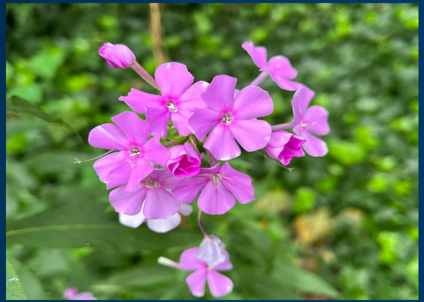
Hormonal Effects on Immune Regulation & Ways to Balance



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Disclosures

Speaker's Bureau:

- Quest Diagnostic Laboratory
- Nestle Health Sciences
- Metagenics
- L-Nutra

Fundamental Premise:

Optimal Hormones are Needed for Optimal Immune and Metabolic Function

PRIME DIRECTIVE OF LIFE:

REPRODUCTION & SURVIVAL OF PRODIGY - TO GROW TO SEXUAL MATURITY

then to repeat the process……

TO THAT END:

Females have a more responsive and robust immune system compared to males

BUT WHEN THINGS GO WRONG:

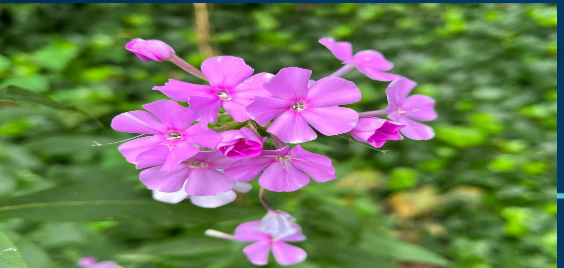
Females respond more aggressively to self-antigens

Females are more susceptible to autoimmune diseases

Estrogen acts on all cellular subsets of immune system through estrogen receptor - dependent & independent mechanisms

- **Women and men have different gut microbiomes**

- **Women and men have different immune systems**



Males & Females have Different Immune Systems

Sex-based immunological differences historically overlooked –
<u>but research & awareness growing!</u>

Khan et al. Front Immunol.2015;6:635
Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38.

Differences driven by:

- X chromosomes
- Epigenetics
- Hormones
- Microbiome
- Circadian Rhythm
- Age and Reproductive status

Sex-based immunological differences not unique to humans - insects, lizards, birds & other mammals also demonstrate immunological differences between sexes

Females Overall do Better than Males in Pandemics

New York City as of April 14, 2020

Deaths: Males 61.8% and Females 38.2%

Prospective trial 1998:

Survival of males vs females with sepsis

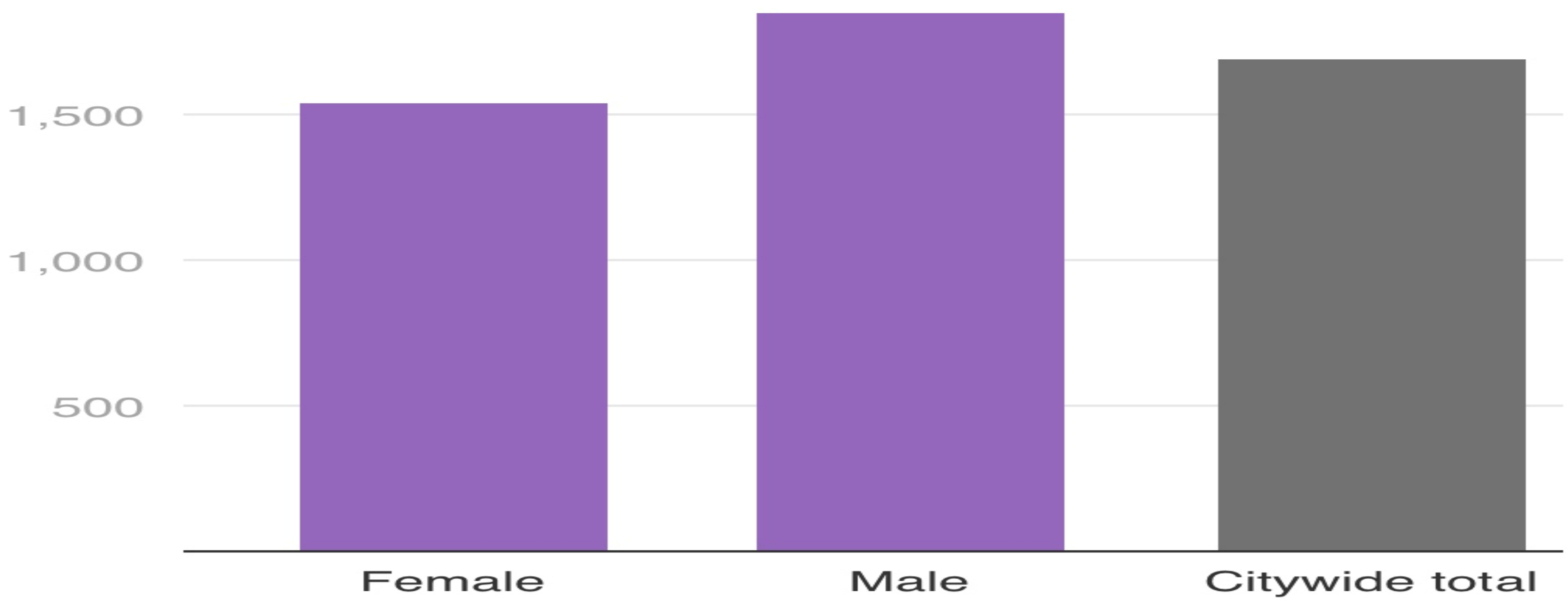
74% survival for women

31% of men survived

Rates by Sex

COVID-19 rate per 100,000 people

Cases Hospitalizations Deaths



Men and Women have Different Genes

- X chromosome contains approximately 1100 genes – many involved in immune functions

- One X chromosome is randomly silenced during X chromosome inactivation but up to 15% of genes escape silencing in humans –

May have large impact

Alebert et al. Nat Rev Immunol. 2010;10(8):594-604

Carrel et al. Nature.2005;434(7031):400-4

Men & Women have Different Genes

Immunological genes – located on sex chromosomes

For example:

Pattern recognition receptors genes

Cytokine receptor genes

Transcriptional factor genes

Non-coding DNA regions

Immune cells

XX sex chromosome mice faired worse than XY mice in autoimmune encephalitis & lupus model, even when gonadal steroid hormones (estrogen or testosterone) depleted

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38..

Men & Women - Different Hormonal Effects

Estradiol acts on all cellular subsets of immune system –
ER dependent & independent mechanisms

Immune System Directly:

- Increases TLR4, TLR7 and TLR9
- Decreases NF-κB activity
- Increases dendritic cell activation
- Increases neutrophil numbers and degranulation
- Increases IFNγ production by NK cells
- Increases in B cells and production of antibodies
- **Testosterone suppresses immune cell activity**

Immune System Indirectly:

- Microbiomes
- Circadian Rhythm

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38..

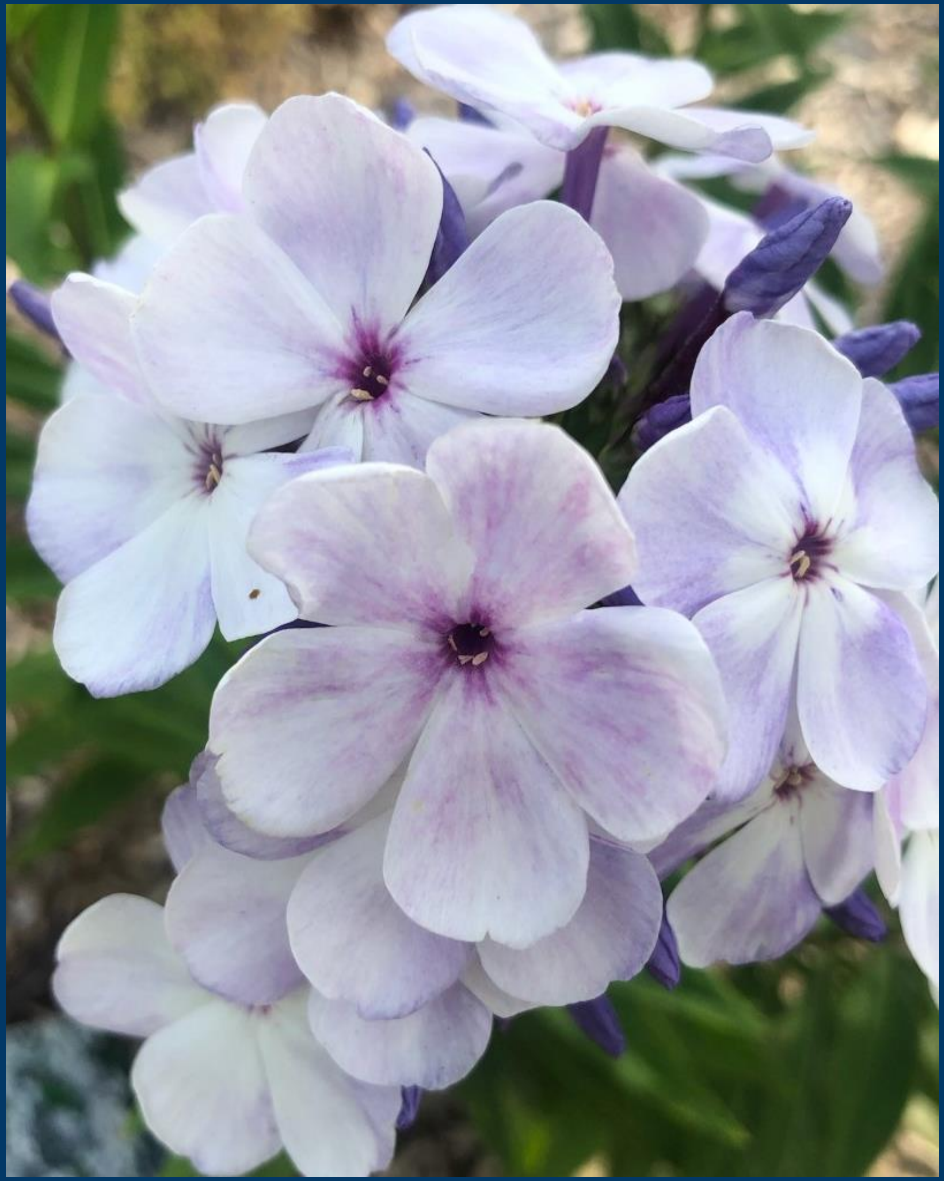
Differences in Adaptive Immunity

- Following invitro stimulation of peripheral monocytes women have higher numbers of activated CD4 and CD8 T cells
- Analysis shows greater cytotoxic T cell activity in adult females
- Stimulation leads to higher upregulation of antiviral genes and pro-inflammatory genes compared to male T cells
- Half of activated genes in female T cells have Estrogen Response Elements (EREs) in their promoters

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38

Differences in CD4 T Cells

- Adult females produce more T helper 1 (Th 1) cytokines (IFN)
- Females have greater antibody responses than males
- Higher basal immunoglobulin levels
- Higher B cell numbers
- Majority of genes differentially expressed are on B cells



Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38.

There are Trade-offs for Each Gender

- Increased vaccine efficacy in females - increased susceptibility to inflammatory & autoimmune diseases
- Energy utilization priorities – energy to mount immune response could be used for growth, maintenance of secondary sex characteristics, reproduction

Theory:

Higher pathogen load & reduced immune function - adverse effect of selection for more successful reproduction & overall survival

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38.

Introducing Estrogen

Estrogen is the

“*MOTHER HORMONE*”

The master of metabolic homeostasis & immune function

Start at the Beginning:
What is the purpose of a Hormone?

An endocrine hormone is any member of a class of signaling molecules produced by glands in multicellular organisms that are transported by the circulatory system to target distant organs to regulate physiology and behavior

What I say:
They are deliverers of important information!!
They deliver a message. It's that simple!

Sex hormones are classically thought of as a reproductive hormone…

But … in the beginning …
it was all about metabolism

Metabolism & Immune Function are One

- Ancestral ER existed in invertebrates lacking sexual reproductive capabilities-played an important role in energy metabolism and survival

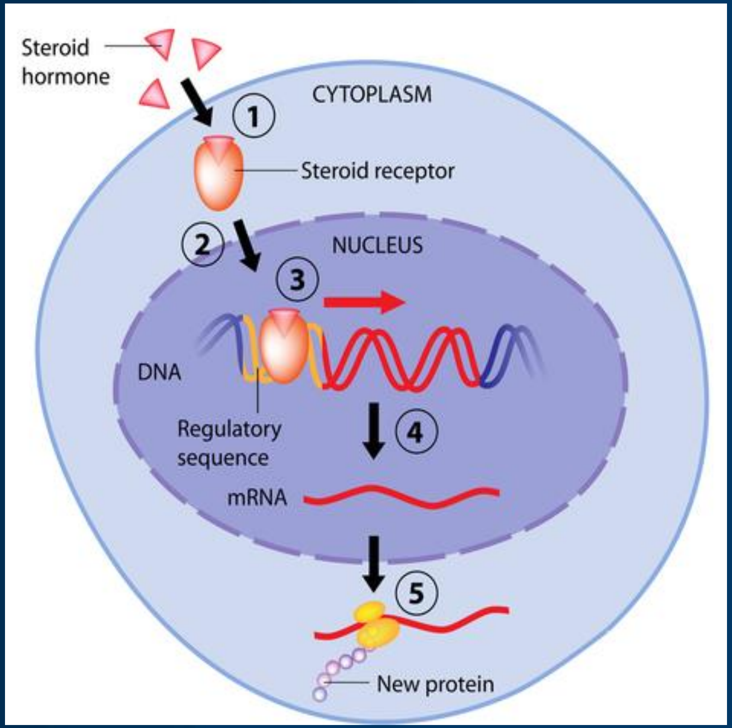
- All members of the steroid receptor family descend from a single ancestral receptor, which separated from the rest of the nuclear receptor family early in animal evolution, 500 million years ago

Mol Cell Endocrinol. Evolution of steroid receptors.2011;334:31-38

Protective Role of Estrogen

- Increases lean body mass
- Reduces abdominal fat
- Improves insulin resistance
- Decreases LDL/HDL ratio
- Decreases blood pressure
- Improves endothelial health
- Maintains bone density
- Improves mood and sleep quality
- Reduces skin wrinkling/hair loss
- Maintains cognition
- Regulates appetite
- Maintains gut & gut microbiome
- Maintains immune system & modulates inflammation
- Modulates endocannabinoid system

Estrogen Basics



- **ER alpha → Regulates genes and membranes**
  - **Expressed in reproductive organs: uterus, ovary, prostate, testes, and breast, and in the hypothalamus of the brain, innate immune cells, T cells**
- **ER beta → Regulates genes and membranes**
  - **Expressed in *non-gonadal* tissues: GI tract, colon, bone marrow, vascular endothelium, lung, bladder, B cells, and much of brain**
- **Membrane-associated ER →**
  - **No effect on genes, but rapid effects on cellular signaling**

High ER beta down-regulates ER alpha

Mendelsohn ME, and Karas RH. *N Engl J Med.* 1999: 340; 1801-1811
Dahlman-Wright et al. *Aspet Pharmacological Reviews.* 2006: 58 (4); 773-781

Introducing the Estrogens

	Forms of Estrogen		
	Estrone (E1)	**Estradiol (E2)**	**Estriol (E3)**
Features	Not beneficial to the brain – dominant in menopause	Dominant estrogen of reproductive aged women	Hormone of Pregnancy
Source	Ovaries, adipose and other hormones	Ovaries	Other estrogens and the placenta
Receptor	ER alpha predominantly	ER alpha ER beta	ER beta predominantly

Estrogen supports wide variety of physiological functions

- Estrogen influences the number, activity and function of immune cells
- Estrogen supports gene activation in immune cells
- Estrogen mediated effects seen in all major innate and adaptive immune cells

Half of all activated genes in female T cells have estrogen response elements in their promoters

Estrogen Receptors are Located…

- Immune System
- Central nervous system
- Skin
- Endothelium
- Lung
- Breast
- Liver
- Gastrointestinal Tract
- Ovary/Uterus
- Bladder/Prostate
- Muscles
- Mitochondria

Beginnings: What is the Immune System & Why Do We Have It?

- Essential for survival
- Keeps us healthy as we drift through a sea of pathogens
- Network of cells, tissues, organs – work together to protect the body
- Distinguishes self from non-self
- Recognizes and clears away dead, damaged, and faulty cells
- Divisions: Innate, Adaptive, Passive

Some of the Genes Regulated by Estrogen

Vasodilation and vasoconstriction

- Endothelial NO synthase
- Prostacyclin cyclooxygenase
- Prostacyclin synthase
- **Renin and angiotensin**
- Endothelin-1

Lipid Metabolism

- Lipoprotein lipase
- Apolipoproteins
- Leptin
- PON 1
- LDL receptors
- HMG-CoAR activity

Immune activity

- **Vascular-cell adhesion molecule**
- **Cytokines (IL1, IL6, TNFα)**
- **Cytokine receptors**
- **Superoxide Dismutase**

Coagulation

- Fibrinogen
- Coagulation factors
- Protein S

Angiogenesis

- Matrix metalloproteinase
- Vascular endothelial growth factor

Non-Genomic Effects

- Fast-acting actions such as NO facilitated vasodilation

Saltiki, K and Alevizaki M.. *Hormones*. 2007; 6(1): 9-24

JVI
Journals.ASM.org

17β-Estradiol Protects Females against Influenza by Recruiting Neutrophils and Increasing Virus-Specific CD8 T Cell Responses in the Lungs

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W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

ABSTRACT
17β-Estradiol (E2) treatment limits the pathology associated with pulmonary diseases caused by pathogens, allergens, and asthma, partly by reducing the production of proinflammatory cytokines and chemokines. To test the hypothesis that E2 protects against influenza A virus (IAV) infection by altering the recruitment and activity of innate immune cells and T cells, chemokine concentrations were measured and innate and adaptive immune cells were enumerated from the lungs of E2- and placebo-treated ovariectomized female C57BL/6 mice following infection. Females treated with E2 experienced less morbidity but had similar lung virus titers to placebo-treated females. Females treated with E2 had lower induction of CCL2 but higher CCL3 and CXCL1 responses in their lungs than placebo-treated females. Pulmonary recruitment of neutrophils, NK cells, macrophages, and dendritic cells was increased following infection, but only neutrophil numbers were greater in E2-treated than placebo-treated females. Neutrophils enhance the responses of influenza virus-specific CD8 T cells to promote virus clearance and improve the outcome of infection. Total numbers of virus-specific CD8 T cells were not altered by treatment with E2, but the proportion of gamma interferon (IFN-γ)- and tumor necrosis factor alpha (TNF-α)-producing, virus-specific CD8 T cells was increased. Neutrophil depletion in E2-treated females increased morbidity, reduced pulmonary production of chemoattractants for neutrophils, and reduced IFN-γ production by virus-specific CD8 T cells. Neutrophils mediate both inflammation and tissue repair during IAV infection and are regulated by E2 to improve the outcome of influenza in females.

IMPORTANCE
Severe influenza is associated with excessive inflammation that leads to tissue damage. We demonstrate that estradiol (E2) is a potent anti-inflammatory hormone that reduces the severity of influenza A virus infection in females. Treatment of female C57BL/6 mice with E2 does not affect virus replication but rather alters the production of chemokines, pulmonary recruitment of neutrophils, and the cytokine responses of virus-specific CD8 T cells to protect females against severe influenza.

OPEN ACCESS Freely available online

PLoS PATHOGENS

Elevated 17β-Estradiol Protects Females from Influenza A Virus Pathogenesis by Suppressing Inflammatory Responses

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Estrogen receptors regulate innate immune cells and signaling pathways

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Abstract

Humans show strong sex differences in immunity to infection and autoimmunity, suggesting sex hormones modulate immune responses. Indeed, receptors for estrogens (ER) regulate cells and pathways in the innate and adaptive immune system, as well as immune cell development. ERs are ligand-dependent transcription factors that mediate long-range chromatin interactions and form complexes at gene regulatory elements, thus promoting epigenetic changes and transcription. ERs also participate in membrane-initiated steroid signaling to generate rapid responses. Estradiol and ER activity show profound dose- and context-dependent effects on innate immune signaling pathways and myeloid cell development. While estradiol most often promotes the production of type I interferon, innate pathways leading to pro-inflammatory cytokine production may be enhanced or dampened by ER activity. Regulation of innate immune cells and signaling by ERs may contribute to the reported sex differences in innate immune pathways. Here we review the recent literature and highlight several molecular mechanisms by which ERs regulate the development or functional responses of innate immune cells.

Neuroscience

Volume 304, 24 September 2015, Pages 328-339

17β-Estradiol up-regulates Nrf2 via PI3K/AKT and estrogen receptor signaling pathways to suppress light-induced degeneration in rat retina

C. Zhu [a, b] … X. Yu [a, d]

βE2 administration up-regulates NRF2 expression.

βE2 regulates NRF2 via two pathways: a nongenomic PI3K/AKT response and a genomic ER response.

Innate and Adaptive Immune System Genes Regulated by Estrogen

List of key selected genes that are regulated by estrogen in cells of innate and adaptive immune system.

Immune cell	List of genes	Reference
Neutrophil	CINC-1, CINC-2β, CINC-3, TNFα, IL-6, IL-1β	(8–10)
Macrophage	iNOS, NO, IL-6, TNFα	(12–16)
Dendritic cells	IL-6, IL-10, CXCL8, CCL2, TGFβ, IL-23, IL-12	(17, 18, 22)
Th1	IFNγ	(23–25)
Th2	IL-4	(26)
Tregs	FoxP3, PD-1, CTLA-4	(27–30)
B cells	Immunoglobulin, CD22, SHP-1, Bcl-2, VCAM-1	(31)

What is the Role of Inflammation?

- **Biological defense mechanism induced by innate immune system against microbial infections**
- **Macrophage Toll-like receptors recognize conserved structures on pathogens - bacteria, parasites, & viruses**
- **TLR4 signaling pathway tightly controlled by *circadian clock* – prepares immune cells for integrated response at time of greatest risk**

White Blood Cells – Leukocytes:
Circulate in Blood and Lymphatic Vessels

Innate Immune System:

First Line Responders

Interface with Acquired Immune System to Perform Phagocytosis

The Players:

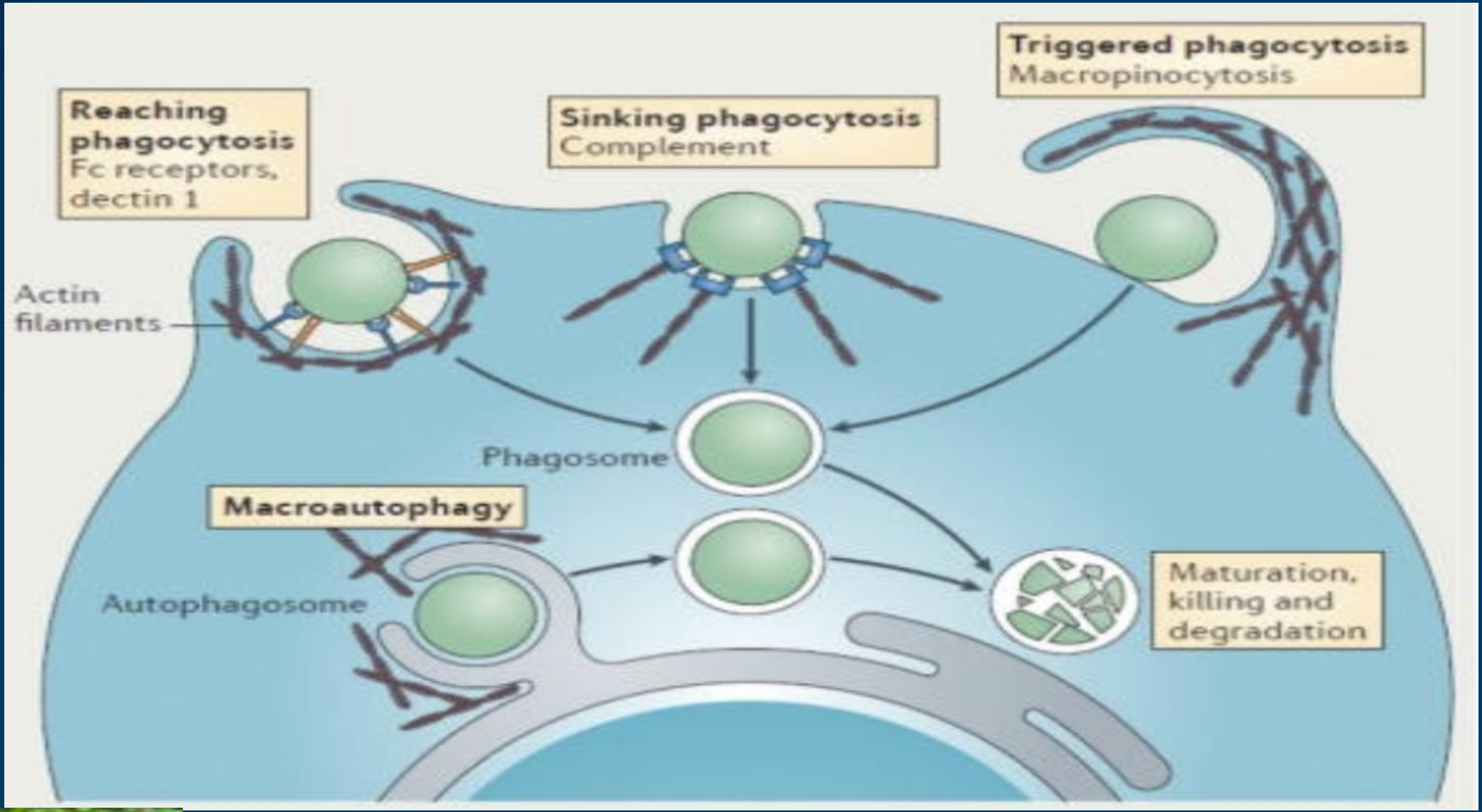
Mast cells

Neutrophils

Monocytes

Macrophages

Phagocytosis



Underhill et al. Information processing during phagocytosis. Nature Review. Immunology. 2012l12(7)L492-502.

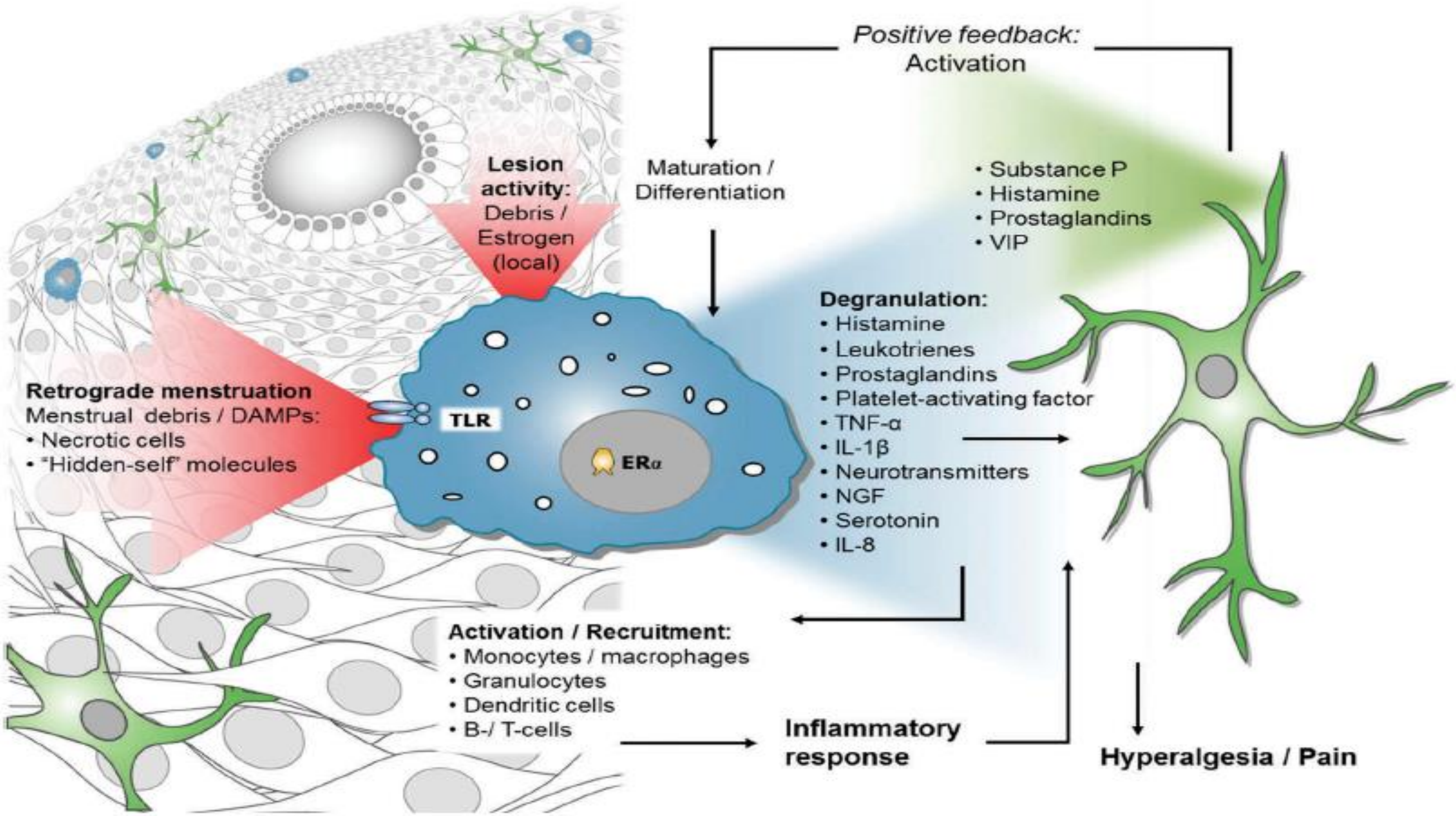
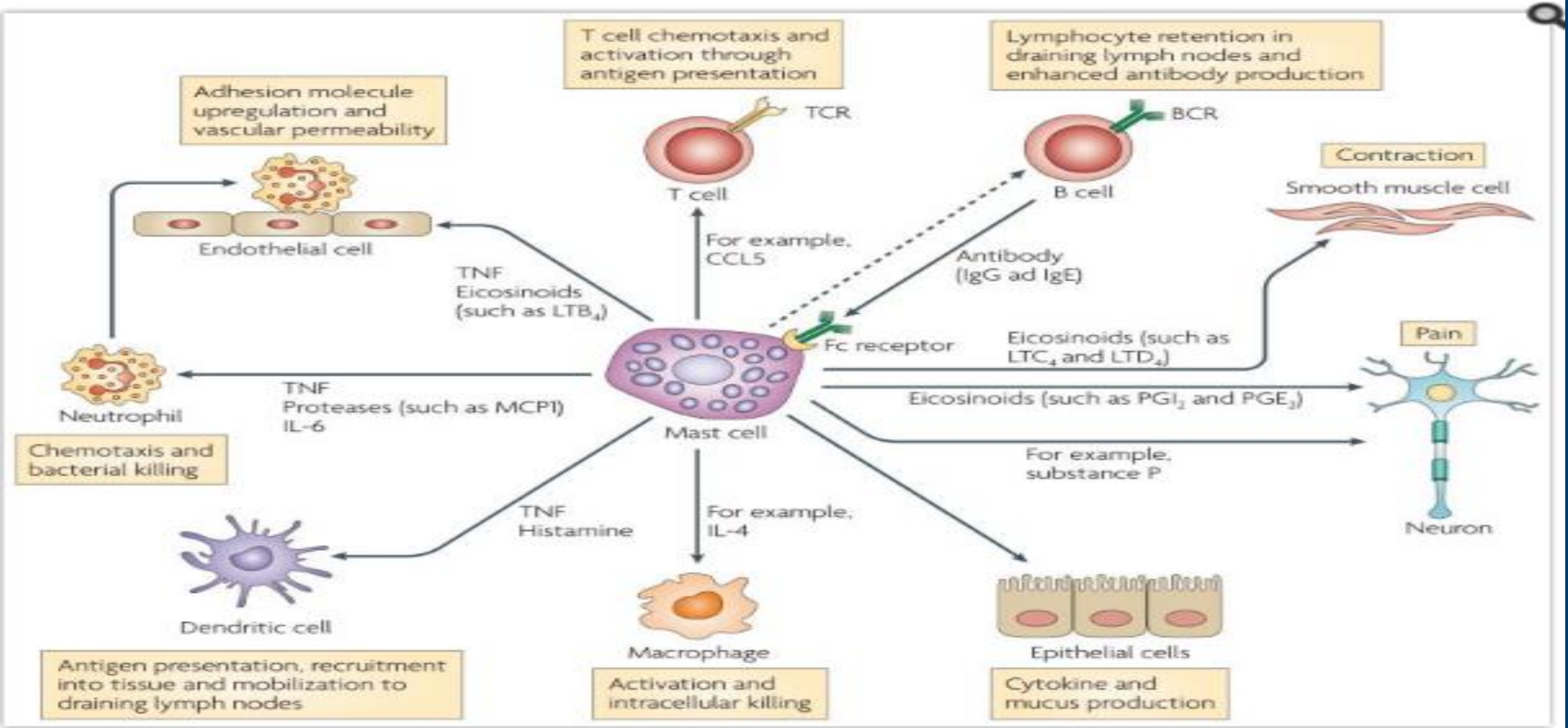


Figure 1. **Mast cells as key players in the pathology of endometriosis.** Kirchhoff D et al. [31] are acknowledged for kindly providing.



Cellular communication by mast cells promotes host defence

Nat Rev Immunol. 2010

Estrogen & the Innate Immune System

Estrogen impacts:
Neutrophils, macrophages/monocytes, natural killer cells, dendritic cells

Neutrophils:

- **Regulate numbers and functions: chemotaxis, infiltration, production of superoxide anion and myeloperoxidase**
- **Induction of chemokines**
- **Induction of cytokines (TNF alpha, IL-6, IL-1beta)**
- **Regulate genes**

Cutolo et al. Lupus.2004;13(9):635

Estrogen & the Innate Immune System

Macrophages:

- Regulate chemotaxis
- Phagocytic activity
- Induction of cytokines, iNOS, and Nitric Oxide
- Regulates macrophages in brain and gut – microglia (involvement in brain health and dementia, gut health)

Karpuzoglu et al. Endocrinology.2009;150(3):1310

Estrogen Affects Innate Immune Cell Signaling

Dendritic Cells:

- Messengers between innate and adaptive immune systems
- Present antigen material to T cells
- Enhance differentiation of immature DC’s into mature functional DCs
- Regulate expression of cytokines and chemokines (IL-6, IL-10, CXCL8, CCL2

Lelu et al. J Immunol.2011;187(5):2386

Estrogen Modulates the Adaptive Immune System

T Cells:

- Modulates all subsets of T cells that include CD+4 (Th1, Th2, Th17, and Tregs)
- Promotes expansion and frequency of T reg cells – critical role in downregulating immune responses via ER alpha mediated signaling

Lelu et al. J Immunol.2011;187(5):2386

Estrogen & Adaptive Immune Cells

T Lymphocytes –

- CD4+ cells have more ER alpha compared to ER beta
- Modulates IFN gamma secreting Th1 cells - E2 driven Th1 cell responsiveness dependent on ER alpha mediated signaling

Khan et al. Front Immunol.2015;6:635.
Lelu et al. J Immunol.2011;187(5):2386

Estrogen Modulates the Adaptive Immune System

B Cells:

- E2 affects B cell differentiation, activity, function, and survival by increasing expression of genes
- Increases plasma cell and autoantibody producing cell numbers
- B Cells – have more ER beta than ER alpha
- Both ER alpha and beta shown to alter B cell maturation, but ER alpha engagement critical for autoimmunity

Think about these issues –

Oral contraceptives
Endocrine disruptors
Menopausal choice of hormone therapy

Lelu et al. J Immunol.2011;187(5):2386

Lymphocytes:
Adaptive (Acquired) Immune System

1. Bone marrow –
B lymphocytes:
Produce antibodies and help alert T lymphocytes

2. Thymus –
T lymphocytes:
Destroy compromised cells in body and help alert other leukocytes
T Helper cells, Killer T cells

Antibodies lock onto antigen but do not kill it –
just mark it for death
Killing is job of phagocytes!

Amount of Estrogen Matters

The concentration of estrogen influences its impact on the immune system

“E2 has a bi-potential effect on monocytes and macrophages – low doses enhance the production of pro-inflammatory cytokines while high doses reduce the production of these cytokines”

Controlling inflammation is a key function of ESTROGEN!

Lateef A and Petri M. J of Autoimm. 2012; 38: J170-J176.

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38..

Estrogen Effects

- Low E2 promotes Th1 type responses and cell mediated immunity
- High E2 augments Th2 type responses and humoral immunity
- Low dose E2 increases production of IFN gamma by T cell & upregulate pro-inflammatory responses mediated by NF Kappa B
- Exogenous E2 enhances expansion of T reg cell populations in mice and healthy women
- High doses E2 lowers IL 17 production by Th 17 cells
- Ovariectomy of mice increases Th17 cells and IL 17 production
- E2 at physiological doses stimulates humoral responses to infection

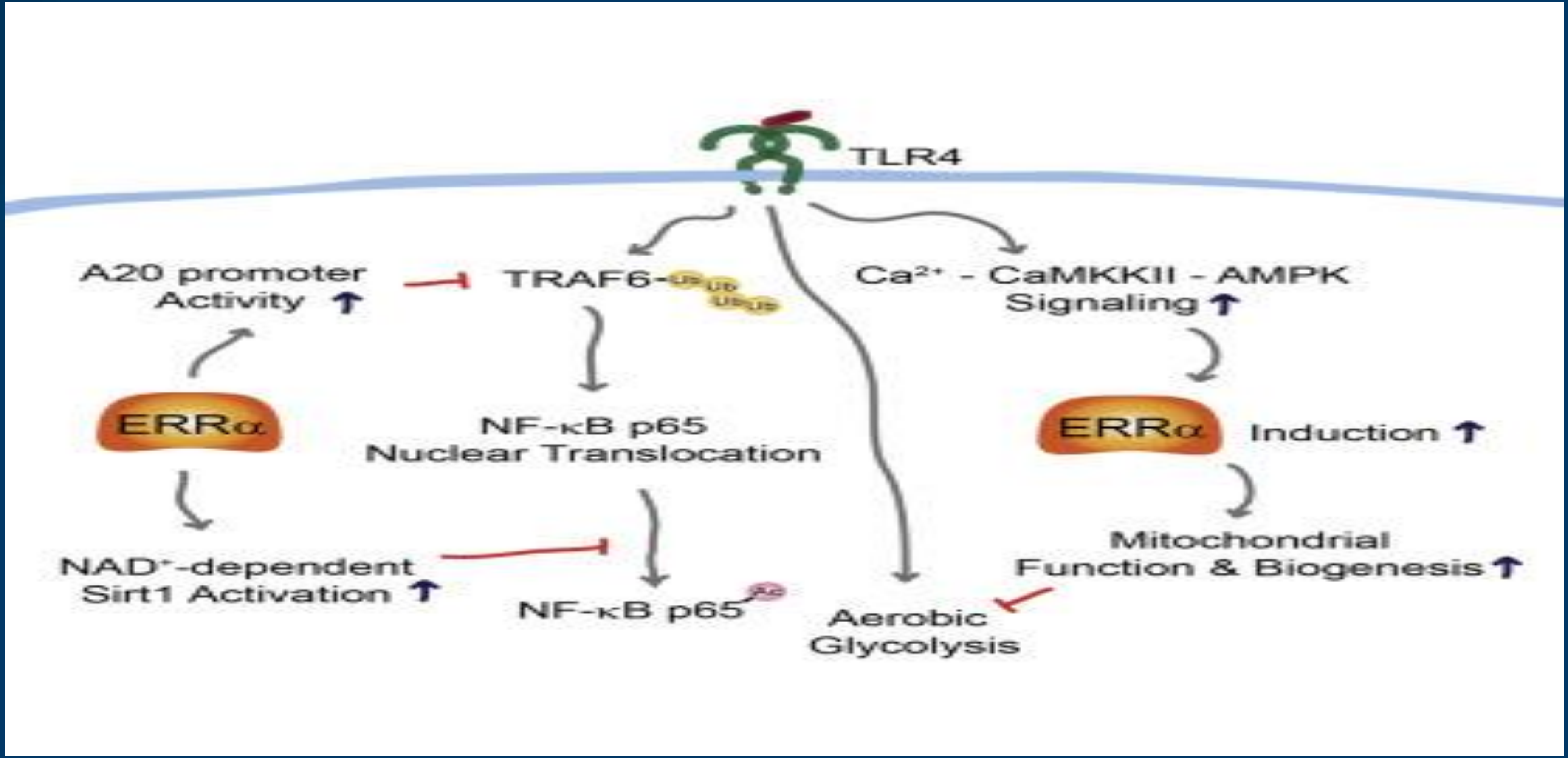
Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38

Progesterone Effects

- Receptors present on many different immune cell types: NK cells, macrophages, DCs, T cells
- P4 has broad anti-inflammatory effects – lower state of activation of macrophages and DCs and reduced inflammatory cytokines
- Reduces production of iNOS and NO
- Antagonizes TLR and NF Kappa B pathways and reduces NK production of IFN gamma
- Pushes from Th1 to Th2 (increased IL-4, IL-5, IL-10)

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38

Estrogen Maintains NAD levels and Activates SIRT1 to Reduce Inflammation



unity. 2015:43(1):80-91

80% of Autoimmune Diseases in Females

Most pronounced for:

- **Sjögren syndrome**
- **Systemic lupus erythematosus**
- **Thyroid diseases**
- **Scleroderma**
- **Myasthenia gravis**

Why?

Khan et al. Front Immunol.2015;6:635

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38

Autoimmune Disease and Hormones

Administration of hormone therapy to patients with autoimmune diseases may have novel therapeutic applications

- **In females with severe MS** – administration of high doses of estrogen suppress cell mediated immune responses and relieves disease symptoms

17 Beta Estradiol - Protective Effects

Estrogen attenuates acute inflammation in sepsis in rat study

- Ovariectomy for 9 weeks then LPS-induced damage
- Long term treatment with E2 prevented the amplified damage (in ovariectomized rats)
- Activation of autophagy contributed to protective effect of long term E2 replacement on multiple organ dysfunction syndrome in endotoxemia

Chung et al. J Cell Mol Med. 2017

Estrogen Indirectly Affects the Immune System - Modulates the Microbiome

- ***Estrogen*** **reduces LPS-induced inflammation by modulating inflammatory pathways, decreasing concentration of pathogenic bacteria**
- ***Estrogen*** **& probiotics might improve gut leakiness & inflammation due to obesity (reduce NAFLD)**

Laaksonen at al. 2004, Diabetes Care. 2004: 27(5) 1036-41
Gradt et al, Obstet Gynecol. 1995; 85(2) 304-13

Pregnancy & Menopause

Pregnancy:

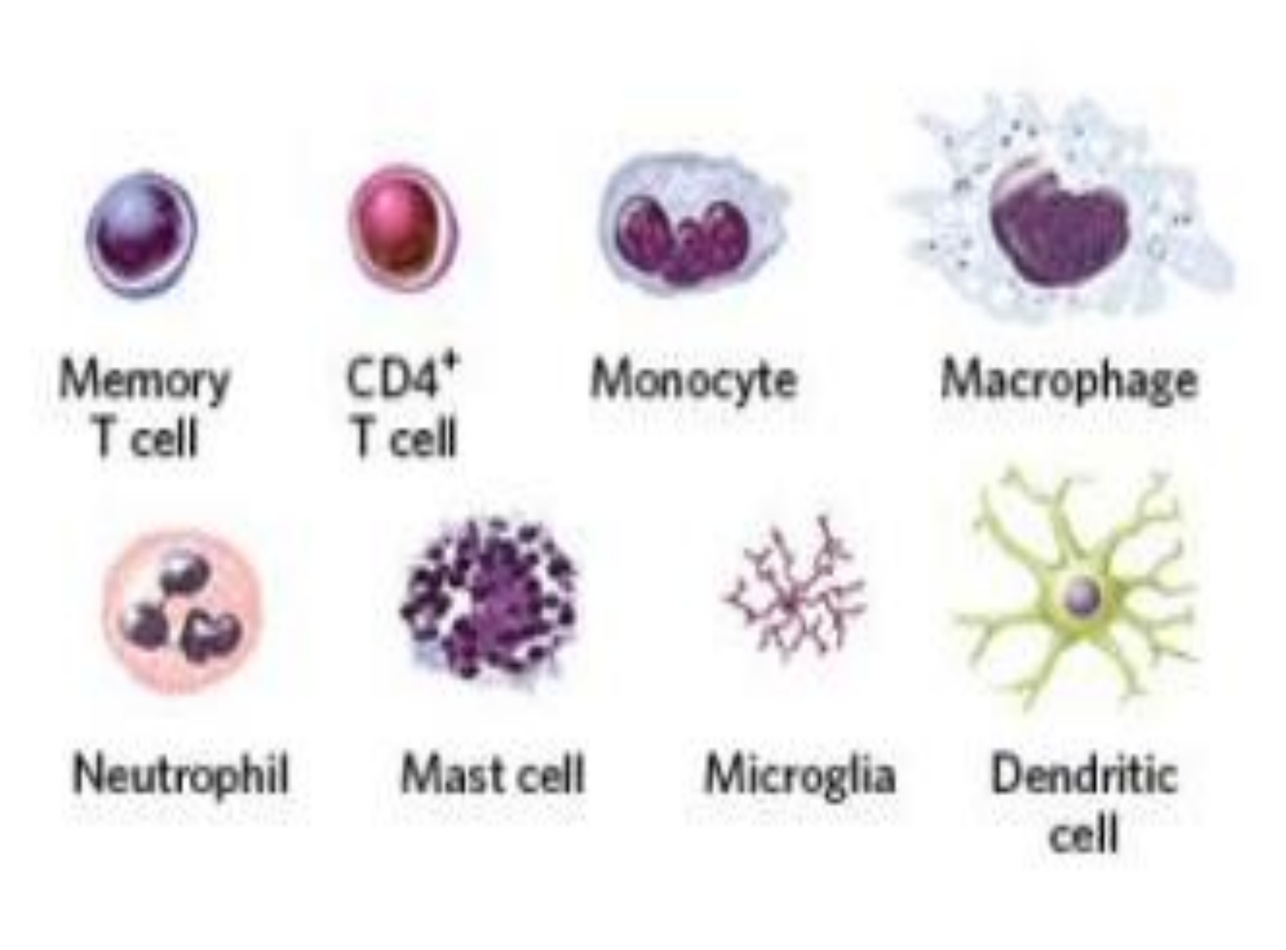
- Immune system skews from Th1 (IFN gamma) to Th2 (IL-4)
- More complex and varies with trimester
- Pregnancy often associated with improved symptoms of autoimmune disease – down-regulation of ER alpha by ER beta

Menopause:

- Associated with increased Th17 – increase in inflammation!!!

Protective effects of estrogen in autoimmune conditions such as MS and RA are believed to be related to estrogen-mediated T reg expansion and activation

Immune Cells of the CNS

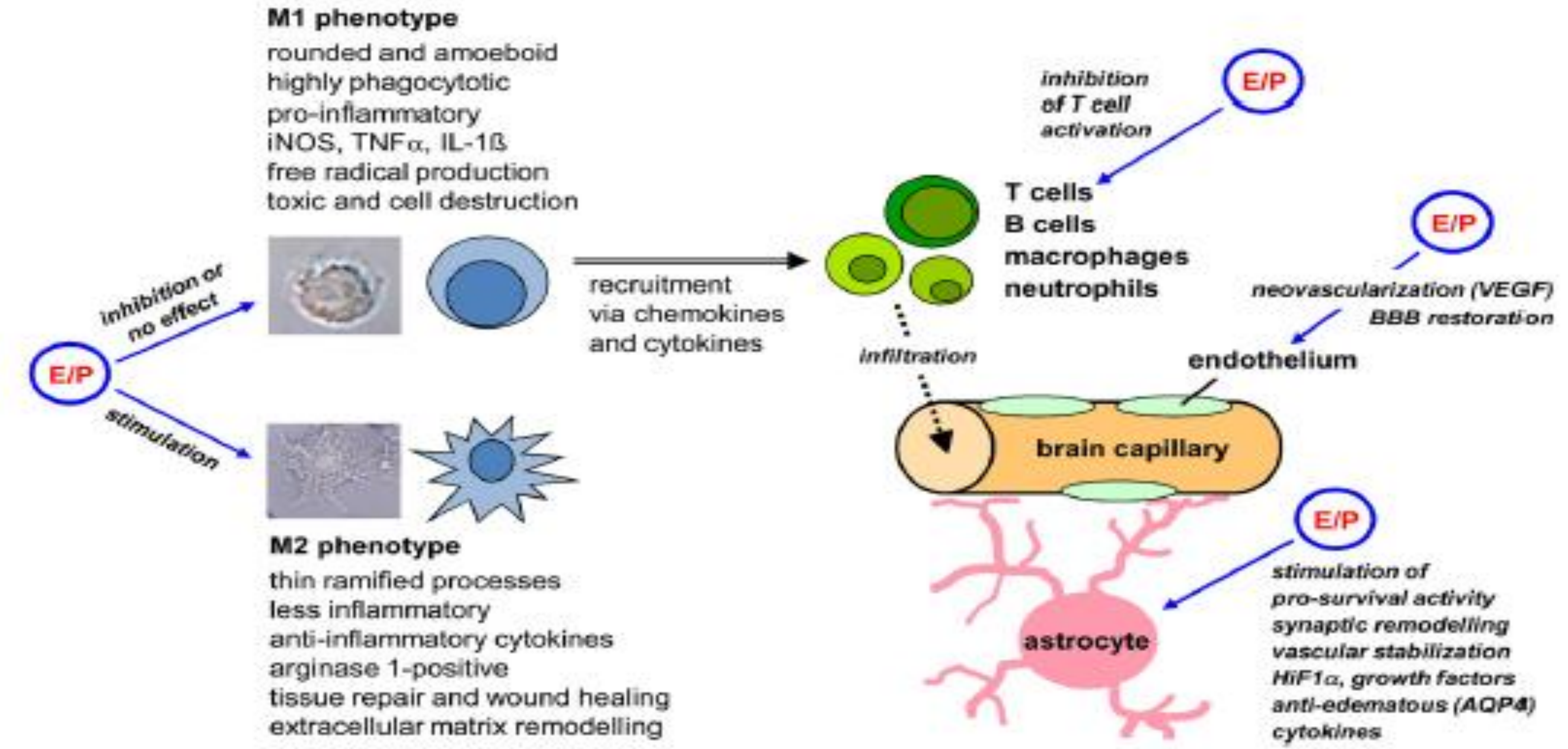


Miraculous Estrogen: Suppression of Local Brain Inflammation

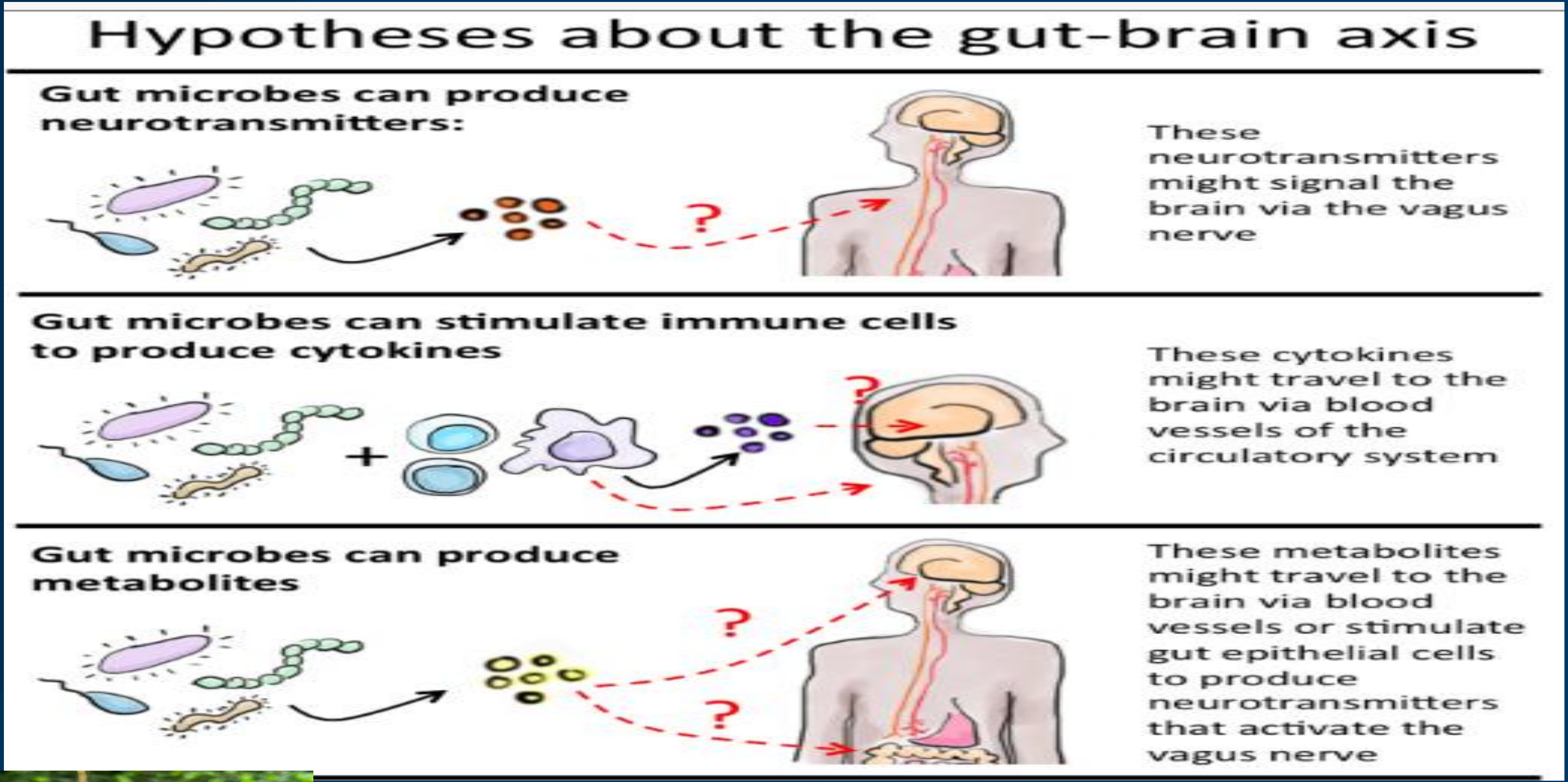
- ER beta: dampens LPS stim of NO production in microglia
- ER alpha: attenuates oxidative damage in hippocampus after hypoxia, inhibits chemokines
- Decreases inflammatory responses (IL 6 and NF kappa B) in neurodegenerative conditions, reduces expression of cyclooxygenase-2
- Antagonize pro-inflammatory glial cytokine responses
- Pro-survival factors (glutamate metabolism and growth factor supply)

Habib et al. J Steroid Biochem and Mol Bio 2014;146:3-14

Innate brain immune activation in the CNS after traumatic brain injury and ischemia and its modulation by gonadal steroid hormones



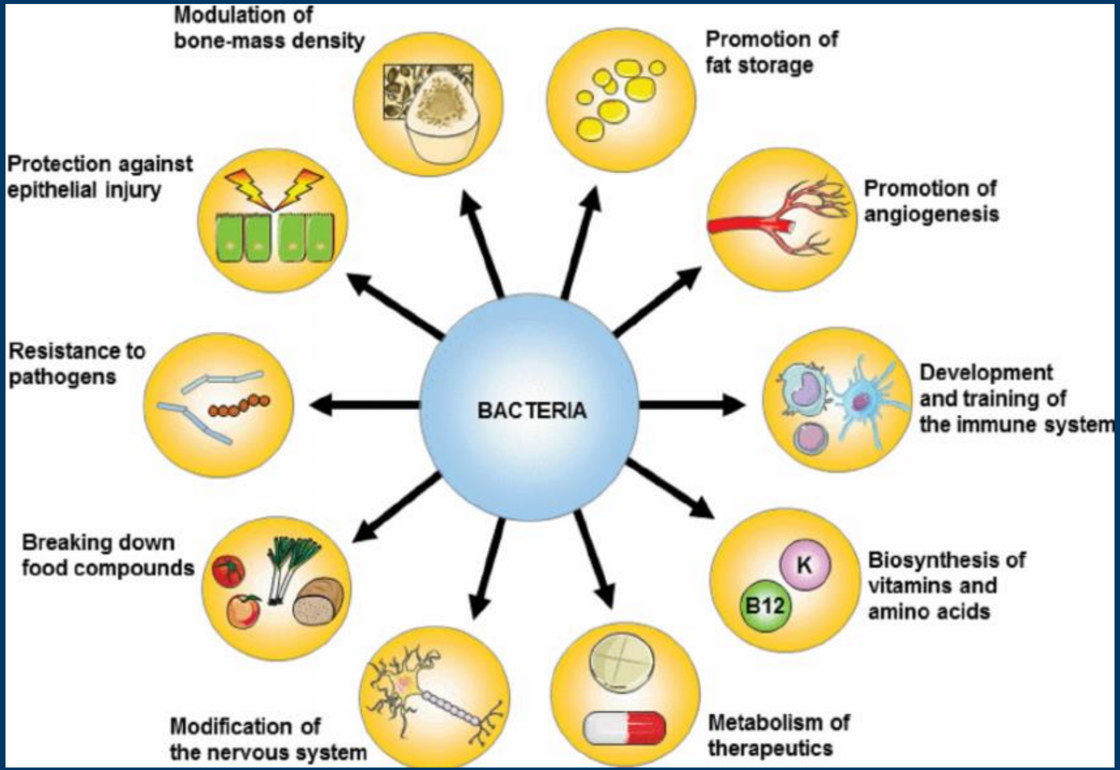
Habib et al. J Steroid Biochem and Molec Bio; 2015:146:3-14



Kanwal, Jess.SITN. 2016

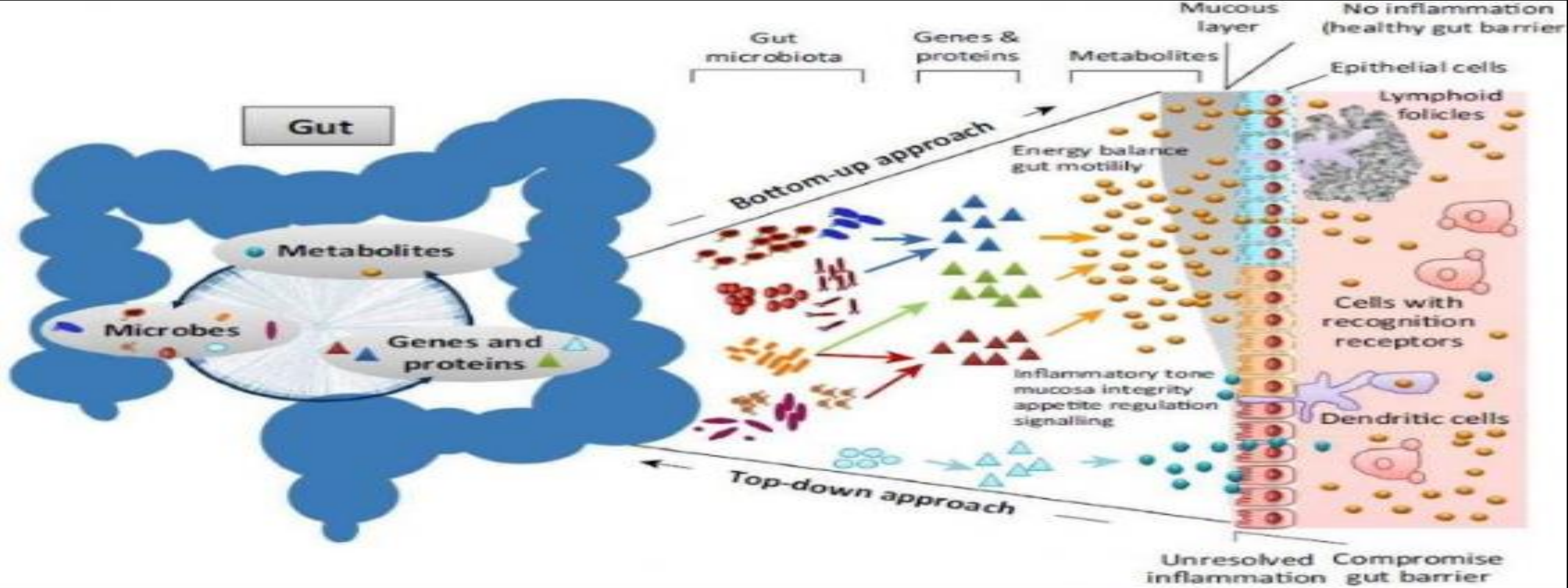
Estrogen & the Gut Immune System

- **E2 has an important role in the immune system involving the gut – to maintain healthy host –microbiota interactions**
- **Peyer's Patches, as a part of the GALT, act as inductive sites of intestinal immune responses- generating immune tolerance and preventing systemic inflammations**



Aug 2015 · FEMS microbiology reviews
Bird et al. Cell Immunol. 2008;252(1-2):57-67

The Complex Immune World of the Gut



This figure shows how microbiota species are interchangeable in terms of functions by means of the metabolites produced by the action of gene products contained in the gut bacteria.

Credit: Moya and Ferrer/Trends in Microbiology 2016

Close

Major Influences on the Microbiome

- Type of birth and infant diet
- Diet, probiotics, and prebiotics
- Medications: antibiotics, NASAIDs, OCPs, PPIs,
- H2 Blockers, laxatives, opioids
- Toxins in meals: Glyphosate, artificial sweeteners, emulsifiers
- Frequency of meals/fasting
- Stress, sleep, hygiene
- **HORMONES: males and females have different gut microbiomes**

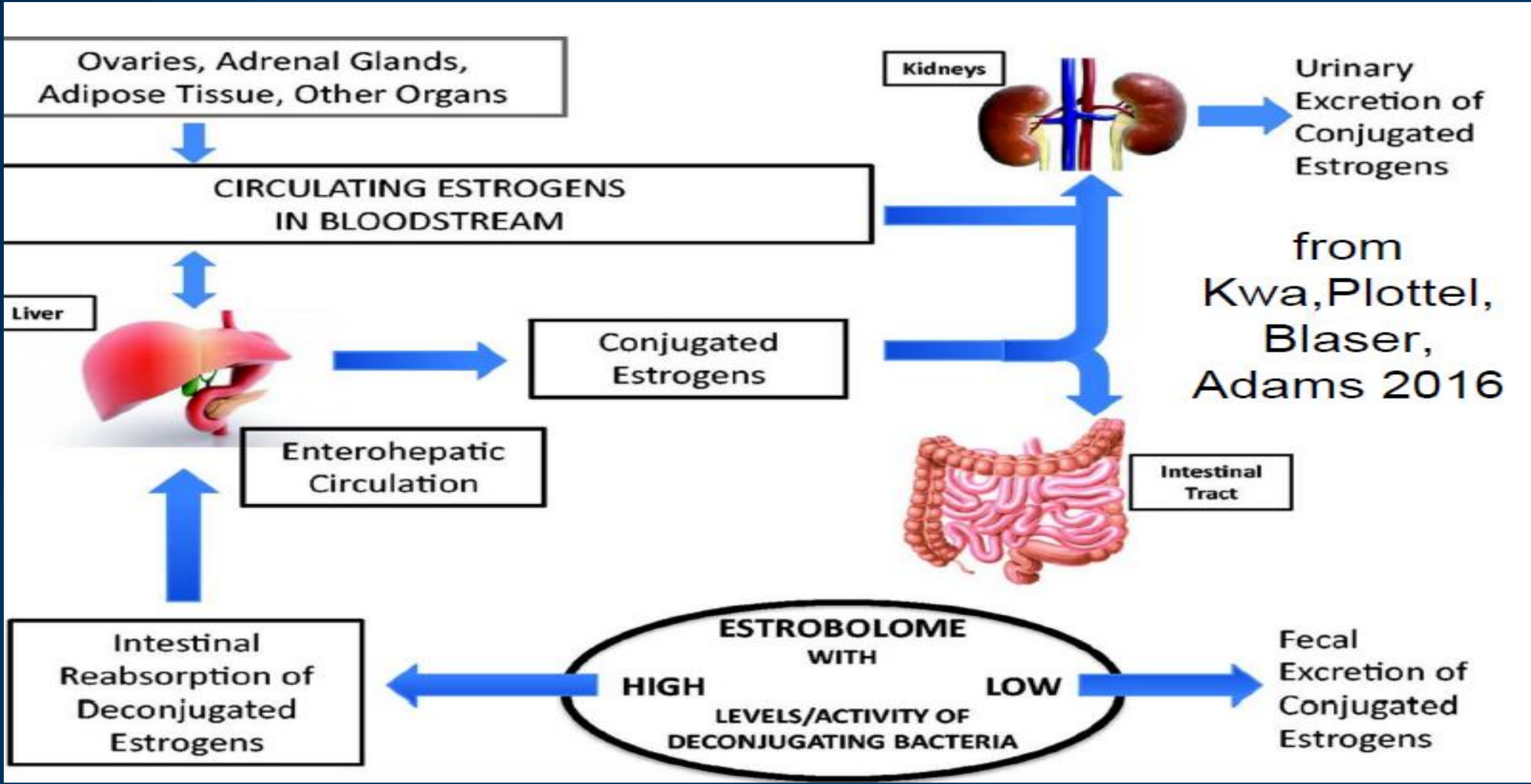
Foerster J et al. PLoS One. 2014; 9(10):e109606

Yurkovetskiy et al. Immunity.2013;39(2):10.1016

Relationship of the Microbiome and Estrogen

- Increased estrogen metabolite concentration associated with microbial diversity, compared to parent estrogens present in fecal specimens
- If the Estrobolome is unhealthy – this process is compromised

Trends in Endo & Metab, 2016 (27);11



Kwa, Plottel, Blaser, Adams, 2016

The Gut Microbiome and Pregnancy

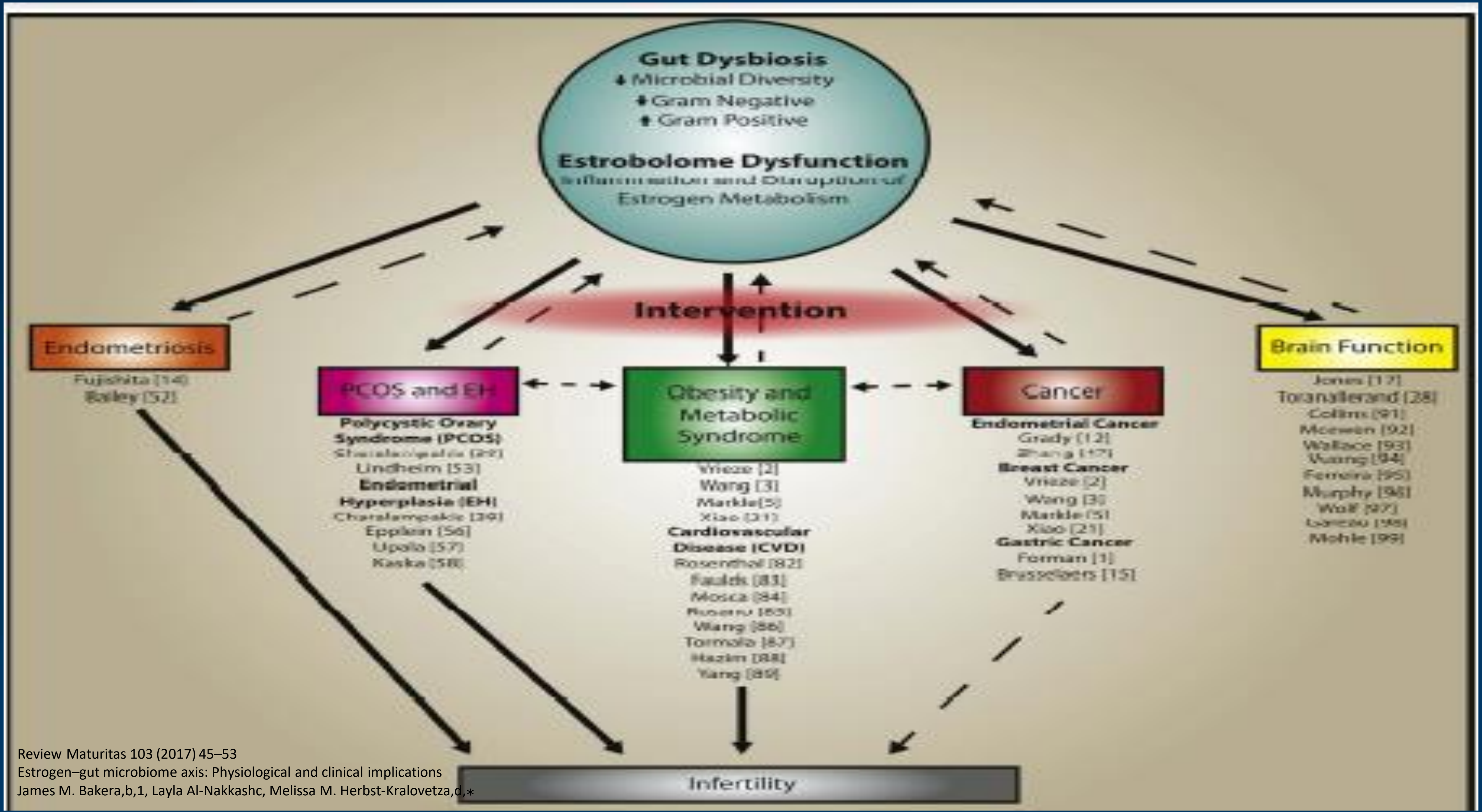
- Unique inflammatory and immune changes, along with estrogen and progesterone – alter maternal gut function and bacterial composition as the pregnancy advances
- Allow for adjustments in maternal metabolism and weight necessary to support the pregnancy
- Composition of gut microbiome contributes to obstetric outcomes with long-term health consequences for mother and child
- Microbial exposure during pregnancy may be critical for preventive effects against allergic disease – possible bacterial translocation during pregnancy – providing a "pioneer" microbiome for the offspring

Abrahamsson et al. Pediatr Res.2015;77(1-2):214-9
Edwards et al.MCN Am J Matern Child Nurs. 2017

Gut Microbiome and Pregnancy

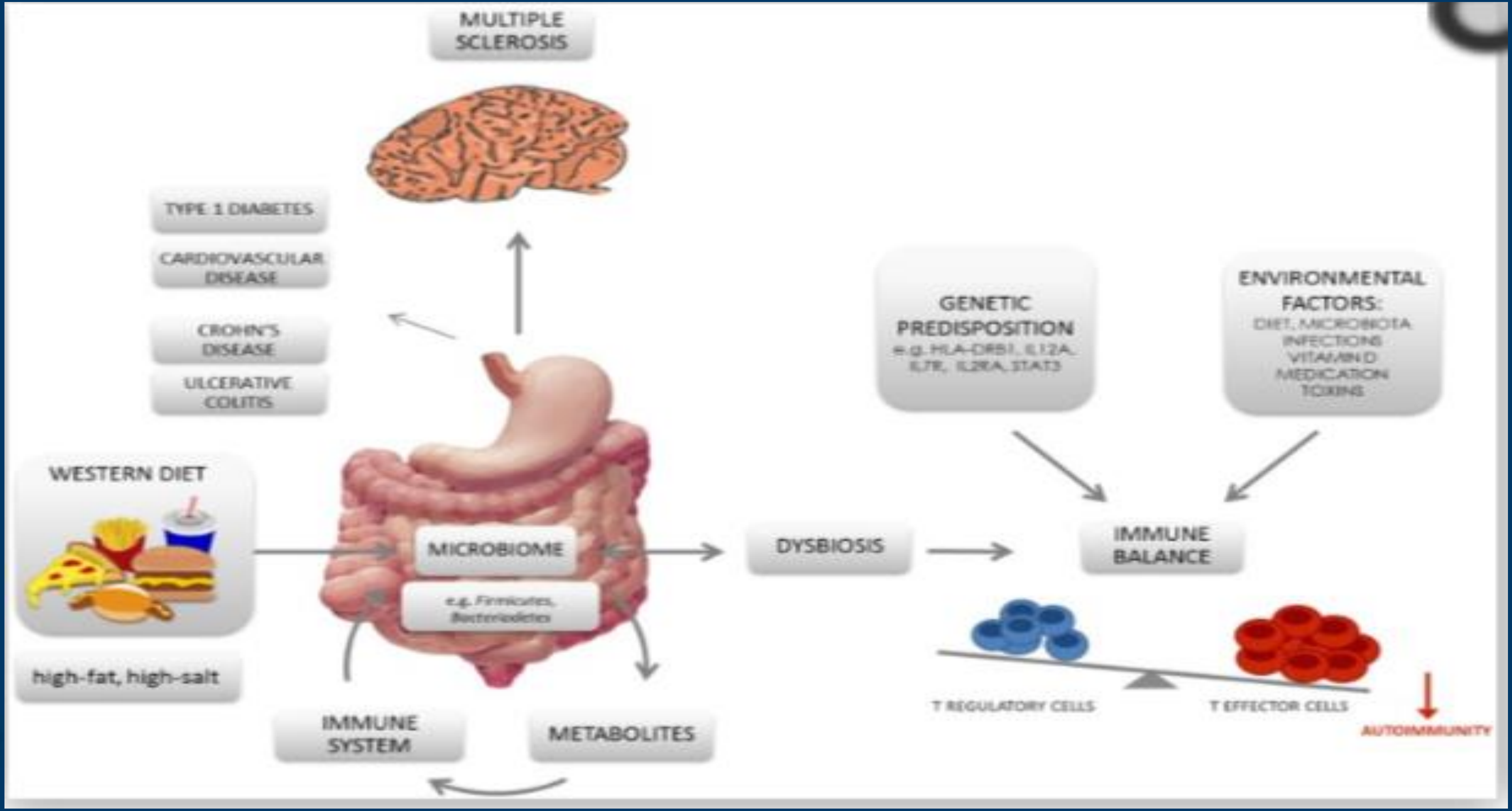
- In pregnancy the initial gut microbiome is the same as a non-pregnant woman
- With no differences in diet or total energy intake, pregnant women found to develop greater adiposity and higher leptin, insulin and insulin resistance measures, cholesterol, and HgbA1C with each trimester of pregnancy, compared with non-pregnant women
- As pregnancy progresses the gut microbiota develop in accordance with an inflammatory state in 70% of women – higher levels of Firmicutes – as seen in obesity
- Rise in pro-inflammatory cytokines

Edwards et al.MCN Am J Matern Child Nurs. 2017



Review Maturitas 103 (2017) 45–53
Estrogen–gut microbiome axis: Physiological and clinical implications
James M. Bakera,b,1, Layla Al-Nakkashc, Melissa M. Herbst-Kralovetza,d,∗

Dysbiotic Microbiome – T reg Deficiency and Activation of Proinflammatory Th17 cells



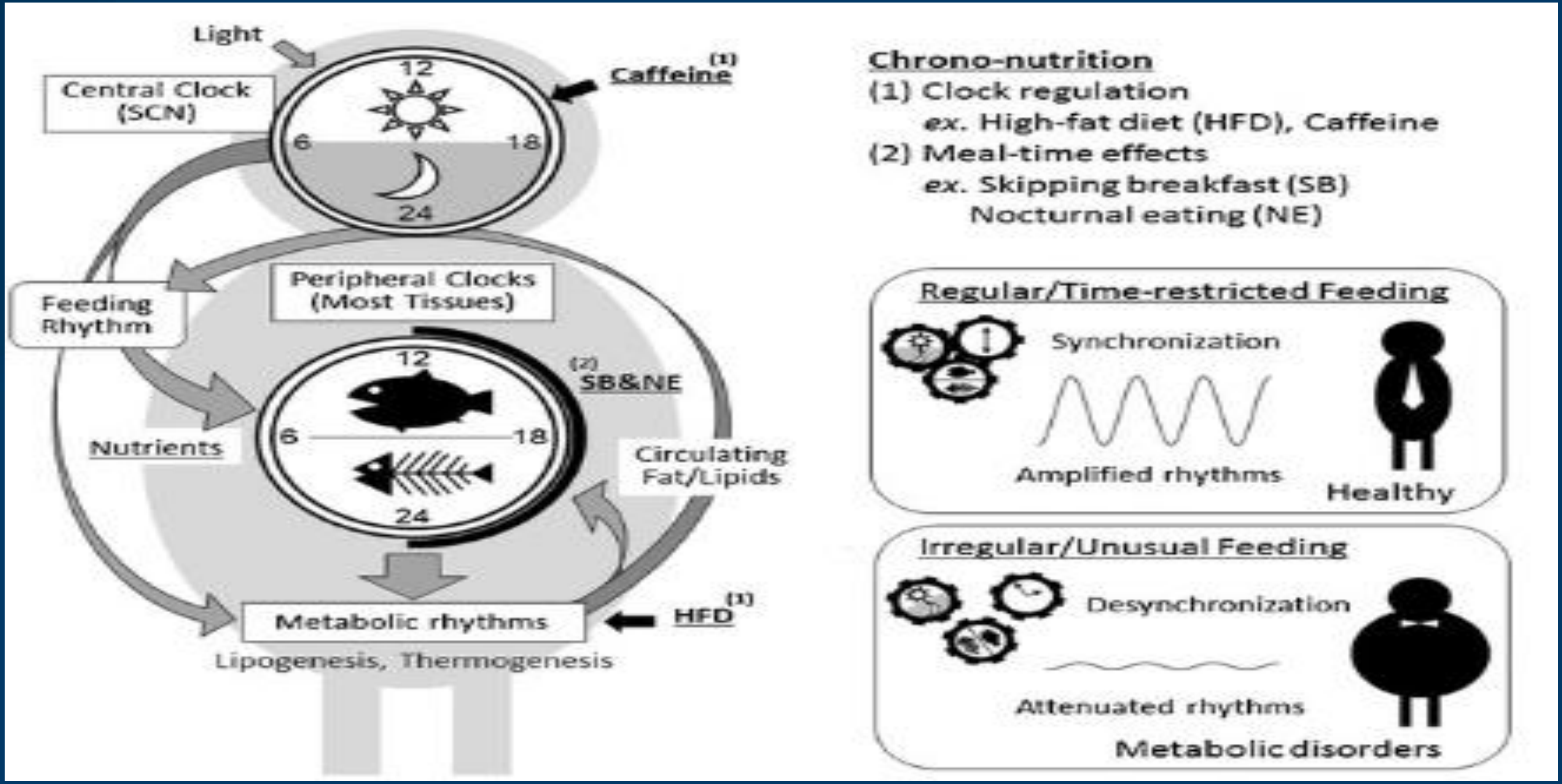
Jorg et al. Cell Mol Life Sci. 2017;73(24):4611-4622

Immune Dysregulation Impacts the Circadian Rhythm
Circadian Rhythm Impacts Immune Function

- **Excessive or chronic inflammatory responses may disrupt biological clock rhythms and trigger various diseases**
- **Disruption of body clocks – impacts autoimmune conditions**

Cirmakian et al. Chronobio Intern.2013;1-19
Butler. J of Circadian Rhythms. 2018;16(1)3
Sutton et al. Nat Com. 2017;8(1)

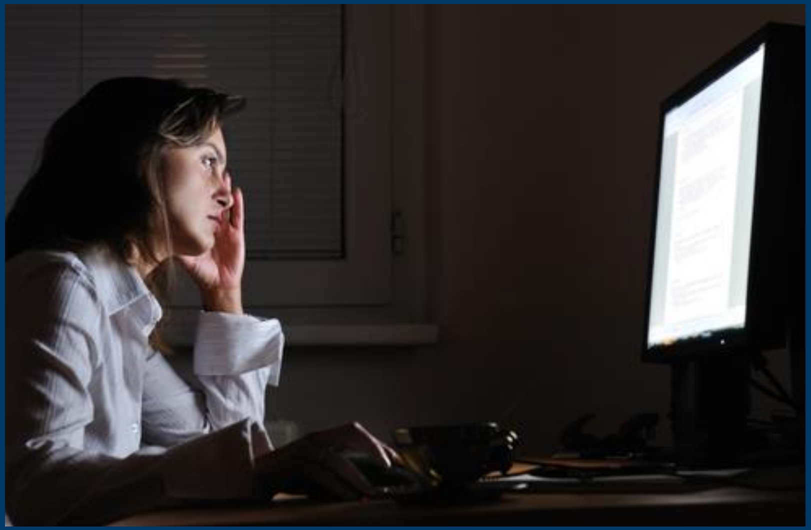
Circadian Rhythm & Feeding

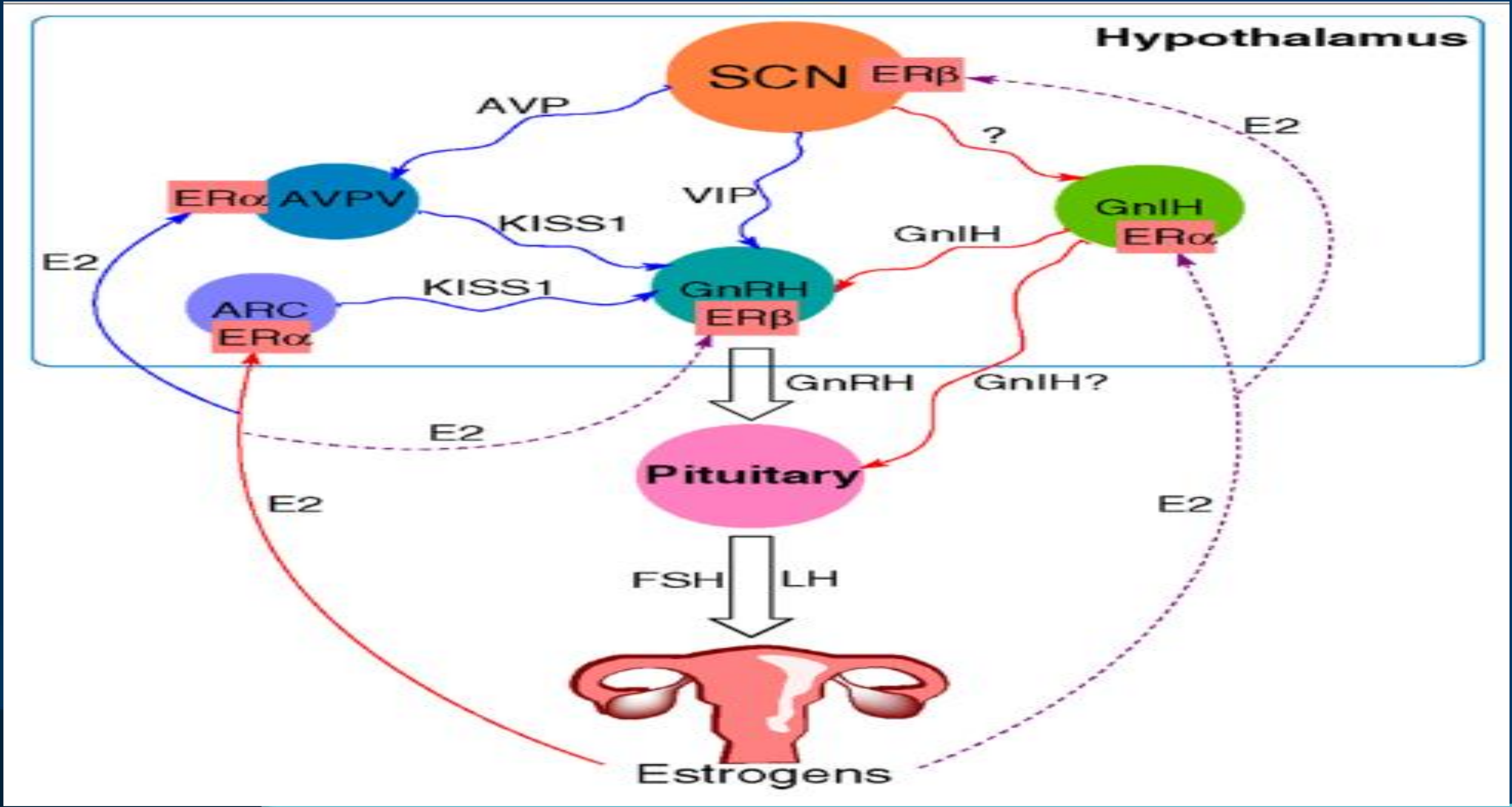


Current Nutrition Reports, Curr Nutr Rep. 2014; 3(3): 204–212

Circadian Disruptors

- Nocturnal light exposure
- Improper meal timing
- Poor or interrupted sleep
- Stress
- Traveling across time zones
- Social jet lag
- Shift work
- Loss of estrogen in women & testosterone in men
- Endocrine disruptors





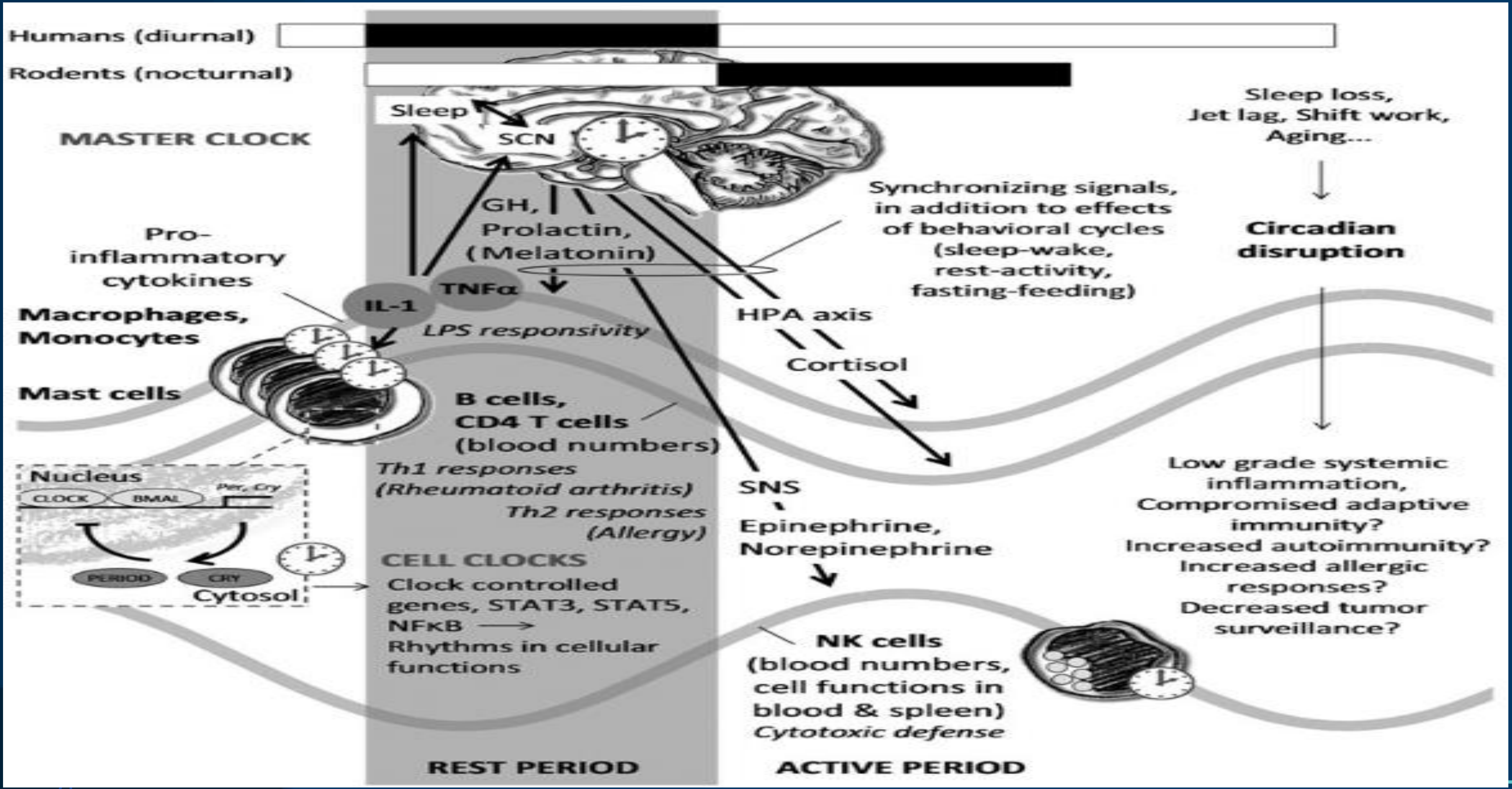
Urlep et al. Front. Endocrinol. 2013. 00111

Menstrual cycles have superimposed circadian rhythm

- **Altered amplitudes of temperature, melatonin, cortisol in women with altered circadian rhythms – shiftwork**

- **Disturbances of menstrual function – irregularity & longer cycles, reproductive dysfunction, mood disorders, sleep function, & increased risk of breast cancer**

Baker et al. Sleep Med.2007;8(6):613-22



Cirmakian et al. Chronobio Intern.2013;1-19

Supporting a Balanced Immune Response in Women

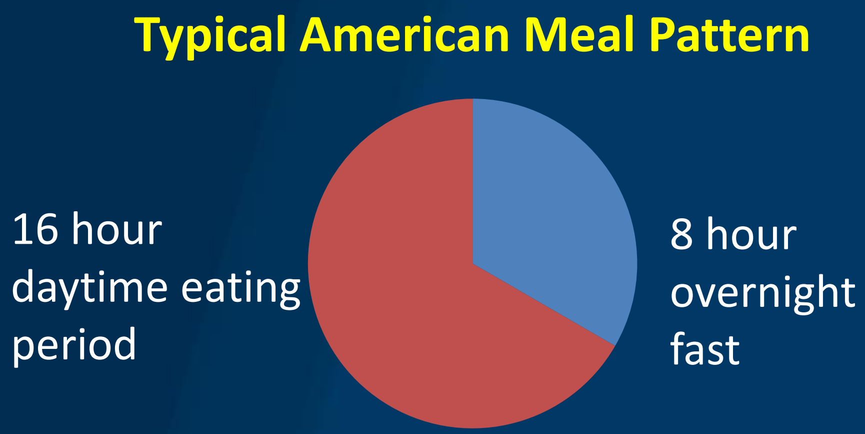
Clinical Objectives

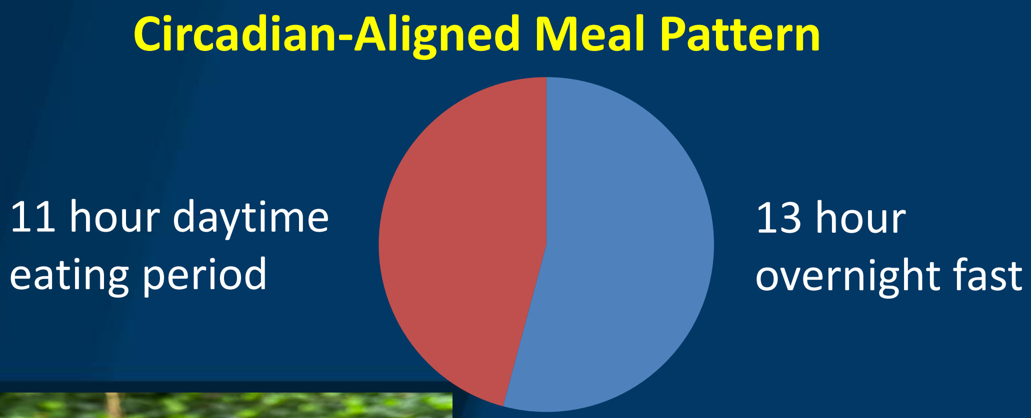
- Maintain healthy, physiologic hormone levels
- Avoid endocrine disruptors
- Keep a healthy gut microbiome
- Maintain a healthy circadian rhythm
- Use targeted supplements
- Consider hormone therapy in menopause

Best Diet to Nourish the Microbiome

INCLUDE	• Complex carbohydrates (70%) whole-grains, all varieties of vegetables, beans, legumes, etc. • Healthy fats (omega’s 3, 6 and 9) from nuts, seeds, olives • Natural fibers, resistant starch, and prebiotic rich foods • Probiotic rich foods – Eat fermented foods! • Green leafy vegetables and root vegetables
LIMIT	• Protein (approximately 12%)
AVOID	• Initially, avoid protein from all animal sources • Avoid all added sugar and refined carbohydrates • Avoid alcohol • Be aware of food intolerances

Correct the Clock: Meal Timing





- Eat a large healthy breakfast
- Eat dinner early
- Eat at approximately the same times each day
- Limit snacking
- Eat dinner to breakfast about 13 hours apart
- Consider periodic fasting or a fasting mimicking diet

Correct the Clock: Sleep Hygiene

3 hours before bed

- Dim the lights
- Minimize computer & electronic use
- Avoid vigorous exercise
- See the sunset if possible

1 hour before bed

- Breathing exercise
- Hot bath
- Calming tea

Sleeping

Cool, dark, quiet room

Upon awakening

15 minutes of sunlight

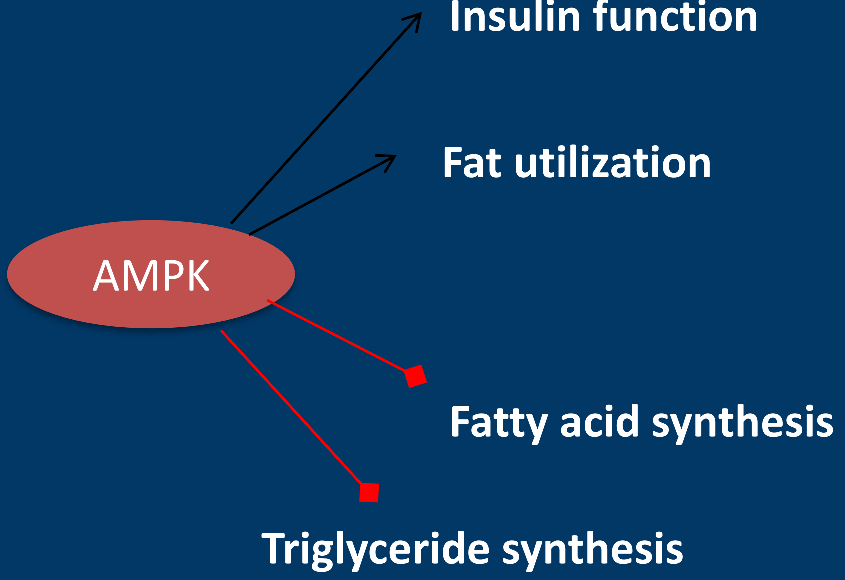
Midday sunlight

Correct the Clock: Meal Timing

The next best thing:
Fasting mimetics

Agents that partially emulate the metabolic benefits of fasting by supporting the AMPK pathway

Resveratrol
Alpha lipoic acid
Berberine
Exercise

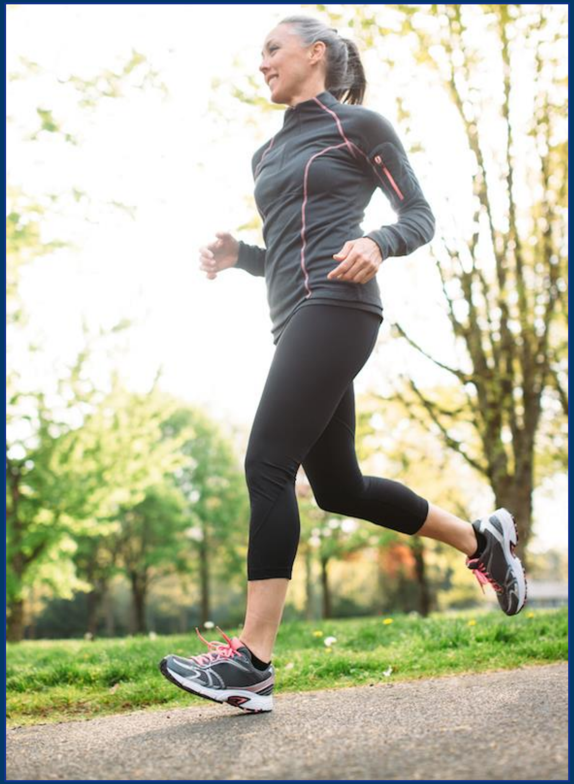


Exercise

- Regular exercise contributes to:
  - Lower blood pressure
  - Lower blood glucose levels
  - Improved lipid profiles
  - Healthy body weight
  - Improves gut microbial

Sedentary elderly adults had decreased NO-mediated vasodilator function, compared to age-matched active adults.

Exercise reversed impaired microvascular NO function in sedentary adults!



Black M, Green D, NT Cable. *J Physiol*. 2008 Jul 15;586(14):3511-24.
Lee V and Foody J. Current Atherosclerosis Reports. 2008; 10:295-302.

Human Papilloma Virus

- One of the most common sexually transmitted agents worldwide
- Estimated prevalence 11.7% in women above 14 years of age
- 2012 – HPV infections estimated as responsible for 8.6% of all cases of cancers in women across the world
- Cervical cancer – 4th most common cancer in women worldwide

Castellsague, X. Gyn Onc. 2008; S4-S7

The Understanding of HPV's Role in Cervical Cancer

- Most women infected with HPV become negative within 2 years
- Women with persistent high-risk infections at greatest risk of developing cervical cancer

Castellsague, X. Gyn Onc. 2008; S4-S7

Increased Risk of Cervical Cancer with Use of Oral Contraceptives

After 10 years of use, risk >4X users vs non-users

Study: case control study of 759 invasive cervical cancer patients and 1430 controls in 4 countries in Central and South America: >5 years use raised risk 70%

- **RR 2.4 for increased risk for adenocarcinomas**
- **Risk not related to number of partners or HPV**

Brinton et al. Internat J of Epidemiology. 1990;19(1)

Oral Contraceptives and Cervical Cancer

- World Health Organization Study: WHO's International Agency for Research on Cancer
- Compelling evidence of link between OCPs and cervical cancer
- Prolonged use of oral contraceptive pill increased risk of cervical cancer up to fourfold in women with HPV

Dyer, Owen. BMJ. 2002;324(7341): 808

WHO links long term pill use to cervical cancer

Dyer, Owen. BMJ. 2002;324(7341): 808

Their results are published in last week's *Lancet* (2002;359:1085-92). Women who had taken the pill for 5-9 years were nearly three times more likely than non-users to develop cervical cancer. Women who had taken the pill for more than 10 years were four times more likely than non-users to develop the disease. These risks did not vary with time since first or last use, or by age at first use.

The study considered only women who were infected with HPV,

Dyer, Owen. BMJ. 2002;324(7341): 808

“These factors appear to act as independent multipliers of risk,” said Silvia Franceschi, one of the studies' authors. HPV positive women who had had more than five full term pregnancies and had taken the pill for more than five years ran nearly 12 times the risk of cervical cancer as HPV positive nulliparous women who had never taken the pill.

Dyer, Owen. BMJ. 2002;324(7341): 808

Nutrition and Cervical Cancer

Increased risk for cervical neoplasia:

High homocysteine

Garcia-Closas. Internat J of Cancer. 2005;117(4):629-637

Nutrition and Cervical Cancer

- Deficiencies of Vitamin C and Carotenoid status relevant to viral persistence
- Folate status relevant to early pre-neoplastic cervical lesions

Potischman et al. Cancer Causes & Control. 1996;7(1):113-126

Nutrition and Cervical Cancer

Possible protective effect on HPV persistence:

Fruits, Vegetables, Vitamin C, Vitamin E, Beta and Alpha Carotene, Lycopene, Luteine/Zeaxanthin, Cryptoxanthin

Garcia-Closas. Internat J of Cancer. 2005;117(4):629-637

Nutrition and Cervical Cancer

Probable protective effect of cervical neoplasia:

Folate, Retinol, Vitamin E, Vegetables, Vitamin C, Vitamin B12, Alpha-Carotene, Beta-carotene, Lycopene, Luteine/Zeaxanthin, Cryptoxanthin

Garcia-Closas. Internat J of Cancer. 2005;117(4):629-637

Green Tea and Soy for Cervical Cancer

- Soy isoflavones and tea catechins – immunomodulating properties relevant to cervical cancer

Study of 30,744 Chinese women over 16.7 years

- High intake soy – 20% reduced risk cervical cancer, not for green tea
- High intake soy + green tea = nearly 60% reduction of risk

Paul et al. Cancer Causes Control. 2019;30(8):847-857

Vaginal Microbiome and Cervical Cancer

Semi-Western Diet:
Higher intake bread, dairy products, eggs, soft drinks, relatively higher fat intake

Dominant Vaginal Microbes:

Lactobacillus crispatus, L iners, Atopobium vaginae, Preveotella vivia

Sang-Soo S et al. Clinical Nut. 2016;35(6):1434-1441

Vaginal Microbiome and Cervical Cancer

- High scoring group – higher risk of CIN (nearly 3.5X)

- Atopodium vaginae + Lactobacillus iners – dominant types with the Western Diet - higher risk for CIN, compared with Lactobacillus crispatus-dominant type

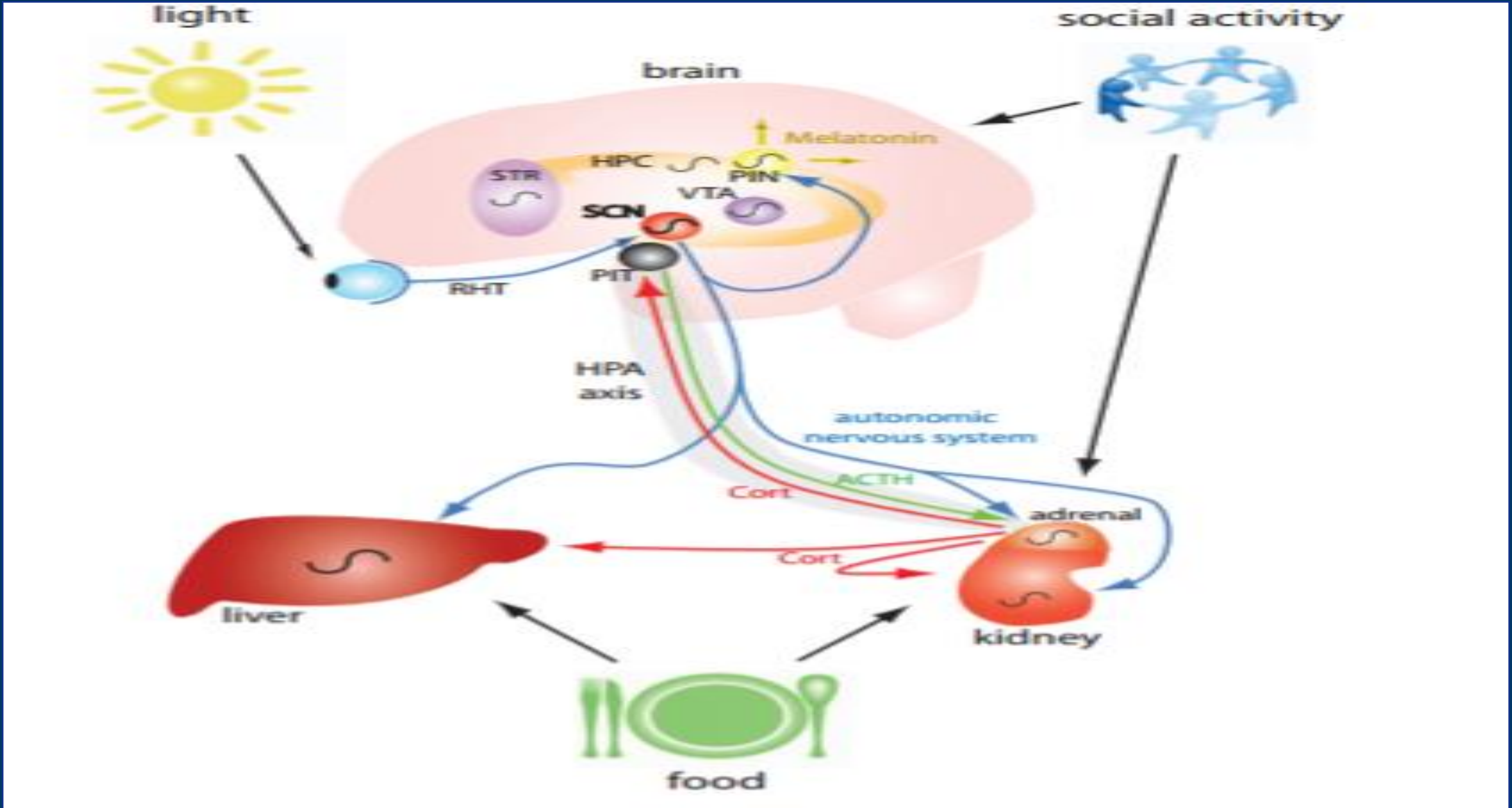
Sang-Soo S et al. Clinical Nut. 2016;35(6):1434-1441

Immunological Differences: Take Home Message

- In general, healthy females at every age have stronger immune responses than males
- Result - faster clearance of pathogens but increased risk of inflammatory and autoimmune diseases when things go wrong

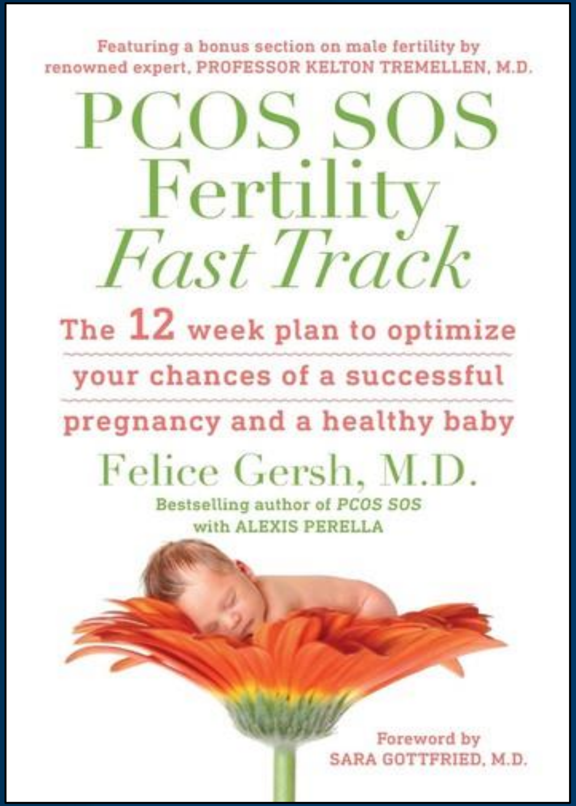
Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38.

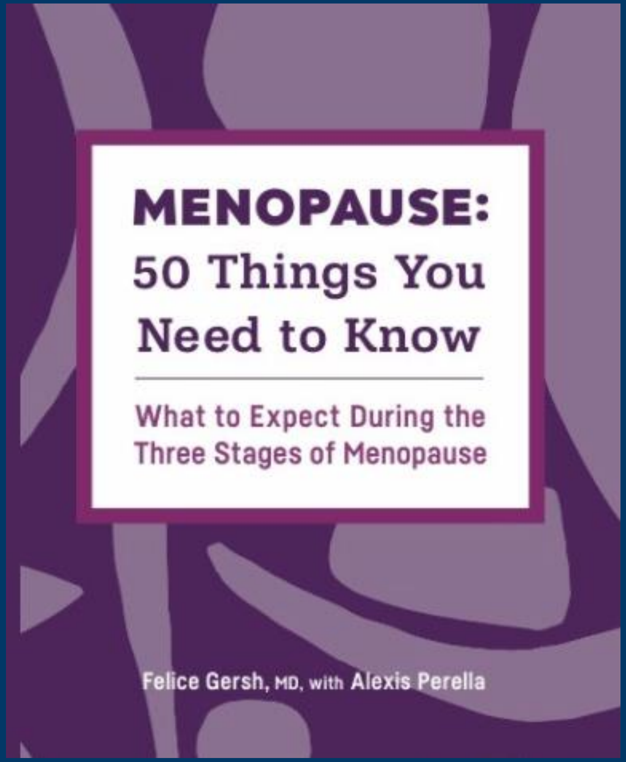
The Path to Immune Health

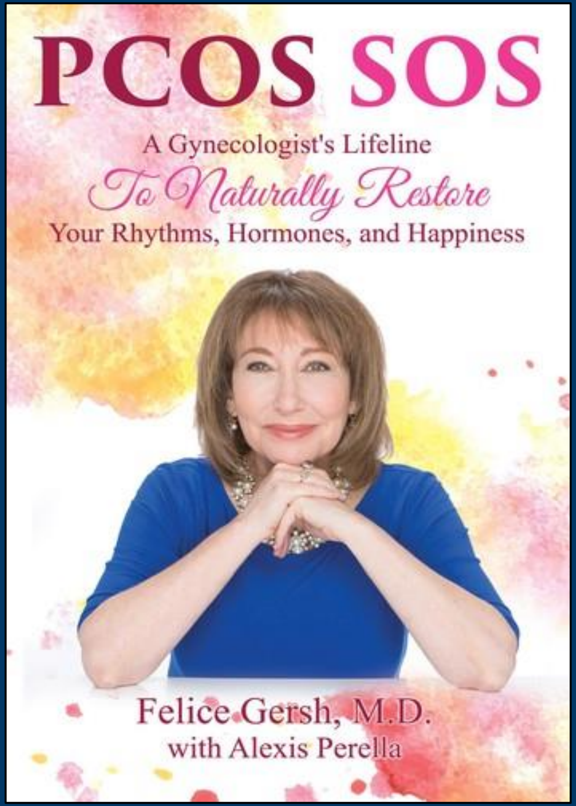


Behav Neurosci 128(3) pg 326-43, 2014.pdf

Thank you!







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