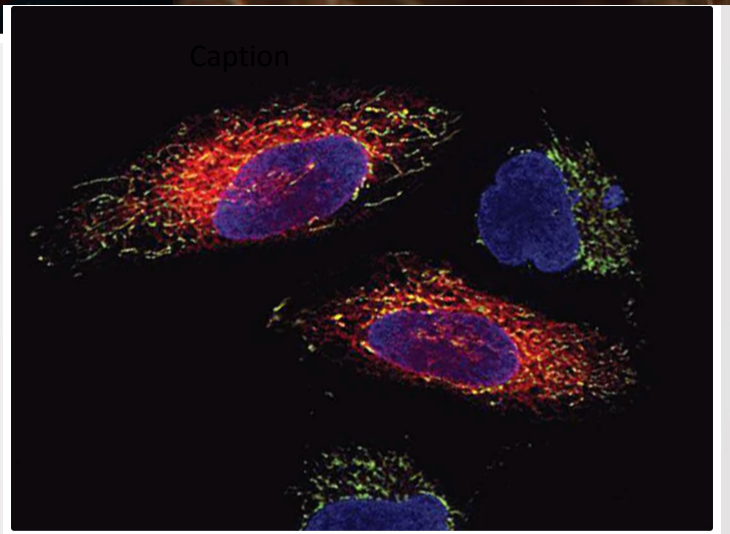
*3-point MACE was a composite outcome of non-fatal myocardial infarction, non-fatal stroke or death from

- Receptors abundantly expressed in the pancreas and central nervous system
  - also detected in lower levels in the gut, kidneys, lungs, liver, peripheral nervous system, bone, immune system, and more…
- Cardiovascular system:
  - sinoatrial node
  - cardiac atria
  - cardiac muscle
  - endothelial cells and vascular cells





https://imgur.com/a/anTwj



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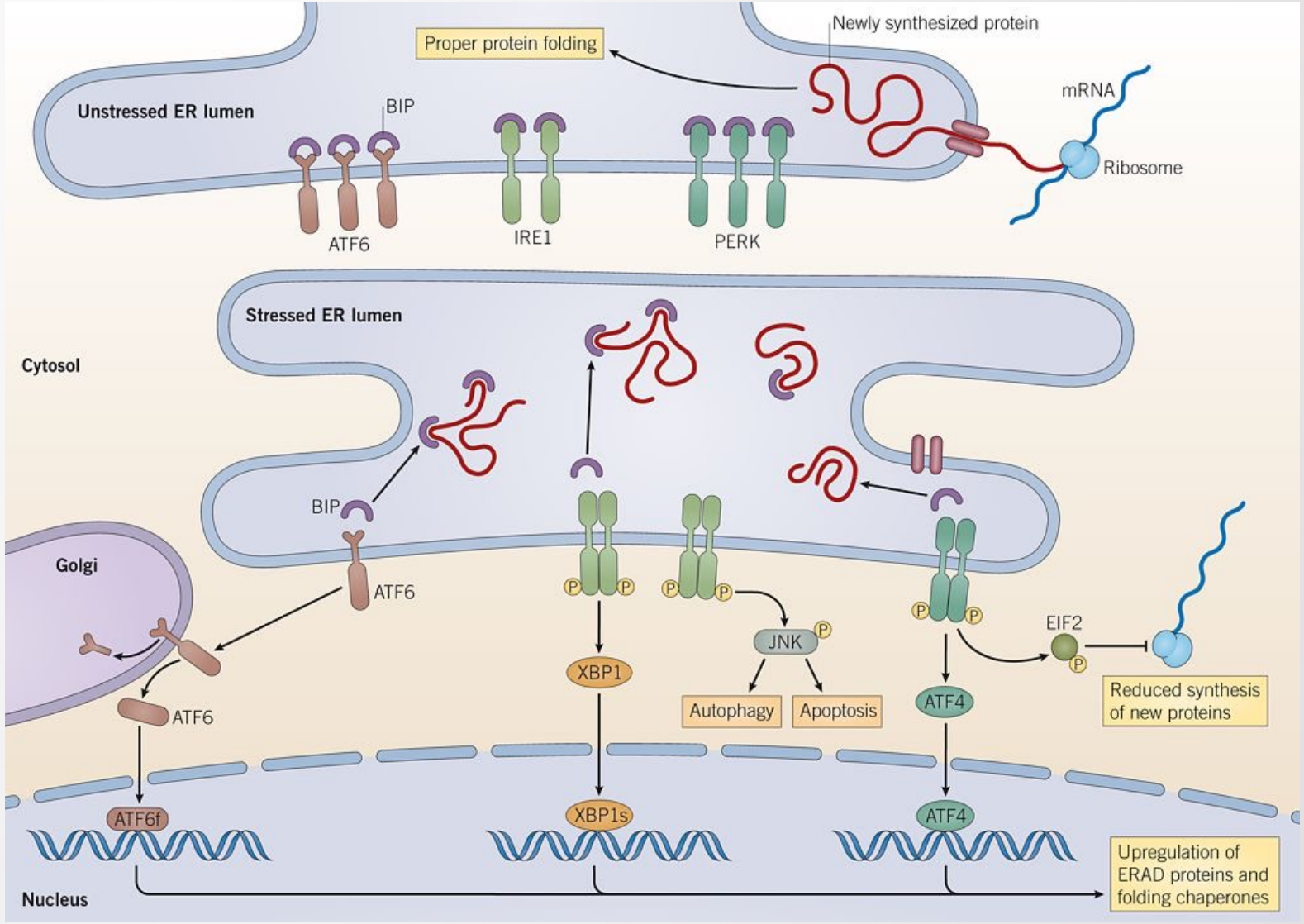

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GLP1-RA —Antioxidant effects

- Direct Effects
  - Upregulates superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities and increase glutathione (GSH)
  - PKA activation following GLP-1R stimulation has been shown to protect against ROS through increased mRNA levels of NADPH dehydrogenase quinone 1 (NQO1) and hemeoxygenase 1 (HO-1).
  - NQO1 and HO-1 both have antioxidant activity and NQO1 also functions as a superoxide scavenger.
- Indirect Effects
  - Reduces plasma concentrations of glucose and free fatty acids(significant drivers of ROS)
  - Reduces superoxide (shown to damage and impair mitochondrial integrity and function)
  - Improves mitochondrial function (reduced ROS leakage from the electron transport chain)

GLP1-RA— Autophagy and Apoptosis

- Via reduction of oxidative stress
- Activates anti-apoptotic genes via cAMP/PKA pathway
- Liraglutide treatment increased SIRT6 expression (DNA repair, telomere maintenance, glycolysis and inflammation) and reduced NF-kB expression
- Autophagy in a **nutrient excess state**!

GLP1-RA— Anti Inflammatory

- Treatment of cultured human islets with exendin-4 suppressed the expression of inflammatory genes such as NFkB1(p105), NFkB2(p100), p65, TNF receptor superfamily member 1A, and receptor-interacting serine/threonine kinase 2

- Liraglutide inhibited the palmitate-induced expression of inflammatory factors and p65 expression.

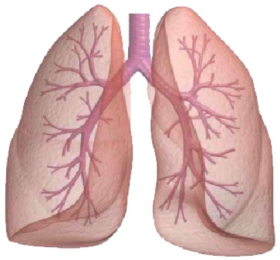
- The inflammatory markers monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor α (TNF-α), were reduced by treatment with exendin-4 in response to **lipopolysaccharide** in cultured peritoneal macrophages harvested from mice

- GLP-1 secretion increases rapidly in response to cytokines (most notably interleukin-6), bacterial metabolites, lipid amides and LPS's in the response to and defense against gut mucosal injury and barrier dysfunction.

GLP1-RA

Functions as a **Senescence** Modulator in all cell types that express a GLP 1 receptor

Young-Sun Lee, Hee-Sook Jun, Anti-Inflammatory Effects of GLP-1-Based Therapies beyond Glucose Control, Mediators of Inflammation, vol. 2016

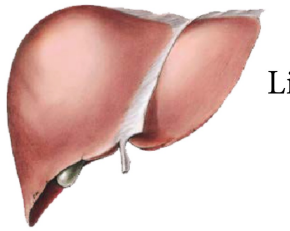


Lung

Asthma:
TNF-α, IL-4, IL-5, IL-13 $\downarrow$

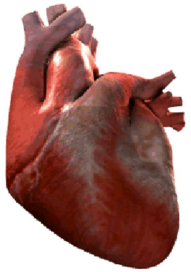
Brain

Alzheimer's disease,
Parkinson's disease:
TNF-α, IL-1β, IL-6 $\downarrow$



Liver

Nonalcoholic steatohepatitis
(NASH):
CRP, TNF-α, IL-1β, IL-6 $\downarrow$

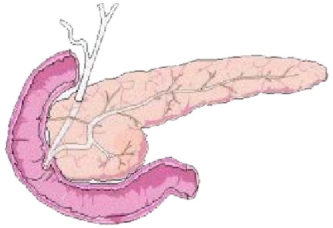


Atherosclerosis
cardiovascular disease:
NOS-2, COX-2, VCAM-1,
TNF-α, IL-6, PAI-1 $\downarrow$

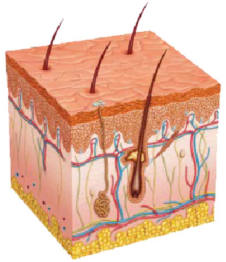
Vascular system

GLP-1
anti-inflammation

Diabetes:
TNF-α, IL-1β, IL-6, IP-10 $\downarrow$

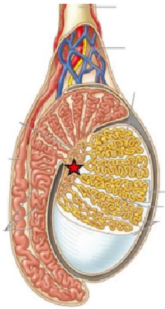


Pancreas



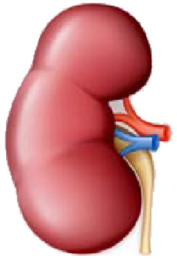
Psoriasis:
iNKT cells $\downarrow$

Skin



Testis:
TNF-α, MCP-1, F4/80 $\downarrow$

Testis



Nephropathy:
TNF-α, IL-1β, ICAM-1 $\downarrow$

Kidney

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GLP1-RA— Anti Inflammatory

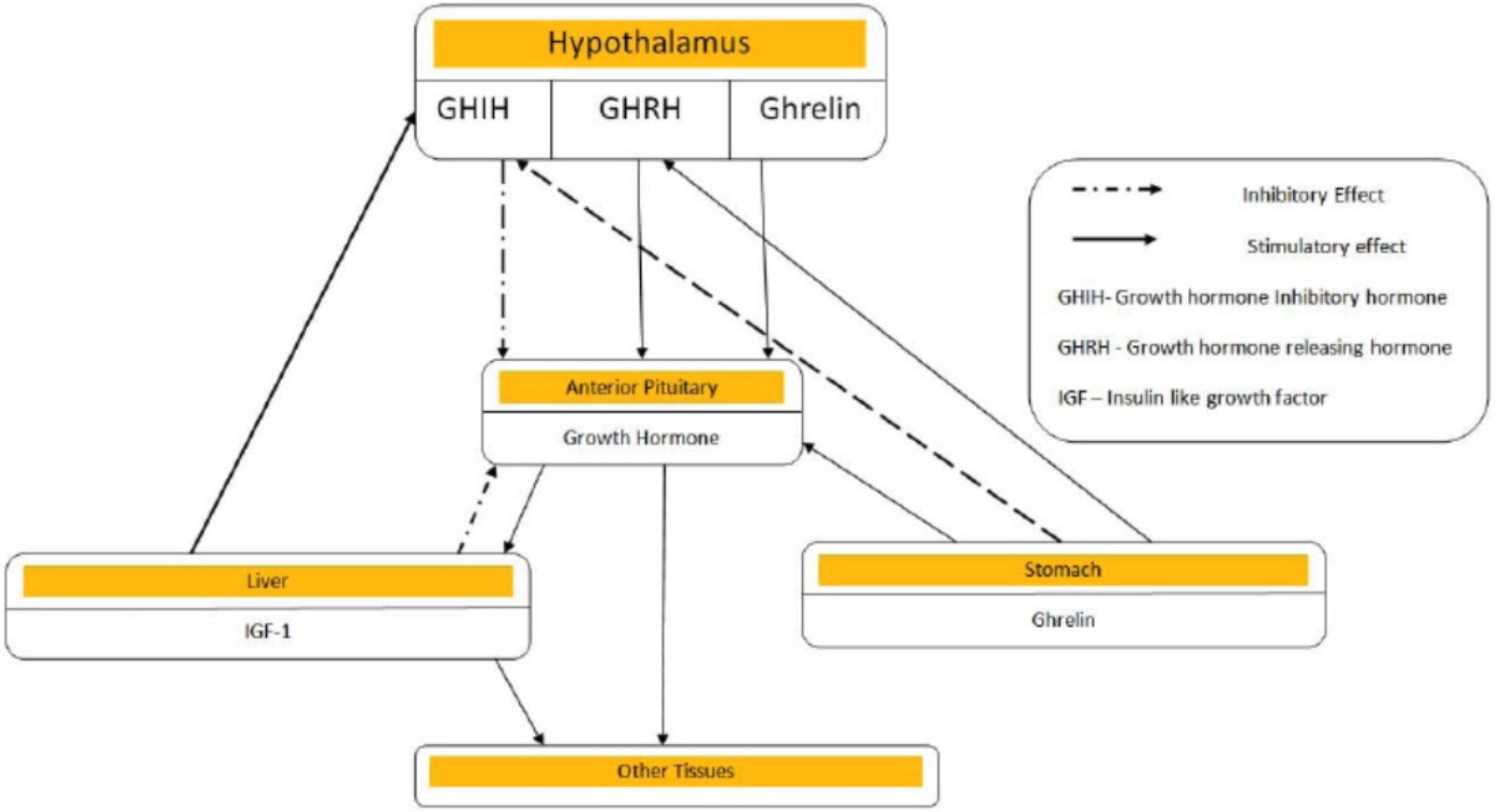
- Vascular function
- Hypertension
- Chronic Stable Heart Failure
- Atherosclerosis stabilization and regression
- Post MI, Post CABG, Acute CHF
- Elevated levels in ICU patients….

GROWTH HORMONE

Replacing Hormones is one of our best tools when Cellular Systems can no longer maintain equilibrium and achieve Peak Efficiency.

Growth hormone is a central regulator in that process.

Growth Hormone Release Anterior Pituitary



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SEEDS SCIENTIFIC RESEARCH & PERFORMANCE

GROWTH HORMONE

- Increase endogenous IGF-1 levels
- Upregulating beta oxidation - FA utilization and metabolic efficiency
- Upregulating PPAR-gamma - FA and cholesterol utilization
- Upregulating PGC-1alpha - AMPK activation
- Upregulating oxidative phosphorylation - efficient ATP production
- Improving mitochondrial efficiency
- Upregulating the SIRT gene - gene regulation and stability
- Activating the FOXO gene - apoptosis, tumor suppression, cell cycle regulation
- Improving the stem cell stress response and maintaining the quiescent state

GROWTH HORMONE

- Anti-fibrotic
- Vasodilation
- Anabolic - appropriate skeletal and cardiac muscle tissue growth
- Inotropic - Improve cardiac contractile function
- Anti-inflammatory
- Improve repair and recovery
- Improve cellular metabolism
- Improve stem cell recruitment - vascular and myocardial

Modulation of Senescent Cells

STATINS!?

The most widely used medication in the world

>30 years of safety data

10-15% of patients get side effects

Internet and media driven controversy

Patient resistance

Definitely not a perfect drug!

The medication that patients and some practitioners love to hate!

WHY?

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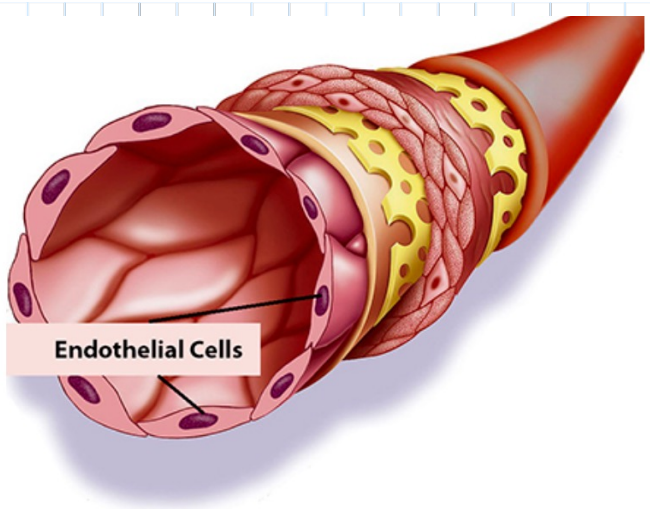
Influence of statins on the aerobic metabolism of endothelial cells



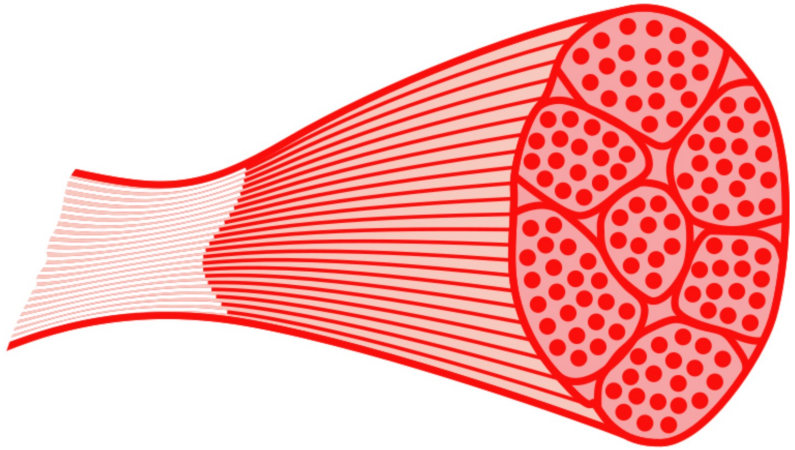
In vitro - endothelial cells exposed to atorvastatin for 6 days:

↓ Mitochondrial Respiration ↓ CoQ10 ↑ Mito and Intracellular ROS ↓ Complex III Activity ↓ Amount of Complex III in Supercomplex IV

These changes could be responsible for observed ↓ mitochondrial membrane potential

Izabela Broniarek, The Influence of Statins on the Aerobic Metabolism of Endothelial Cells Received: 31 January 2020; Accepted: 20 February 2020; Published: 21 February 2020

Simvastatin on Human Skeletal Muscle.



Altered mitochondrial function:

- ↑ cytoplasmic Ca2+
- SR Ca2+ overload
- Ca2+ waves
- ↑ mitochondrial NADH and induced mitochondrial membrane depolarization

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WHY USE STATINS AT ALL?

- Trials suggest a
  - 54% reduction in MI
  - 48% reduction in CVA
- Can Reduce LDL 50% or more
  - LDL is a driver for multiple pathways of inflammation
- Reduces need for revascularization
- Reduces follow up event rates

STATINS- PLEIOTROPIC EFFECTS

- increase endothelial NO production by stimulating and upregulating endothelial NO synthase (eNOS).
- restore eNOS activity in the presence of hypoxia and oxidized LDL
- increase the number of circulating endothelial progenitor cells
- Reduces systemic inflammation in LDL **independent** manner
- Improved bone health
- Improved kidney health

- Decreases **senescence** of endothelial cells and neurons!

STATINS- PLEIOTROPIC EFFECTS

- Inhibits senscence in different cell types, including endothelial progenitor cells (EPC), endothelial cells (EC), VSMC and chondrocytes. At the molecular level, the effect of statins on cellular senescence could be mediated by their interaction with the ***telomere/telomerase*** system.
- Recent evidence suggests that the anti-aging effects of statins are linked to their ability to inhibit telomere shortening by reducing either directly and indirectly oxidative telomeric DNA damage, as well as by a telomere capping proteins dependent mechanism

Current Vascular Pharmacology, Volume 10, Number 2, 2012, pp. 216-224(9)

Table 1. Effect of Statins on Low-Density Lipoprotein-Cholesterol–Independent Diseases

Kidney disease	↓ Creatinine with normal and abnormal renal function[19,20]
Pneumonia	↓ Incidence[22]
	↓ Mortality[21]
Venous thromboembolism	↓ Incidence[31]
Multiple Sclerosis	↓ Whole brain atrophy[23]
	↓ Disability[23]
Bone strength	↓ Hip fracture in postmenopausal women[24]
Gastrointestinal	↓ Cholecystectomy for gallstones[25]
	↓ Pancreatitis with normal triglycerides[26]
Erectile dysfunction	↑ Function in sildenafil nonresponders[27]
Periodontal disease	↓ Periodontal inflammation[28]
Rheumatoid arthritis	↓ Mortality[29]
	↓ Inflammatory markers and improved disease activity score[30]

PCSK9 INHIBITORS

- Emerging evidence suggests a direct involvement of PCSK9 in the pathogenesis of cardiovascular disease independent of hyperlipidemia and liver LDLR
- PCSK9 expression was induced by the inflammatory stimulus lipopolysaccharide and hemodynamic shear stress, mediating a pro-apoptotic effect through mitochondrial DNA damage.
- PCSK9 induces apoptosis and senescence in vascular and neural cells

23 Nov 2021, Arteriosclerosis, Thrombosis, and Vascular Biology. 2022;42:67–86

PCSK9 INHIBITORS

- Inhibition can increase plaque stability and reduce inflammation in a ***LDL independent*** manner

- PCSK9 inhibitors block formation of unstable atherosclerotic plaque in a multitude of ways including the reduction of vascular senescence and apoptosis.

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- The treatment of atherosclerosis will require a re-thinking of the lipid-centric approach. LDLs stimulate a multitude of pathways driving atherosclerosis but it is no longer the only focus of preventative treatment. No singular agent will be the solution but it will require multiple agents that influence multiple cellular processes.

A "Cellular Medicine" approach is needed.

- Multiple tools are already available to treat ASCVD in this manner but are underutilized. And… more are on the horizon.

Thank you for your attention!

Abid Husain, MD, FACC, ABAARM

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