The Circadian Rhythms in Our Lives

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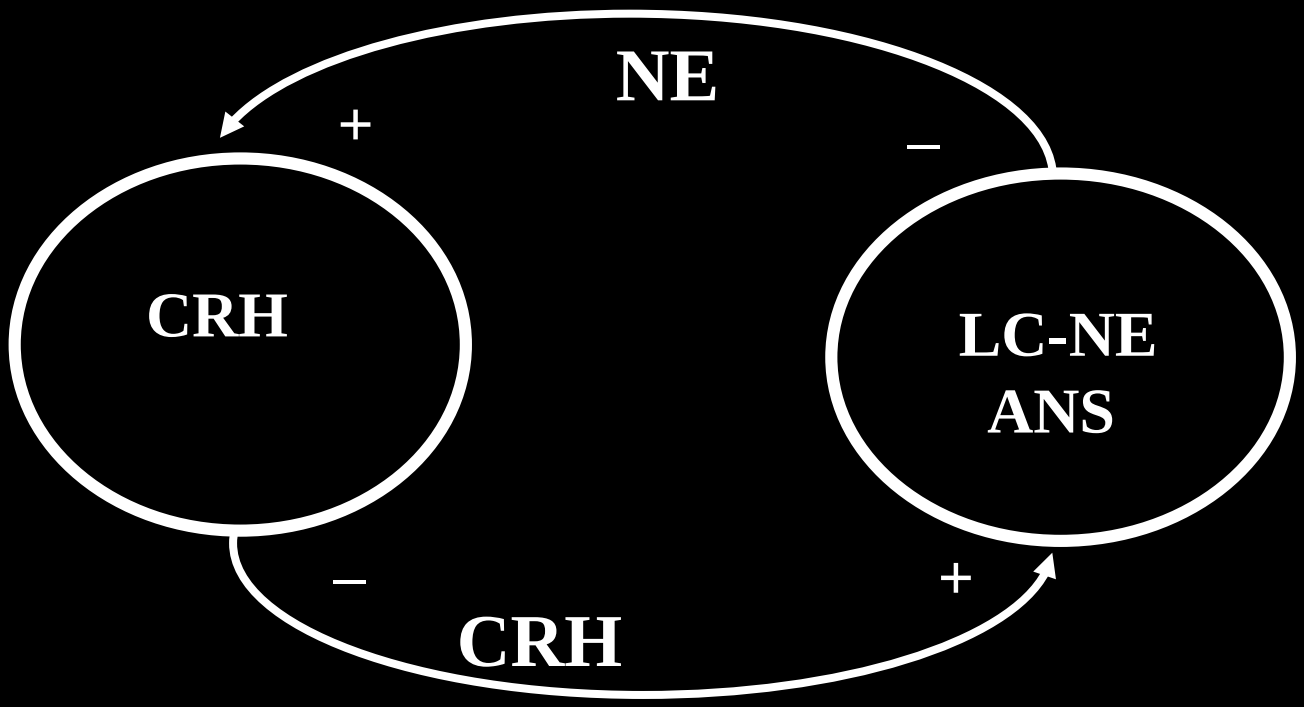
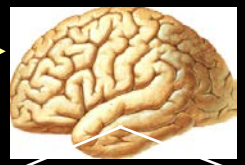
(No Disclosures)

Stress System



G.P. Chrousos JAMA 1992

STRESS SYSTEM= HPA axis + LC/NE



Amygdala CRH

HIPPOCAMPUS

CRH/AVP

LC/NE +ANS

ACTH

F/B

NE/E

iCRH

GH/IGF-1
LH/T/E2
TSH/T_3

IL-6

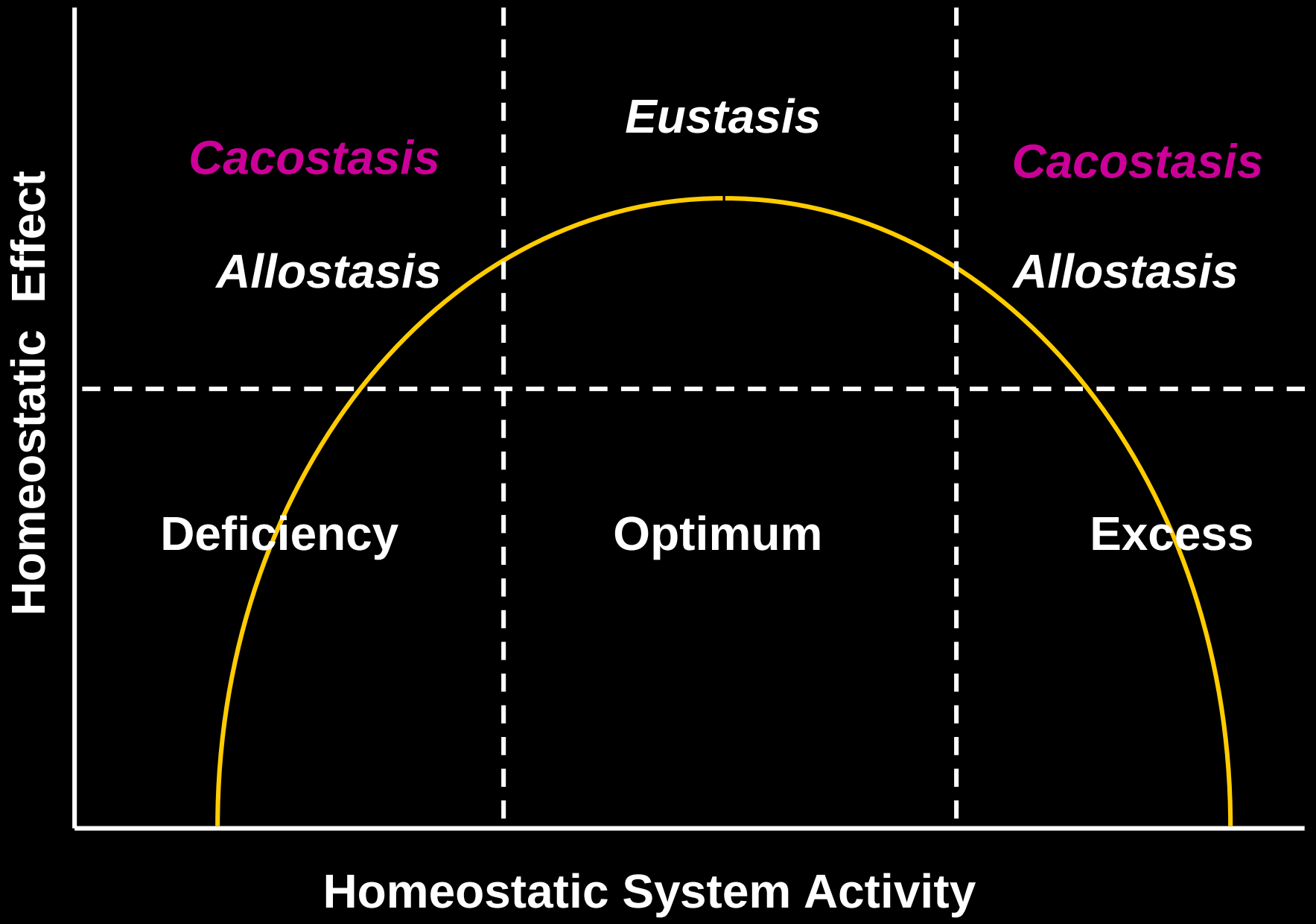
Immune Response
Inflammation

Chrousos JAMA 1992

«Μηδέν άγαν»

‘Everything in moderation’

Inscription at the Oracle of Delphi


Eustasis
Cacostasis
Cacostasis
Allostasis
Allostasis
Deficiency
Optimum
Excess
Homeostatic Effect
Homeostatic System Activity

HUMAN COMPLEXITY: POST(EPI)GENOMIC ERA

Human genome:

- **About 3 billion bases ("Non-junk" DNA)**
- **About 20 thousand protein-coding genes**
- **About 22 thousand ncRNA-coding genes**
- **About 16 thousand pseudogenes**
- **About 200 thousand transcripts (mRNA, ncRNA)**
- **About 200-260 thousand proteins**

Single nucleotide polymorphisms (snp's or snv's), microsatellites or copy number variants (cnv's) :

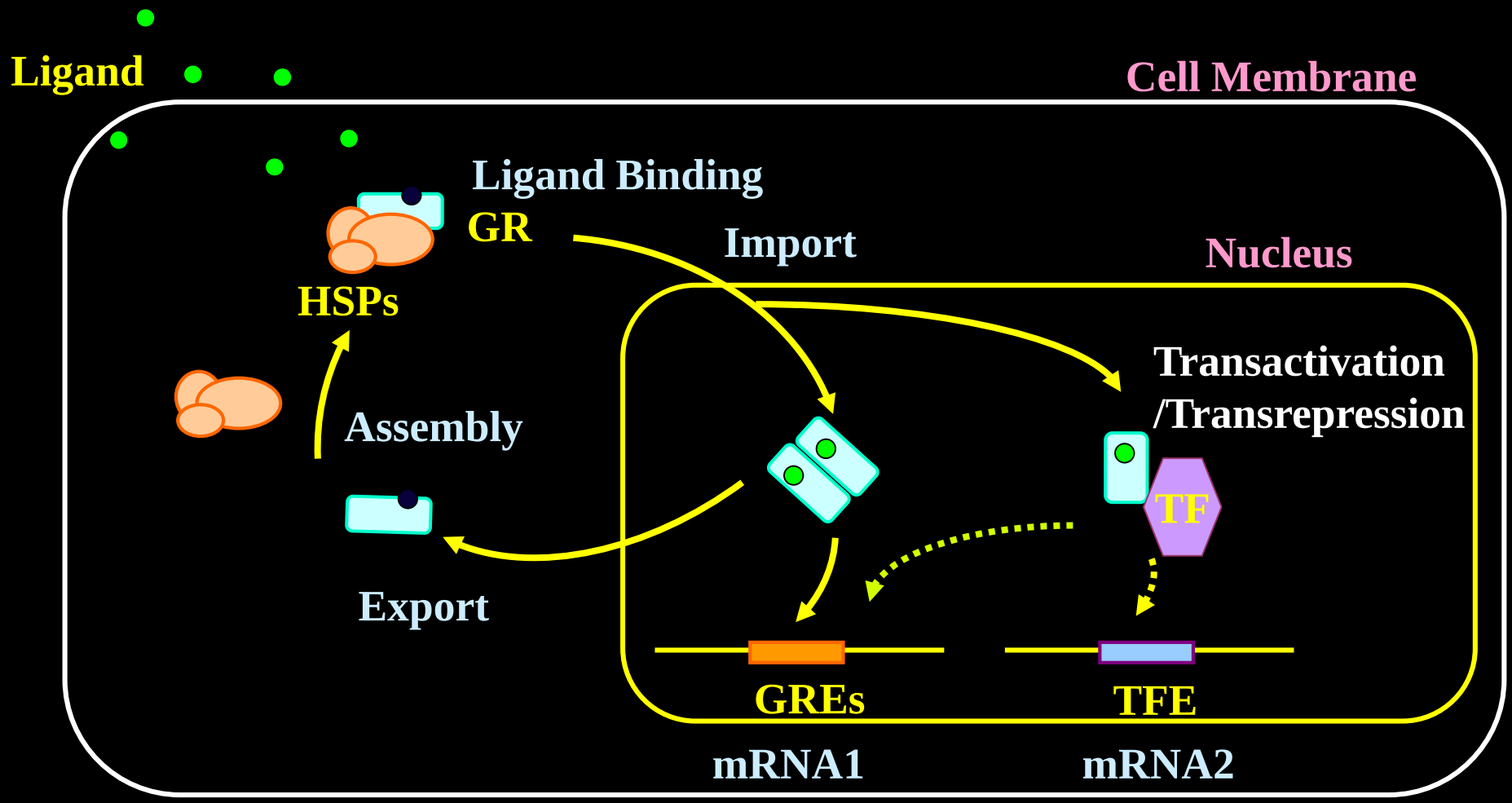
- **About >25 million snv's, 1.5 million indels**
- **About 20 million microsatellites**
- **>5000 cnv's (many million bases) ~0,9 % difference**
- **Over 100 k disease-related mutations**

60% of promoters have CpG islands, > million regul. regions

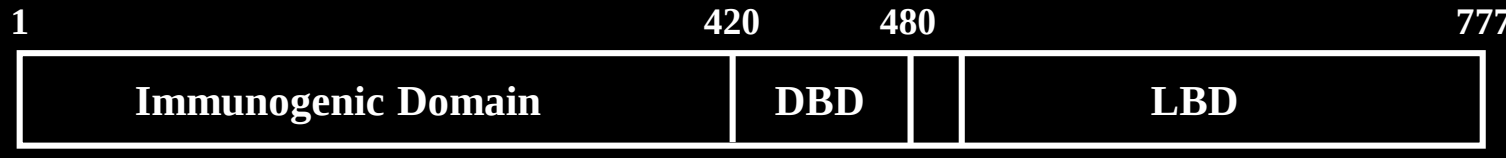
↓

EPIGENETICS/EPIMUTATIONS

Glucocorticoid Receptor Signaling



Functional Domains of Human Glucocorticoid Receptor α



HSP Binding 568 653

Dimerization 456 777

Nuclear Translocation

NL1 479 506

NL2 526 777

Transactivation

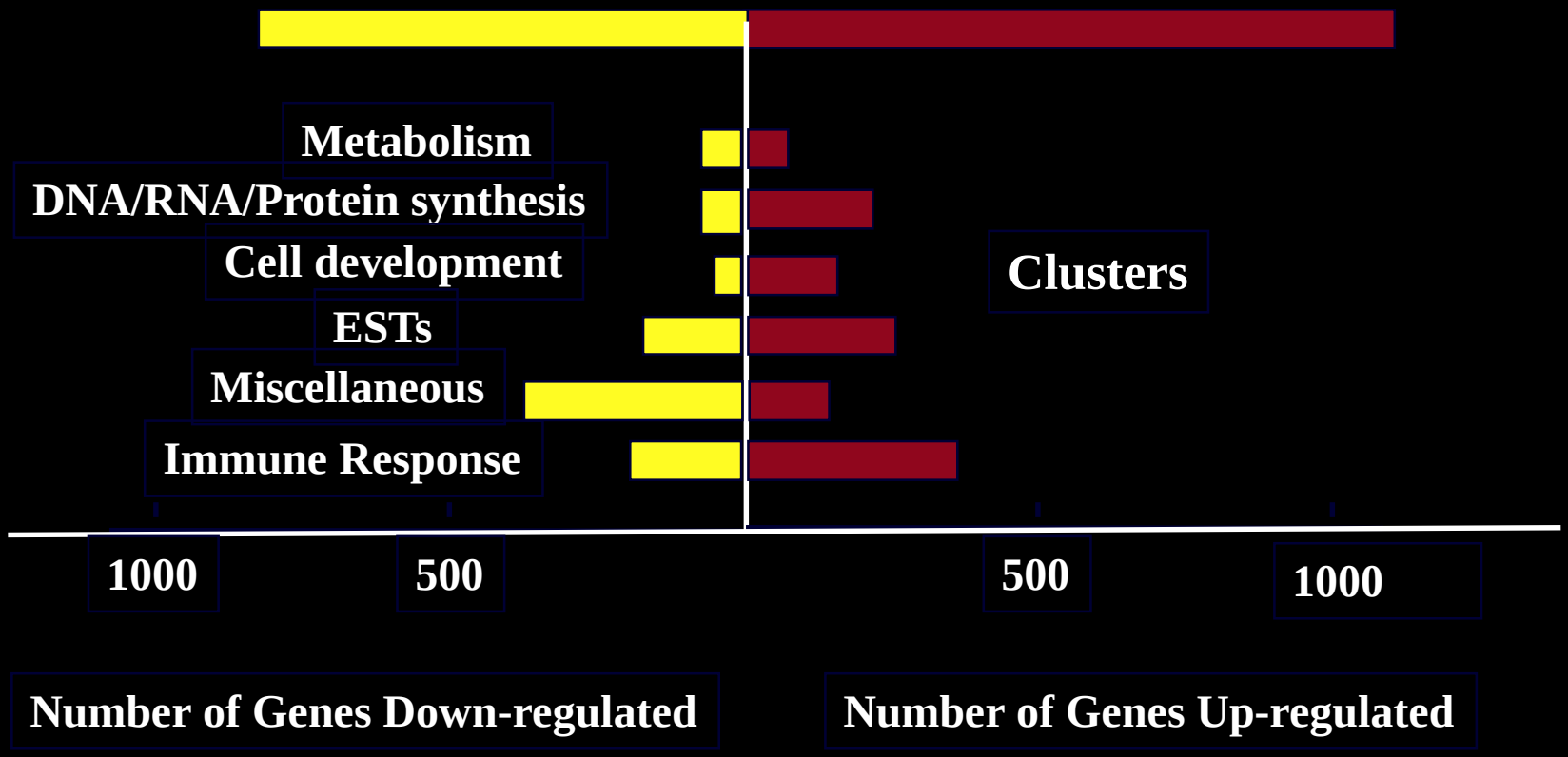
AF1 77 262

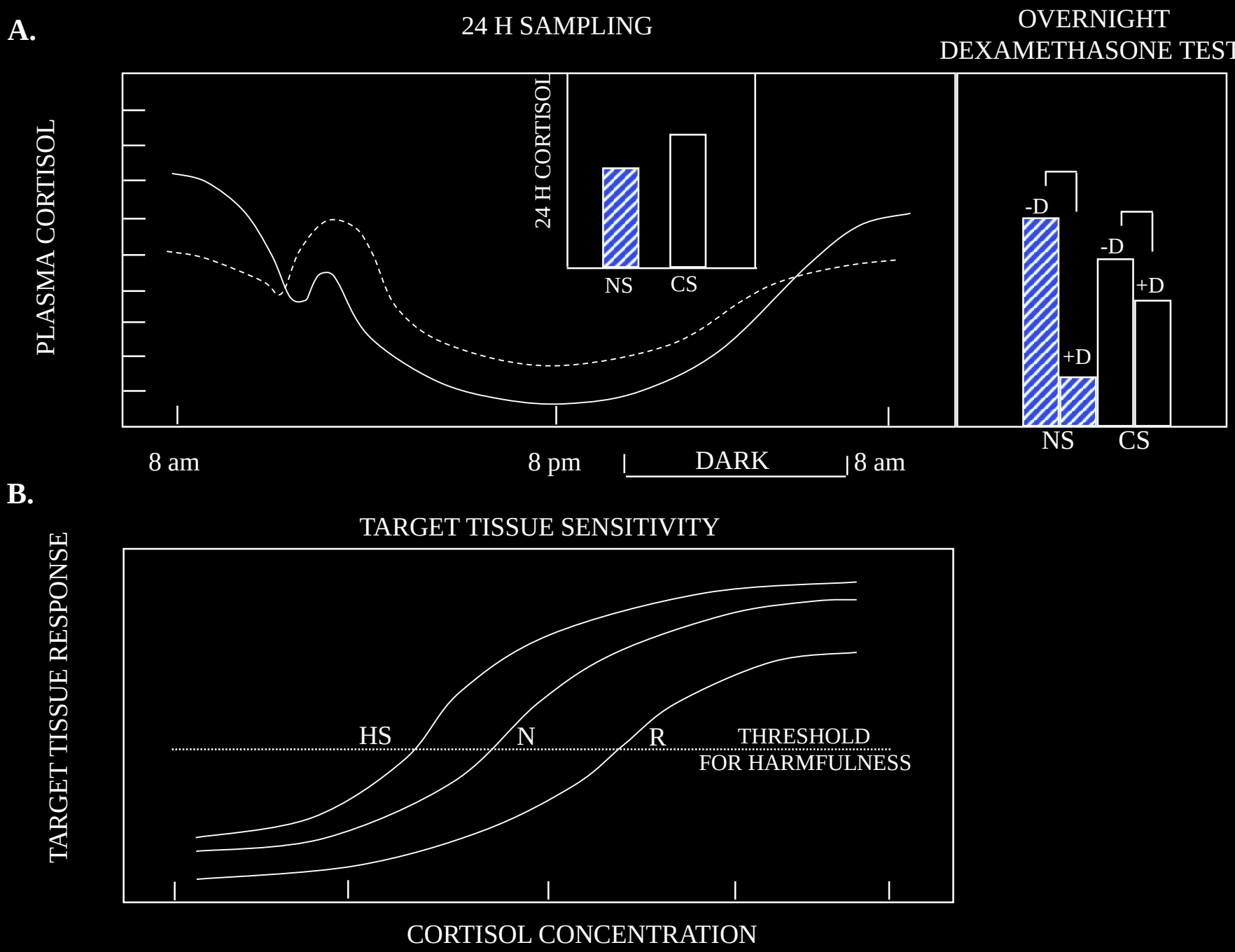
AF2 526 556

- **~20 % of the human leukocyte transcriptome responds to glucocorticoids.**
- **Almost 2 thirds of these are stimulated, the rest suppressed.**
- **Glucocorticoid-controlled genes and downstream output genes are involved.**

Galon et al. FASEB J 2002

Glucocorticoid Regulated genes



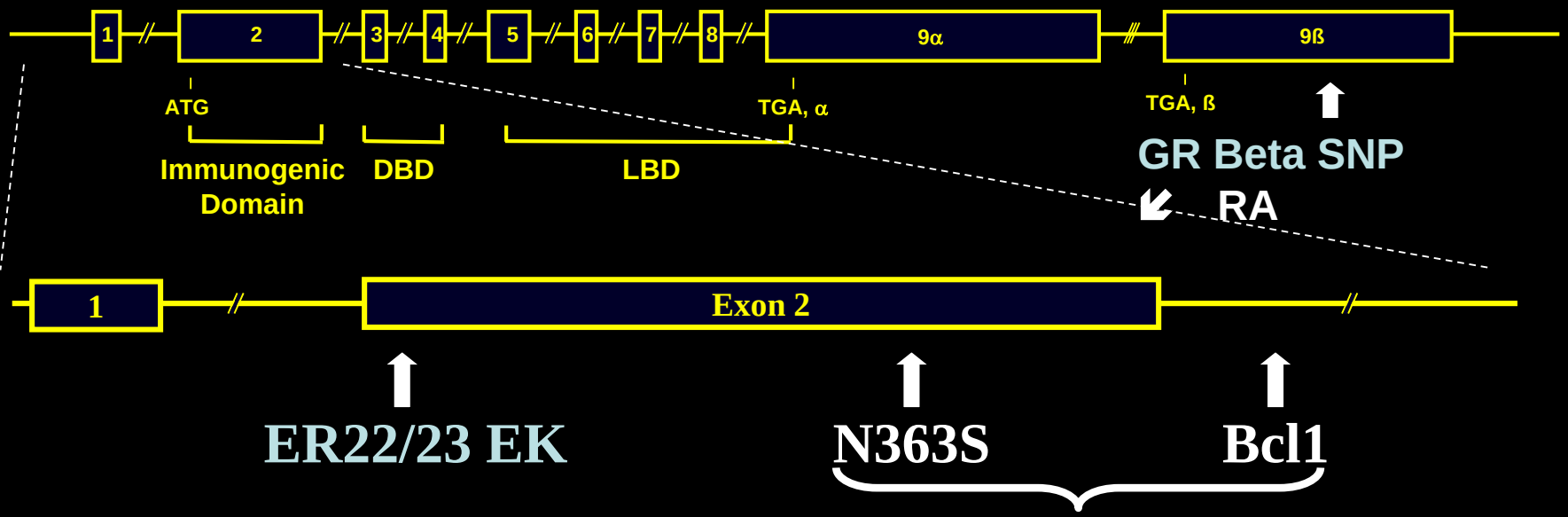

A.
24 H SAMPLING
OVERNIGHT
DEXAMETHASONE TEST
PLASMA CORTISOL
24 H CORTISOL
NS
CS
-D
+D
-D
+D
NS
CS
8 am
8 pm
DARK
8 am
B.
TARGET TISSUE SENSITIVITY
TARGET TISSUE RESPONSE
HS
N
R
THRESHOLD
FOR HARMFULNESS
CORTISOL CONCENTRATION

Clinical Manifestations in Tissue Hypersensitivity or Resistance to Glucocorticoids

	Glucocorticoid Excess	*Glucocorticoid Deficiency*
AFFECTED AREA	**GLUCOCORTICOID HYPERSENSITIVITY**	**GLUCOCORTICOID RESISTANCE**
Central Nervous System	Insomnia, anxiety, depression, defective cognition	Fatigue, somnolence, Malaise, defective cognition
Liver	Increased gluconeogenesis* and liposynthesis, insulin resistance	Hypoglycemia, increased insulin sensitivity
Fat	Accumulation of visceral* fat	Loss of weight
Muscles	Insulin resistance*	Increased insulin sensitivity
Blood Vessels	Hypertension*	Hypotension
Bone	Stunted growth, osteoporosis	
Inflammation/ Immunity	Immune suppression, suppressed inflammation	Increased inflammation/ autoimmunity

*dysmetabolic syndrome

Alteration of Tissue Glucocorticoid Sensitivity in Pathologic States: Glucocorticoid Receptor Polymorphisms

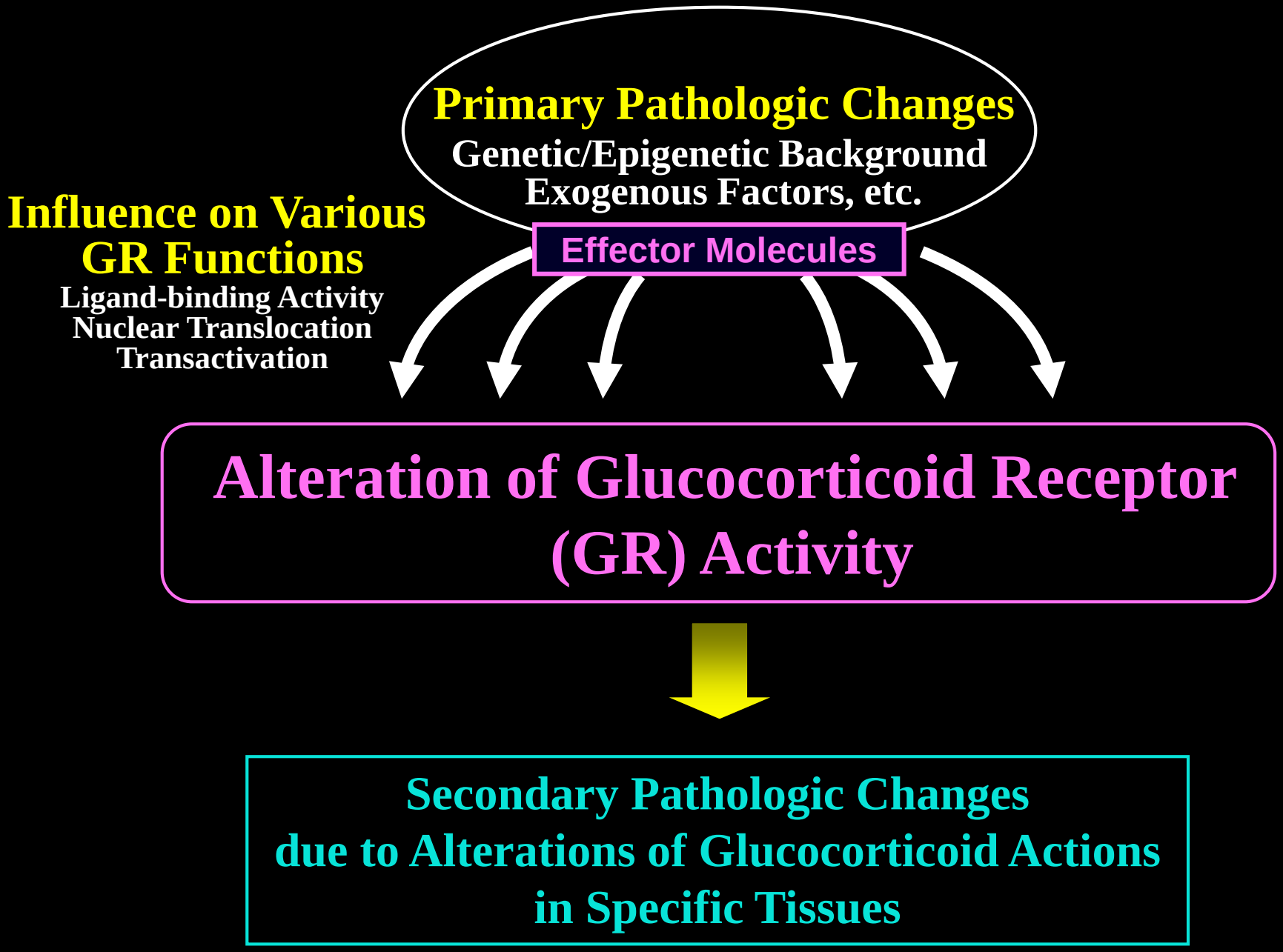


"Glucocorticoid Resistant"

- Better Body Composition
- Healthier Metabolic Profile
- Better Survival
- Lower Risk of Dementia
- Depression

"Glucocorticoid Hypersensitive"

- More Body Fat, Less Lean Body Mass
- Insulin Resistance
- Metabolic s. manifestations
- Depression


Primary Pathologic Changes
Genetic/Epigenetic Background
Exogenous Factors, etc.
Effector Molecules
Influence on Various GR Functions
Ligand-binding Activity
Nuclear Translocation
Transactivation
Alteration of Glucocorticoid Receptor (GR) Activity
Secondary Pathologic Changes due to Alterations of Glucocorticoid Actions in Specific Tissues

Politically Correct 1980's

- **Hypothesis-driven Research**

ANATHEMA

- **"Shotgun Research"**
- **"Fishing expedition"**

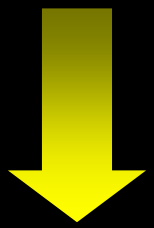
Politically Correct 2000's

- Discovery-driven Research

NO LONGER ANATHEMA

- "Shotgun Research"
- "Fishing Expedition"

Finding Molecules that Potentially Alter GR Action

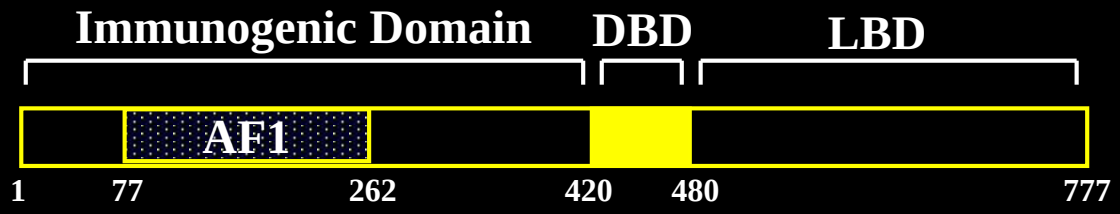


Yeast Two-hybrid Screening

Using GR Fragments as Baits

Yeast Two-hybrid Screening Using GR LBD as Bait

Human GR



Bait Fragment

LexA System/Human Jurkat Cell cDNA Library

CLOCK transcription factor

Nader N. et al. *FASEB J* 2009

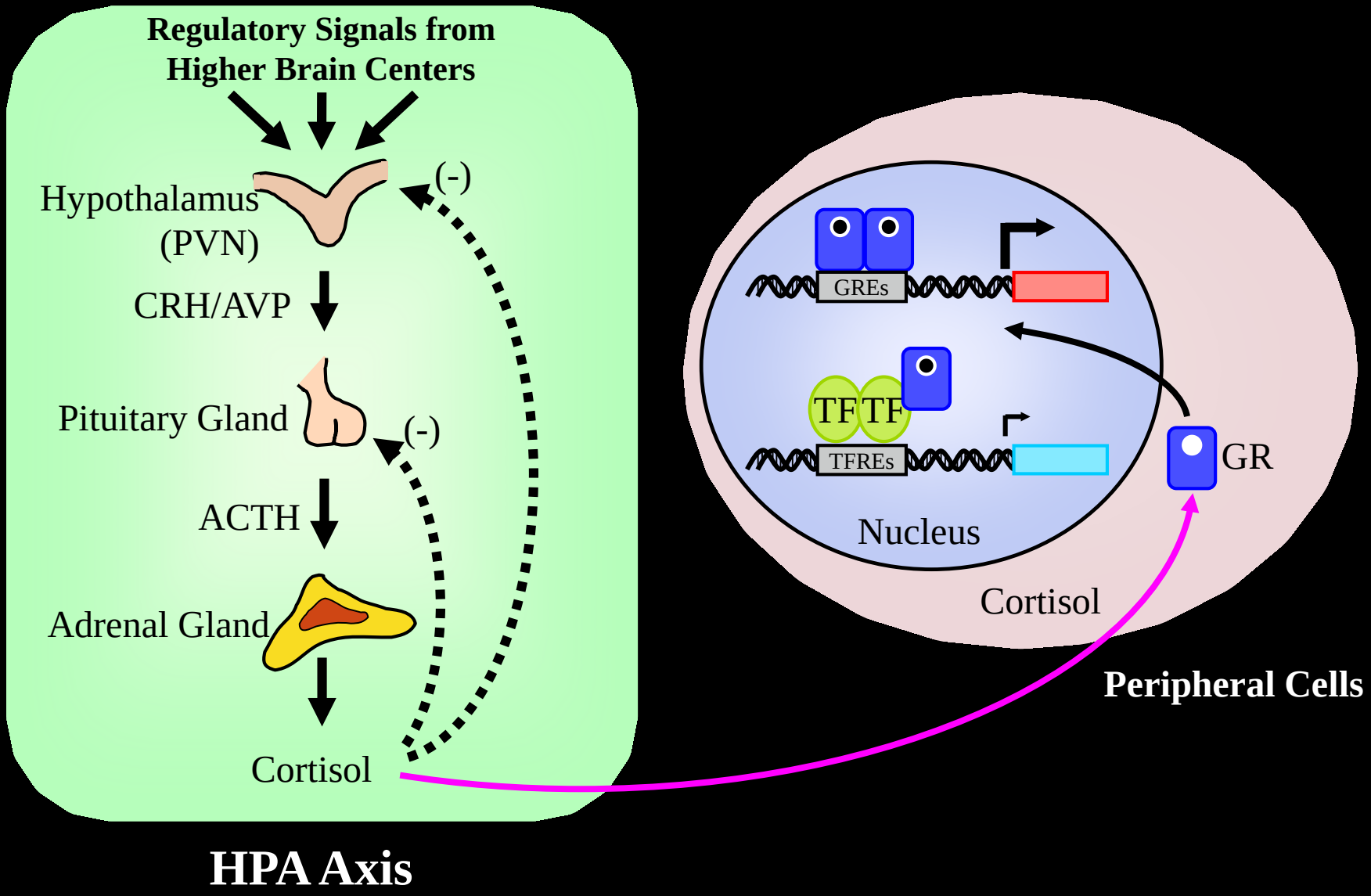
- **15 % of the mammalian transcriptome oscillates with a 24 h rhythm.**

- **Clock-controlled genes and downstream output genes are involved.**

Life on Planet Earth

Organisms continuously face unforeseen random short- and long-term changes in the environment called “stressors.”

HPA Axis and Glucocorticoid Signaling System.



Life on Planet Earth

In addition to fighting against various unforeseen stressors…..

organisms live under recurrent changes associated with the rotation of the planet around itself and its revolution around the sun, which are daily, predictable and nonrandom *(circa diem).*

Major Regularly Recurrent Environmental Changes on the Earth.

Rotation of the Earth:

Day/Night Changes: Circadian

Revolution of the Earth:

Seasonal Changes

Revolution of the Moon:

Lunar Changes

Major Regularly Recurrent Environmental Changes on the Earth.

Day/Night Changes:
Circadian

Seasonal Changes(3 mo),
Lunar (28d) Changes:
Infradian

Brief Recurrent Changes <24 h:
Ultradian, q90min, other

Adjustment of internal homeostasis and synchronization of physical activities to Day/Night changes.

Day

**Work, Exercise,
Food Intake and Other
Activities: High**

Night

**Rest and Sleep
Activities: Low**

Circadian CLOCK System

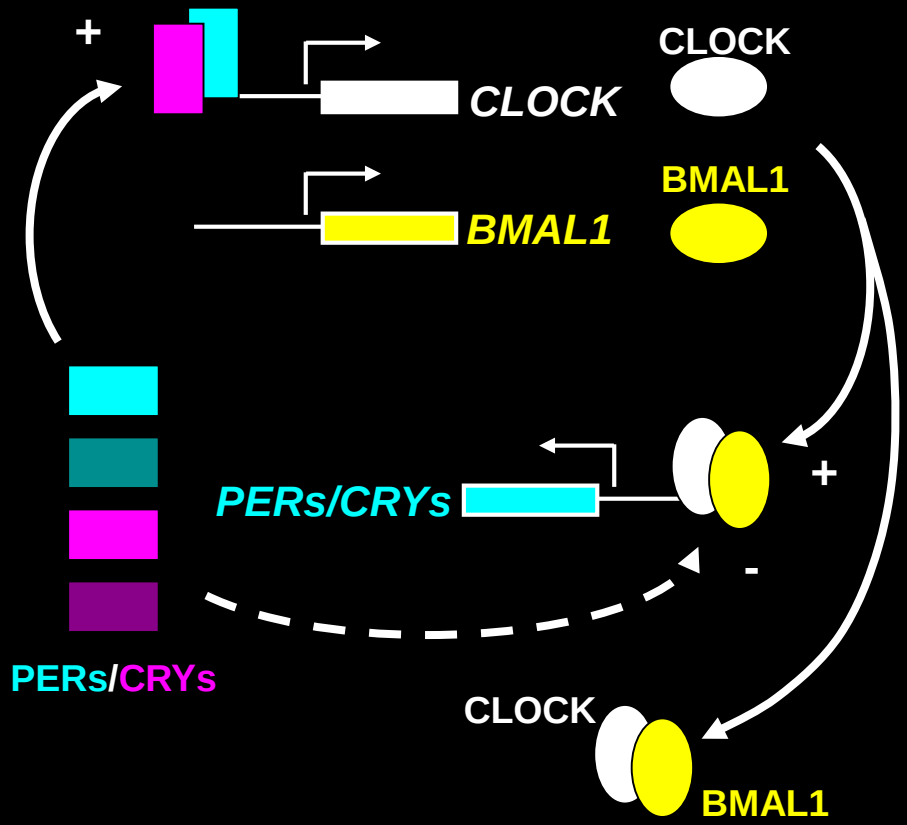
1. A highly conserved and ubiquitous molecular “CLOCK”, which creates internal circadian rhythmicity under the influence of light/dark information (retino-hypothalamic track).

2. The central “master” CLOCK is located in suprachiasmatic nucleus (SCN) of the hypothalamus, while the peripheral “slave” CLOCKs are found virtually in all organs and tissues.

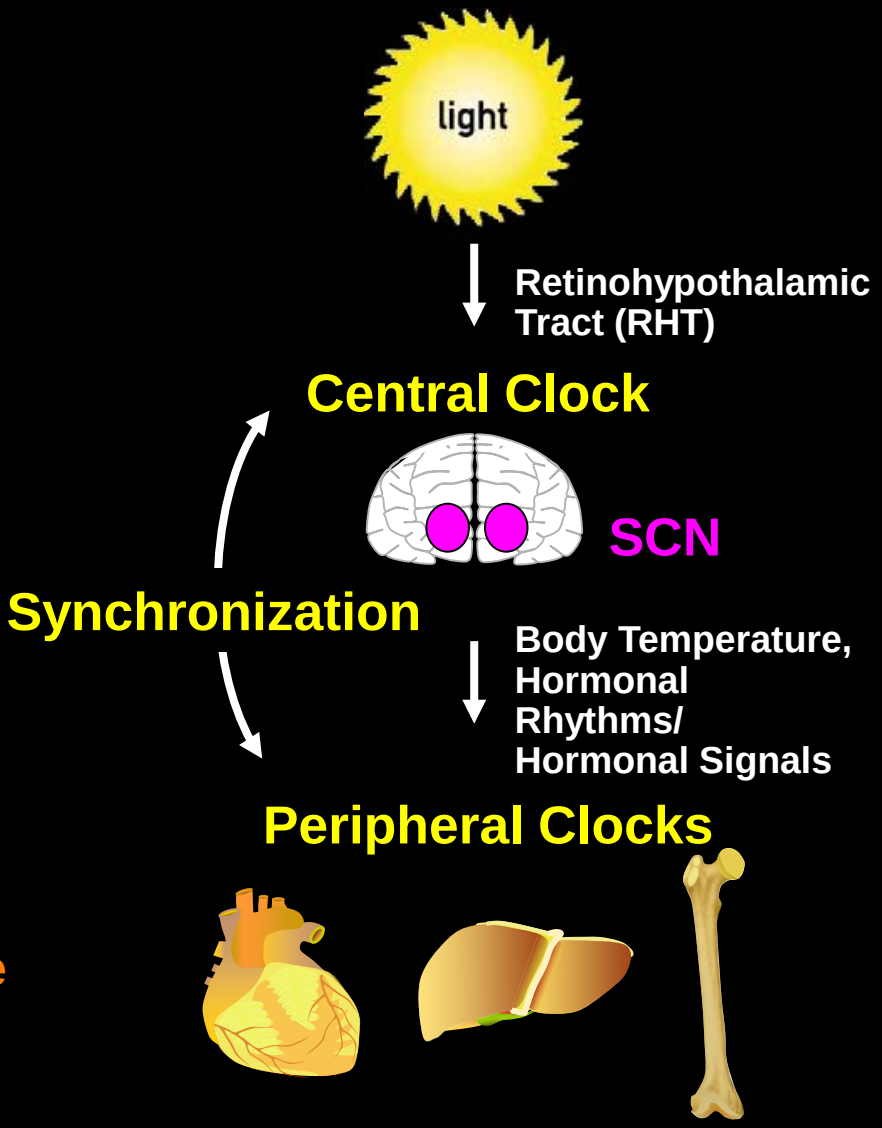
1. Circadian rhythm of the peripheral CLOCKs are synchronized to that of the central master CLOCK by as yet unknown mechanisms.

CLOCK/BMAL1: Circadian Rhythm Transcription Factors

Self-oscillating Transcription Loop

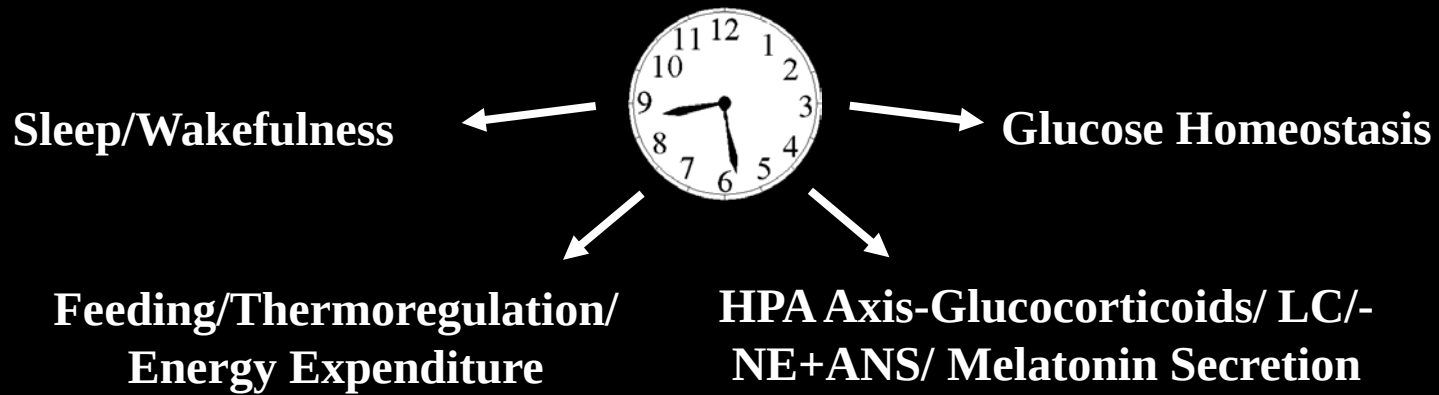


How does CLOCK/BMAL1 influence GR transcriptional activity?

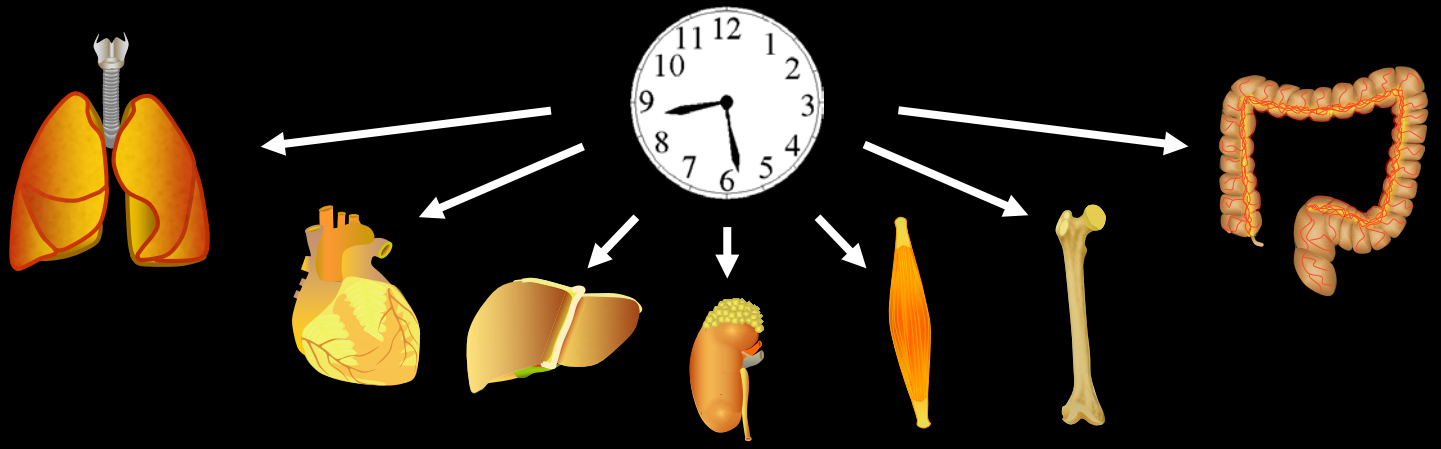


Circadian CLOCK Influences Virtually All Physiologic Functions/Organs

Central CLOCK Output



Peripheral CLOCK Outputs

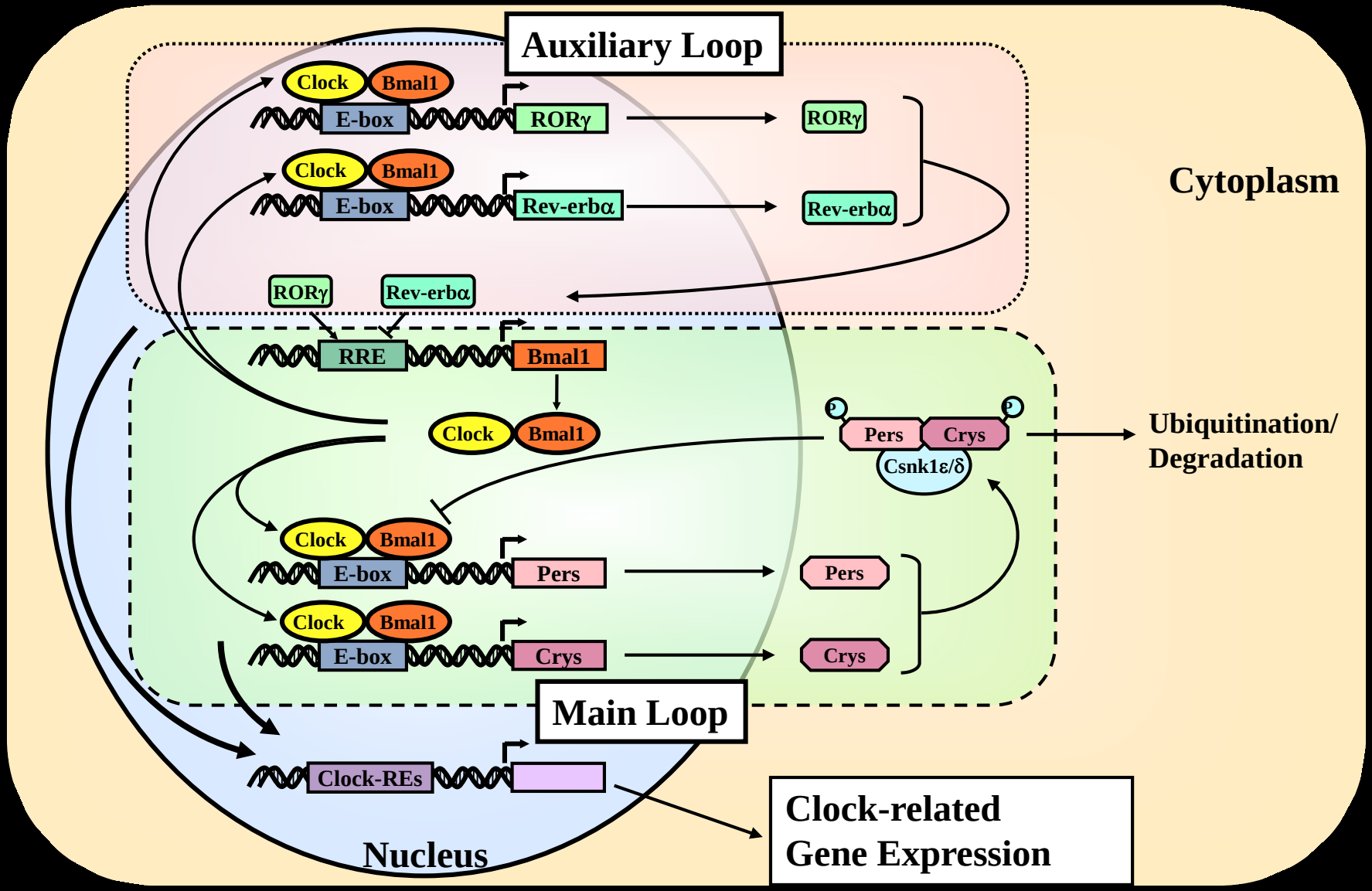


Circadian CLOCK System

1. A **transcriptional loop** creating an intrinsic, self-oscillating circadian rhythm in both central and peripheral CLOCKs.

2. Composed of the **Clock/Bmal1** heterodimer and other negative transcription factors (such as the ***Periods*** (*PER1*, *PER2* and *PER3*) and ***Cryptochromes*** (*CRY1* and *CRY2*) genes.

CLOCK Circadian Transcriptional Loop



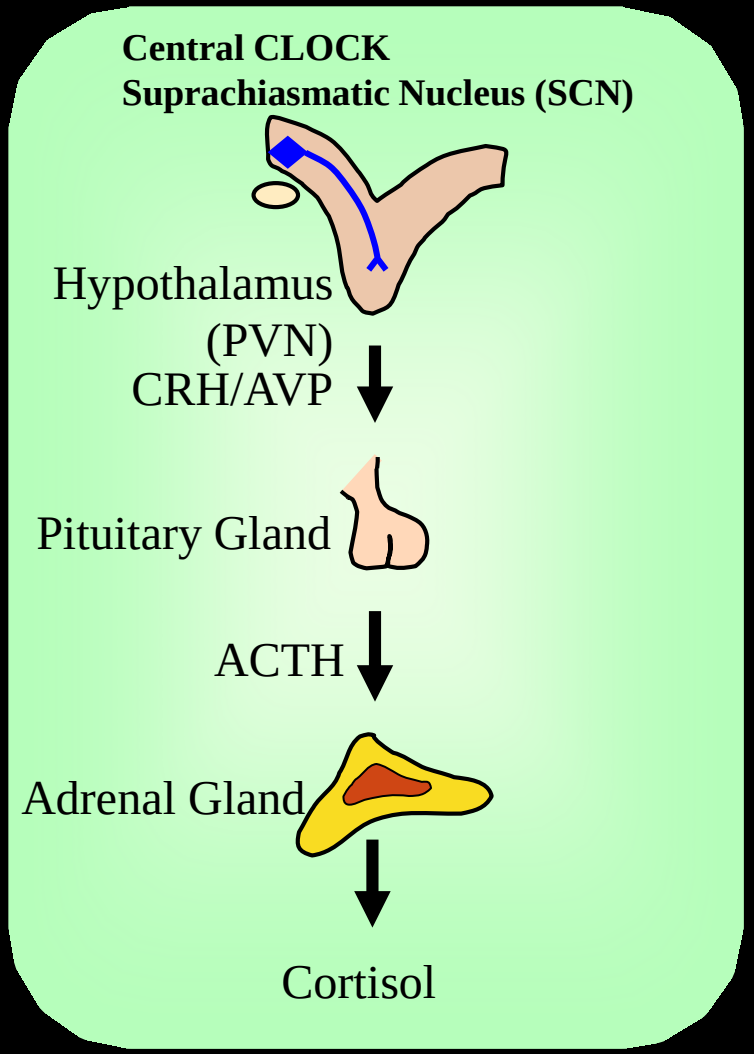
Interaction of the Circadian CLOCK System and the Stress System/HPA Axis

Circadian CLOCK –related Stress System:
Adaptation to the regularly recurrent Day/Night change

Stressor-related Stress System/HPA Axis:
Adaptation to recurrent Day/Night, but also to unforeseen and random environmental changes

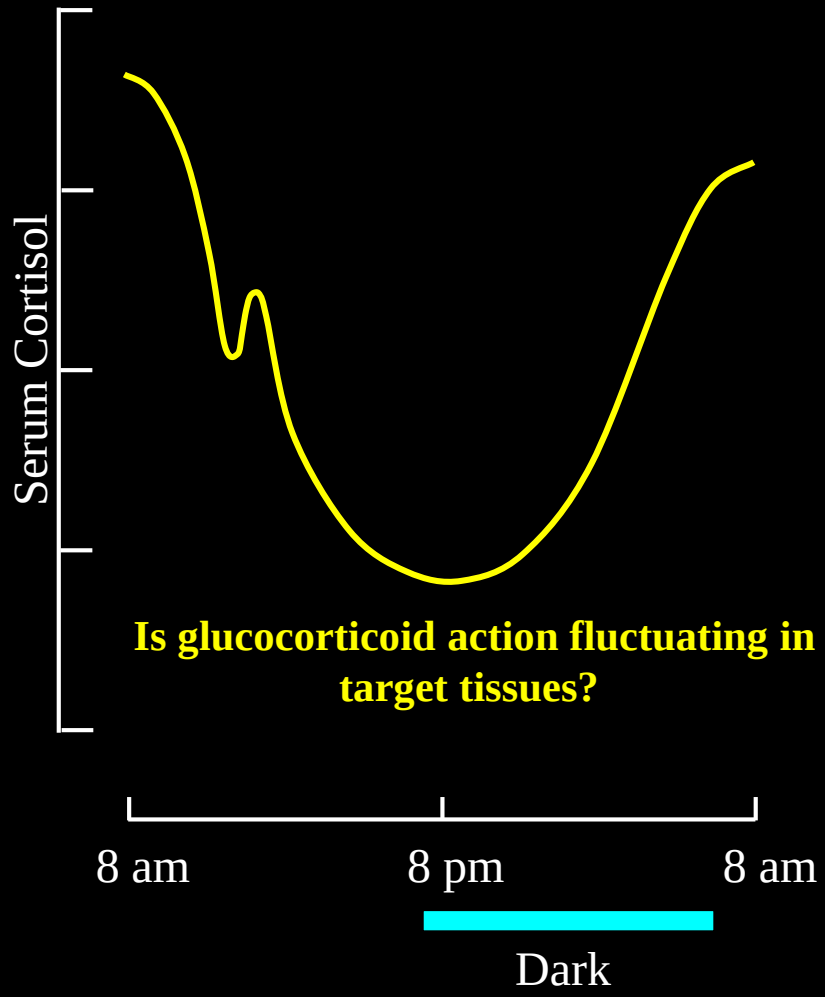
There are strong links between these two systems

Plasma Cortisol Circadian Rhythm



HPA Axis

24 hr Circulating Cortisol



Interaction between Clock/Bmal1 and GR at the Transcriptional Level

Circadian Rhythm Transcription Factor CLOCK/BMAL1 Regulates the Transcriptional Activity of the Glucocorticoid Receptor through Acetylation

Nancy Nader[1], George P. Chrousos[2] and Tomoshige Kino[1]

1: Program in Reproductive and Adult Endocrinology, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

2: First Department of Pediatrics, Athens University Medical School, Athens 11527, Greece

FASEB J 2009

Clock/Bmal1:
Circadian Rhythm Transcription Factors

1. Master regulators of the circadian rhythms both in the central nervous system and peripheral tissues/organs.
2. The basic helix-loop-helix (bHLH)-PER-ARNT-SIM (PAS) superfamily of transcription factors.
3. Clock is a histone acetyltransferase (HAT).

Clock Displays High Sequence Similarity to p160 Nuclear Receptor Coactivator ACTR

Circadian Regulator CLOCK Is a Histone Acetyltransferase

Masao Doi,[1,3] Jun Hirayama,[1,2] and Paolo Sassone-Corsi[1,2,*]
[1] Institut de Génétique et de Biologie Moléculaire et Cellulaire, B.P. 10142, 67404 Illkirch, Strasbourg, France
[2] Present address: Department of Pharmacology, University of California, Irvine, 360D MedSurge II, Irvine, CA 92697, USA
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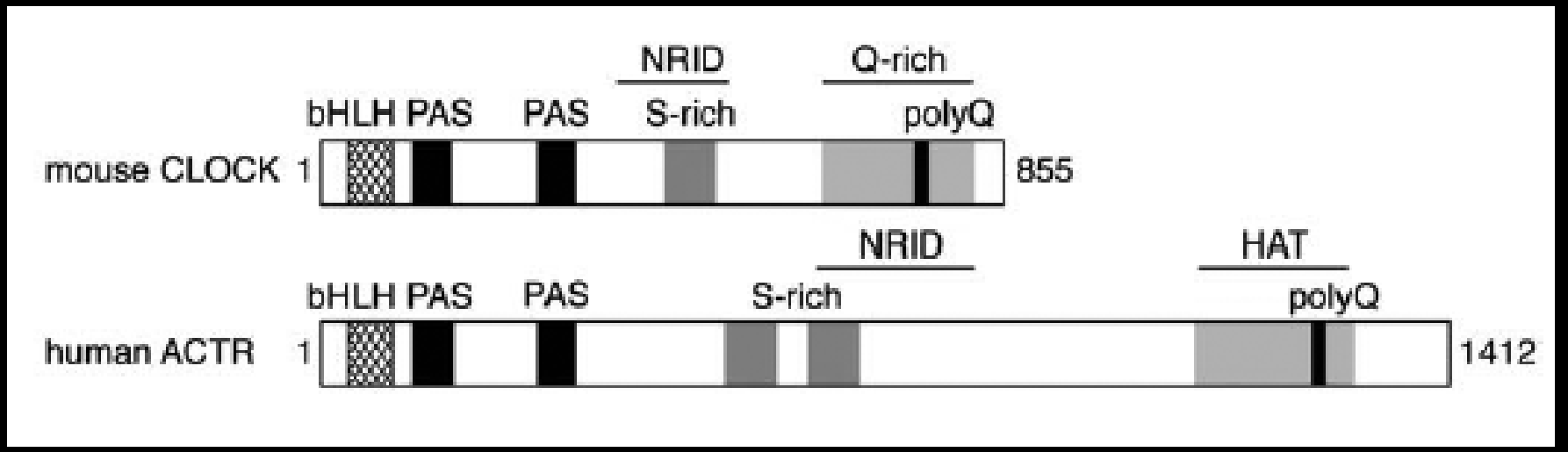
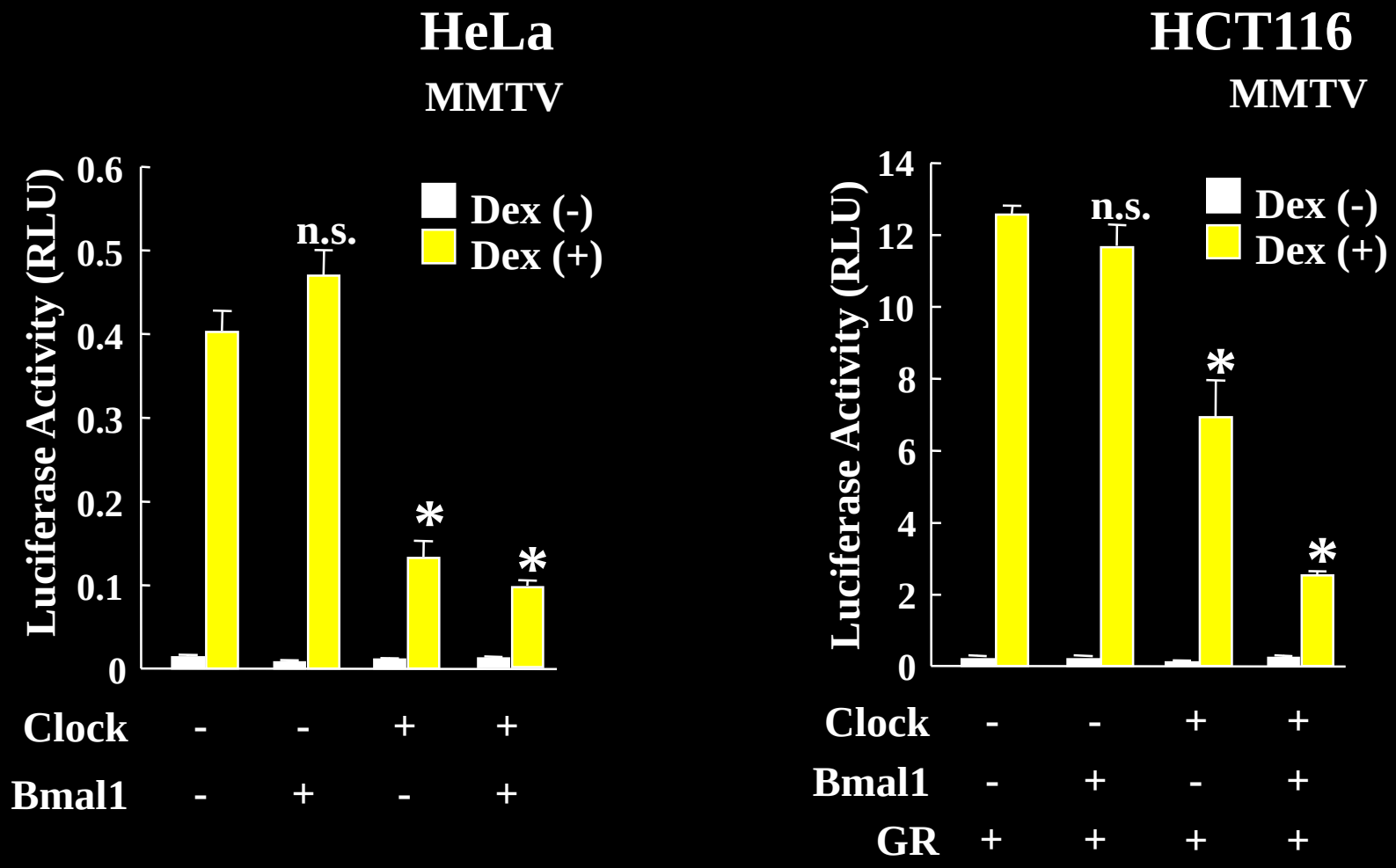
*Contact: paolosc@igbmc.u-strasbg.fr
DOI 10.1016/j.cell.2006.03.033



Doi et al. *Cell* 2006, 125: p496

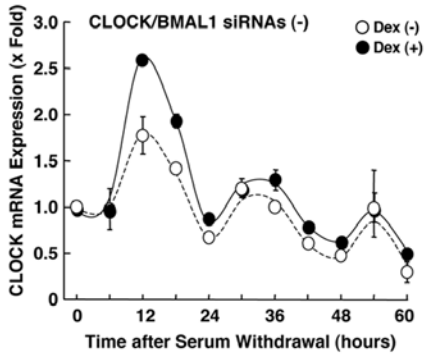
Clock/Bmal1 Represses GR-induced Transcriptional Activity

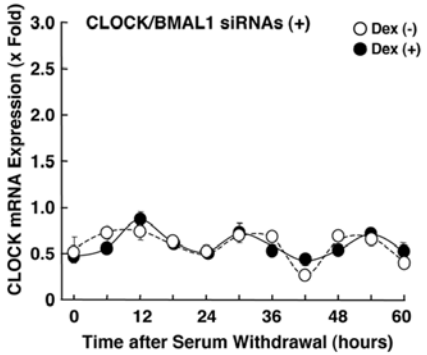


Nader N. et al. *FASEB J* 2009

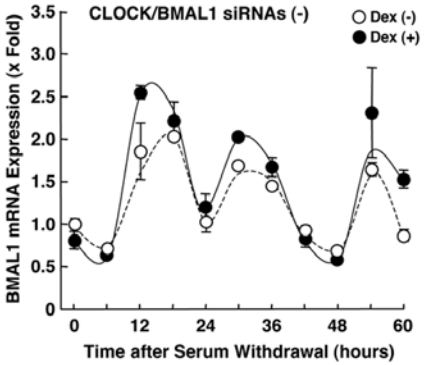
HeLa

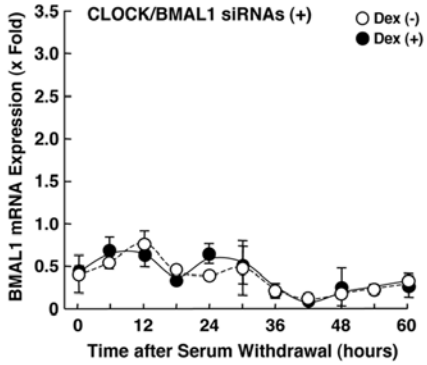
CLOCK mRNA



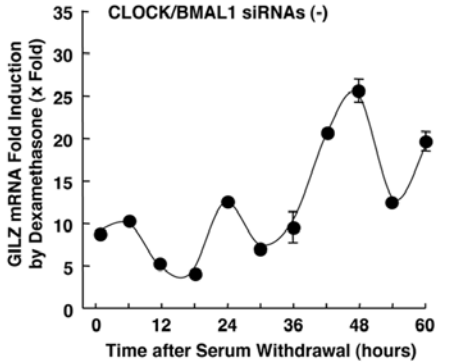


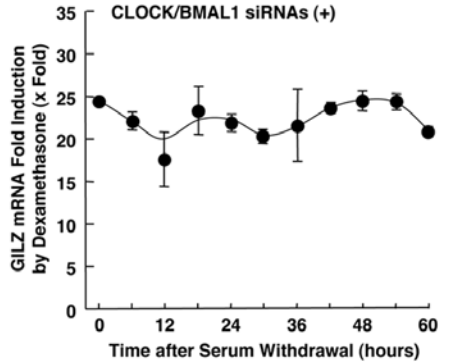
BMAL1 mRNA





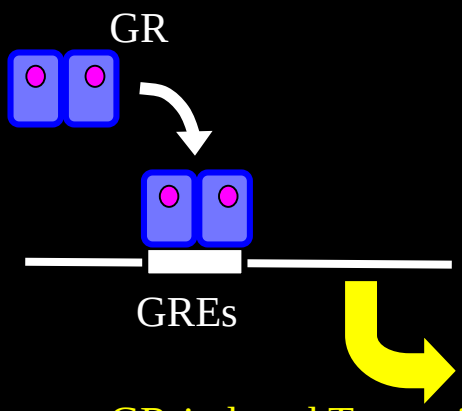
GILZ mRNA Fold Induction by Dexamethasone



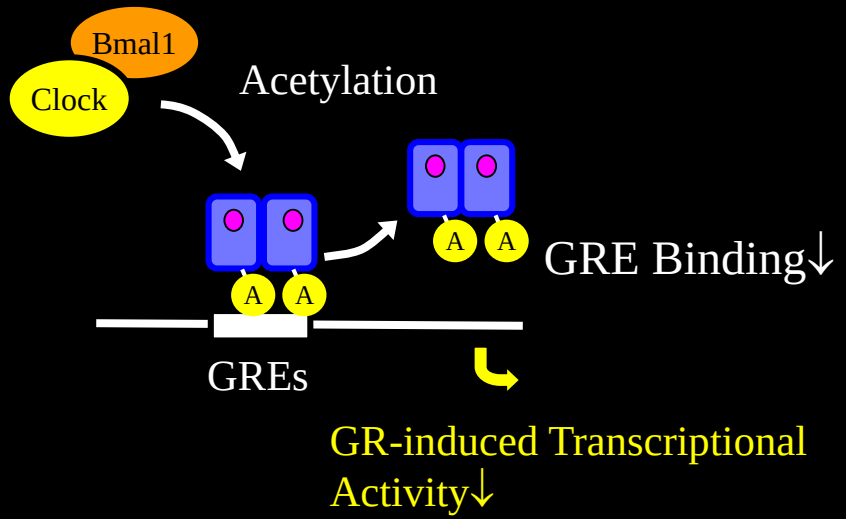


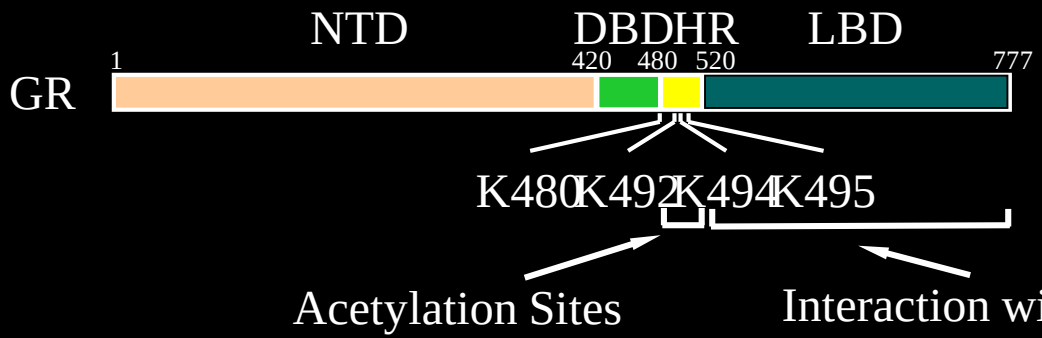
Results #1: Clock/Bmal1 Represses GR Transcriptional Activity through Acetylation

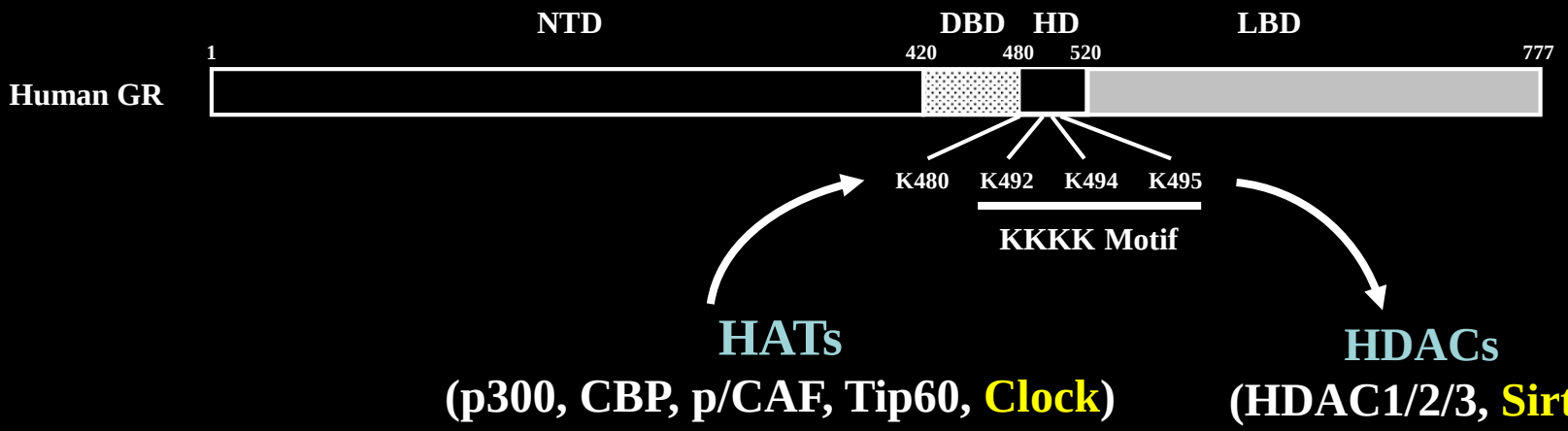
In the Absence of Acetylation by CLOCK



In the Presence of Acetylation by CLOCK

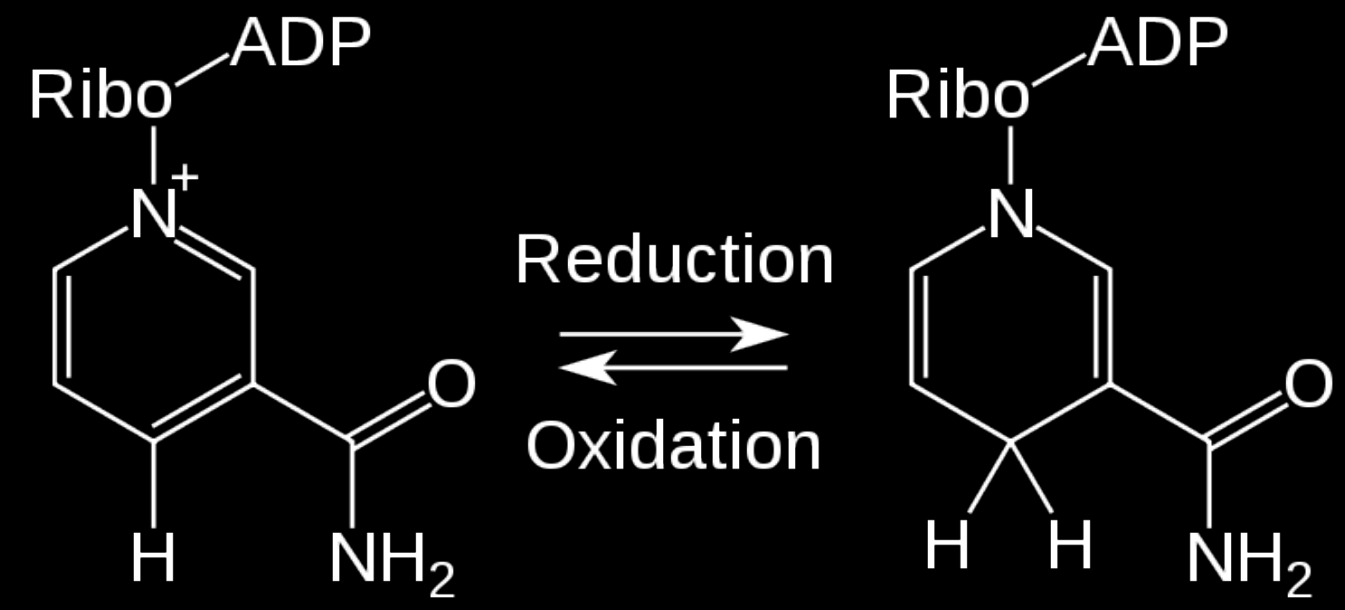




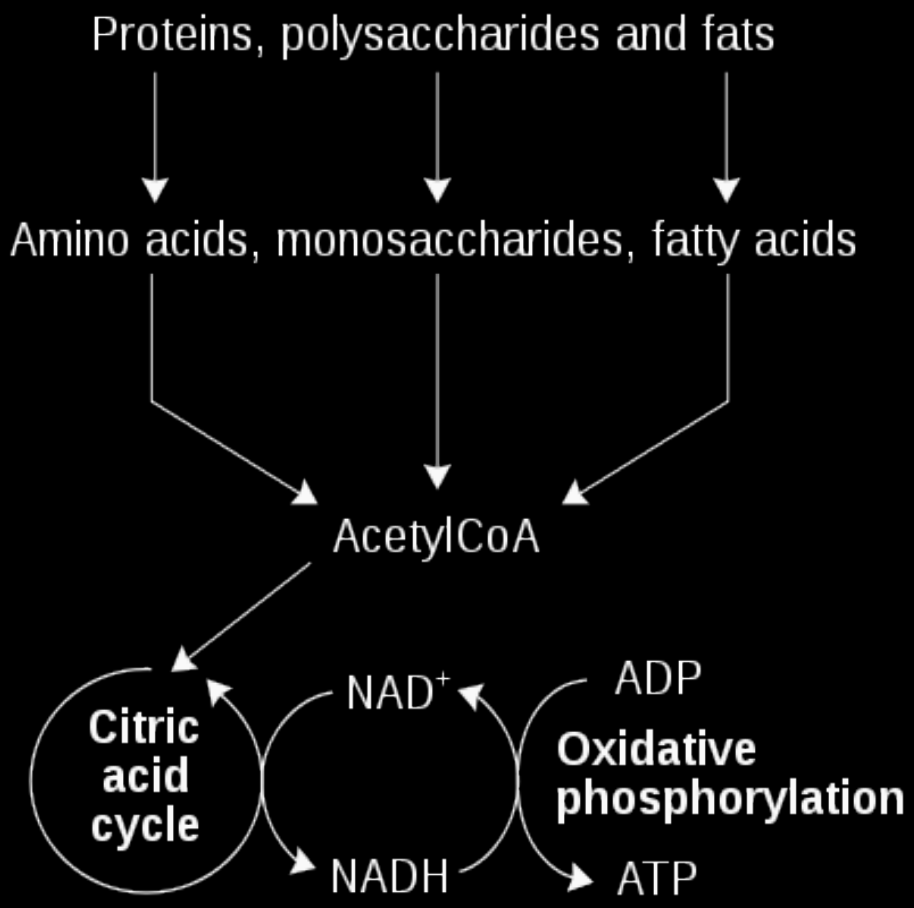


NAD

Nicotinamide phosphoribosyltransferase (NAMPT) = Visfatin



$NAD^+ + H^+ + 2e^- \longrightarrow NADH$


Proteins, polysaccharides and fats
Amino acids, monosaccharides, fatty acids
AcetylCoA
Citric acid cycle
NAD+
NADH
ADP
Oxidative phosphorylation
ATP

Clock-mediated Regulation of GR Transcriptional Activity in Humans

Peripheral CLOCK Regulates Target-tissue Glucocorticoid Receptor Transcriptional Activity in a Circadian Fashion in Man

Charmandari E, Chrousos GP, Lambrou GI, Pavlaki A, Koide H, Ng SSM and Kino T

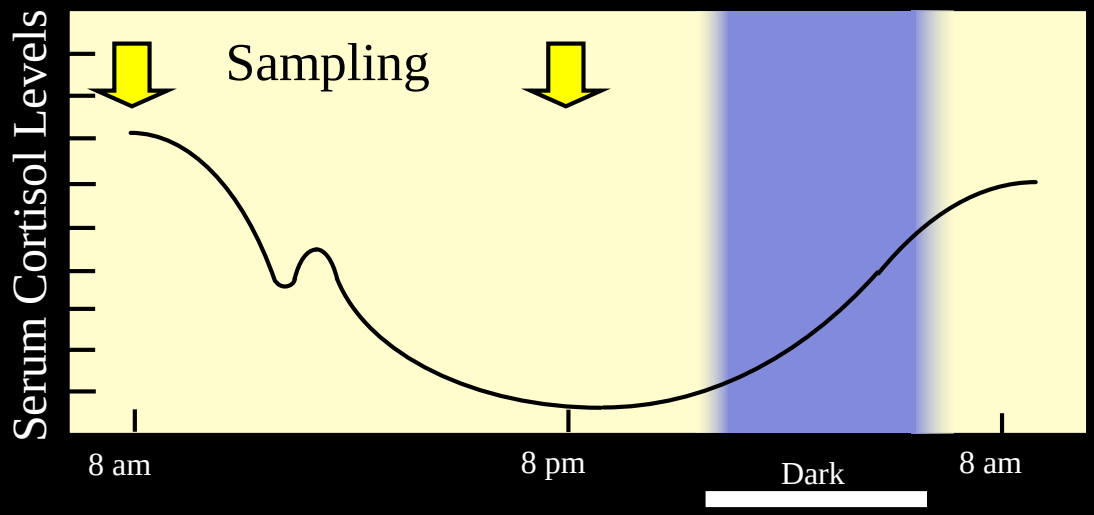
PLoS ONE 6(9): e25612, 2011

Recruitment of healthy volunteers

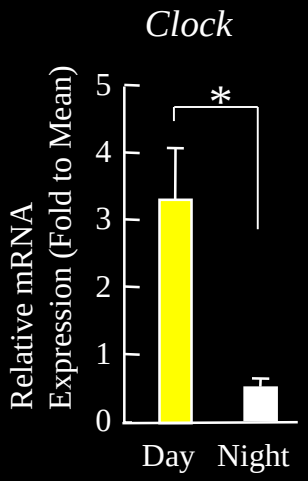
Blood sampling at 8 am and 8 pm for PBMC

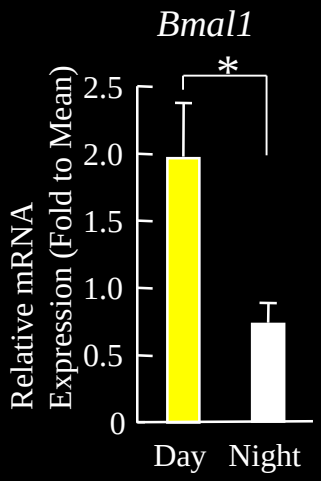
1. Evaluation of GR acetylation
2. mRNA Expression of known glucocorticoid-responsive and CLOCK-related genes

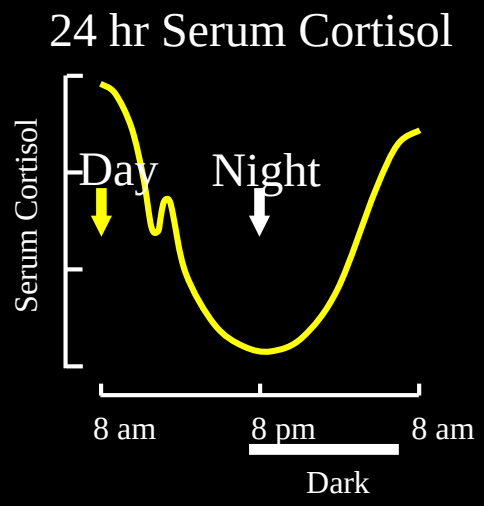
Treatment of EBV-transformed lymphocytes with hydrocortisone (HC) for 5 hours

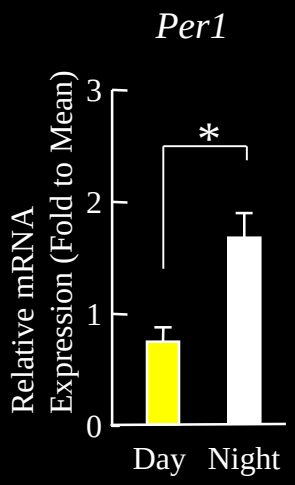


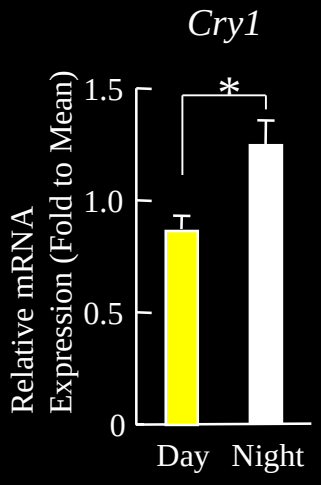
Circadian mRNA Expression of CLOCK-related Genes in PBMCs *in vivo*

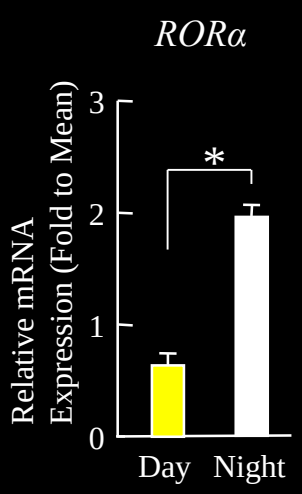




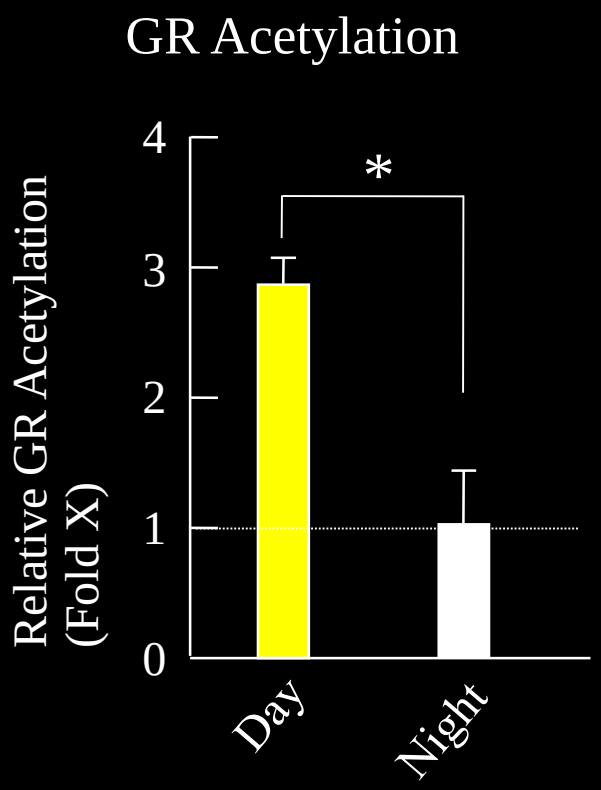


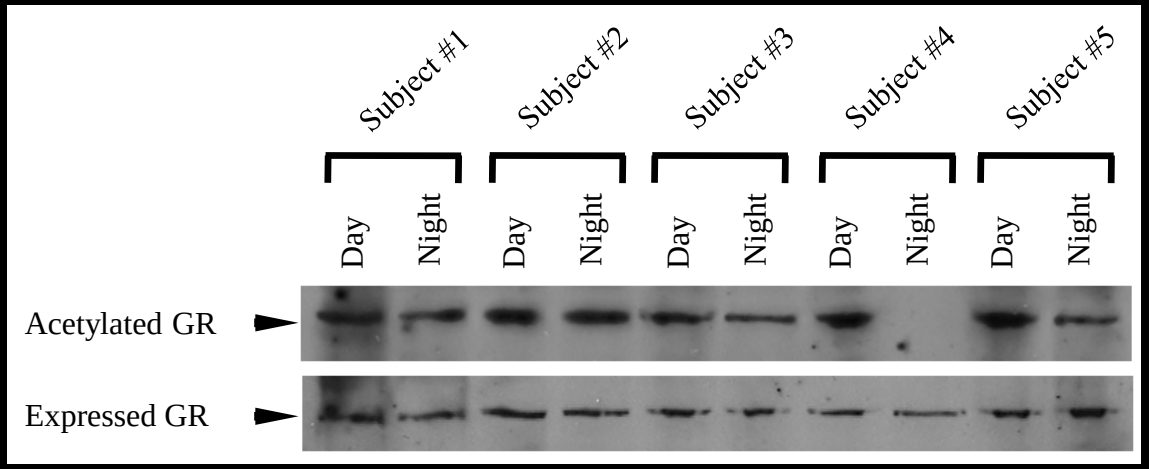




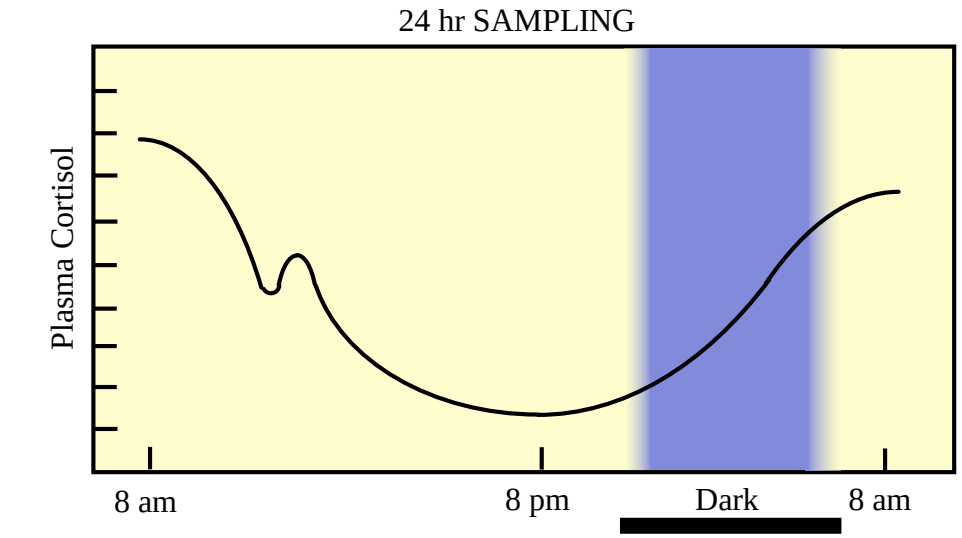


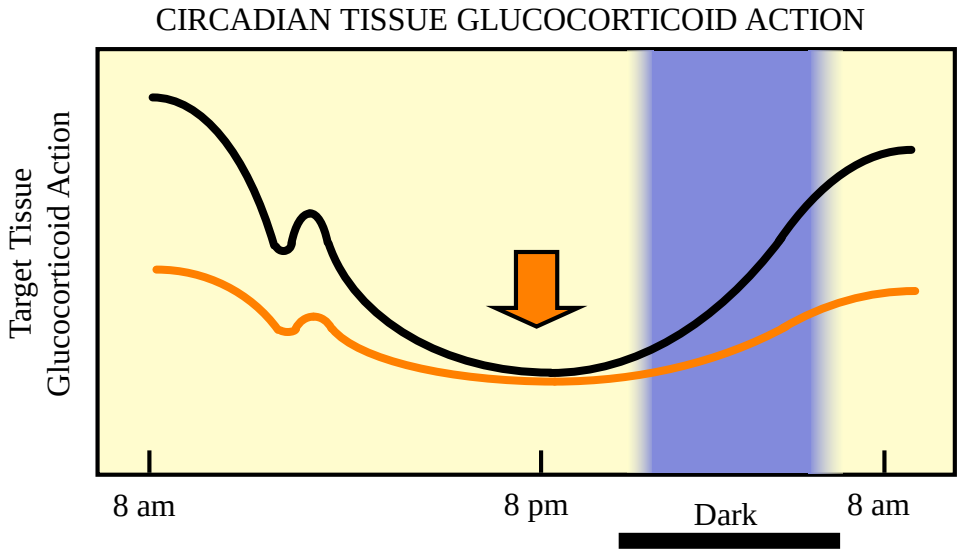
GR is Acetylated in a Circadian Fashion in PBMCs *in vivo*





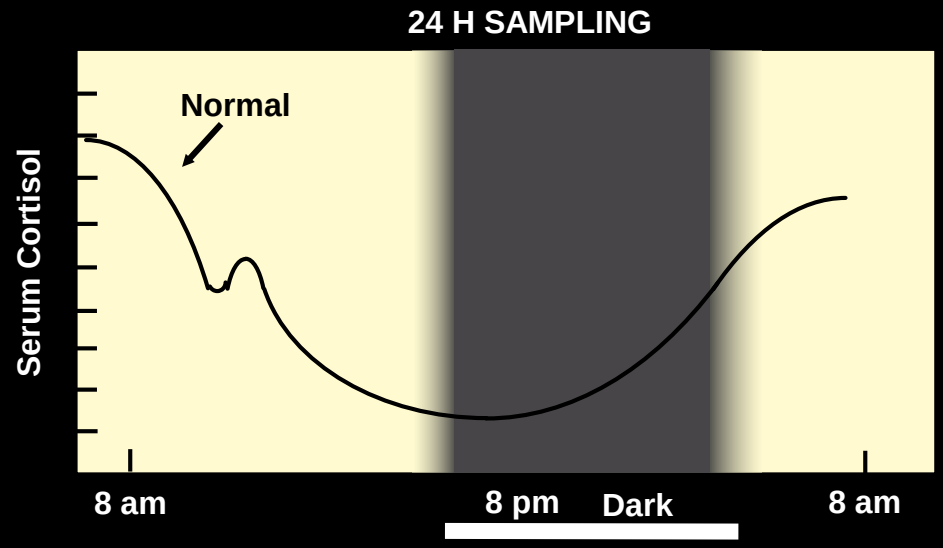
CLOCK-mediated Gene-specific Regulation of Glucocorticoid Action at Peripheral Tissues

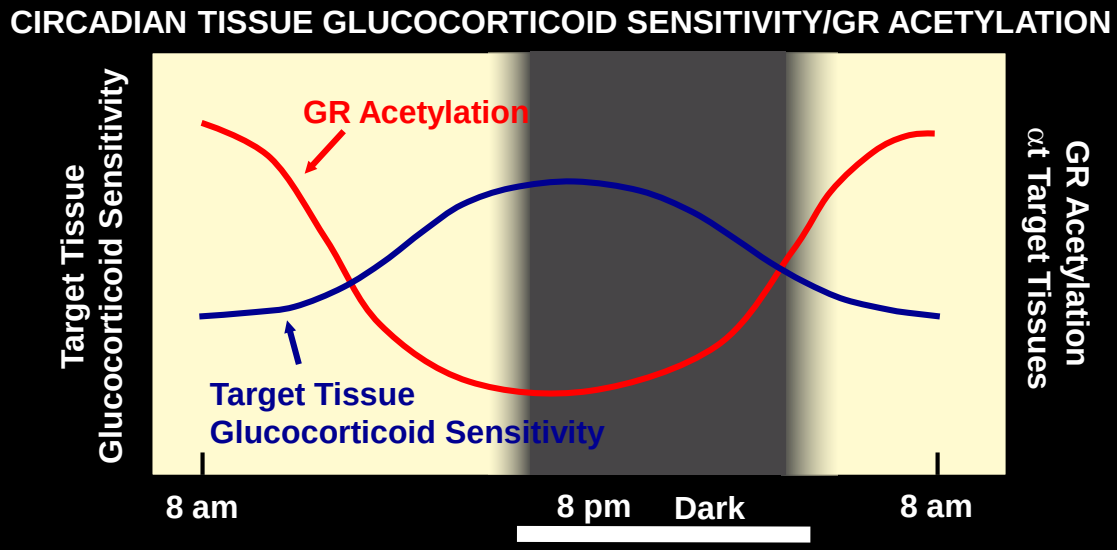




Gene- (and Tissue-) specific Moderation of Glucocorticoid-Responsive Gene Action (Transactivation or transereprssion)

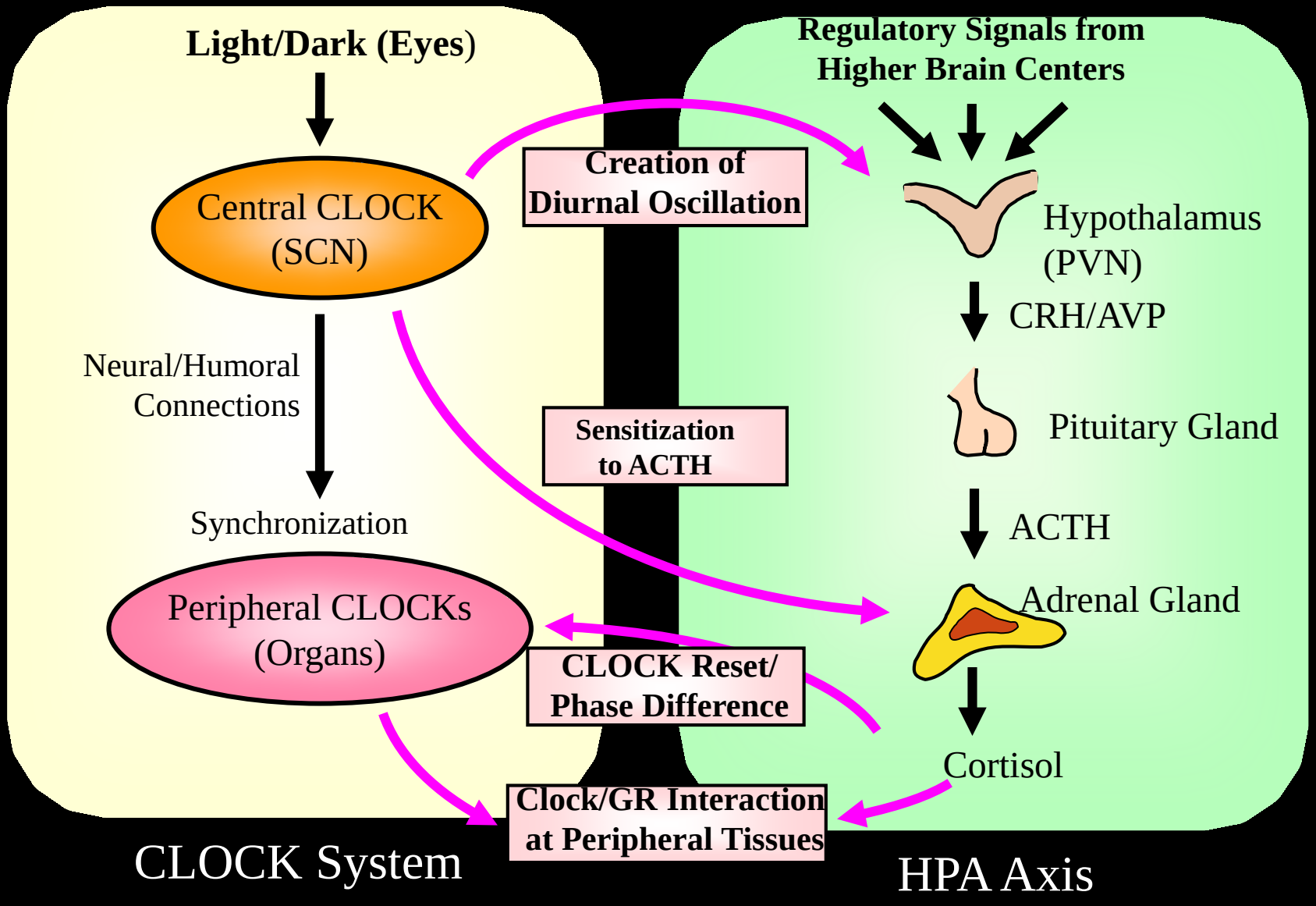
CLOCK-mediated Gene-specific Regulation of Glucocorticoid Action at Peripheral Tissues





Gene- (and Tissue-) specific Repression of Glucocorticoid-Responsive Genes

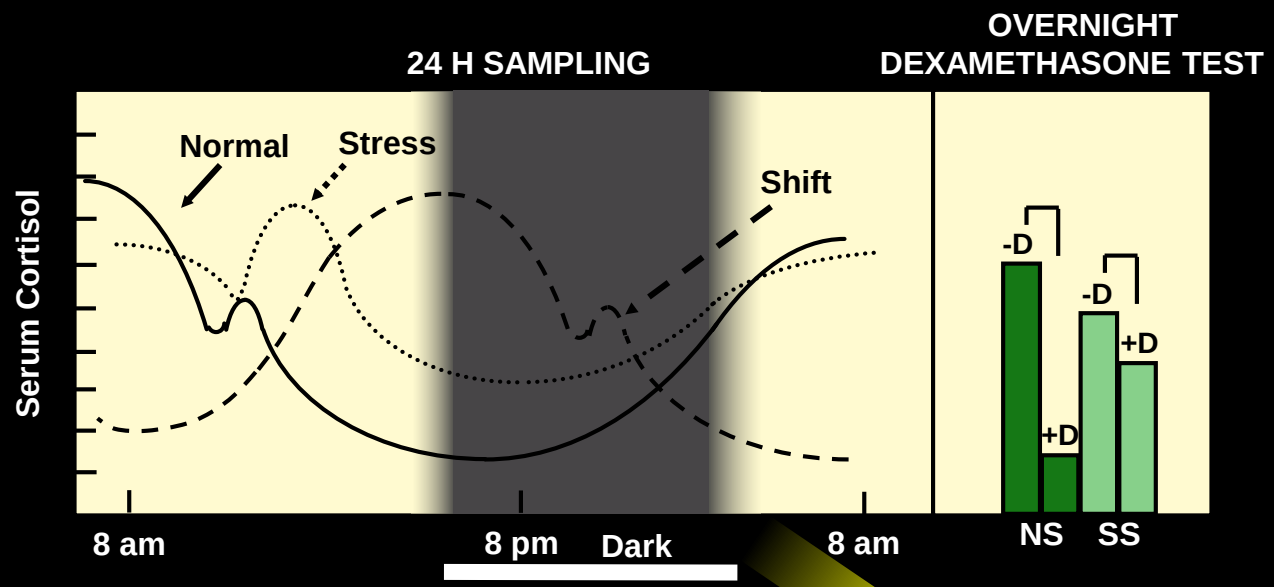
Multiple Interactions between the Circadian CLOCK System and the HPA Axis

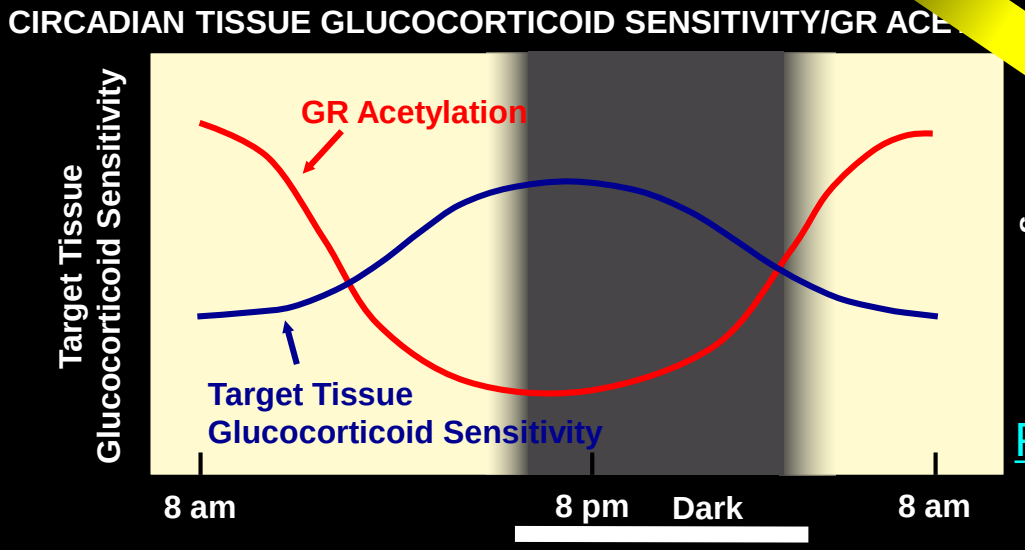


Loss of Circadian Rhythm and Glucocorticoid Excess Cause Similar Metabolic Disturbances

Signs & Symptoms	Loss of Circadian Rhythm	Glucocorticoid Excess
Glucose Metabolism		
Hyperglycemia	++	++
Insulin Resistance	++	++
Fat Metabolism		
Hyperlipidemia	++	+
Fatty Liver	+	+
Central Obesity	++	++
Hypertension	+	+
Appetite	↑	↑
Immunity (Th1➔ Th2)	↑	↑

Uncoupling between Circadian Rhythm of Cortisol and Tissue Glucocorticoid Sensitivity





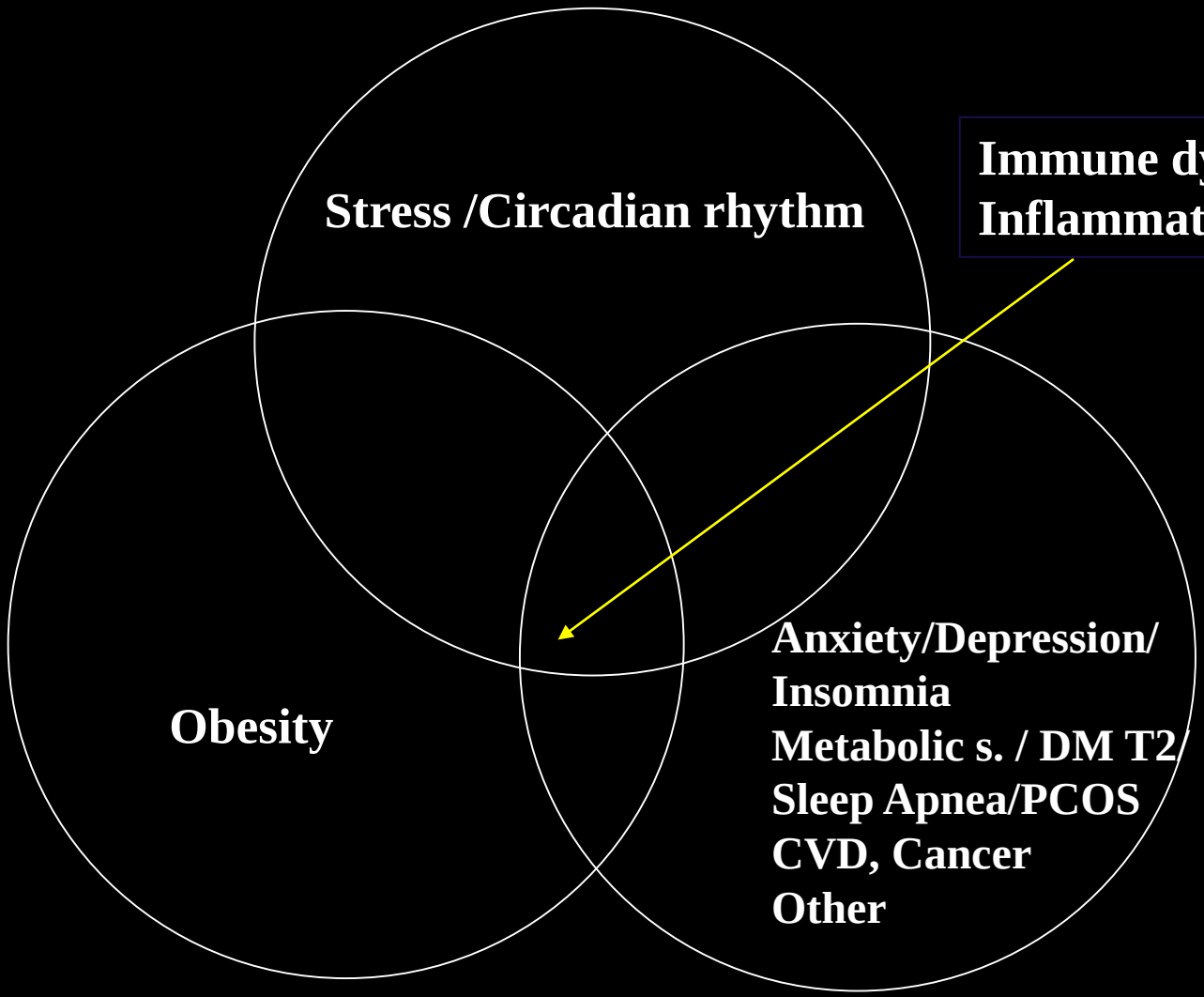
Functional Glucocorticoid Hypersensitivity

Pathologic Consequences?

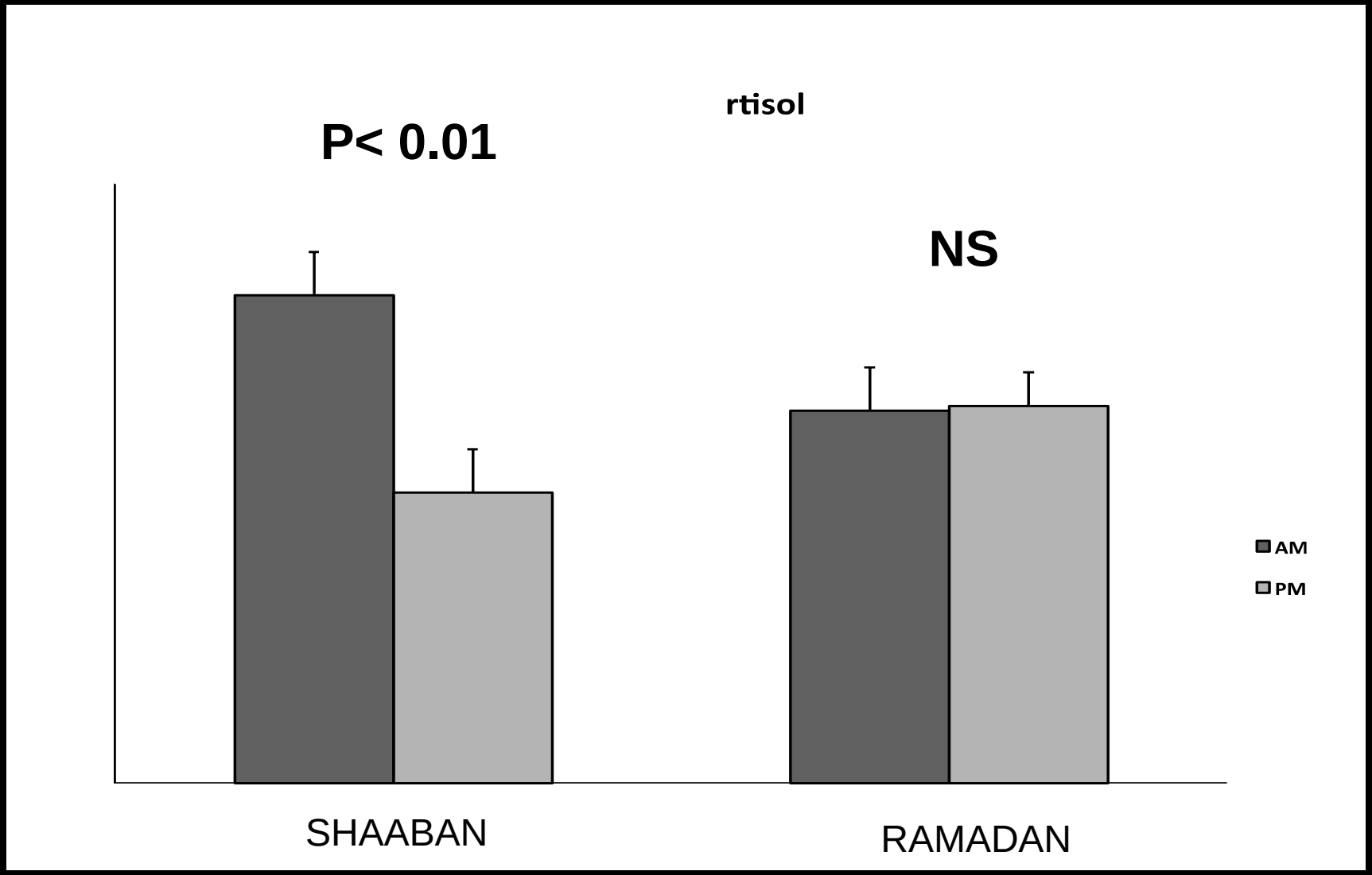
Examples of Pathologies Due to Aberrant Coupling of CLOCK and HPA Axis

1. Chronic Stress-associated Evening Cortisol Elevations.
2. Endogenous/Exogenous Cushing Syndrome.
3. Trans-time-zone Travel.
4. Nightshift Work.

All above conditions are associated with a high risk for cardiovascular diseases and immune dysfunction.


Stress /Circadian rhythm
Immune dysregulation/
Inflammation
Obesity
Anxiety/Depression/
Insomnia
Metabolic s. / DM T2/
Sleep Apnea/PCOS
CVD, Cancer
Other

Serum cortisol Level before and through fasting month:



We compared AM/PM serum cortisol ratios to mRNA expression of ~160 GR action-regulating or glucocorticoid-responsive genes in the subcutaneous fat obtained from 25 obese subjects.

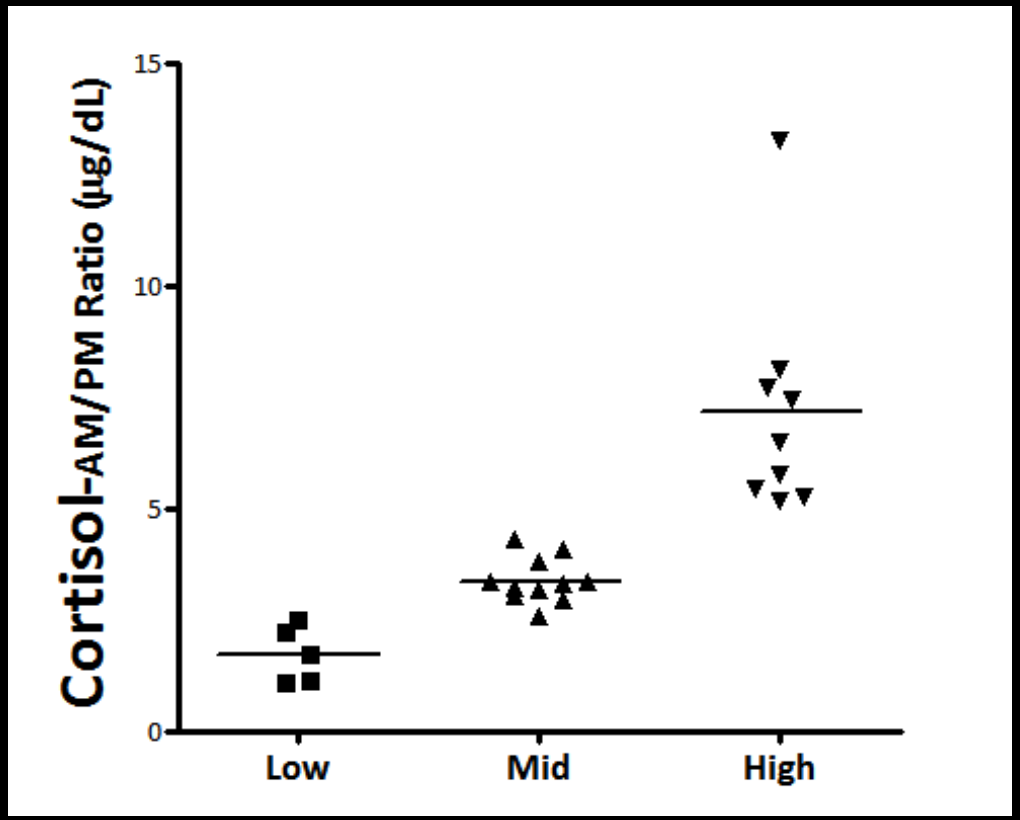
Results (3): Focus on Glucocorticoids

AM/PM Serum Cortisol Ratio

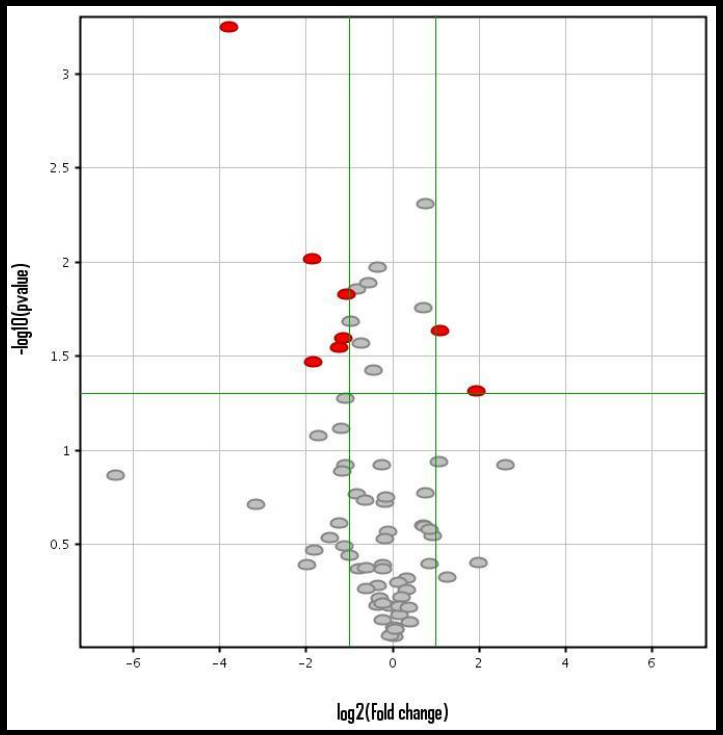
Low: <2.5

Middle: 2.5 ~ 4.5

High: >4.5



AM/PM Cortisol Ratio Influences mRNA Expression in Human Subcutaneous Fat



Gene Symbol	p-value	Fold Change
NAMPT	5.60E-04	-13.83
FTO	0.009687231	-3.67
CDKN1A	0.034129716	-3.60
KDM3A	0.028344488	-2.37
VHL	0.02523294	-2.21
PRKAA1	0.014816636	-2.10
CEBPB	0.02314632	2.14

Potential Mediators for Cortisol Circadian Rhythm to Fat Physiology/Pathology

NAMPT

(1)Nicotinamide phosphoribosyltransferase (Visfatin)
(2)Rate-limiting enzyme for NAD synthesis

FTO

(1)Fat mass and obesity-associated protein
(2)Involved in demethylation of DNA
(3)Associated with susceptibility to diabetes mellitus type 2

KDM3A

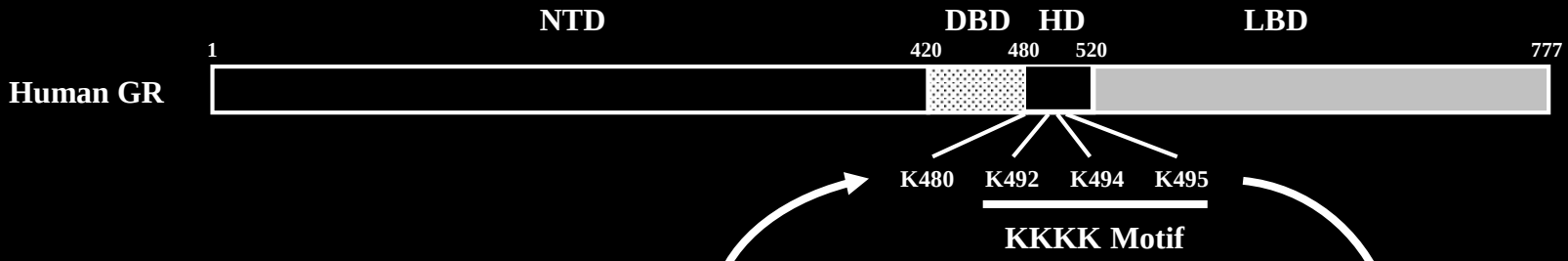
(1)Lysine-specific demethylase 3A

PRKAA1

(1)AMP-activated protein kinase (AMPK) a1 catalytic subunit

CEBPB

(1) C-EBPβ: transcription factor important for adipocyte proliferation/differentiation and lipid metabolism



HATs
(p300, CBP, p/CAF, Tip60, Clock)

HDACs
(HDAC1/2/3, Sirt1)

NAD

NF-kB (-)

Nicotinamide phosphoribosyltransferase
(NAMPT) = Visfatin

Metabolism

Inflammation

MUSCLE MASS

Best predictor of morbidity and life expectancy

DEFINITIONS

- **Osteosarcopenia *vs.* (Lean) Paradoxic Obesity *vs.* Osteosarcopenic Obesity**

Decreased bone mass:

Osteopenia *vs.* Osteoporosis

T-scores from -1 to -2.5 *vs.* <2.5

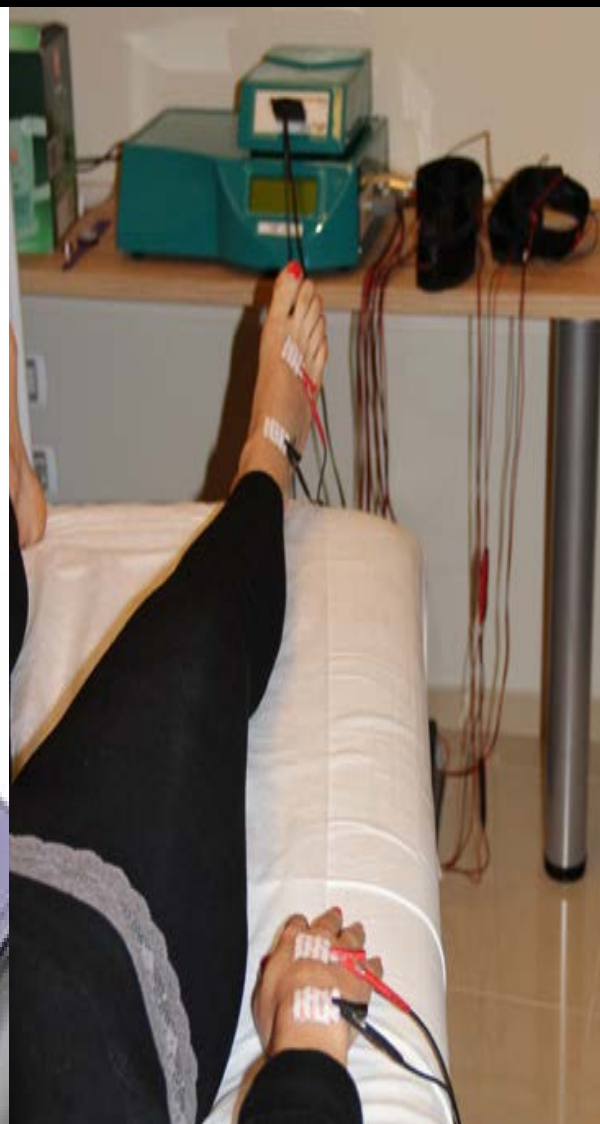
Decreased muscle mass:

Sarcopenia *vs.* Sarcasthenia (frailty)

Weight + Height

Hologic -DXA

BIA-ACC



DOI: 10.1111/eci.12388

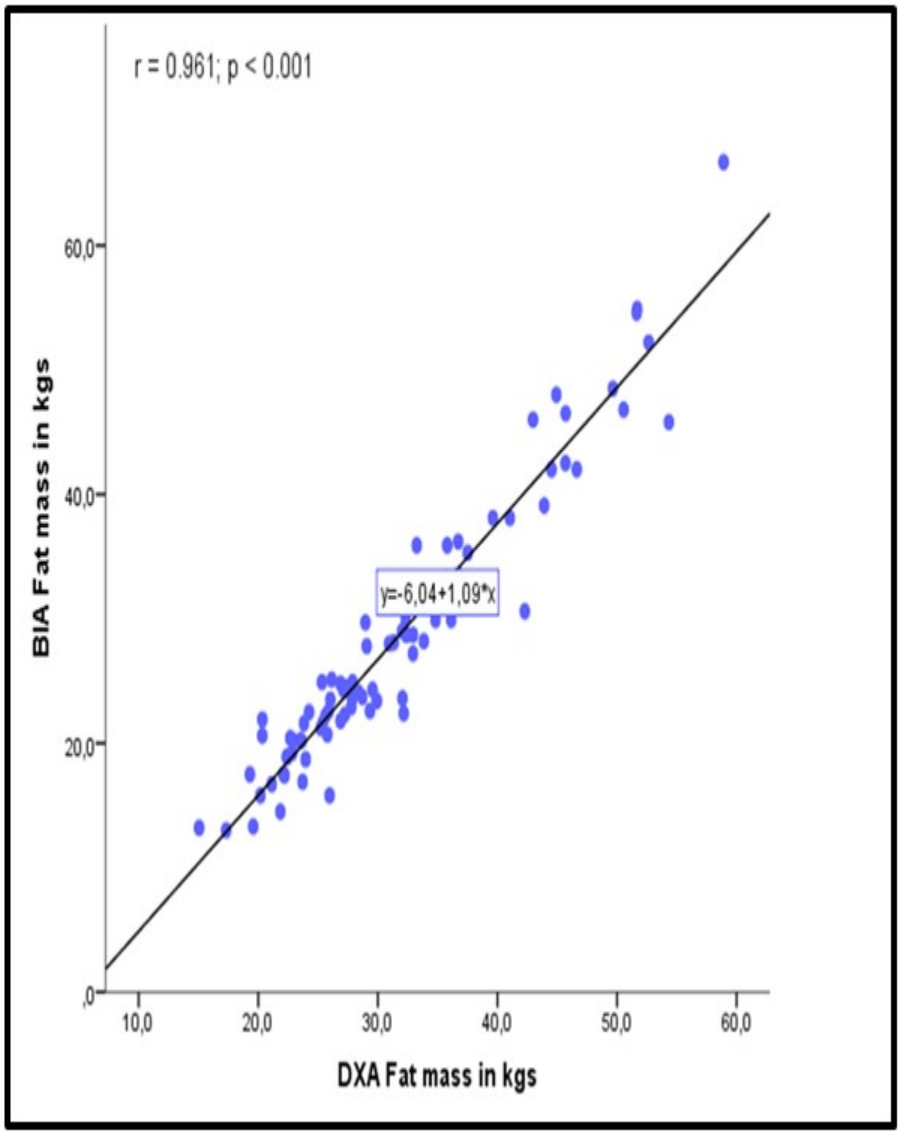
ORIGINAL ARTICLE

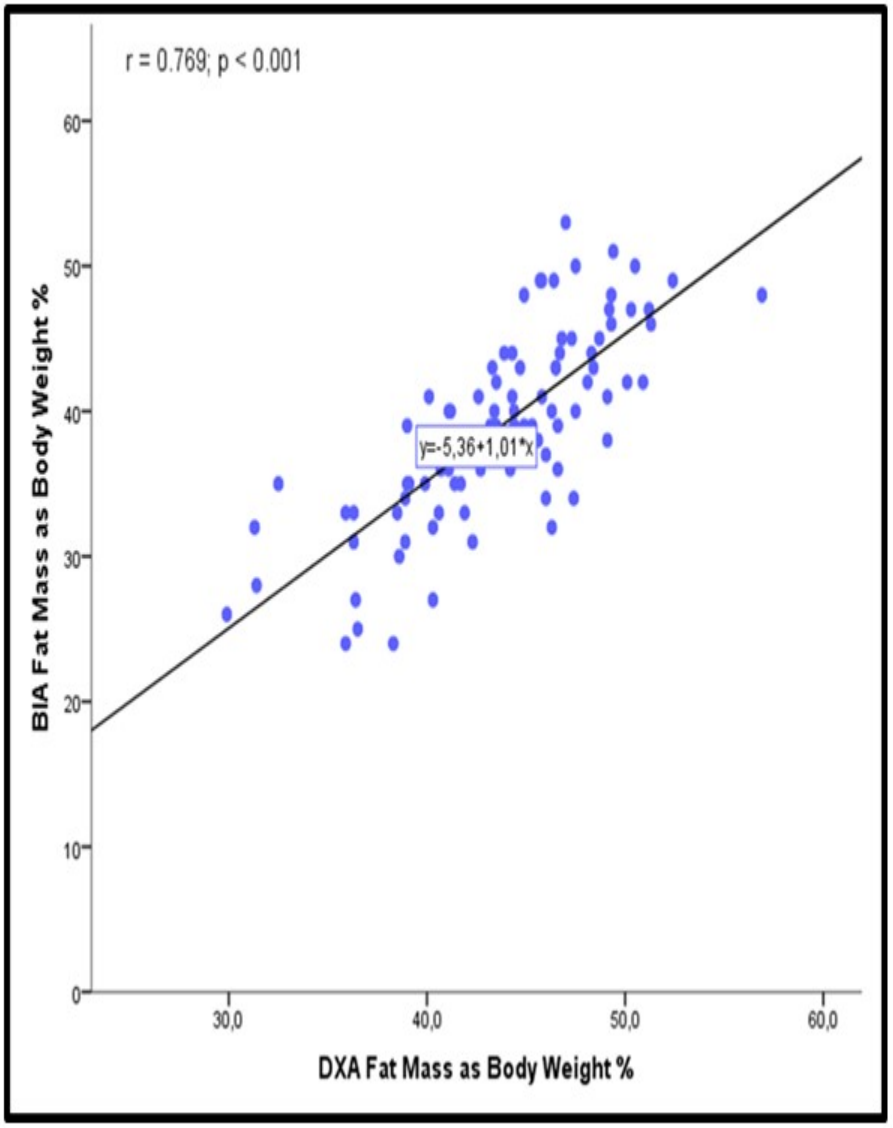
Stress and inflammatory biomarkers and symptoms are associated with bioimpedance measures

Constantine Tsigos[*], Charikleia Stefanaki[†], George I. Lambrou[‡], Dario Boschiero[§] and George P. Chrousos[†,‡,¶]

[*]School of Health Sciences and Education, Harokopio University of Athens, [†]Division of Endocrinology, Metabolism and Diabetes, University of Athens Medical School, "Eugenideion" Hospital, [‡]First Department of Pediatrics, Choremeio Research Laboratory, University of Athens, [§]BIOTEKNA Co., Venice, Italy, [¶]Biomedical Research Foundation, Academy of Athens, Athens, Greece

Pearson's correlation co-efficient for BIA and DXA Fat Mass in kg & as Body Weight %





Participants were 99571 adult Caucasians (29624 males and 60047 females), ages 20-80 y, grouped by:

- **BMI: Lean, Overweight, Obese**
- **Presence of Medically Unexplained Symptoms = MUS**
- **Body Composition**

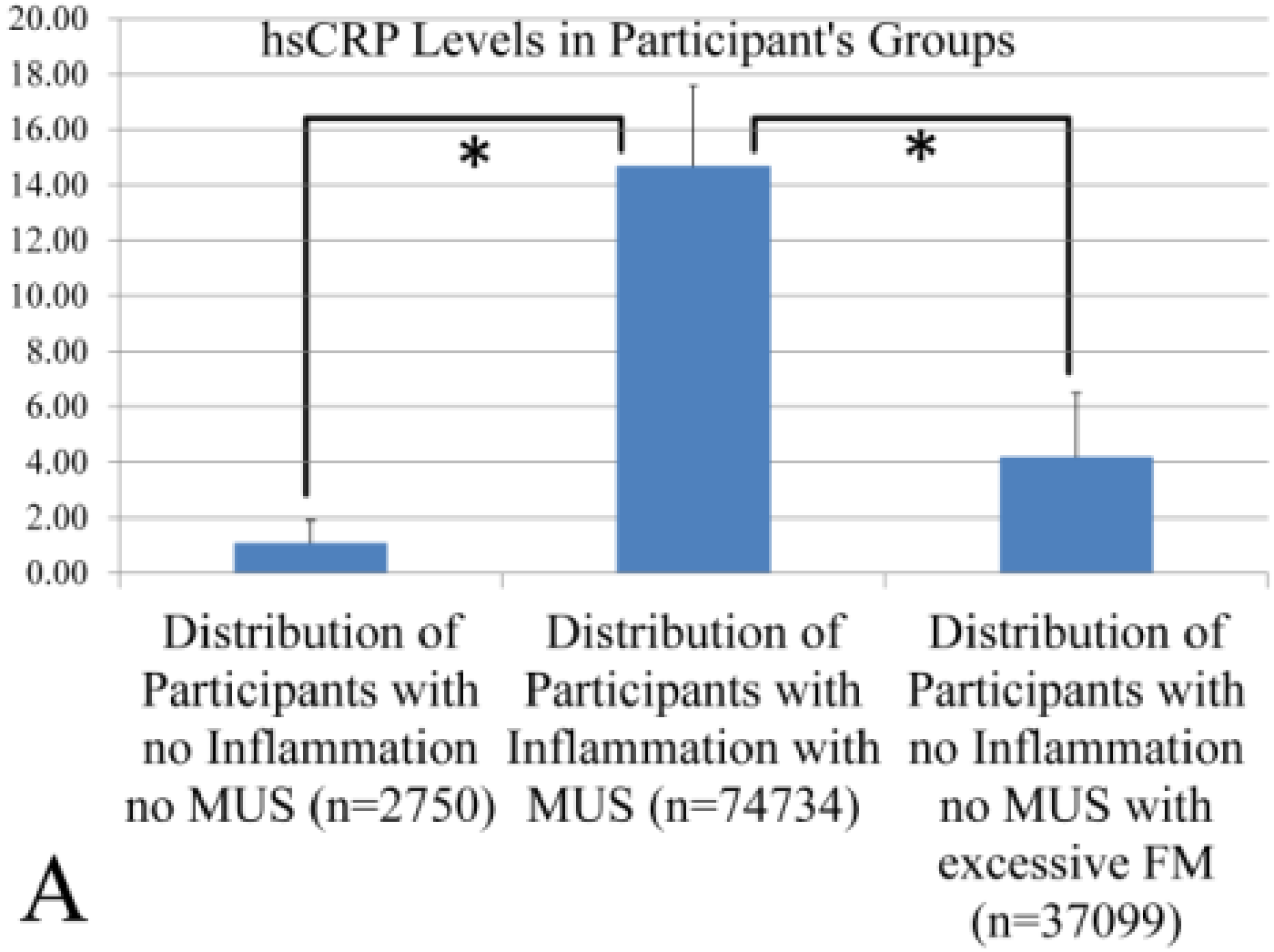
The MUS symptoms examined*:

(i) persistent tiredness or fatigue,
(ii) depressive symptomatology,
(iii) persistent insomnia or night awakenings,
(iv) persistent drowsiness during the day,
(v) anxiety,
(vi) apathy,
(vii) panic attacks,
(viii) changes in heart rate (arrhythmias/tachycardia) at rest,
(ix) changes in appetite (appetite loss or excessive hunger),
(x) night binge eating,
(xi) stomach cramps, bloating or gastro-esophageal reflux disease (GORD),
(xii) presence of irritable bowel syndrome (IBS) symptoms,
(xiii) cold hands and feet,
(xiv) sweating during sleep.

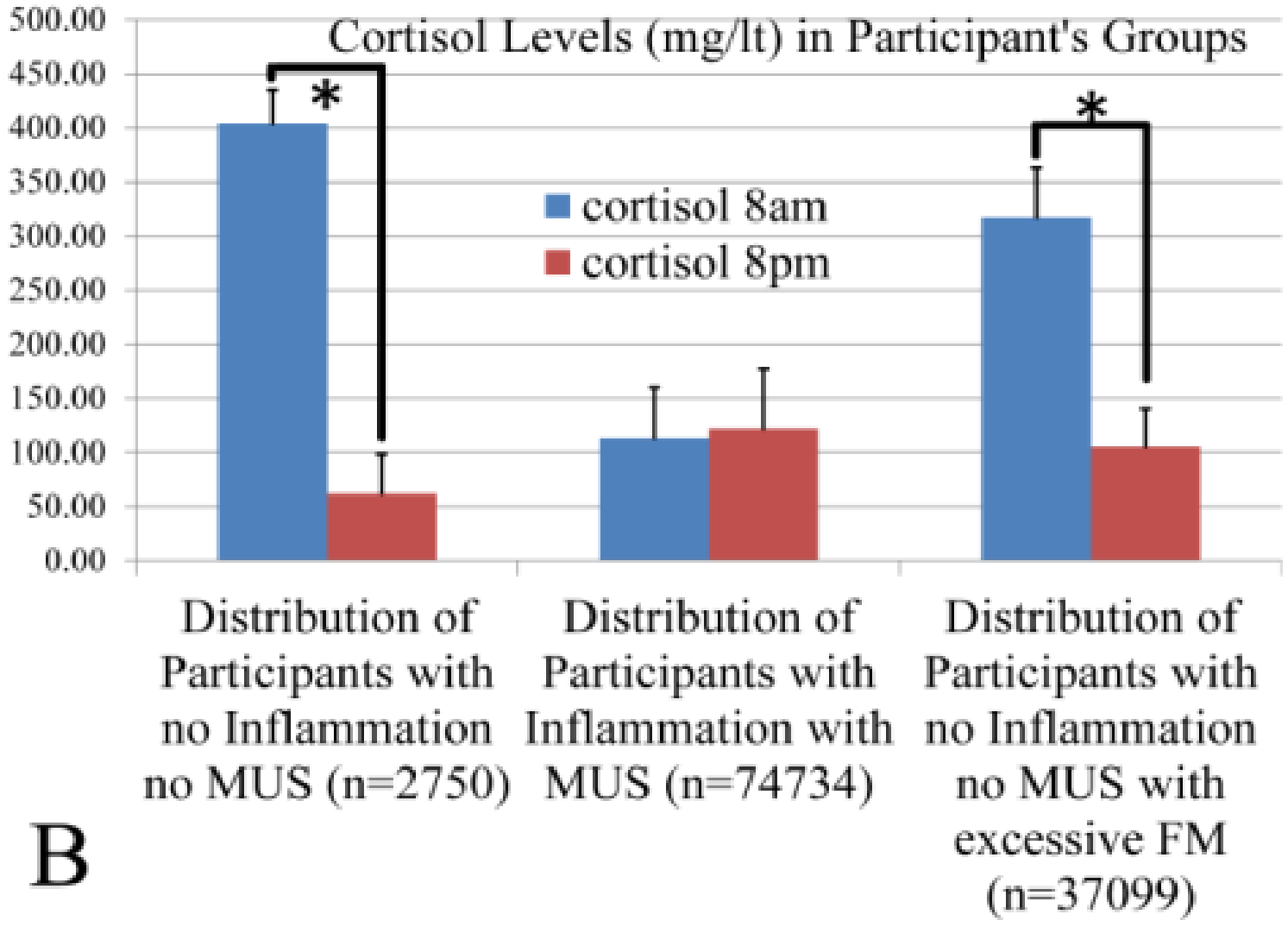
***Presence of MUS was defined as a positive answer to more than 3 of the above 14 questions.**

Evaluation included:

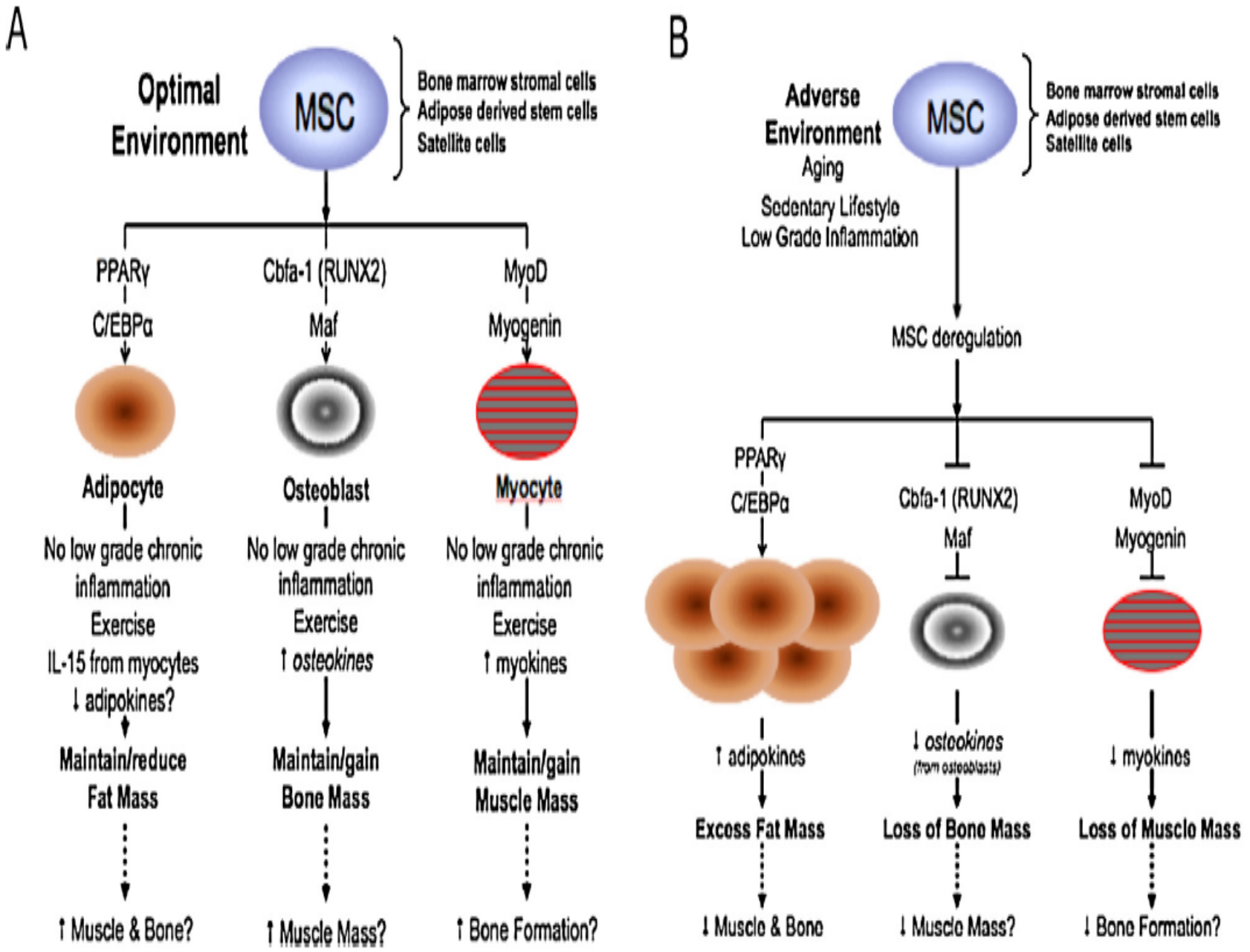
- **Advanced Body Composition Analysis by BIA-ACC *vs.* DEXA**
- **Indices of Inflammation: hsCRP, Interleukin-6**
- **Indices of Stress: am and pm Salivary Cortisol, Delta-Cortisol (am-pm)**


hsCRP Levels in Participant's Groups
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
*
*
Distribution of Participants with no Inflammation no MUS (n=2750)
Distribution of Participants with Inflammation with MUS (n=74734)
Distribution of Participants with no Inflammation no MUS with excessive FM (n=37099)

A


Cortisol Levels (mg/lt) in Participant's Groups
500.00
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
cortisol 8am
cortisol 8pm
*
*
Distribution of Participants with no Inflammation no MUS (n=2750)
Distribution of Participants with Inflammation with MUS (n=74734)
Distribution of Participants with no Inflammation no MUS with excessive FM (n=37099)

B



Interrelationship among muscle, fat, and bone: connecting the dots on cellular, hormonal, and whole body levels. Ageing Res Rev. 2014 May;15:51-60.

Healthy Overweight/Obese Youth: Early Osteosarcopenic Obesity Features.

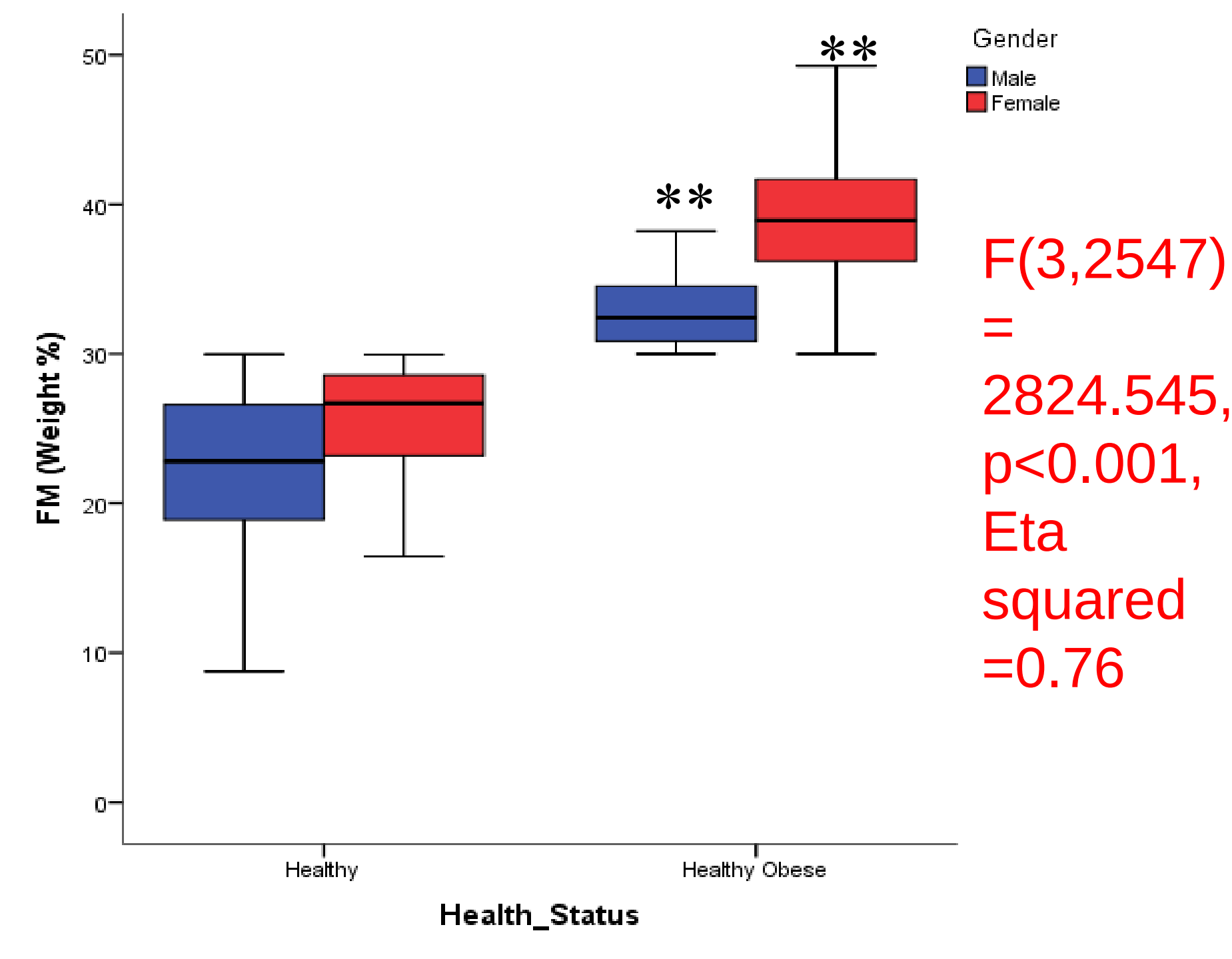
Stefanaki C, Peppa M, Boschiero D, Chrousos GP.

Eur J Clin Invest. 2016 Jul 19. doi: 10.1111/eci.12659. [Epub ahead of print]

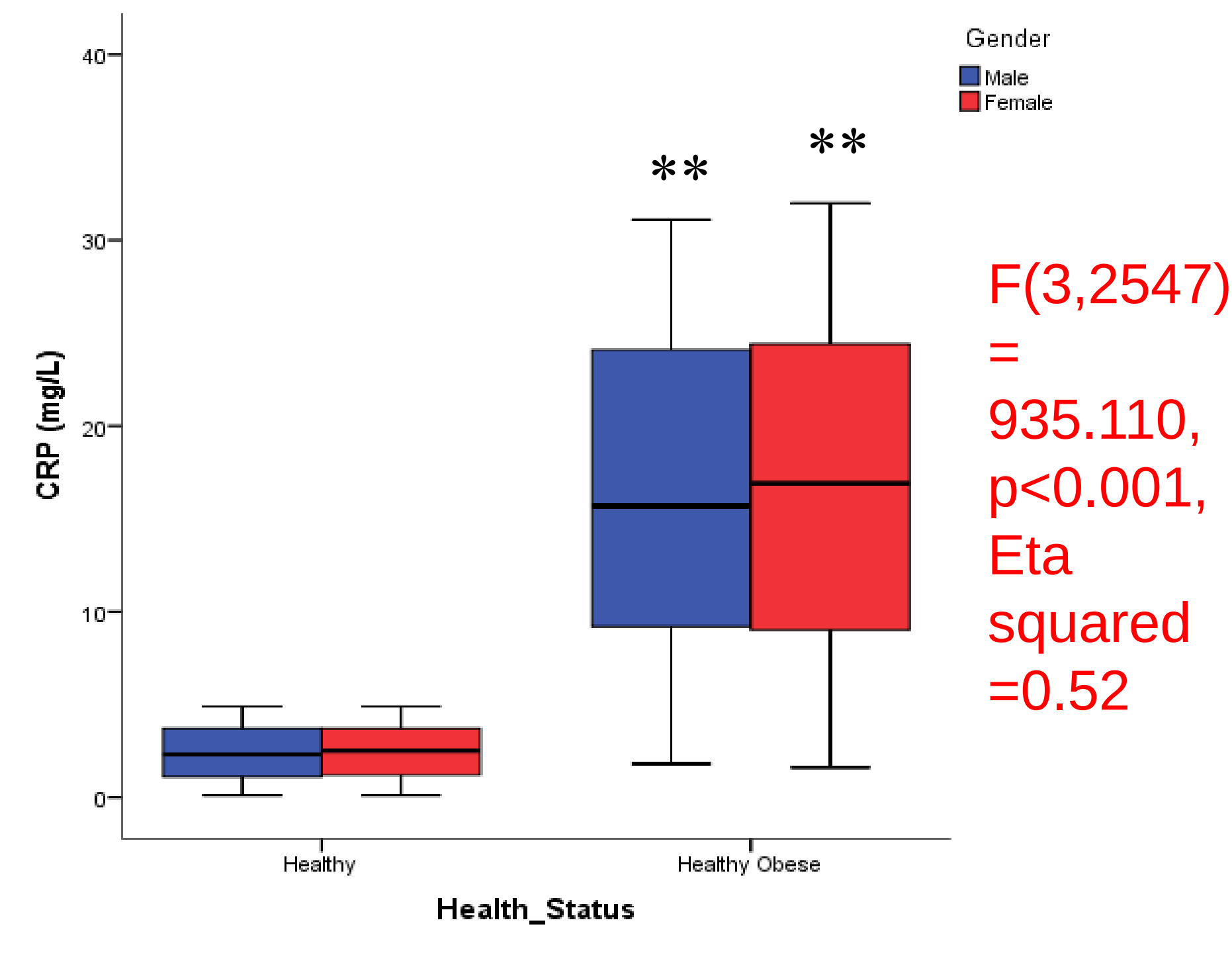
Overall, 2551 subjects (974 males) aged 18–21 years participated in the study.

The healthy lean group included 1072 participants [900 males (84%) and 172 females (16%)].

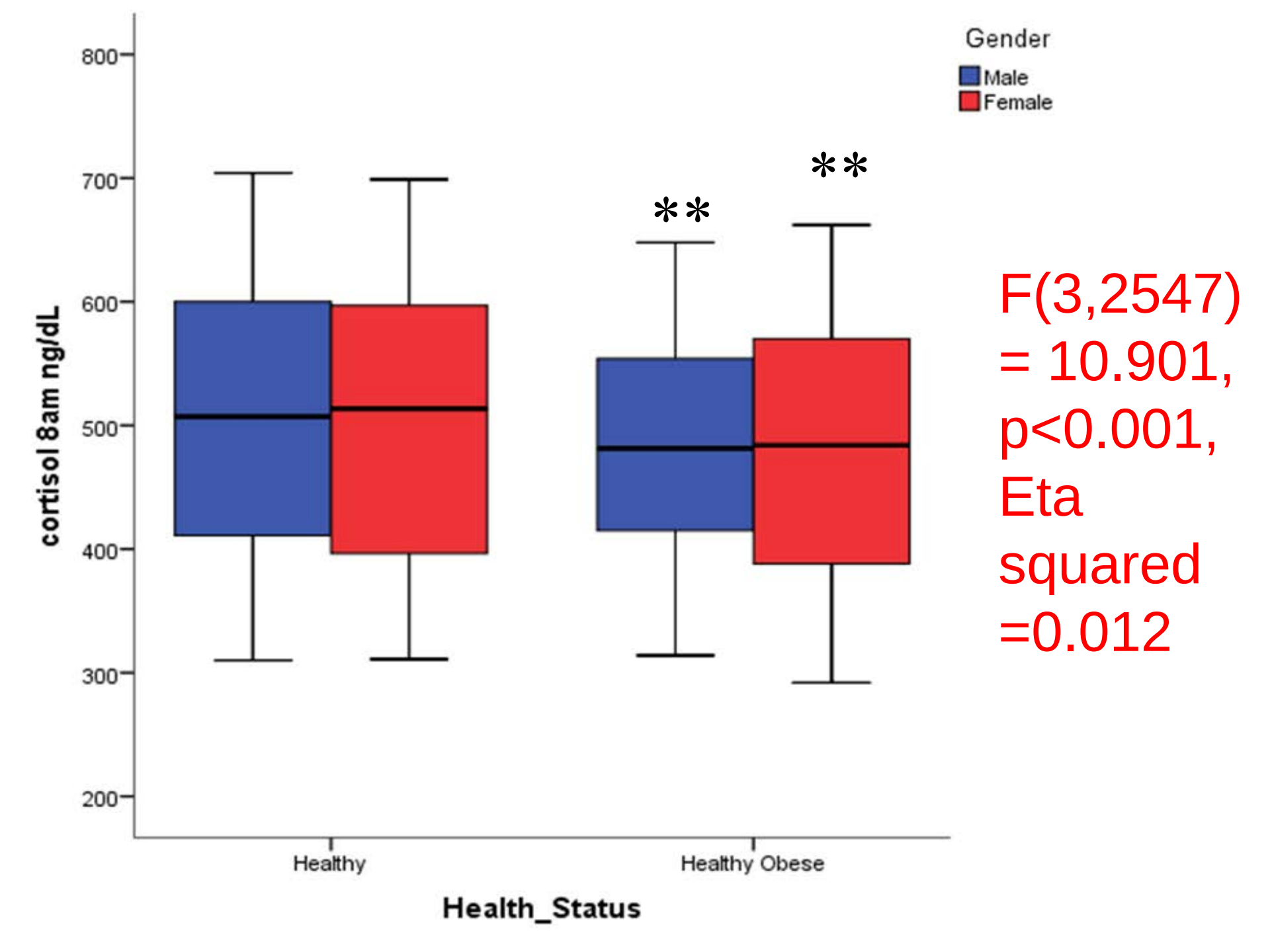
The healthy overweight/obese group included 1479 participants [74 males (5%) and 1405 females (95%)].



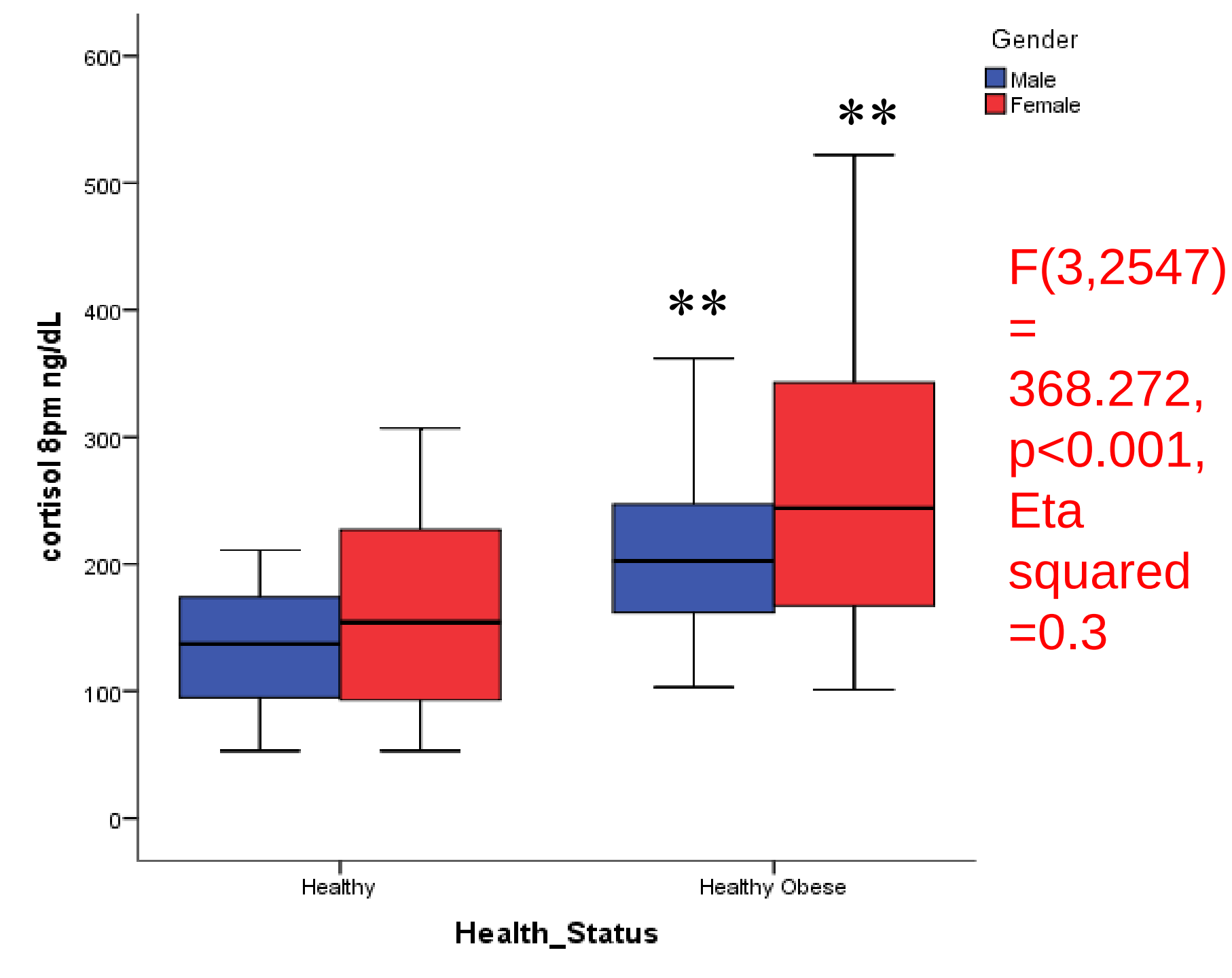
F(3,2547) = 2824.545, p<0.001, Eta squared =0.76



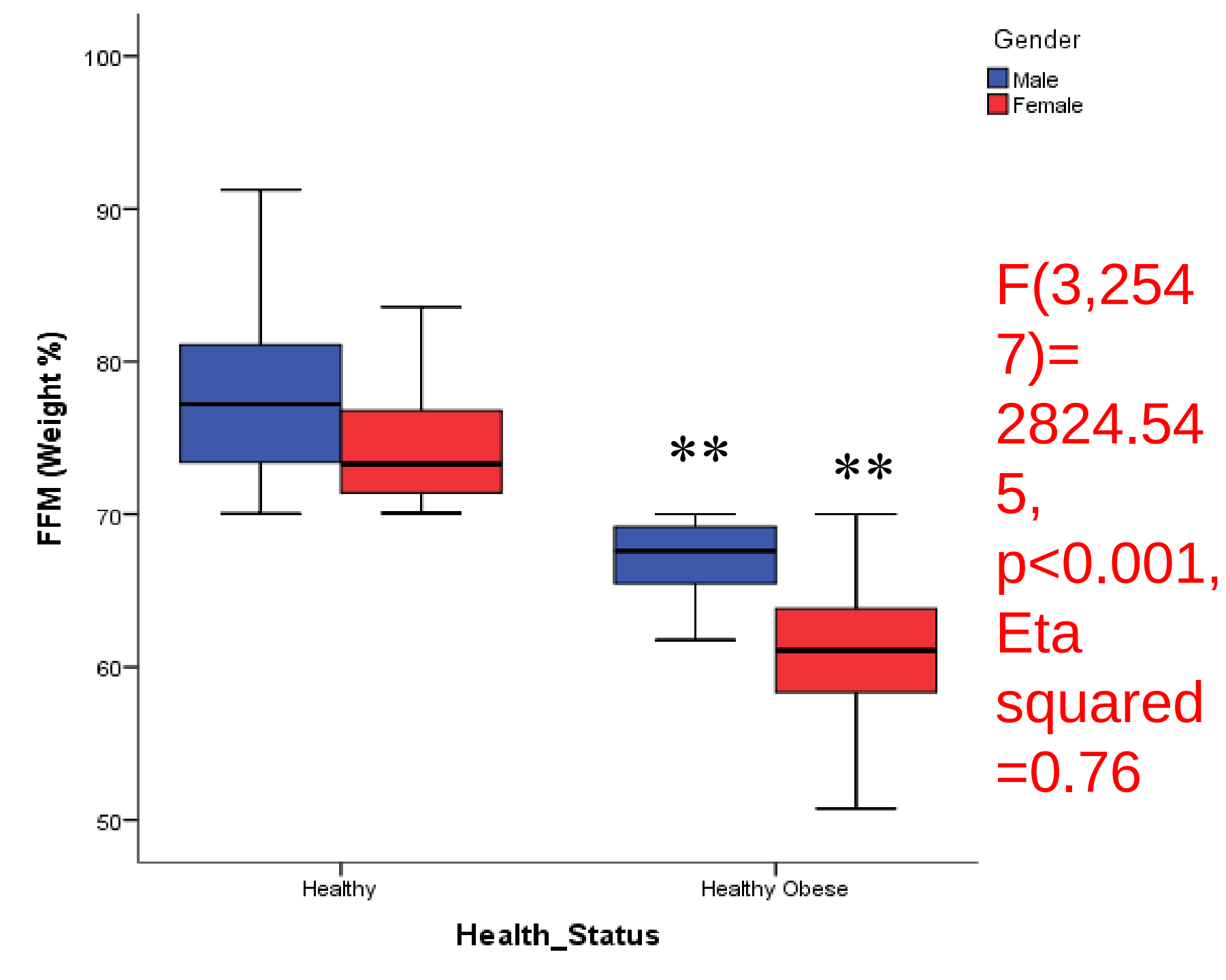
F(3,2547) = 935.110, p<0.001, Eta squared =0.52



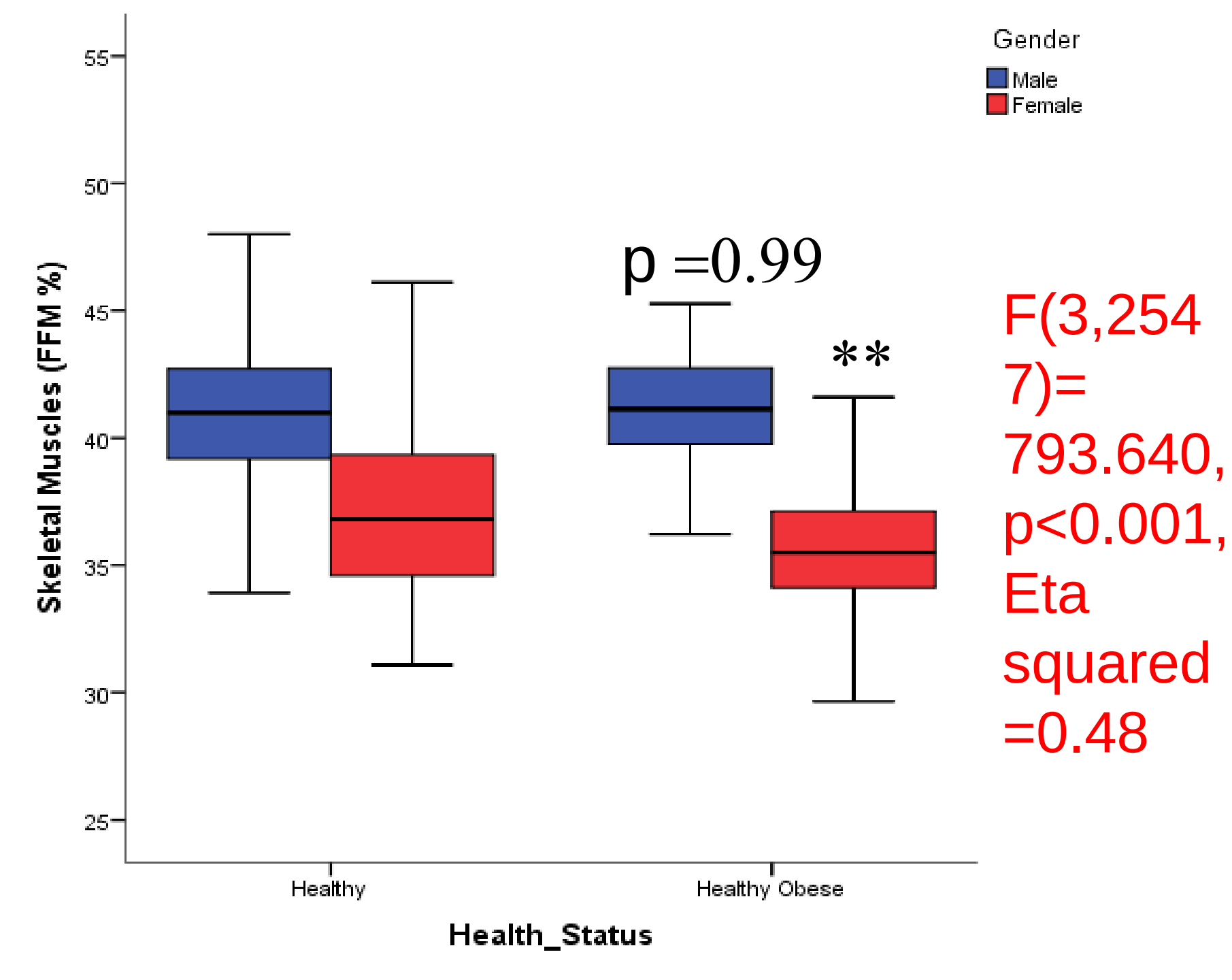
F(3,2547) = 10.901, p<0.001, Eta squared =0.012



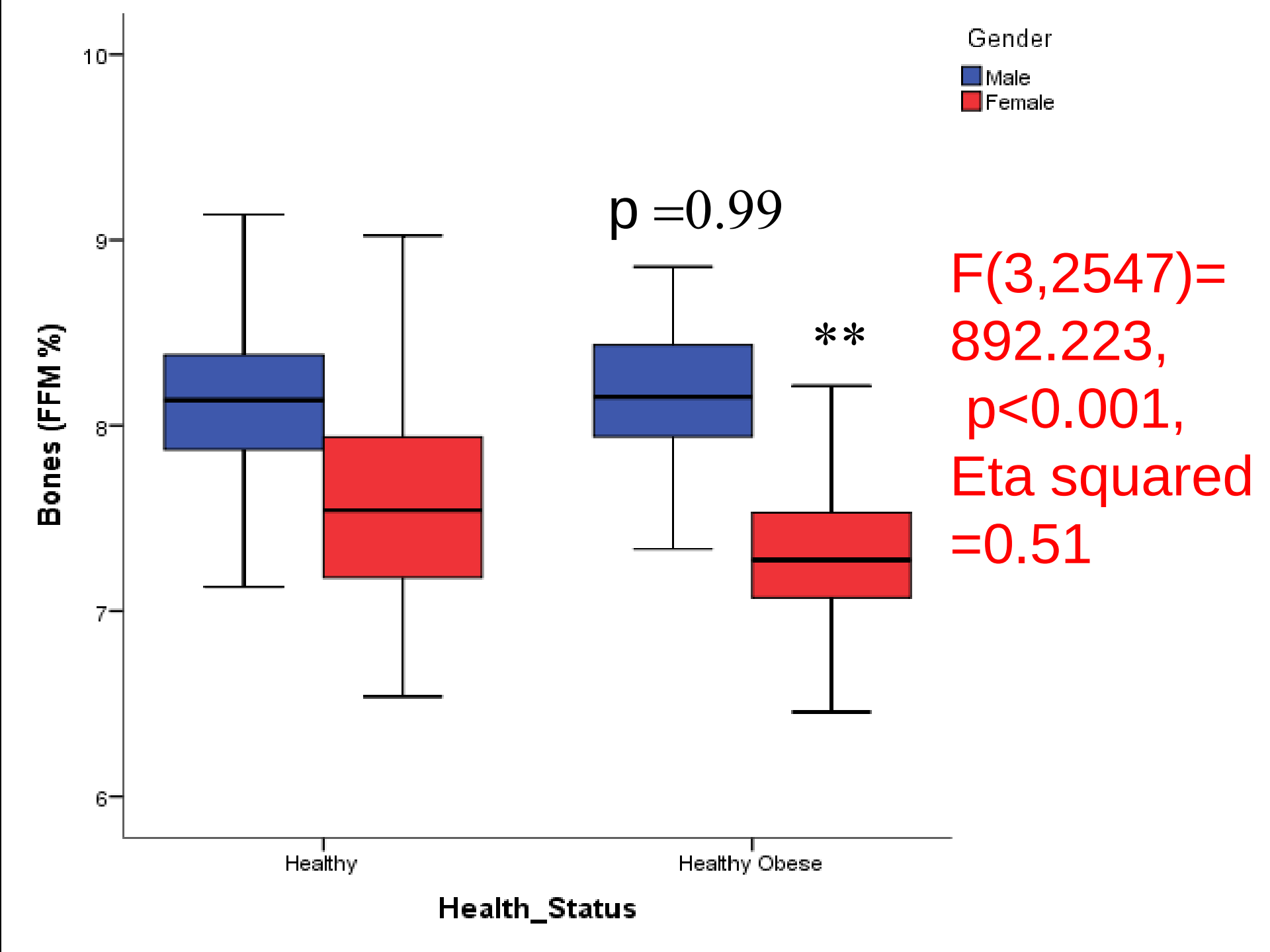
F(3,2547) = 368.272, p<0.001, Eta squared =0.3



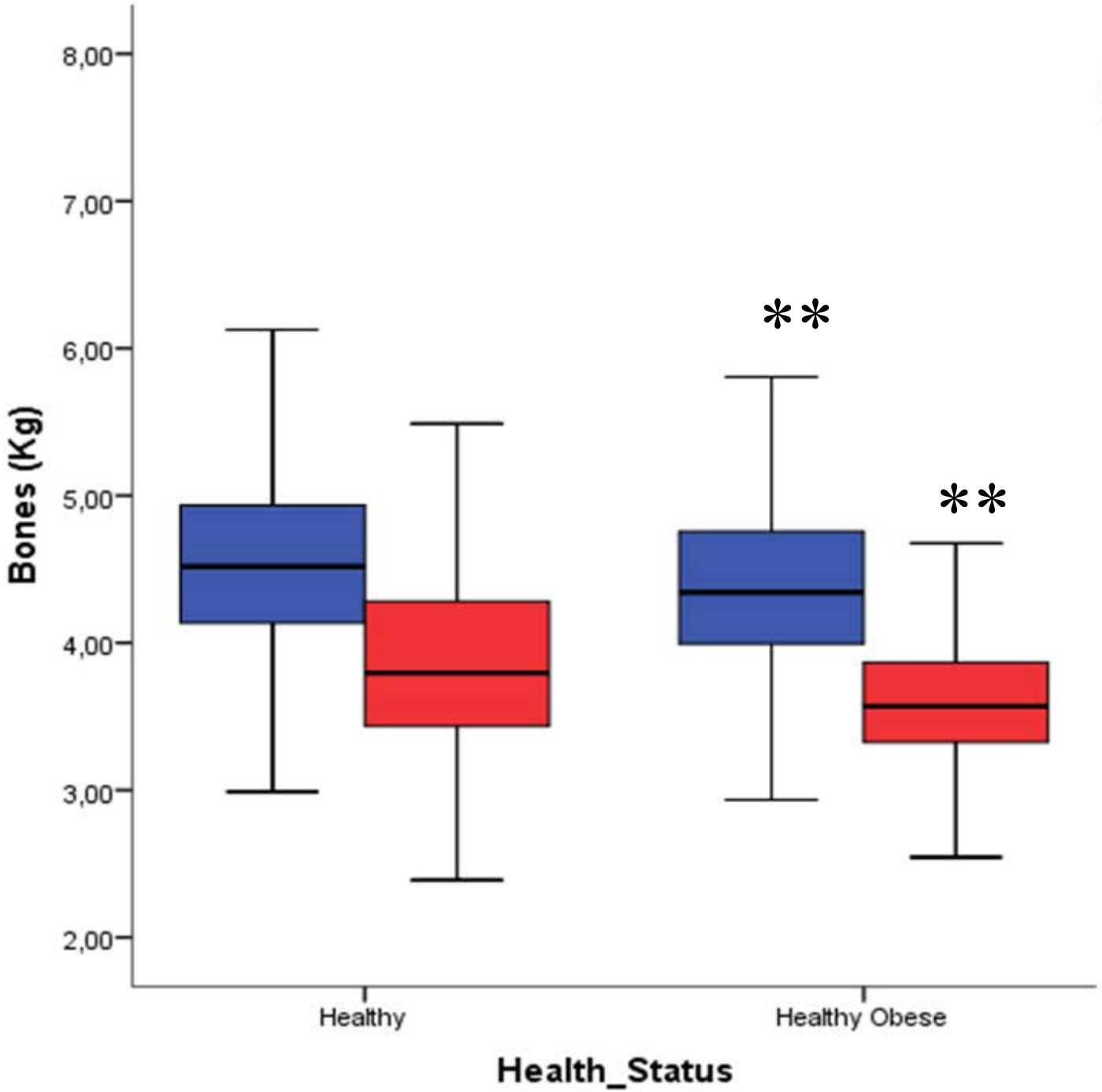
F(3,2547)= 2824.545, p<0.001, Eta squared =0.76



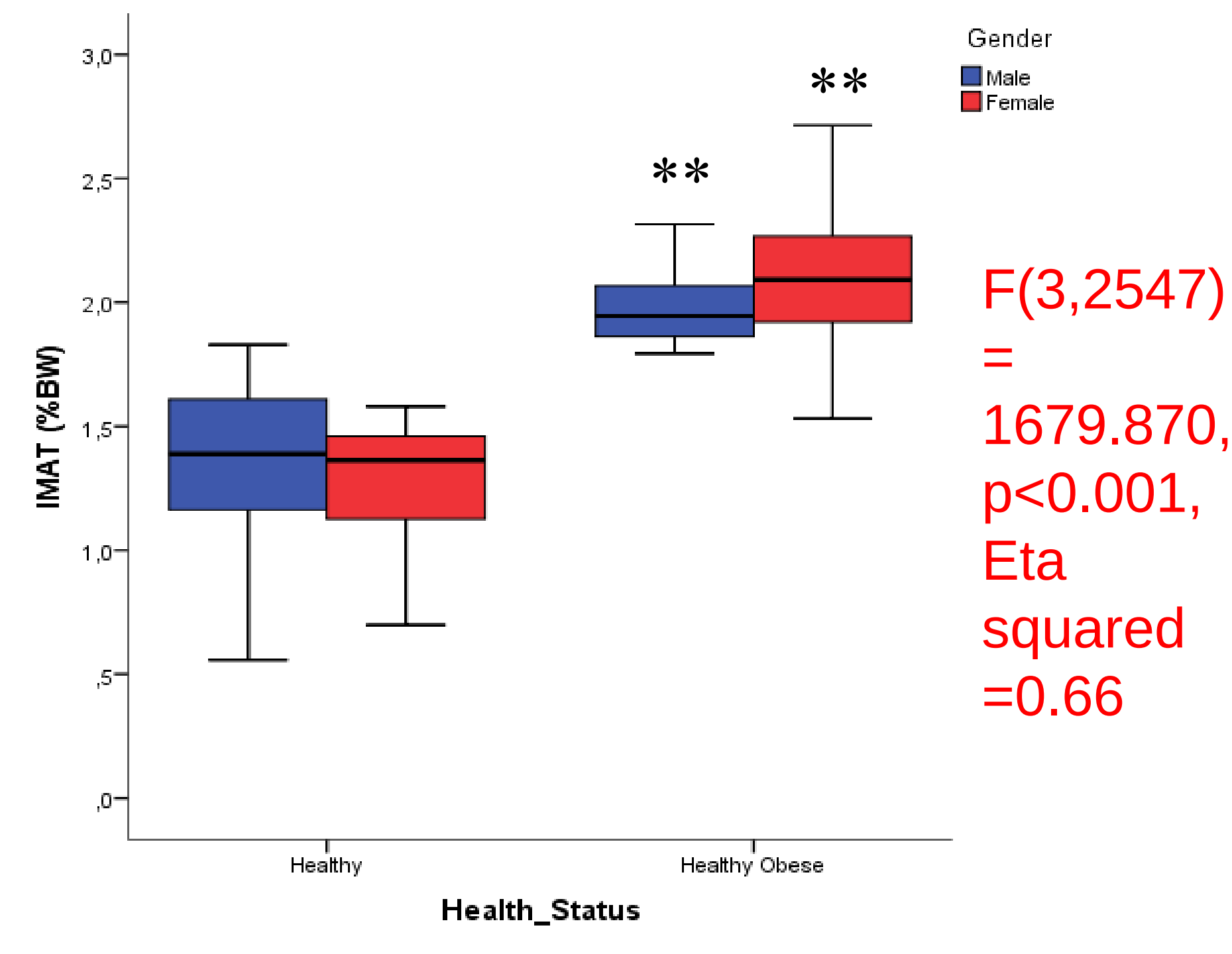
F(3,2547)= 793.640, p<0.001, Eta squared =0.48



F(3,2547)= 892.223, p<0.001, Eta squared =0.51



F(3,2547)=554.679, p<0.001, Eta squared=0.39



F(3,2547) = 1679.870, p<0.001, Eta squared =0.66

CONCLUSIONS

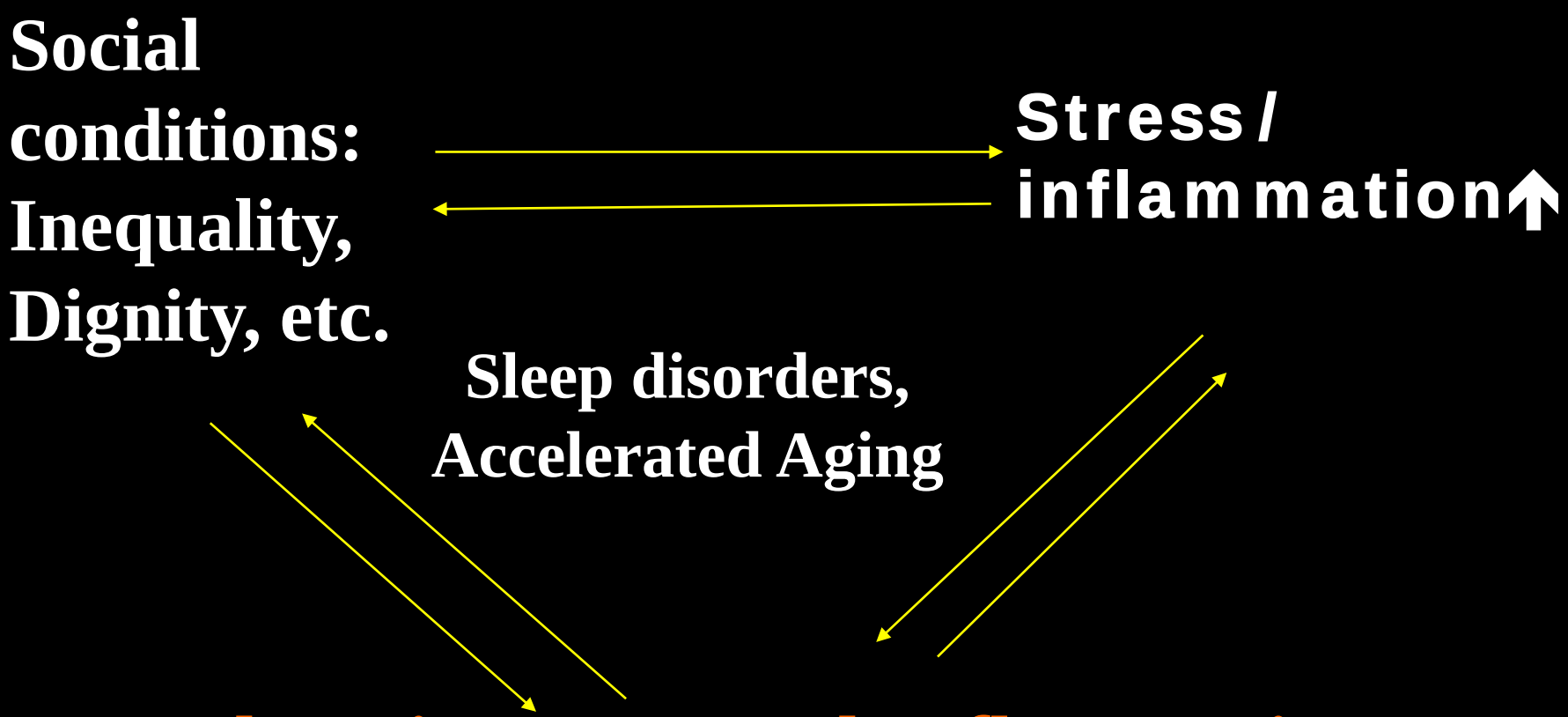
- **Osteosarcopenic phenotype is common and** exists even in the young, suggesting early start of prevention and treatment.

- **"Healthy" lean, overweight or obese populations may demonstrate:**

1. **Decreased bone mass;**
2. **Decreased muscle mass;**
3. **Increased hsCRP concentrations;**
4. **Flattening of cortisol circadian rhythm;**
5. **MUS**

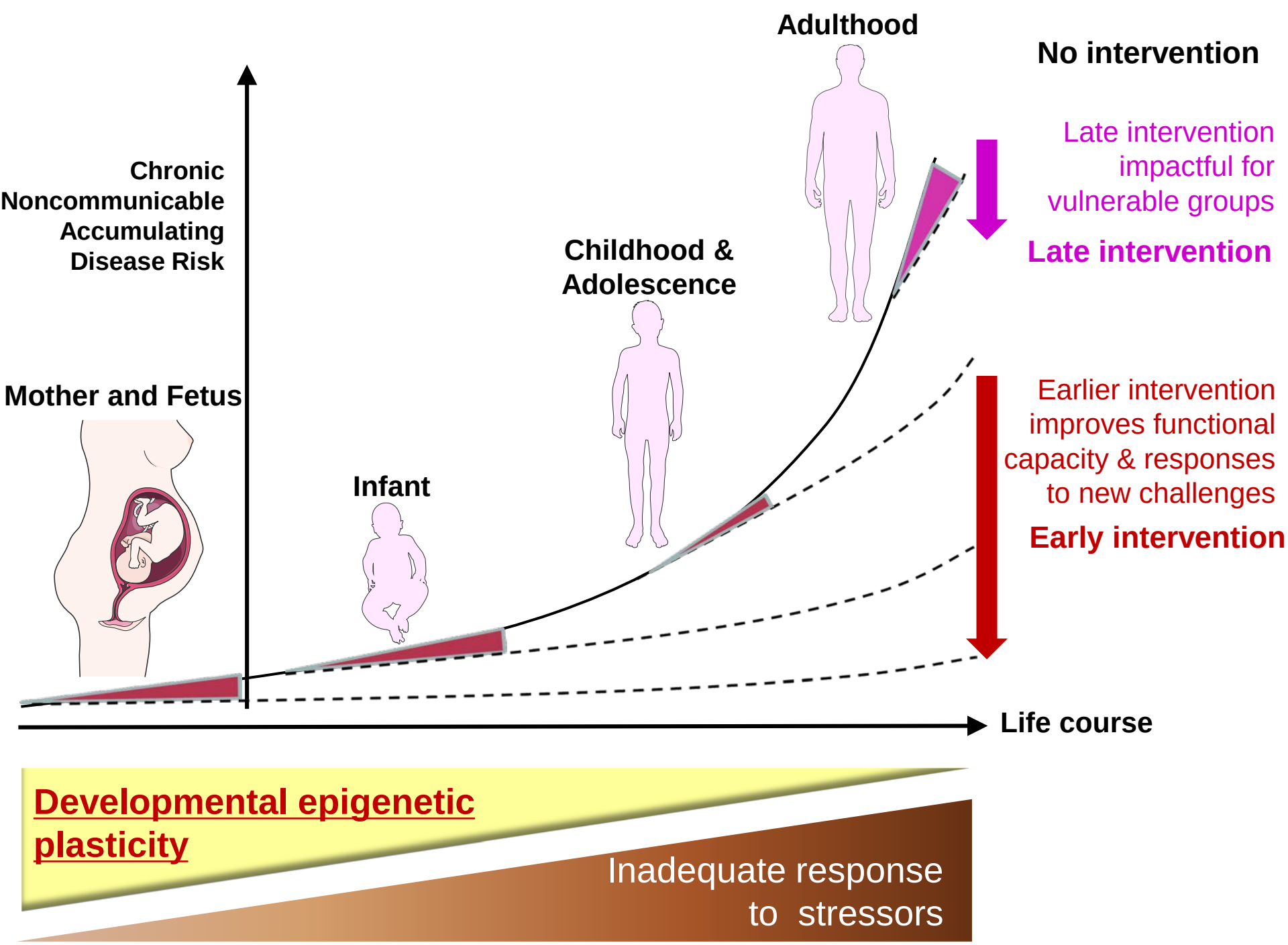
- **BIA-ACC is a highly potent device that may detect osteosarcopenic phenotypes, and may be used for early intervention**.

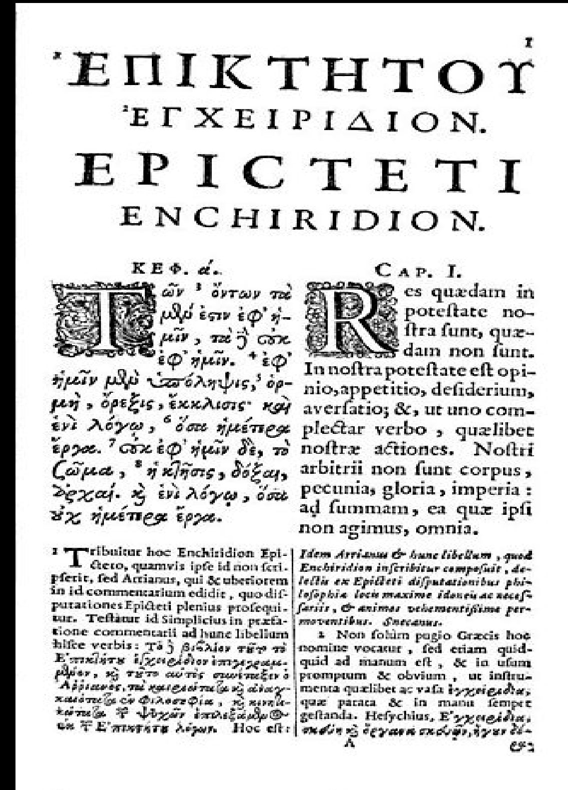
- **Future cohort studies are needed to establish the definite causative factors behind the negative relations between fat, bone & muscle mass.**



"Chronic Stress and Inflammation Syndrome" (CSIS)

Psychologic and somatic manifestations:
Obesity, Osteosarcopenia, MUS, Anxiety, Depressive symptomatology, etc.


Adulthood
No intervention
Chronic
Noncommunicable
Accumulating
Disease Risk
Late intervention
impactful for
vulnerable groups
Late intervention
Childhood &
Adolescence
Mother and Fetus
Infant
Earlier intervention
improves functional
capacity & responses
to new challenges
Early intervention
Life course
Developmental epigenetic
plasticity
Inadequate response
to stressors



ΕΠΙΚΤΗΤΟΥ
ΕΓΧΕΙΡΙΔΙΟΝ.
EPICTETI
ENCHIRIDION.

ΚΕΦ. α.

Τῶν [1] ὄντων τὰ μὲν ἐστιν ἐφ' ἡμῖν, τὰ δὲ οὐκ ἐφ' ἡμῖν. [4] ἐφ' ἡμῖν μὲν ὑπόληψις, [5] ὁρμὴ, ὄρεξις, ἔκκλισις· καὶ ἑνὶ λόγῳ, [6] ὅσα ἡμέτερα ἔργα. [7] οὐκ ἐφ' ἡμῖν δὲ, τὸ σῶμα, [8] ἡ κτῆσις, δόξαι, ἀρχαί. καὶ ἑνὶ λόγῳ, ὅσα οὐχ ἡμέτερα ἔργα.

CAP. I.

Res quædam in poteſtate noſtra ſunt, quædam non ſunt. In noſtra poteſtate eſt opinio, appetitio, deſiderium, averſatio; &, ut uno complectar verbo, quælibet noſtræ actiones. Noſtri arbitrii non ſunt corpus, pecunia, gloria, imperia: ad ſummam, ea quæ ipſi non agimus, omnia.

1 Tribuitur hoc Enchiridion Epicteto, quamvis ipſe id non ſcripſerit, ſed Arrianus, qui & uberiorem in id commentarium edidit, quo diſputationes Epicteti plenius proſequitur. Teſtatur id Simplicius in præfatione commentarii ad hunc libellum hiſce verbis: Τὸ δὲ βιβλίον τοῦτο τὸ Ἐπικτήτου ἐγχειρίδιον ἐπιγεγραμμένον, καὶ τοῦτο αὐτὸς συνέταξεν ὁ Ἀρριανὸς, τὰ καιριώτατα καὶ ἀναγκαιότατα ἐν φιλοσοφίᾳ, καὶ κινητικώτατα τῶν ψυχῶν ἐπιλεξάμενος ἐκ τῶν Ἐπικτήτου λόγων. Hoc eſt: *Idem Arrianus & hunc libellum, quod Enchiridion inſcribitur compoſuit, delectis ex Epicteti diſputationibus philoſophiæ locis maxime idoneis ac neceſſariis, & animos vehementiſſime permoventibus. Snecanus.*

2 Non ſolum pugio Græcis hoc nomine vocatur, ſed etiam quidquid ad manum eſt, & in uſum promptum & obvium, ut inſtrumenta quælibet ac vaſa ἐγχειρίδια, quæ parata & in manu ſemper geſtanda. Heſychius, Ἐγχειρίδια, σκεύη καὶ ὄργανα σκευῶν, ἤγουν δό-

A

ρα:

Νάφε και μέμνασο απιστείν’

‘Be equanimous and remember not to believe easily’