

Stress, Cardiovascular Disease and Longevity

**George P. Chrousos, MD,
National and Kapodistrian University
of Athens,
Athens, Greece
(*No disclosures*)**

SN

SCIENCE NEWS MAGAZINE
SOCIETY FOR SCIENCE & THE PUBLIC

MARCH 7, 2015

Rethinking
Antioxidants
for Athletes

Pacific Plate's
Slippery Slope

Genetics
of Darwin's
Finches

Teeny Tiny
Temps

STRESSED OUT

How chronic stress wreaks havoc



Physical and Emotional Stress

- **Stress Concepts-Complexity**
 - **Stress Mechanisms**
 - **Effects of Stress on Metabolism, cardiovascular disease and longevity**
-

***“Πολλά τα δεινά κ’ουδέν ανθρώπου
δεινότερον πέλλει....”***

***‘There are many wonderful things and
nothing is more wonderful than the
human ...’***



**Σοφοκλής
Sophocles
496-406 BCE**

HUMAN COMPLEXITY: **POST(EPI)GENOMIC ERA**

Human genome:

About 3 billion bases

About 20 thousand protein-coding genes

About 18 thousand ncRNA-coding genes

**About 100-140 thousand transcripts
(mRNA, ncRNA)**

About 200-260 thousand proteins

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

About >25 million snp' s (snv' s), 1.5 million indels

About 20 million microsatellites

>5000 cnv' s (many million bases)

Over 10 k disease-related mutations

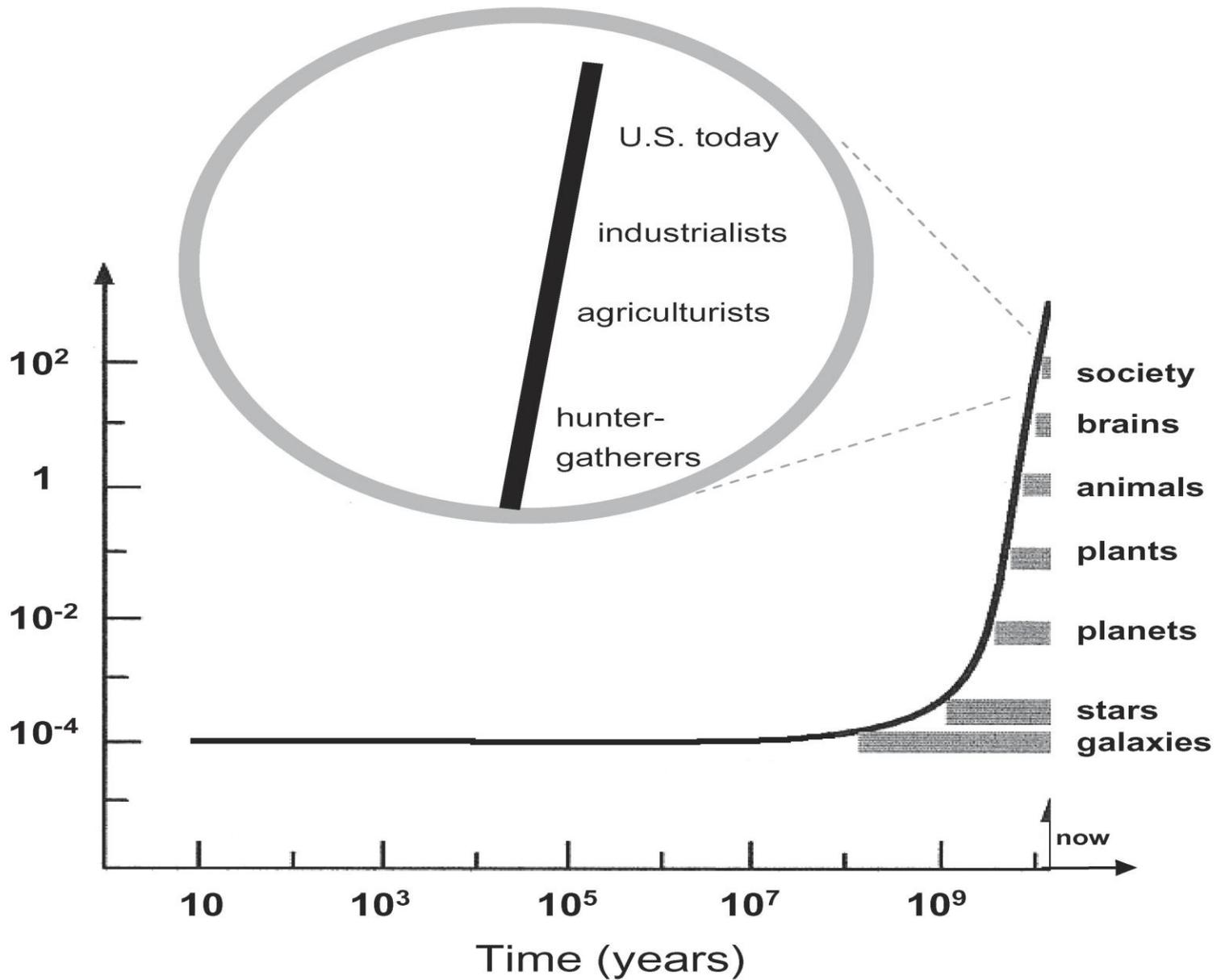
60% of promoters have CpG islands

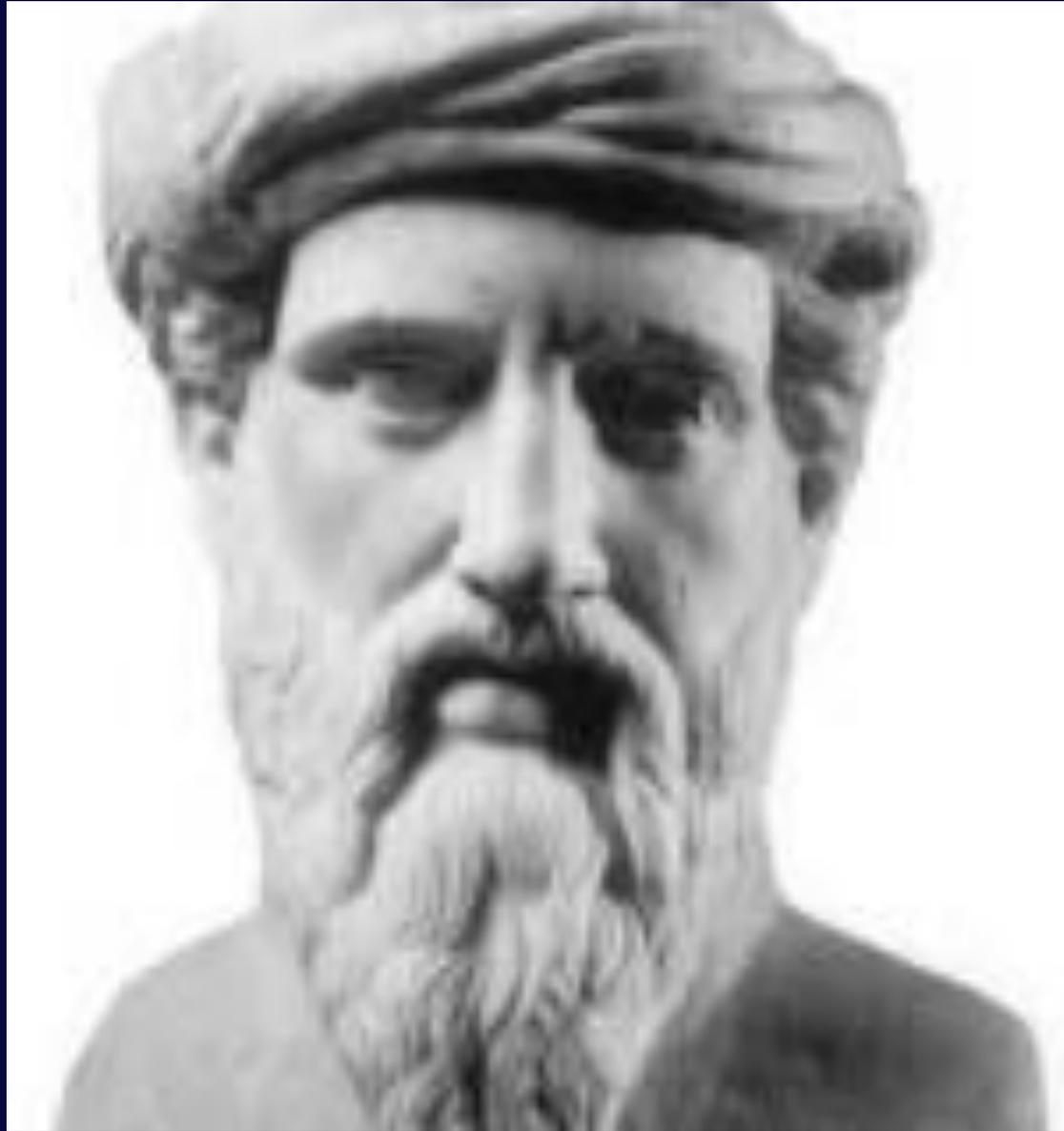
EPIGENETICS-EPIMUTATIONS

HUMAN COMPLEXITY: **SOME HUMAN BRAIN NUMBERS**

- ~ 100 billion neurons (100×10^{12}) x >10.000 synapses per neuron = $>10^{18}$ synapses)
 - ~ 100.000 km of fibers
 - ~ 1 trillion or more glial cells
 - ~ 1.25 terabytes
 - ~ 15 Watt lamp (2% of BW uses 20% energy)
-

Power density
(W/kg)

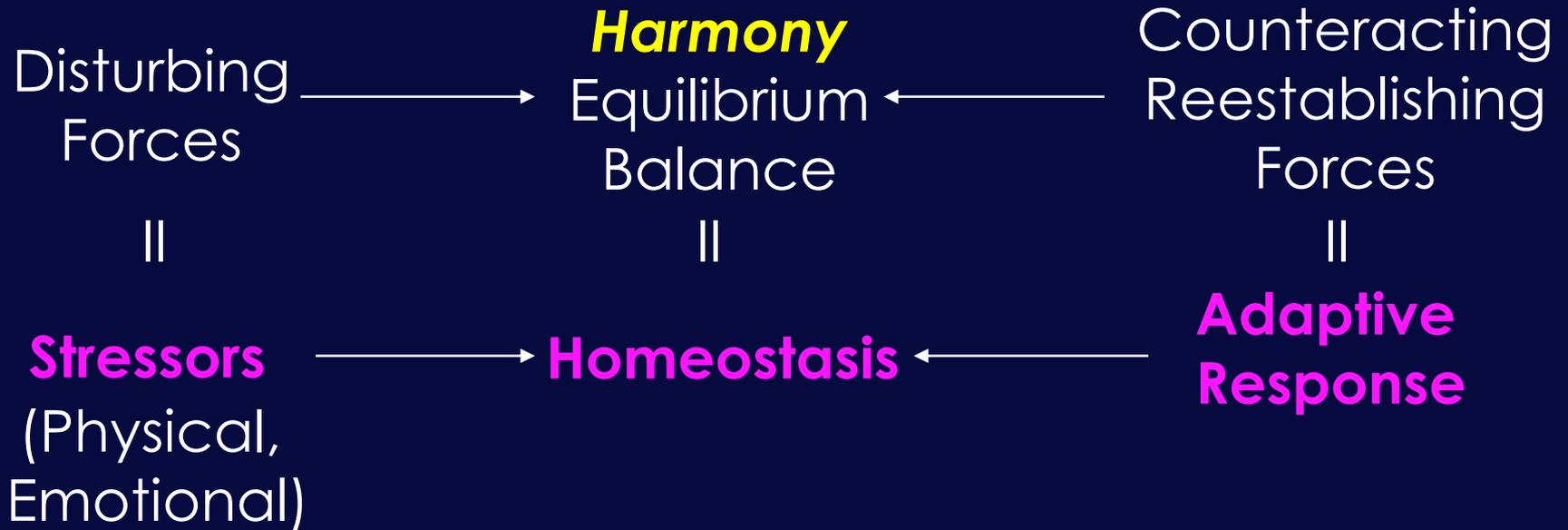




G.P. Chrousos

Pythagoras 6th century BCE

Complex Systems Theory

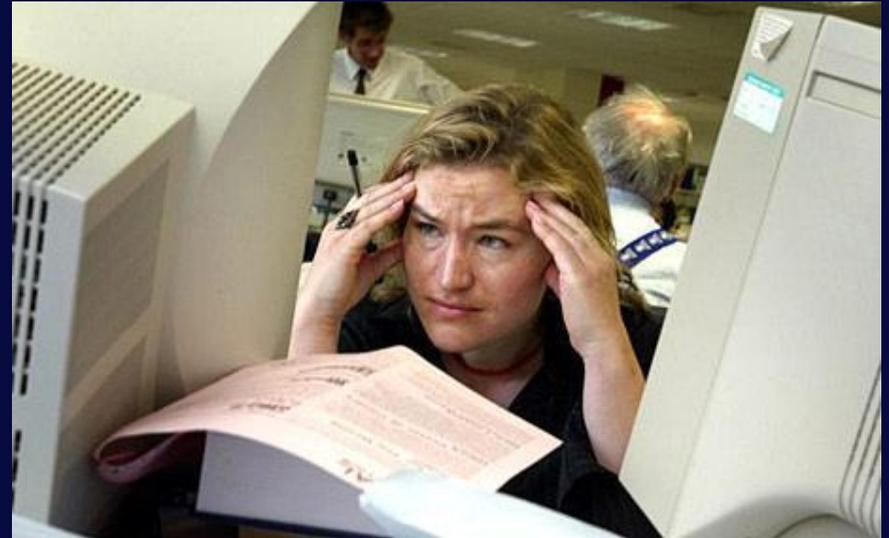


Pythagoras= Harmony

Alcmaeon= Iso-nomia

Walter Cannon= Homeostasis

**Stress is the State of
Threatened (or Perceived
as Threatened)
Homeostasis**



STRESS CONCEPTS

Pythagoras (580-489 BC)	The Harmony of the Cosmos
Alkmaion (c. 500 BC)	The intellect is based in the brain
Empedocles (500-430 BC)	Health is the equipose of opposing forces: « Isonomia » Matter consists of essential elements and qualities in opposition or alliance to one another
Hippocrates (460-375 BC)	A harmonious balance of the elements and qualities of life is health-Dysharmony is disease « Nouson physeis iatroi =Vis medicatrix naturae » “ Eudaimonia ”
Aristotle	
Stoics/Sceptics	Ataraxia (imperturbability of mind, equanimity)
Epicurus (341-270 BC)	Ataraxia (imperturbability of mind), Aponia (no pain) and “ Hedone ” (tranquil, non sensual pleasure) as desirable states « Eustachius » = Good balance, Carpe diem= seize the day
Thomas Sydenham (AD 1624-1689)	Symptoms and signs of a disease arise also from the reaction of the patients system
Claude Bernard (1813-1878)	The “ milieu interieur ”
Walter Cannon (1871-1945)	Homeostasis/Stress Bodily responses to emotions Fight or flight and freeze reaction
Hans Selye (1907-1982)	The general adaptation syndrome (the stress syndrome) Diseases of adaptation, Distress vs. Eustress

Human Stressors

Daily hassles

Work stress (Effort Reward Imbalance,ERI)

Life transitions

Natural and unnatural catastrophies

Starvation, Excessive nutrition, Deficient exercise, Excessive exercise, Obesity

Socioeconomic status, Minority status (*Dignity*)

Job loss, Downsizing, Loss of control

Bereavement

Caretaking/ Pathologic empathy/*Unprincipled compassion*

Addictions/ Toxic substances

Inflammations (Traumatic, Infectious, Autoimmune, Allergic)

Anxiety, Depression, Personality disorder

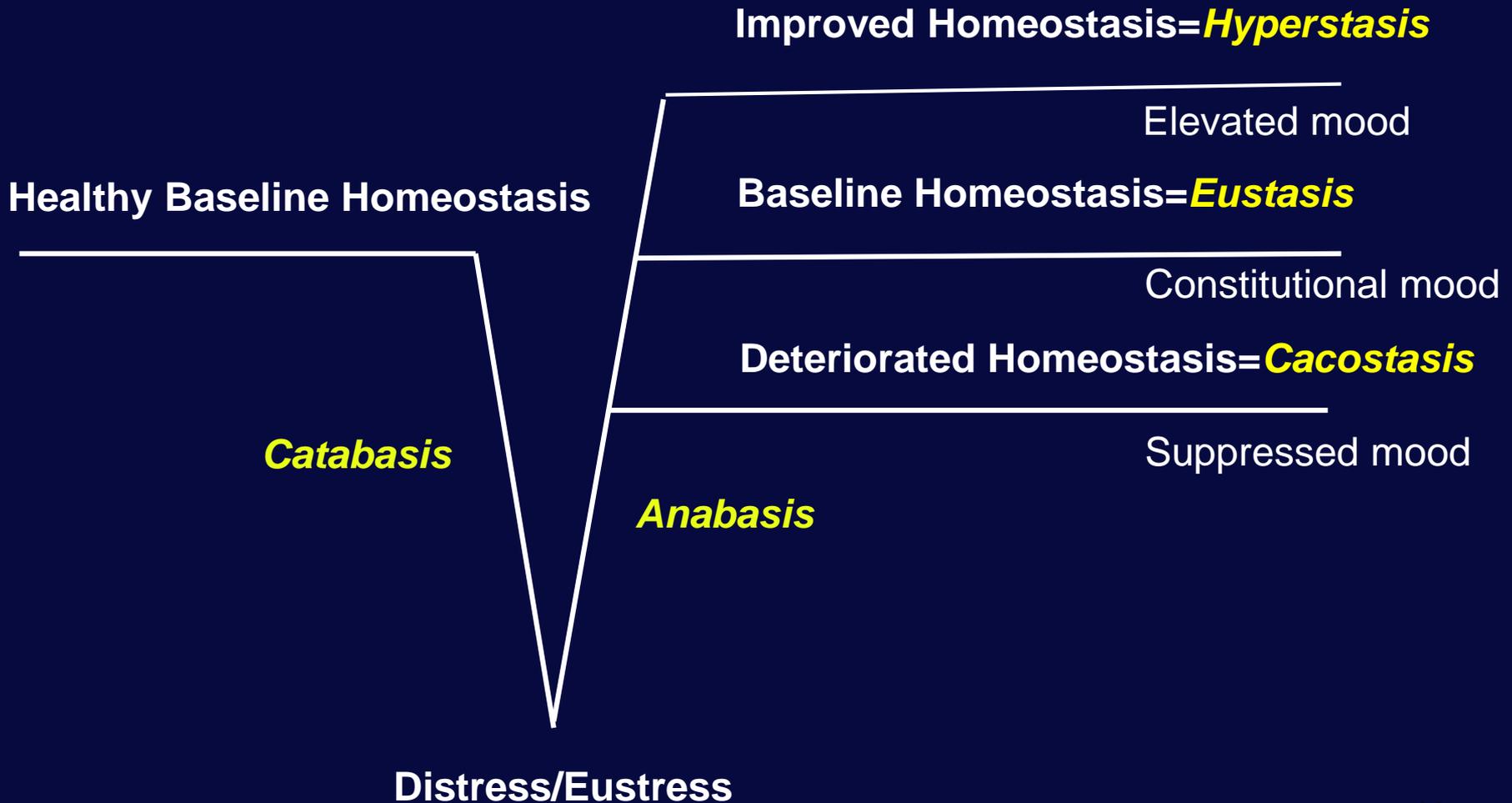
Sleep deficiency, Sleep excess

Uncoupling of the Clock: Jet-lag, “Social-jetlag”

Behavioral addictions: “Meta-modern” or “Cyborg stress”



Homeostasis over Time



“CRITICAL” PERIODS OF LIFE

Prenatal, Early Childhood, Puberty
(Human brain ontogeny complete at 25-27 y)



Organizational Effects of Hormones

(CRH, glucocorticoids, sex steroids, cytokines)

HOMEOSTATIC SYSTEMS

Pre-/Frontal Lobe/Logos

- Amygdala, fear/anger
- MCLS, reward/punishment
- **Stress - CRH/LC-NE**
- Cardiorespiratory
- **Metabolic**
- **Immune/Inflammatory**
- Fatigue - Pain
- Wakefulness/Sleep
- Clock

Physical and Emotional Stress

- **Stress Concepts-Complexity**
 - **Stress Mechanisms**
 - **Effects of Stress on Metabolism, Cardiovascular Disease and Longevity**
-

What Mediates the Adaptive Response?

The Stress System

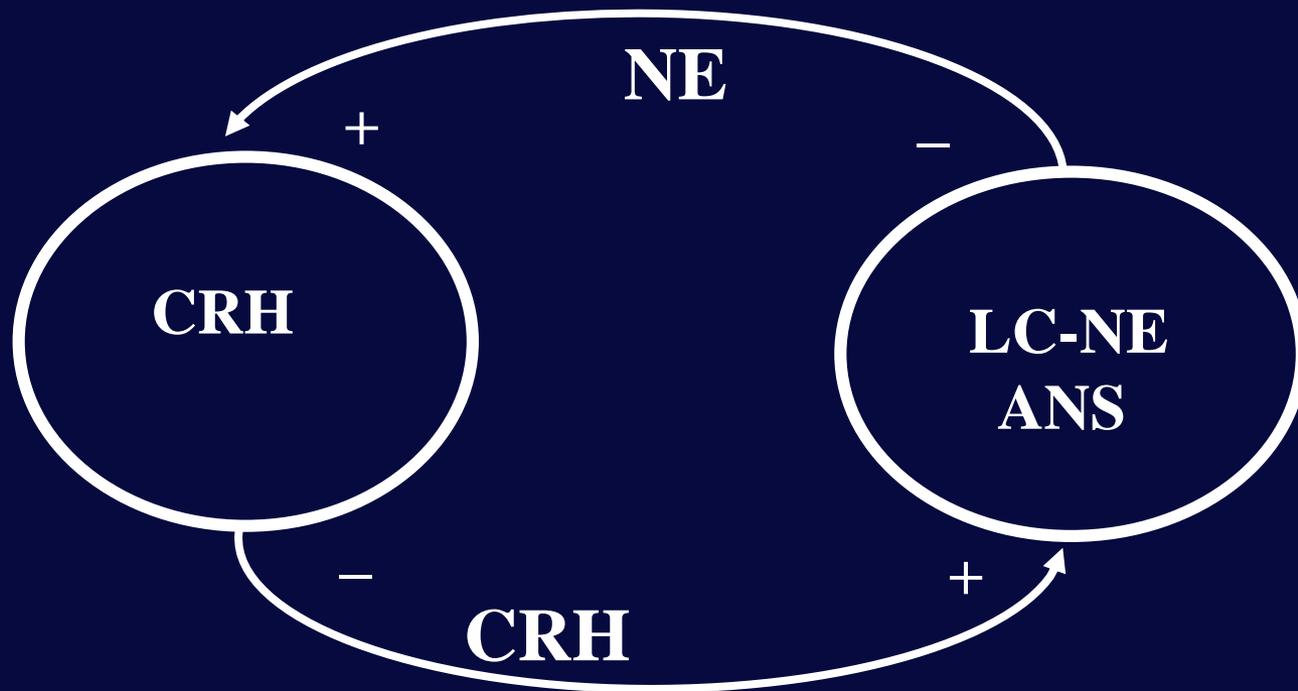
a. CNS

1. CRH system
2. *Locus caeruleus* (LC)-norepinephrine (NE)/autonomic (sympathetic) systems

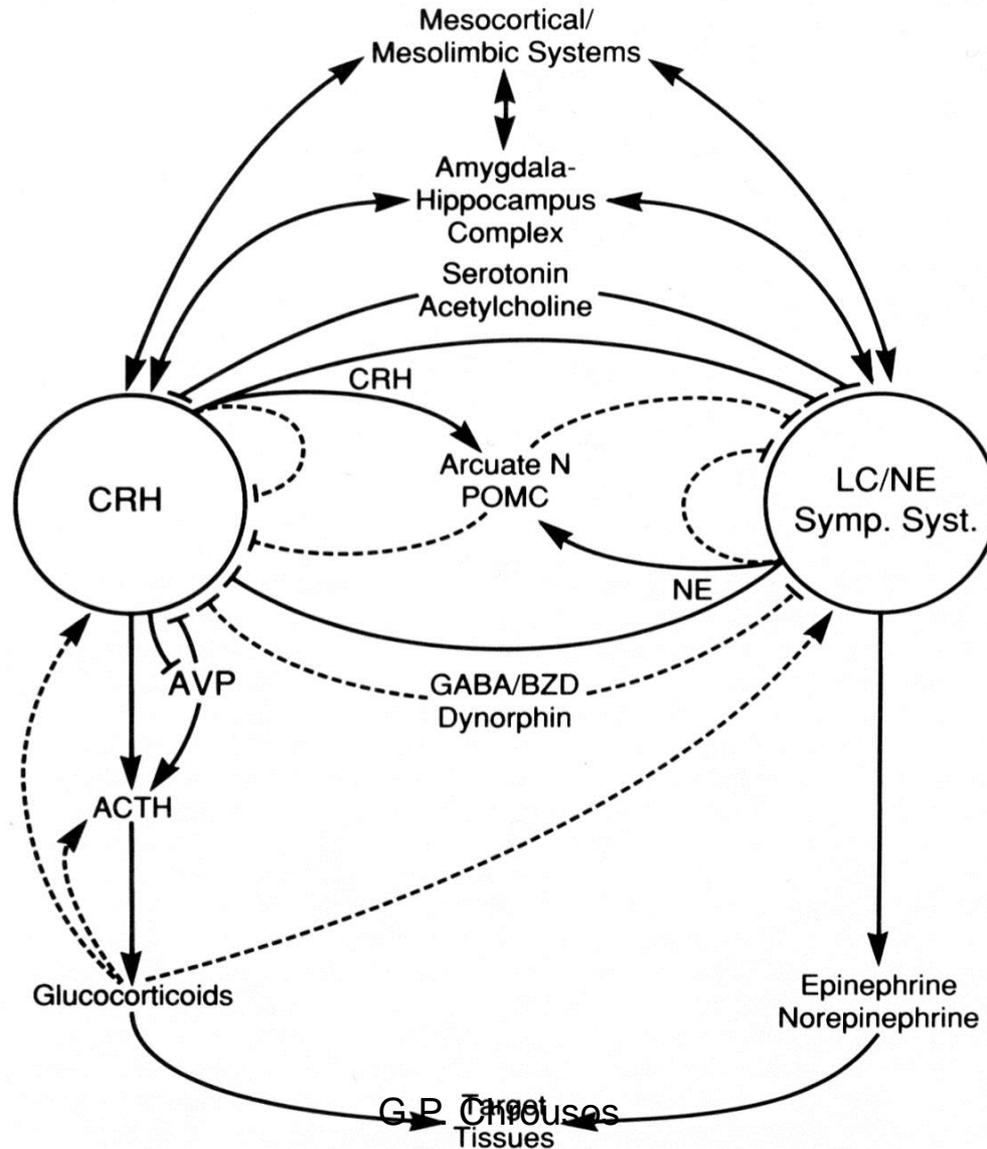
b. Periphery

1. HPA axis
 2. Autonomic (sympathetic) systems
-

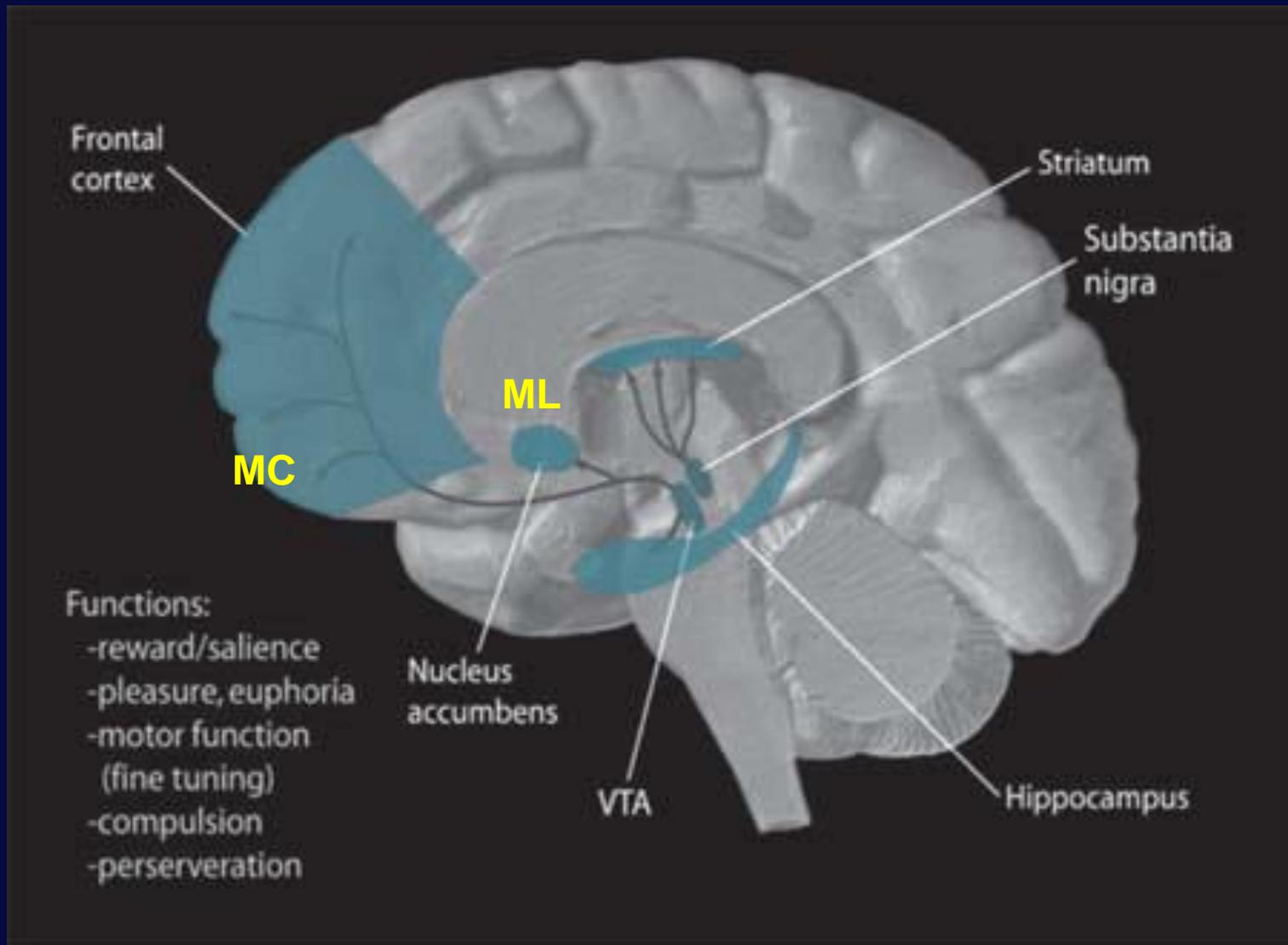
Stress System



Stress System



The Reward System



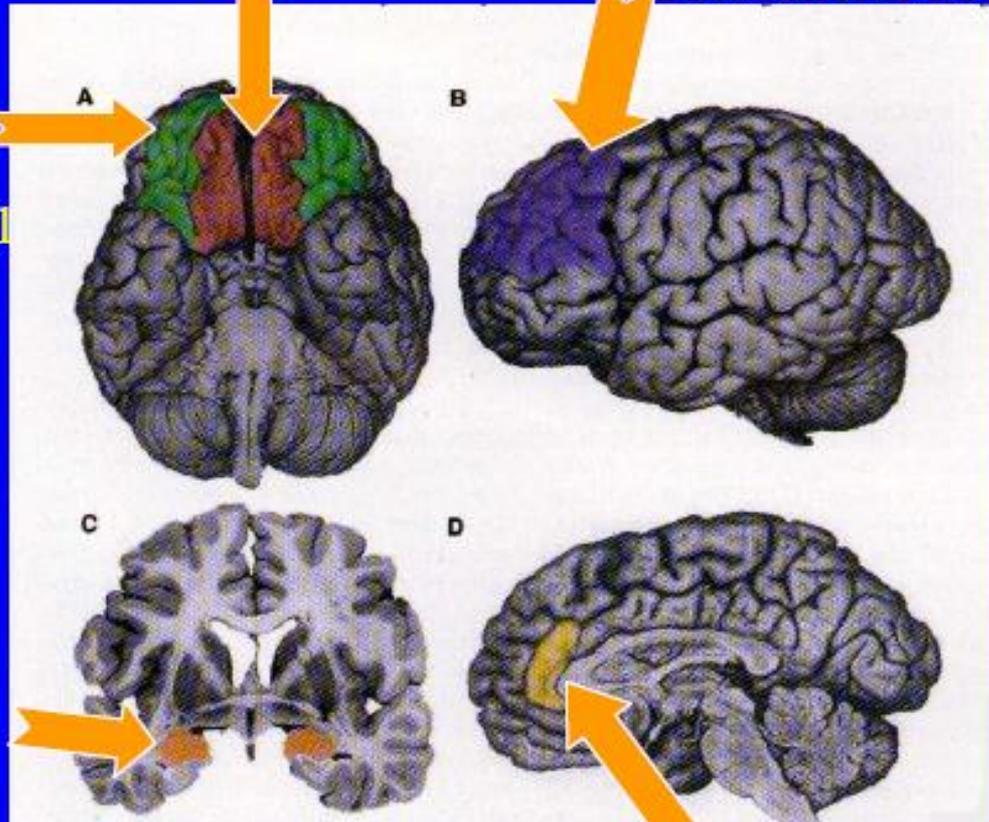
Structures related to emotional regulation

Ventral Prefrontal Cortex

Dorsolateral Cortex

Orbital Prefrontal Cortex

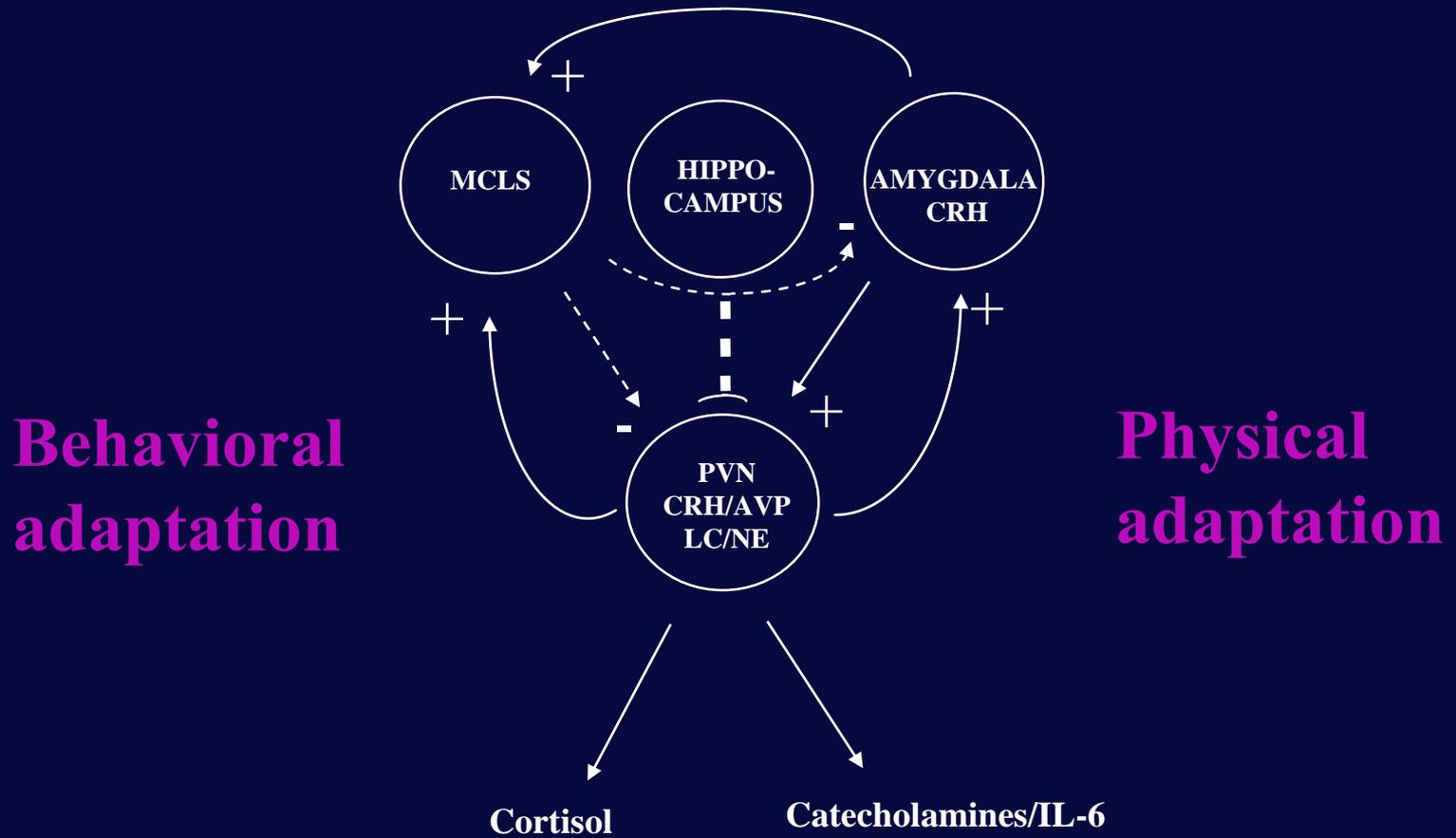
Amygdala



Science Vol 289, p 592

Anterior Cingulate
OhioCanDo4Kids.org - 2006

Acute Stress



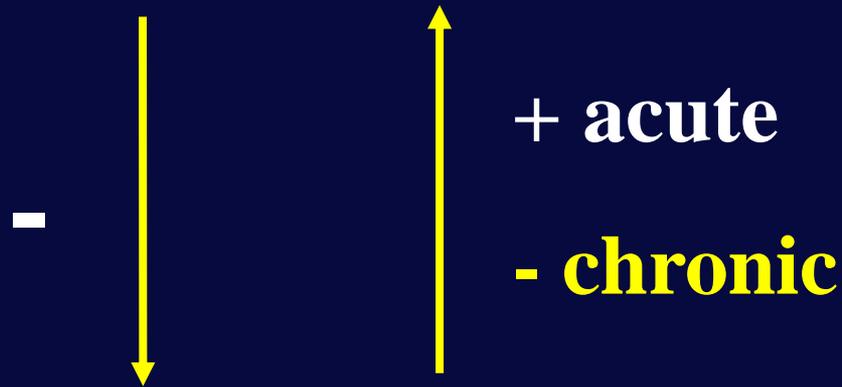
**Behavioral
adaptation**

**Physical
adaptation**

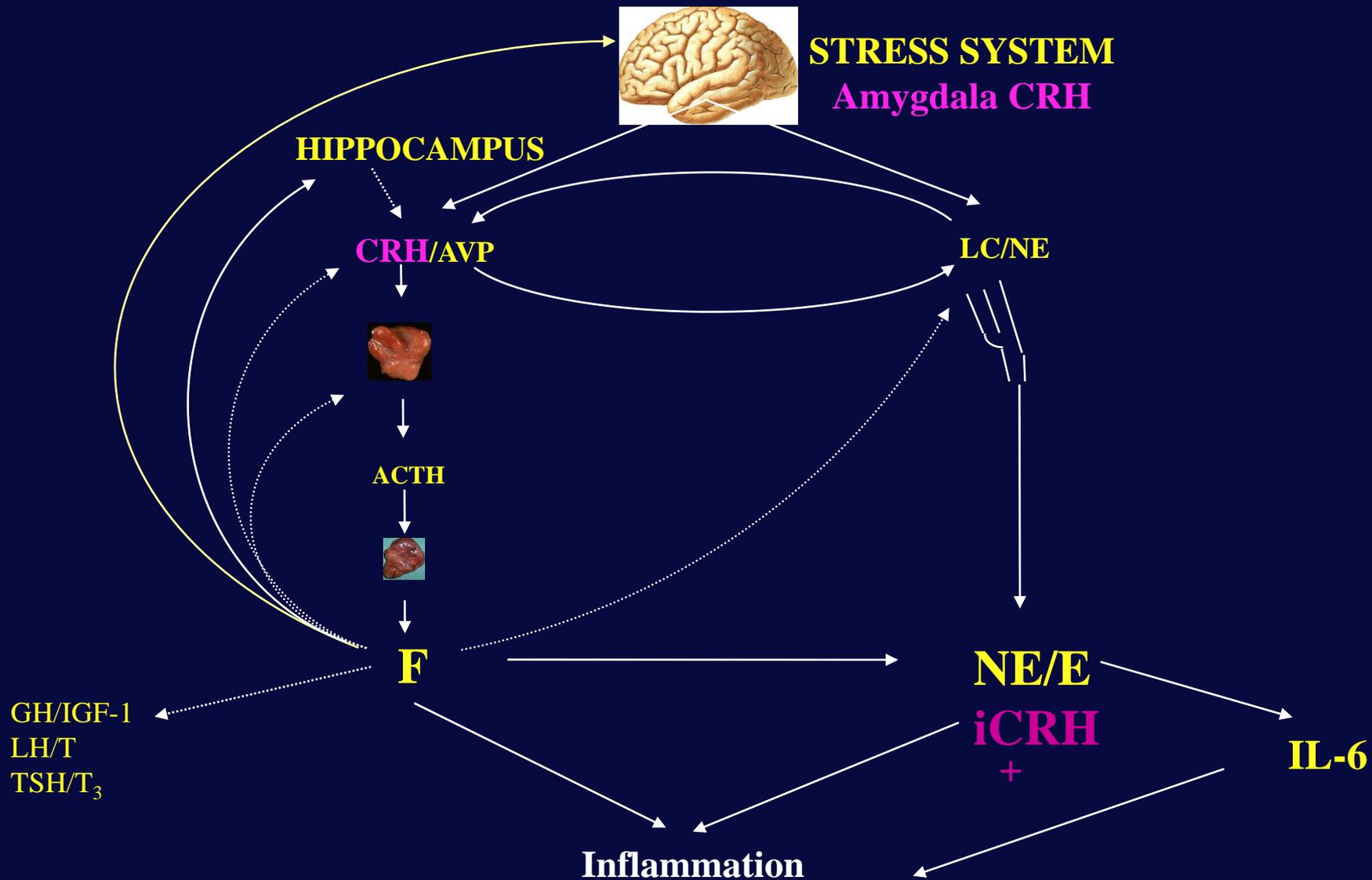
Cortisol

Catecholamines/IL-6

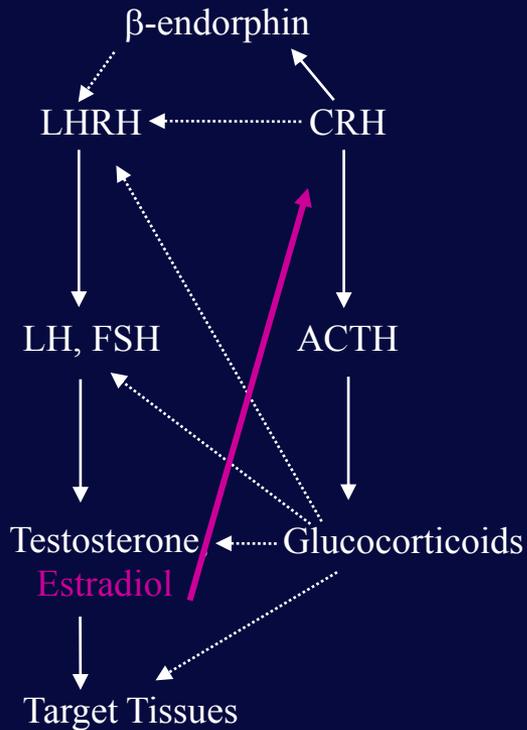
MC/ML System Tone



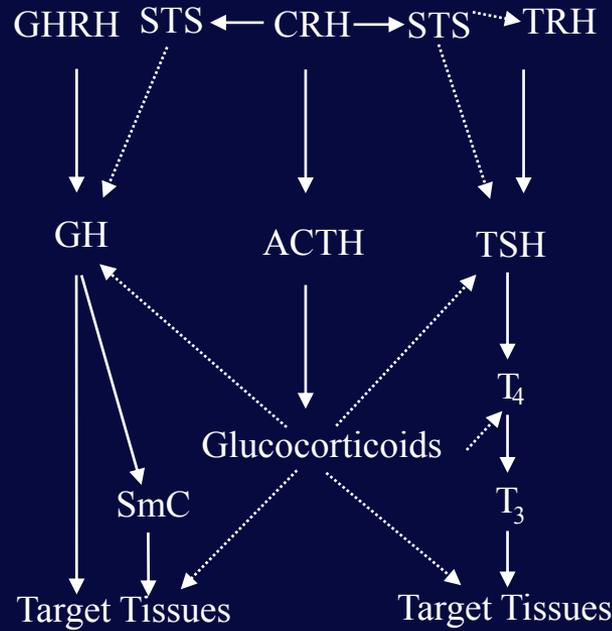
Stress System Tone



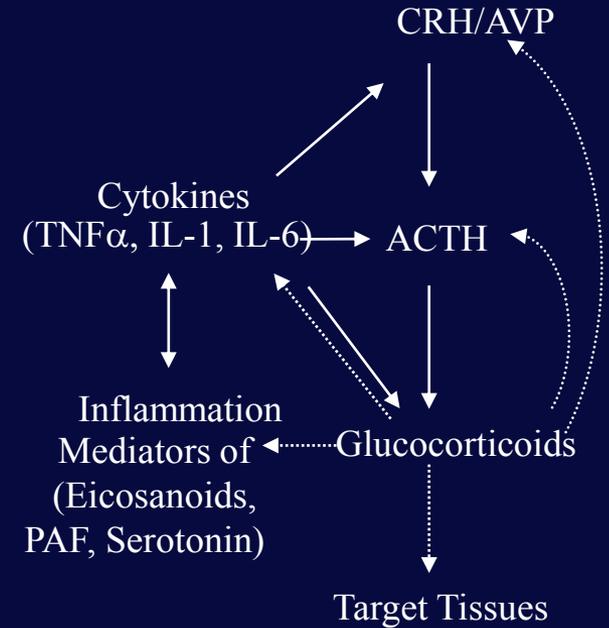
Reproduction



Growth and Thyroid Function



Immune Function



SICKNESS SYNDROME

ANOREXIA/NAUSEA

FATIGUE AND/OR DEPRESSED AFFECT

SOMNOLENCE

HYPERALGESIA \pm HEADACHE

ELEVATED TEMPERATURE/ FEVER

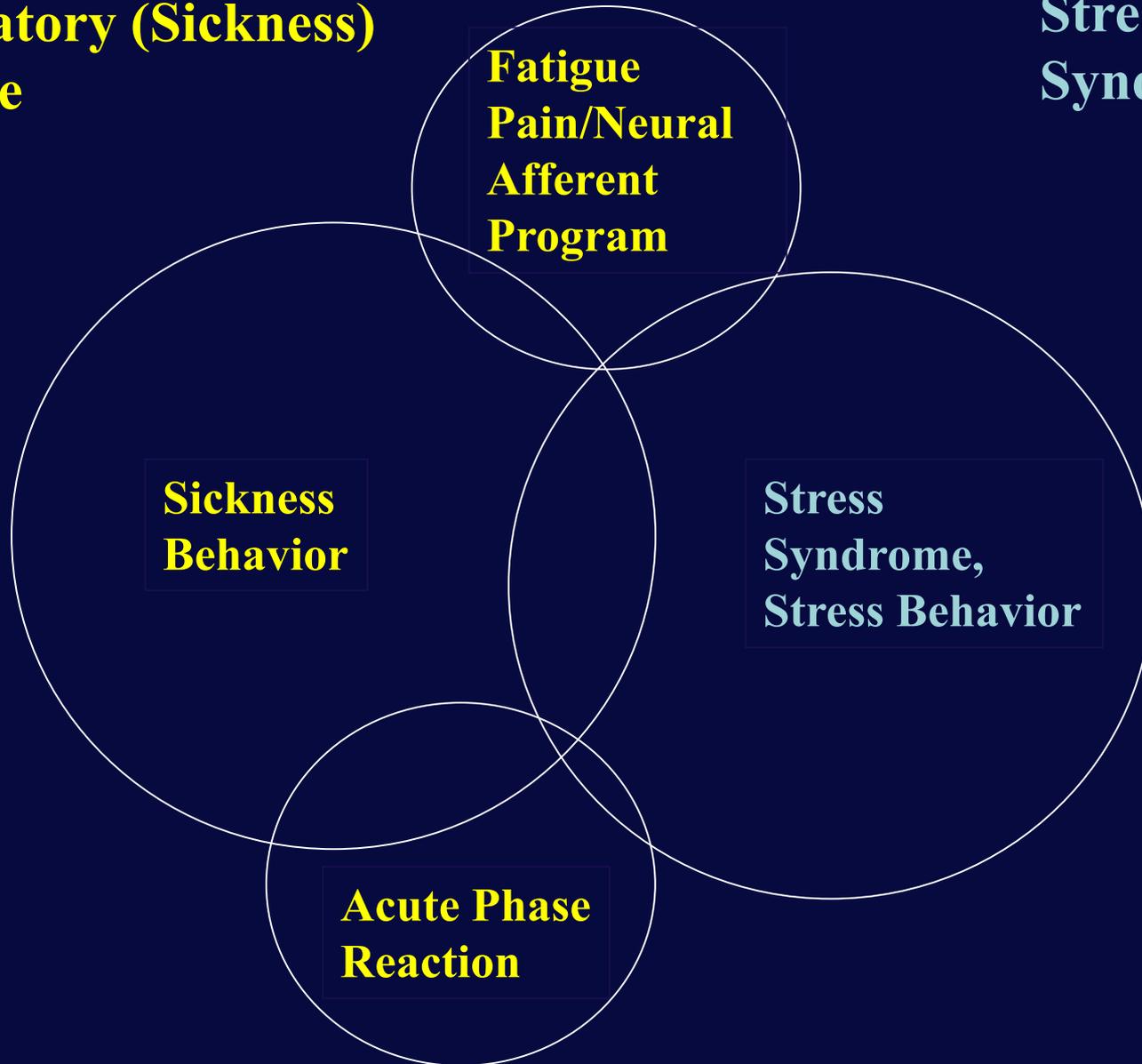
INCREASED METABOLIC RATE

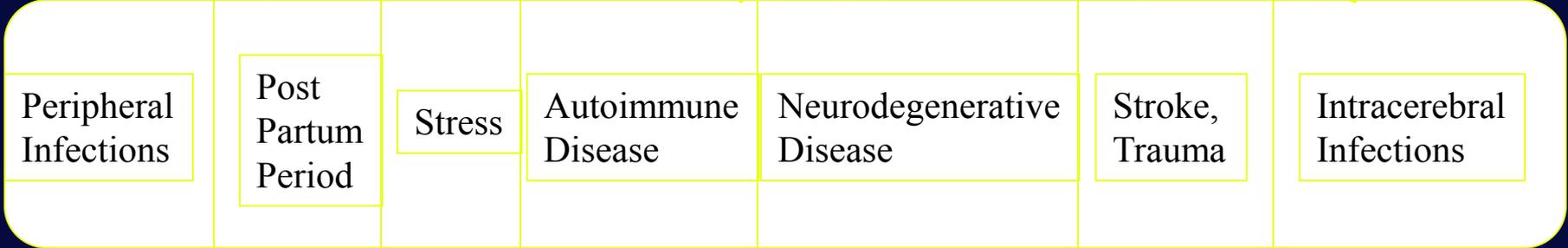
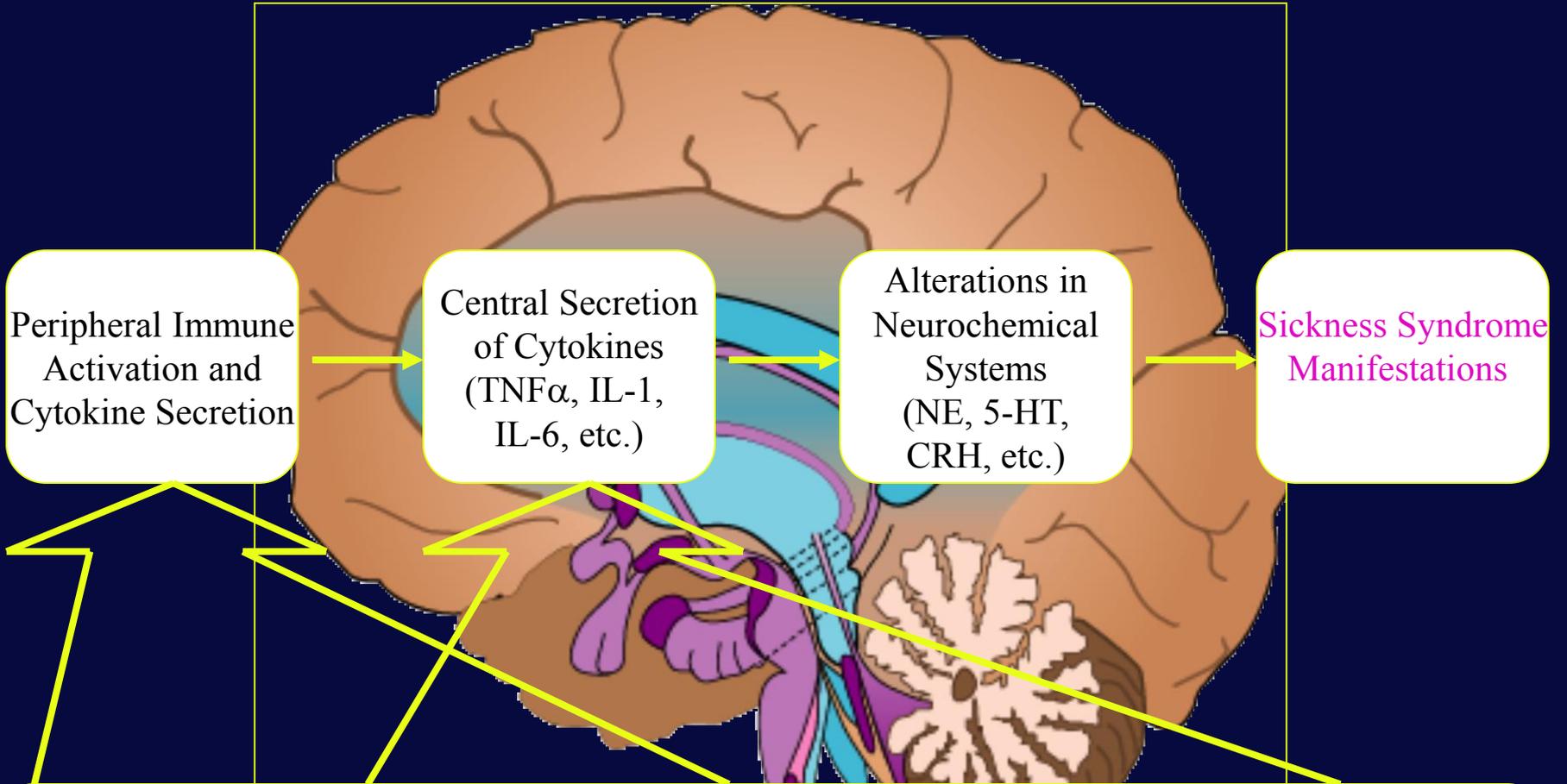
MOLECULAR EFFECTORS

INFLAMMATORY CYTOKINES/MEDIATORS

Inflammatory (Sickness) Syndrome

Stress Syndrome





Physical and Emotional Stress

- **Stress Concepts-Complexity**
 - **Stress Mechanisms**
 - **Effects Stress on Metabolism, Cardiovascular Disease and Longevity**
-

Physical and Emotional Stress

- **Timing** (Critical periods=prenatal, first 5 y and adolescence)
 - **Acuity**
 - **Chronicity**
-

THE STRESS SYSTEM

Pathophysiology

Acute effects of stress system activation

- **Asthma, eczema, urticaria**
- **Migraine and tension headache**
- **Gastrointestinal pain**
- **Hypertensive episode, CVA, death (compromised host)**
- **Cardiac ischemia, MI, Arrhythmia, death (compromised host)**
- **Psychotic episode**

THE STRESS SYSTEM

Pathophysiology

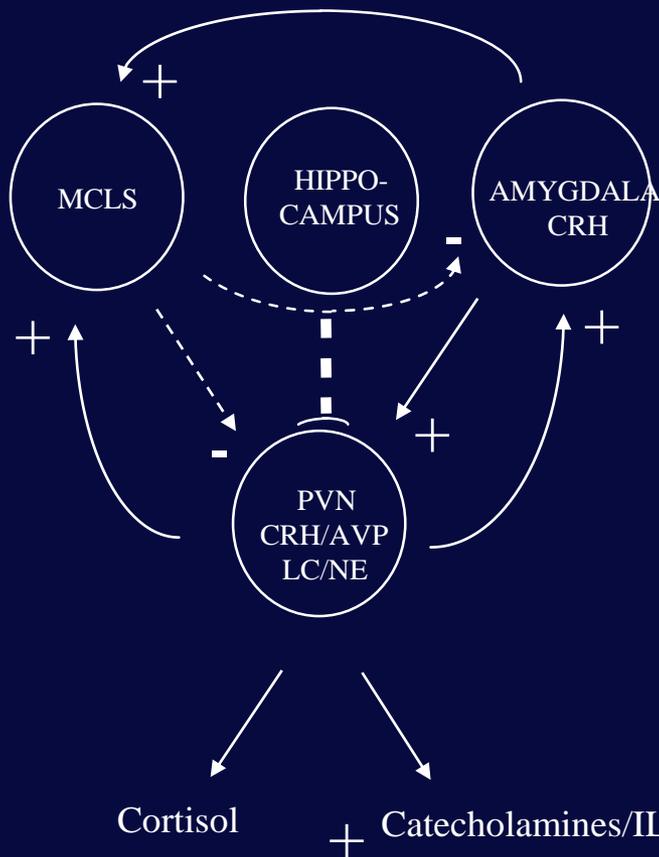
Chronic effects of stress system activation:

- Anxiety, depression, addiction, anti-social behavior, psychosomatic disorders, fatigue, pain
- Loss of weight, poor growth, **obesity, metabolic syndrome, T2DM, smoldering inflammation, Immune dysfunction, atherosclerosis, CVD**
- Osteoporosis
- Premature aging of all vulnerable organs, including the brain (neurodegeneration) and the skin
- Vulnerability to infections and cancer



CHRONIC NONCOMMUNICABLE DISEASES

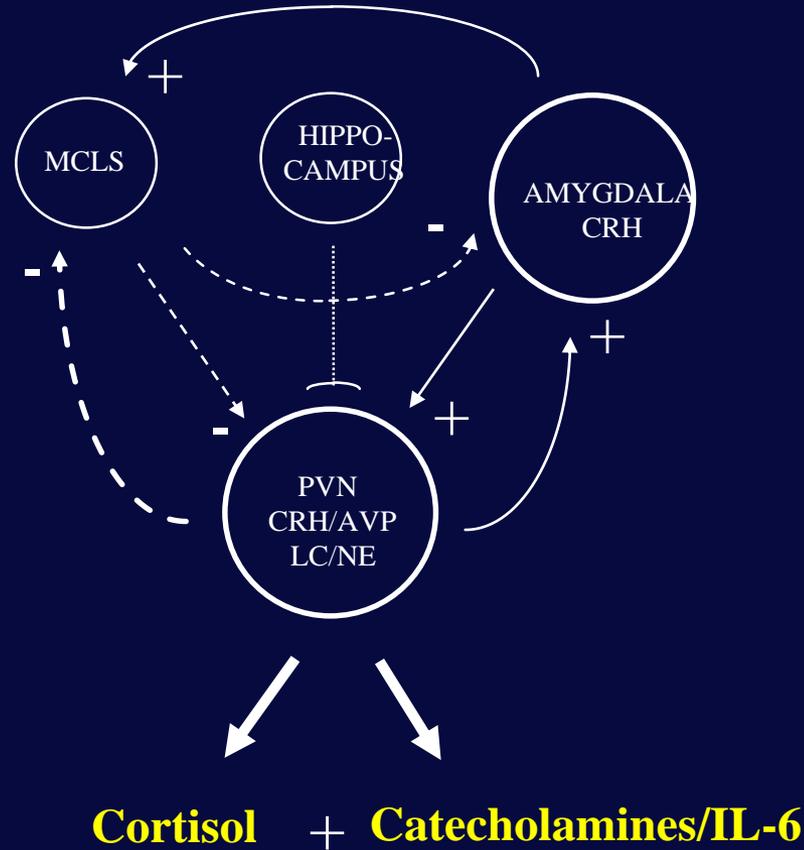
Acute Stress



**Adjustment vs.
Maladjustment**

G.P. Chrousos

Stress Hyper-responsive Child/Adult



Cortisol + Catecholamines/IL-6
Behavioral consequences + Somatic consequences
 Maladjustment disorders + Growth retardation
 Anxiety, Depression + Metabolic syndrome X
 Personality disorders + /T2DM
 Addiction, Psychosomatics + Cardiovascular disease
 Osteoporosis

Sickness s.

HYPERCYTOKINEMIA

TRAUMA/ BURNS

INFECTIOUS ILLNESSES

AUTOIMMUNE INFLAMMATORY DISEASES

ALLERGIC INFLAMMATIONS

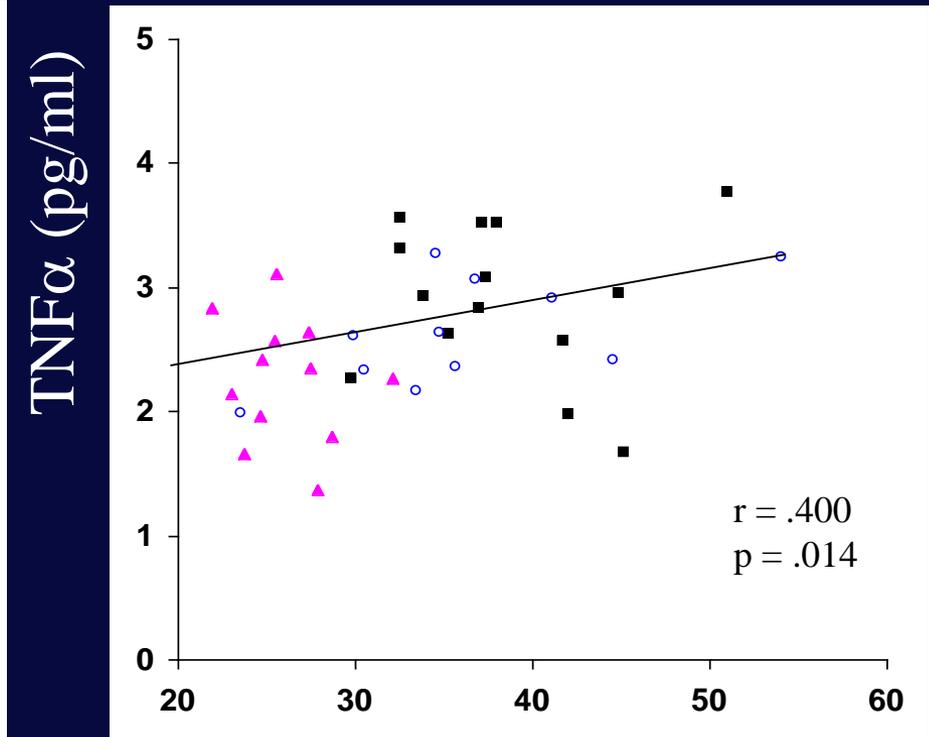
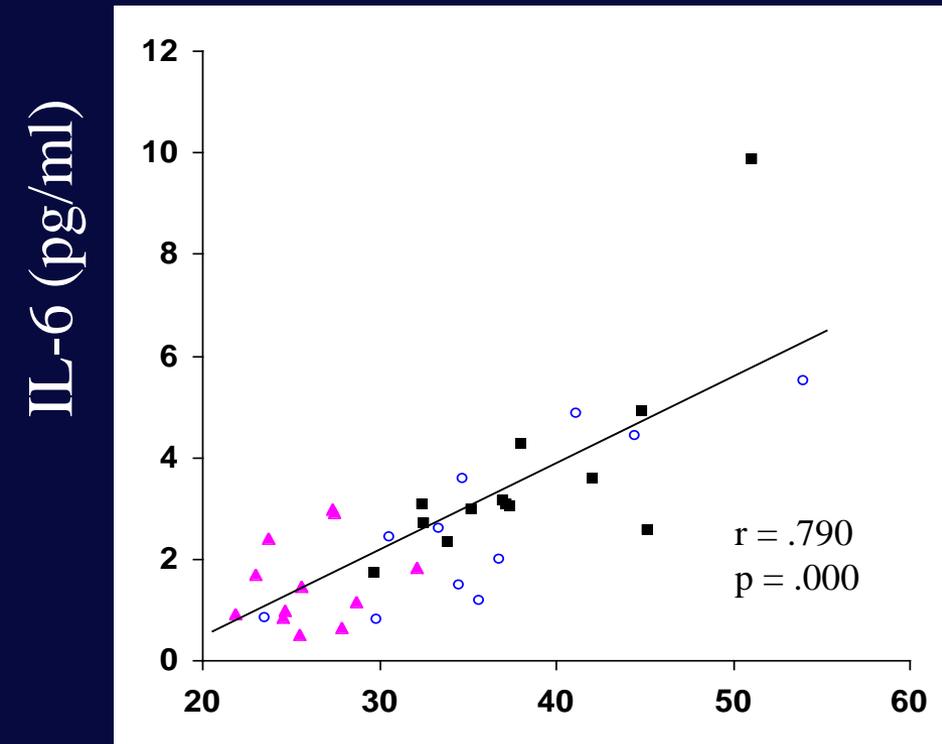
CNS INFLAMMATIONS

NONINFLAMMATORY STRESS

OBESITY/VISCERAL OBESITY

AGING

Both IL-6 and TNF α correlate with BMI



BMI

Vgontzas *et al.* JCEM 1997

DEVELOPMENTAL HISTORY

STRESS

Real or perceived

GENETIC VARIATION



NUTRITION

AGING

HPA axis

Stress system
CRH/AVP-LC/NE

Systemic Sympathetic
Adrenomedullary Systems

↓ GH/IGF-1
↓ LH, T, E2
↓ TSH, T₃

↑ Cortisol

Target Tissues

NE, E, IL-6 ↑

Insulin resistance

↻ Visceral Obesity/Sarcopenia ↻

=Metabolic
Syndrome, DM type2

ABP ↑

Sickness
Syndrome

↑ TG
↑ LDL
↓ HDL

APR ↑

Cytokines ↑

Dyscoagulation

Sleep Apnea

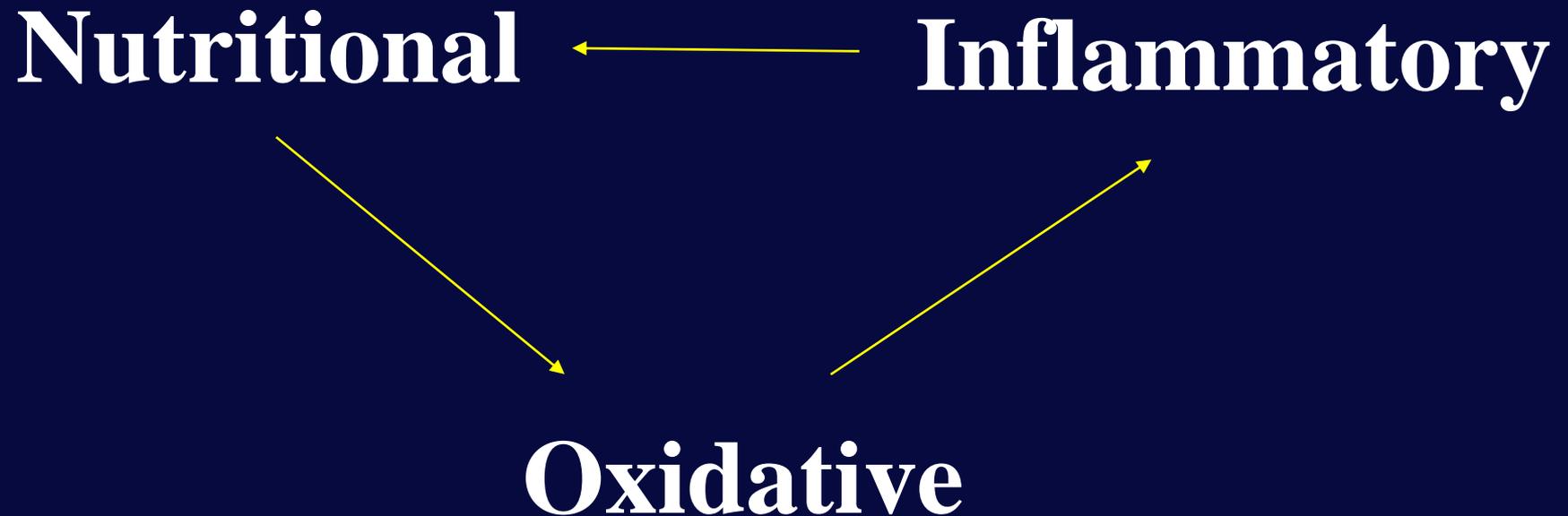
PCOS

Osteoporosis

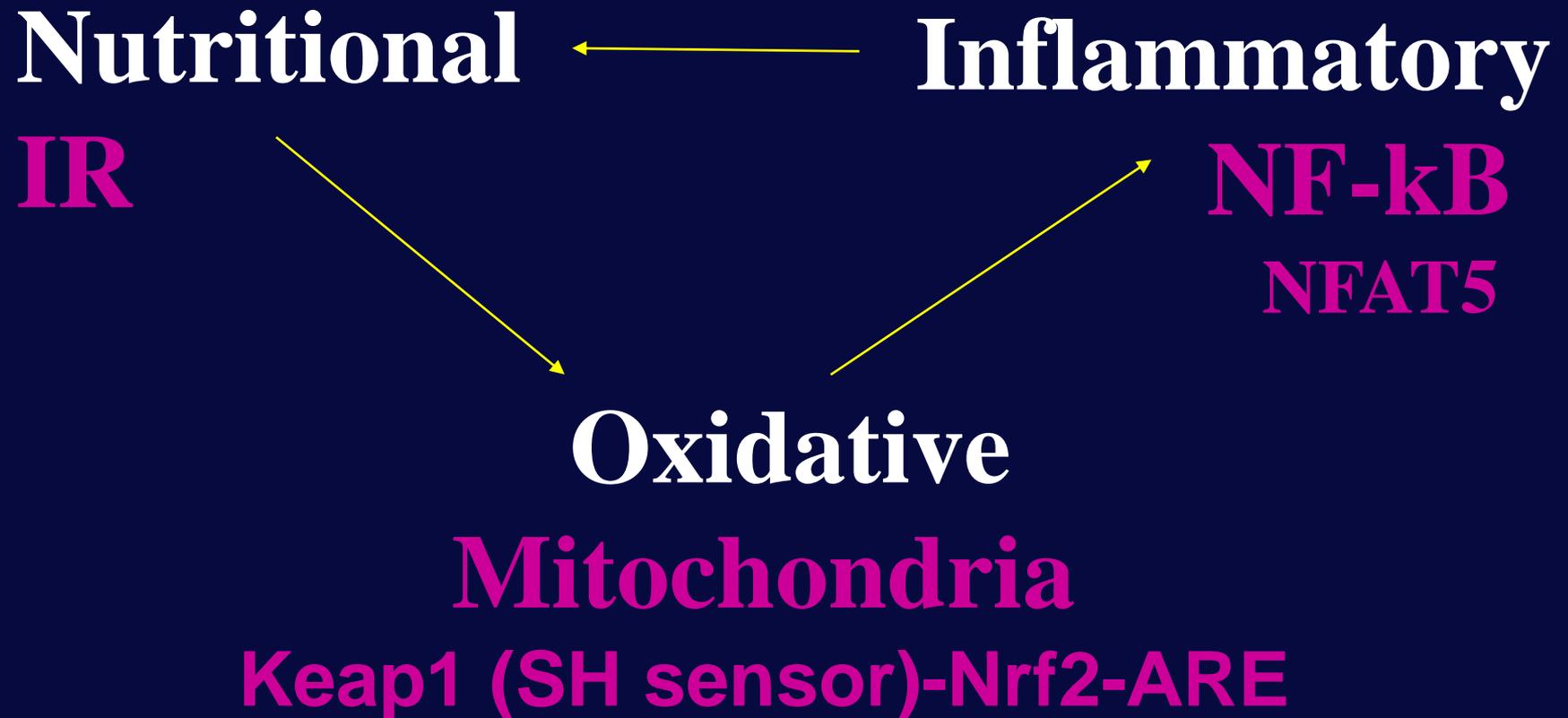
Endothelial Dysfunction/Inflammation

Atherosclerosis
Cardiovascular Disease

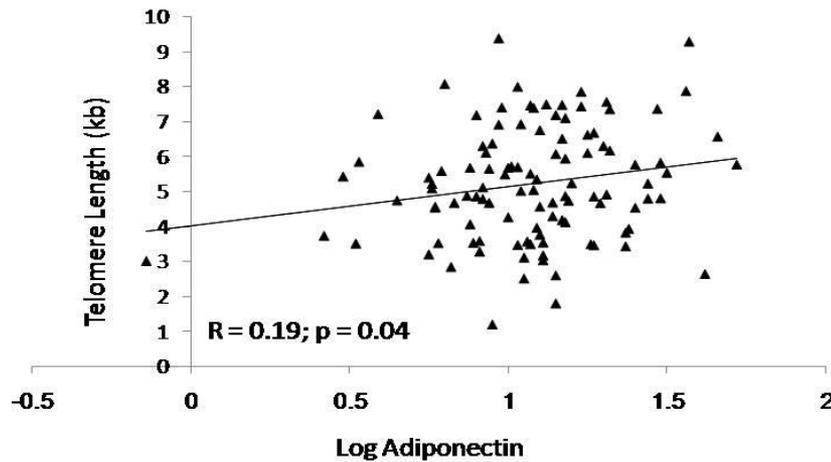
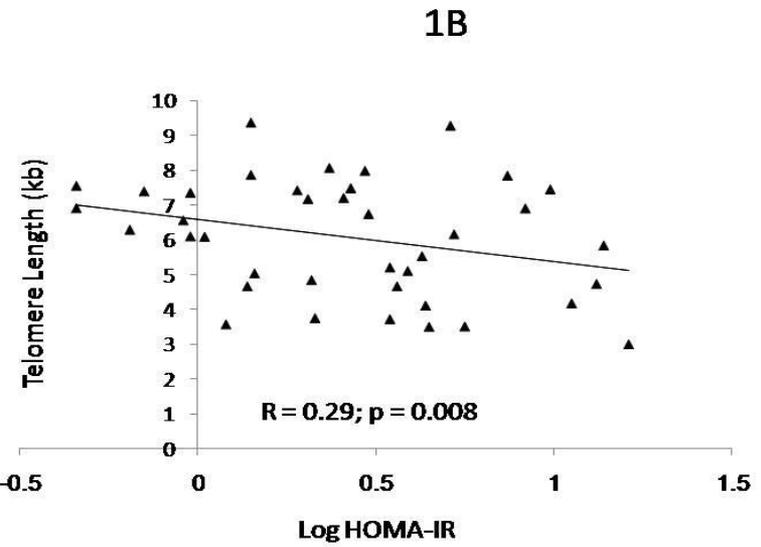
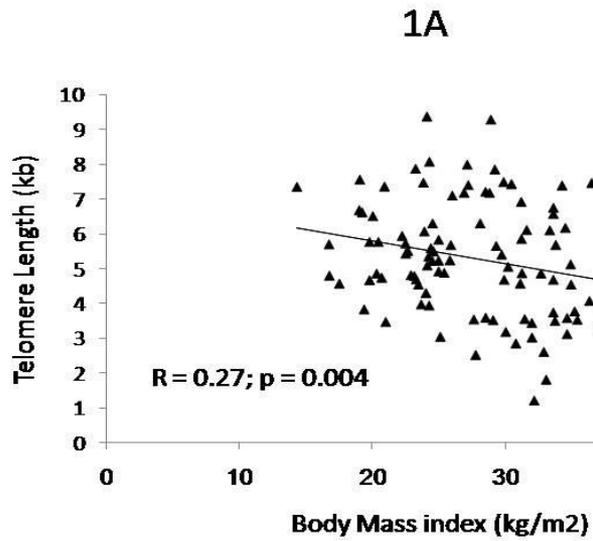
Cellular Stress



Cellular Stress







1C

SPECIAL REPORT

THE NEXT CYBER ATTACK
COULD COME FROM YOUR COFFEEMAKER

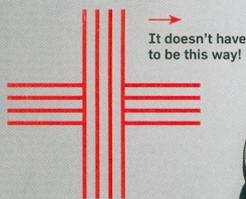
PLUS!
INSIDE AMAZON'S
DRONE LAB PG.19

POPULAR SCIENCE

STRESS

IT'S KILLING YOU

How to Beat It with Science



DIY HOVERCRAFT

TAKE OFF IN AN HOUR

WHY IS EVERYONE SO SCARED OF ARTIFICIAL INTELLIGENCE?

RELAX, STEPHEN HAWKING



MARCH 2015

INTRODUCING THE VIZZIES

PG. 52

15
MUST-HAVE GADGETS

RISE OF THE DWARF PLANETS

PG. 32

MARCH 2015
WWW.POPSCI.COM

938
135#
735

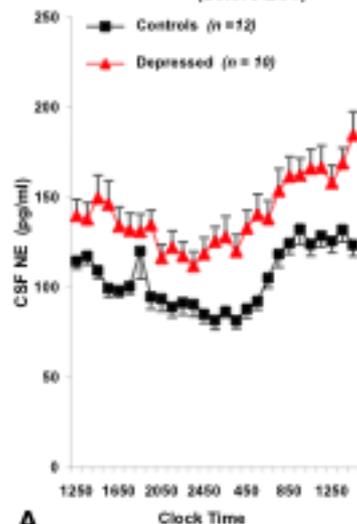
#BXGDCHX *****SCH 5-DIGIT 20814 FSS
#PS0008218455/5# MAR15 A99 FEB19 0014
GEORGE P CHROUSOS
4977 BATTERY LN APT 820N
BETHESDA MD 20814-4923

“Grief and fear when lingering provoke melancholia”

Hippocrates 460-479 BCE

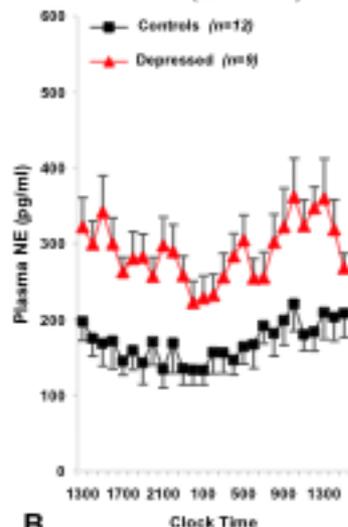
**STRESS SYSTEM
HYPERACTIVITY AND
PARADOXIC OBESITY
IN DEPRESSION**

Diurnal Variations in CSF NE in Controls and Depressed Patients (before ECT)



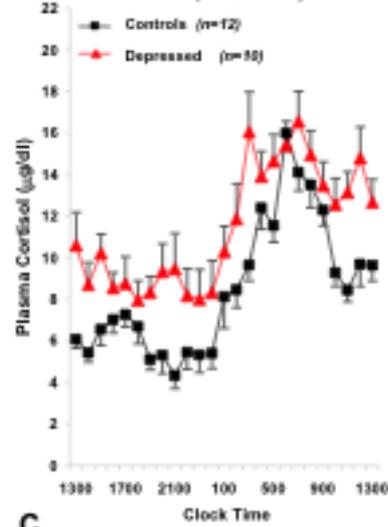
A

Diurnal Variations in plasma NE in Controls and Depressed Patients (before ECT)



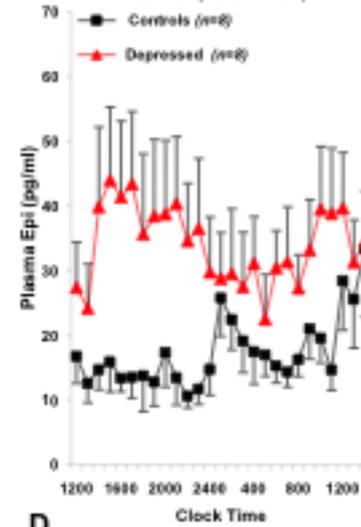
B

Diurnal Variations in plasma Cortisol in Controls and Depressed Patients (before ECT)



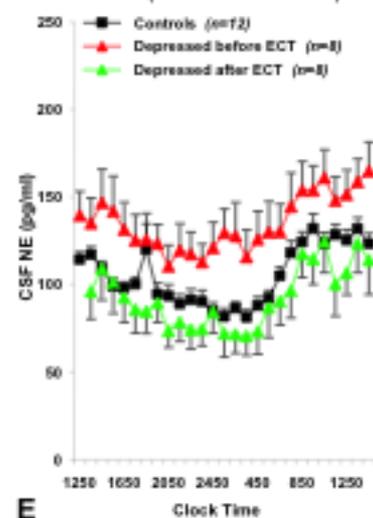
C

Diurnal variations in plasma Epi in Controls and Depressed Patients (before ECT)



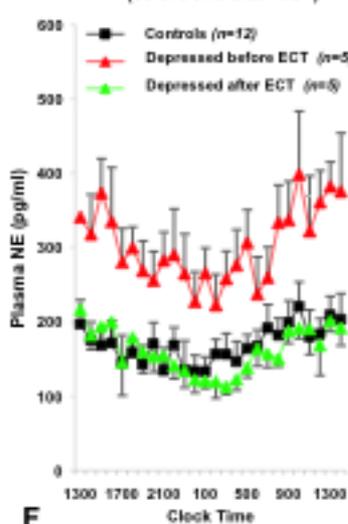
D

Diurnal Variations in CSF NE in Controls and Depressed Patients (before and after ECT)



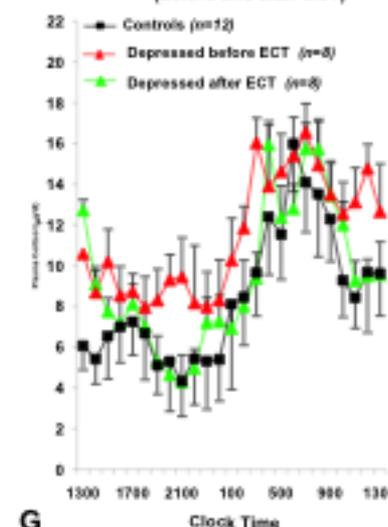
E

Diurnal Variations in Plasma NE in Controls and Depressed Patients (before and after ECT)



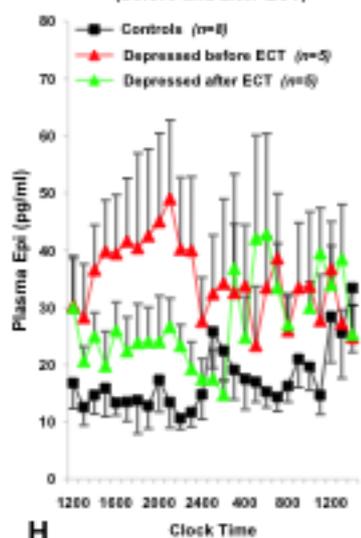
F

Diurnal Variations in Plasma Cortisol in Controls and Depressed Patients (before and after ECT)



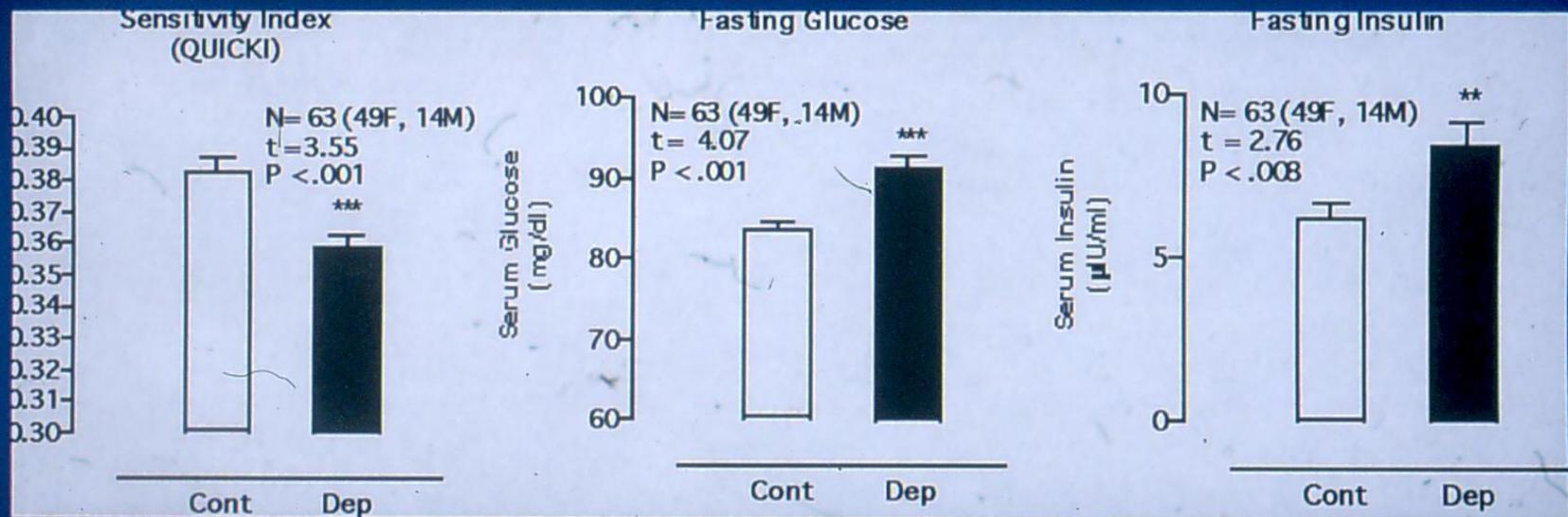
G

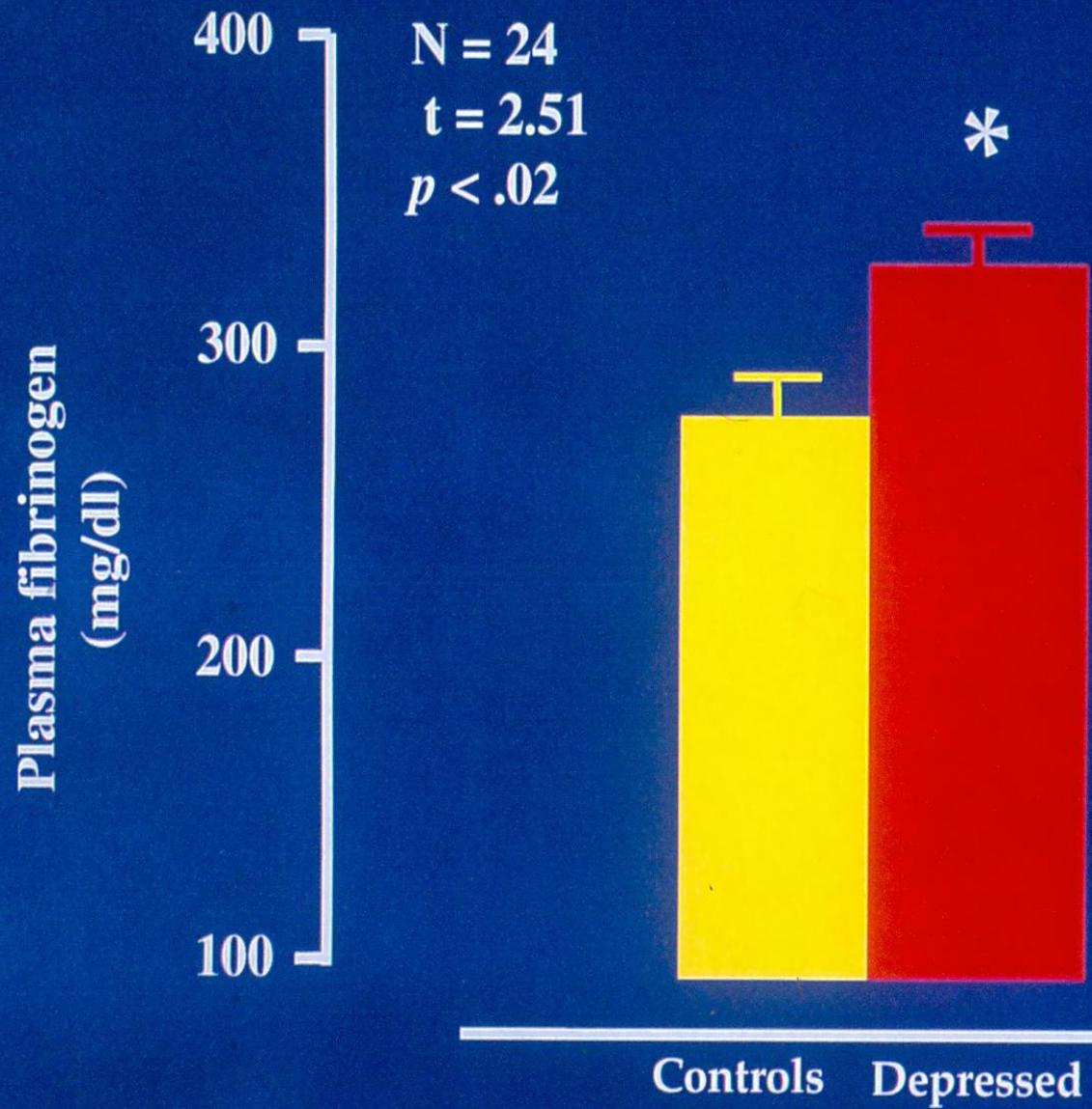
Diurnal variations in plasma Epi in Controls and Depressed Patients (before and after ECT)

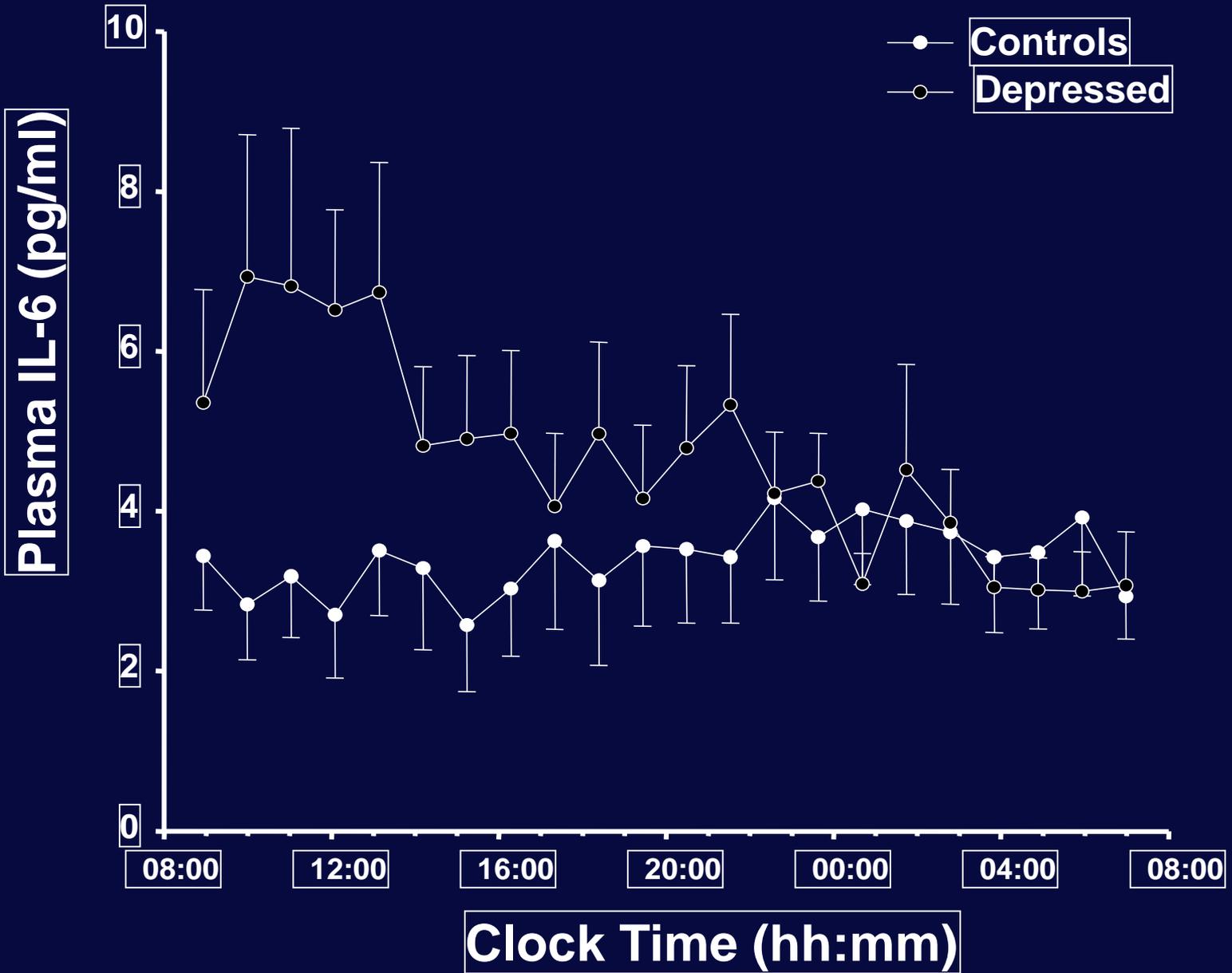


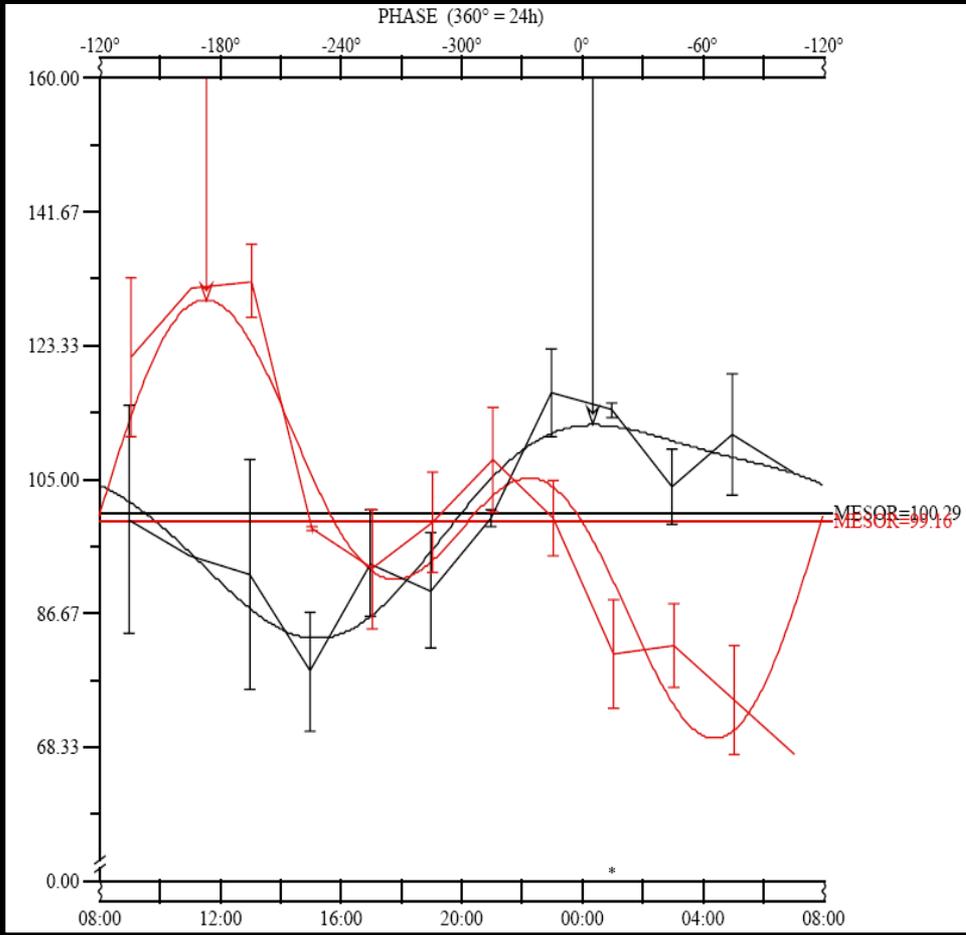
H

	Age	BMI	Fasting Serum Insulin	Fasting Serum Glucose	Insulin Sensitivity
Depressed	39.016 ± 1.218	24.6 ± 0.5	8.43 ± 0.71	91.16 ± 1.491	0.354 ± .004
Controls	38.079 ± 1.222	24.4 ± 0.4	6.21 ± .49	83.54 ± 1.1	0.381 ± .006
<i>p</i> value			<i>p</i> = .008	<i>p</i> = .0001	<i>p</i> < .0008



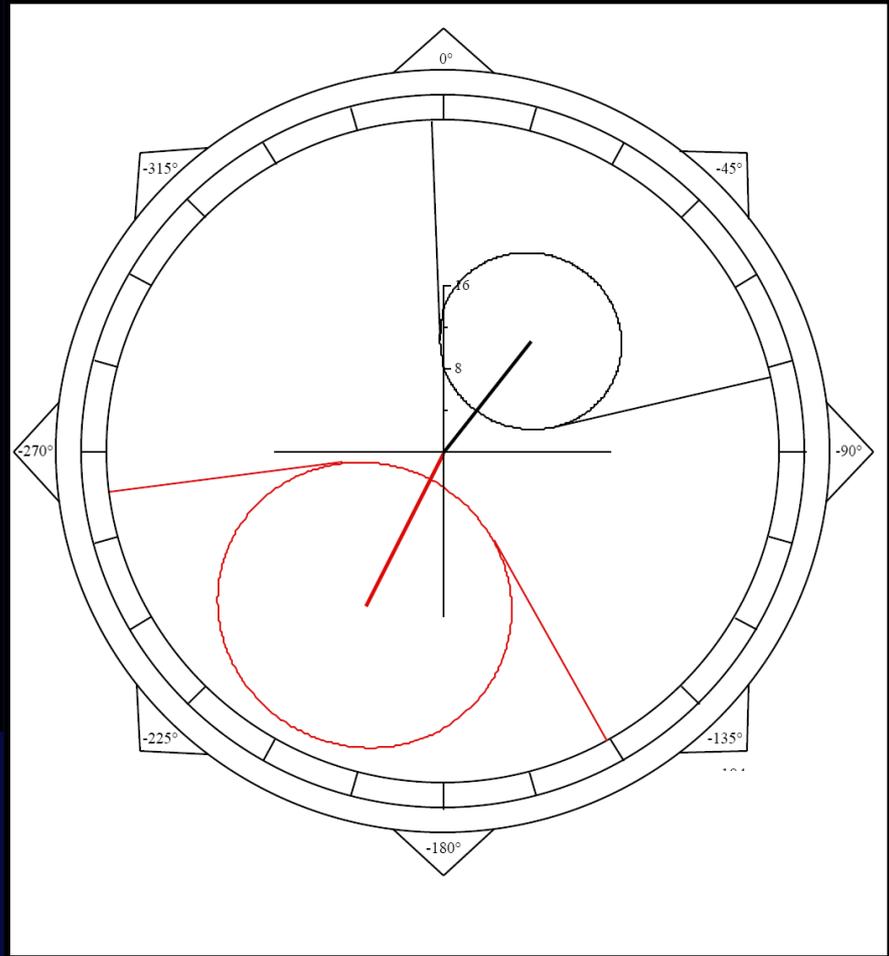






Clock time (hh:mm)

— Controls
 — Depressed

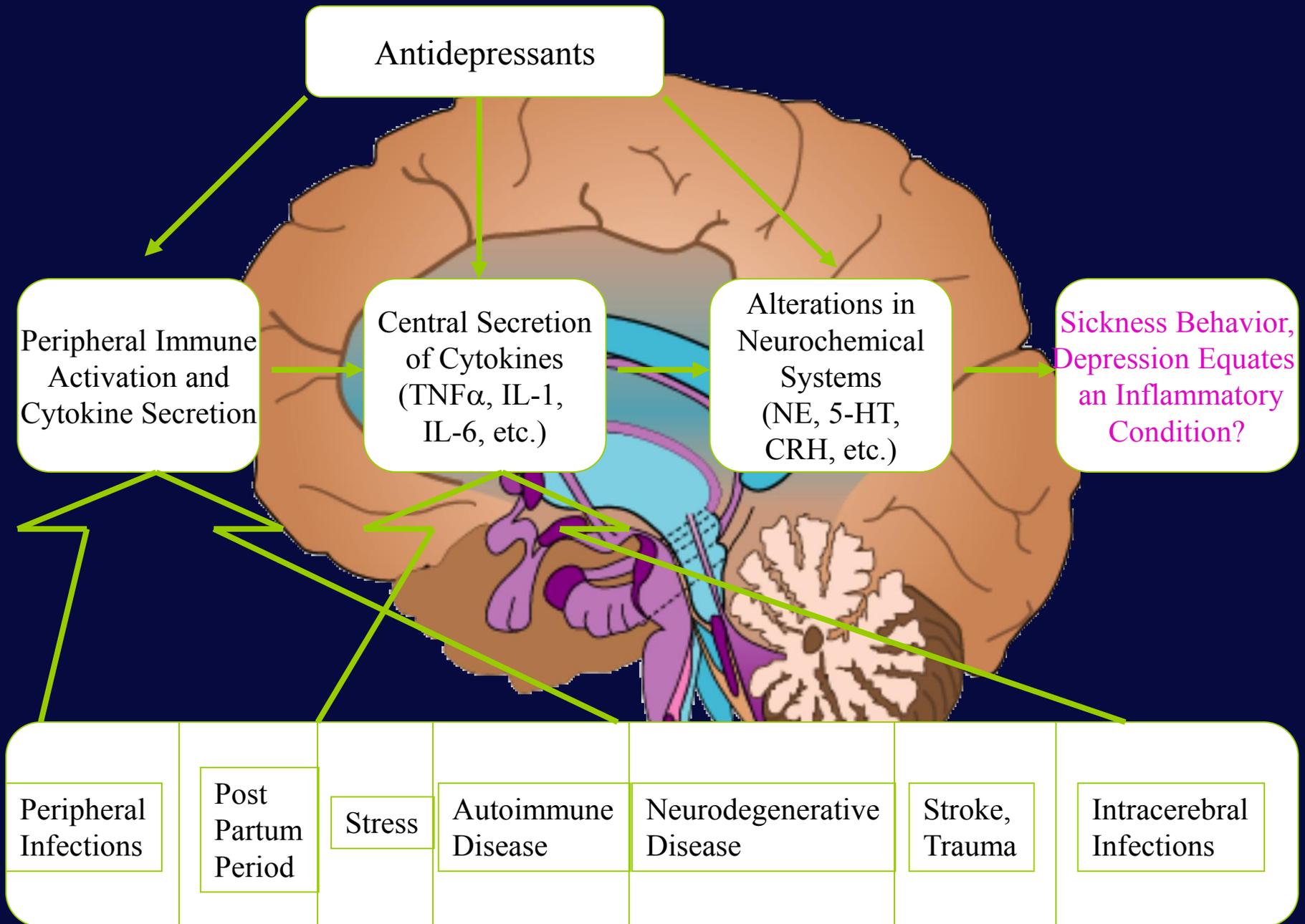


Pearson's correlations between mean 0800-2300 h plasma IL-6 levels and MVAS scores.

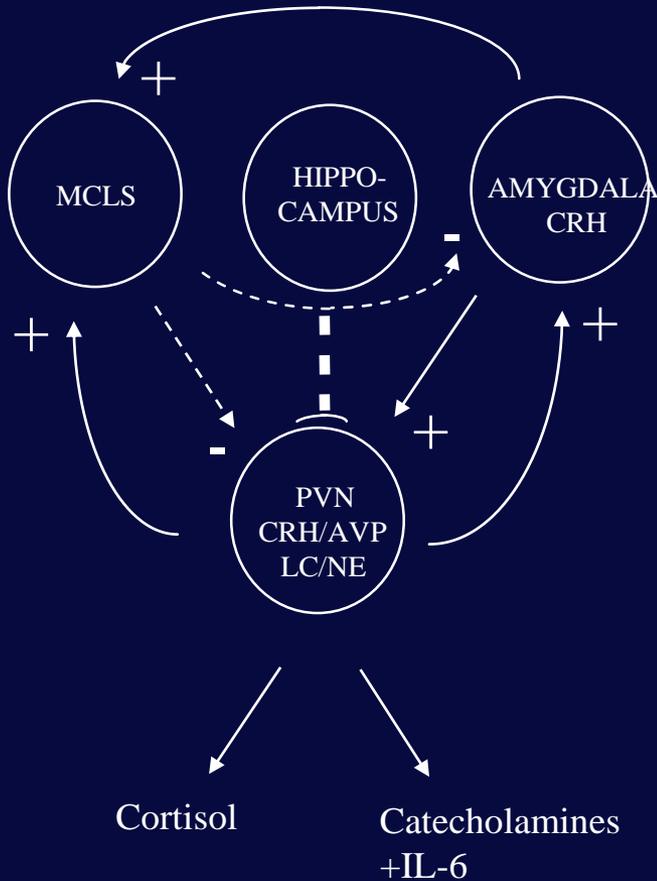
	Correlation coefficient	Probability value
Appetite	-0.61	0.07
Concentration	-0.64	0.05
Craving	-0.45	0.19
Guilt	-0.82	0.004*
Physical discomfort	-0.35	0.32
Sadness	-0.72	0.02
Self-esteem	-0.86	0.002*
Suicidal thoughts	-0.88	0.0007*
Tiredness	-0.75	0.02
Withdrawal	-0.21	0.56

Note: For each measure, a higher VAS score denoted better feelings.

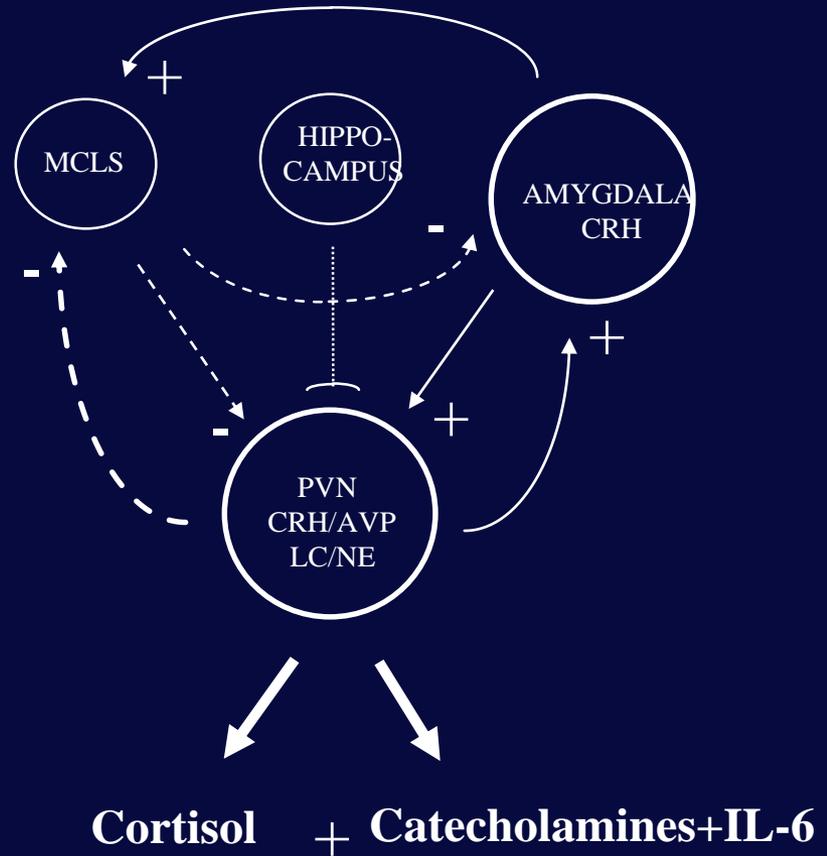
*Correlations of IL-6 with guilt, self-esteem and suicidal thoughts remained significant after Bonferroni correction.



Acute Stress

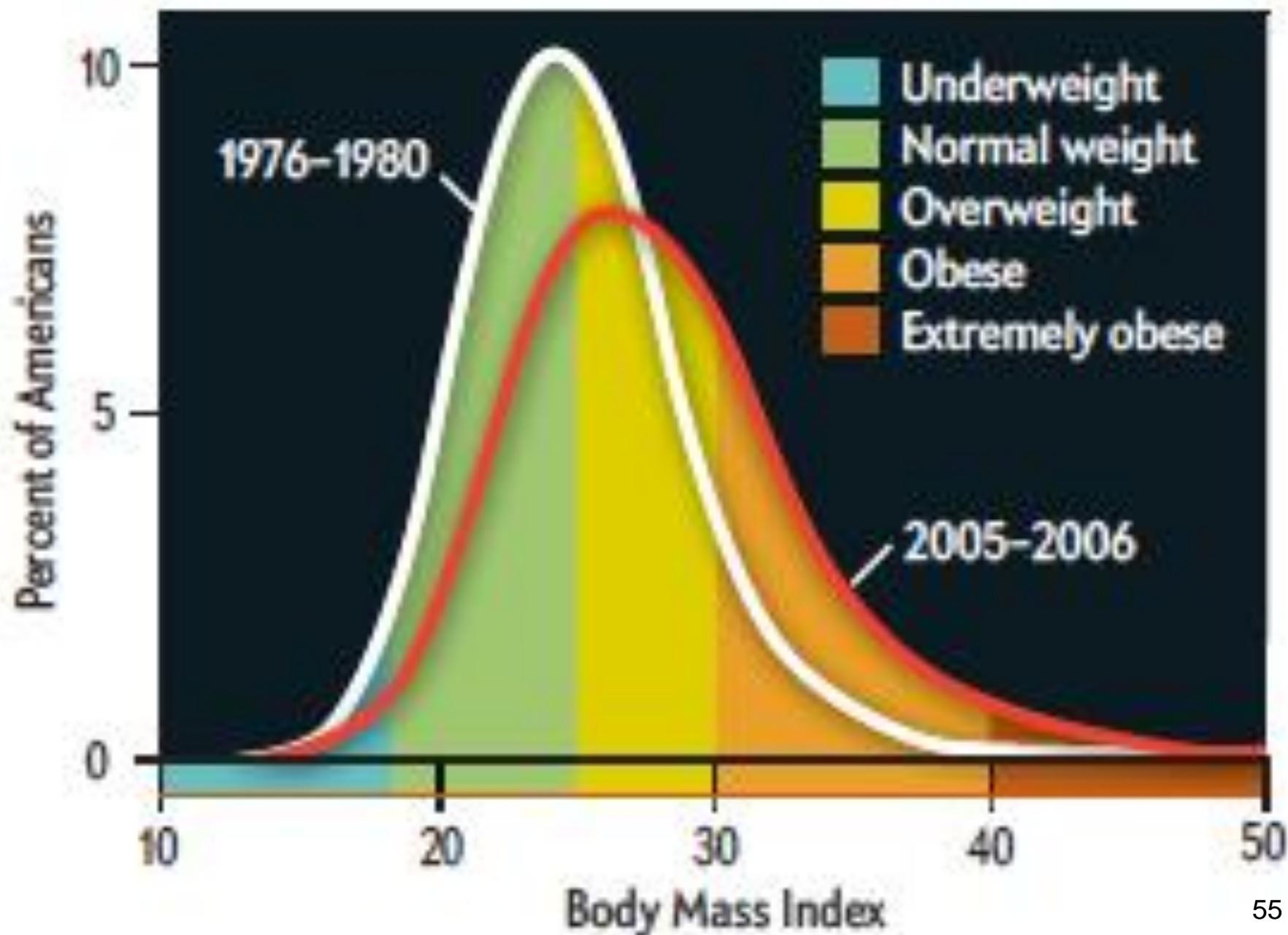


Melancholic Depression



Behavioral consequences + Somatic consequences
 Anhedonia, Fatigue, **Metabolic syndrome X**
 Insomnia, Anorexia. Cardiovascular disease
 Loss of libido Osteoporosis

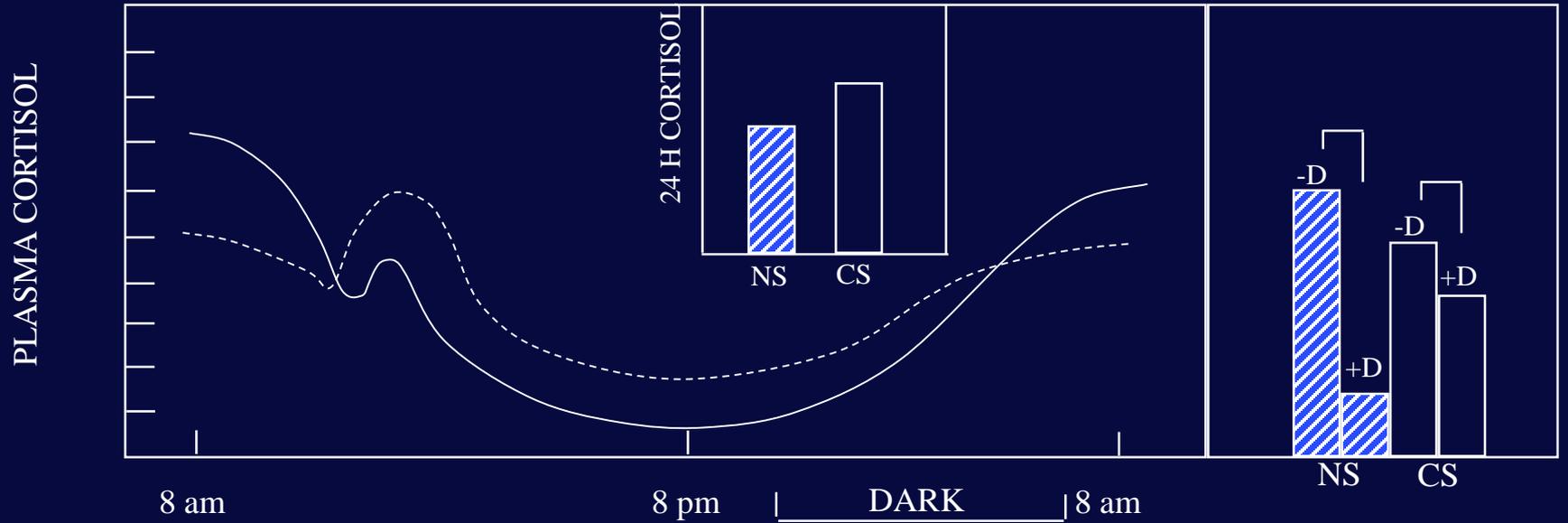
Sickness syndrome



A.

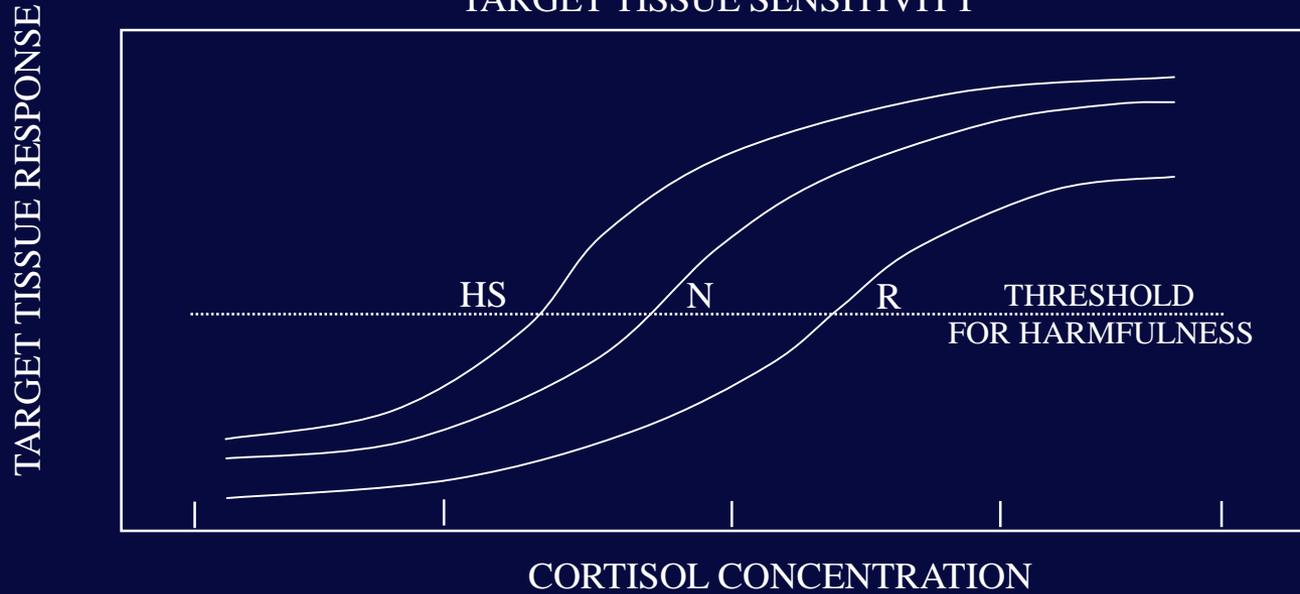
24 H SAMPLING

OVERNIGHT
DEXAMETHASONE TEST



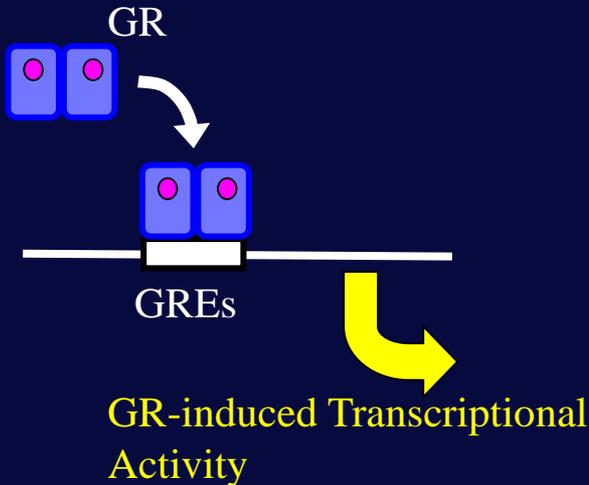
B.

TARGET TISSUE SENSITIVITY

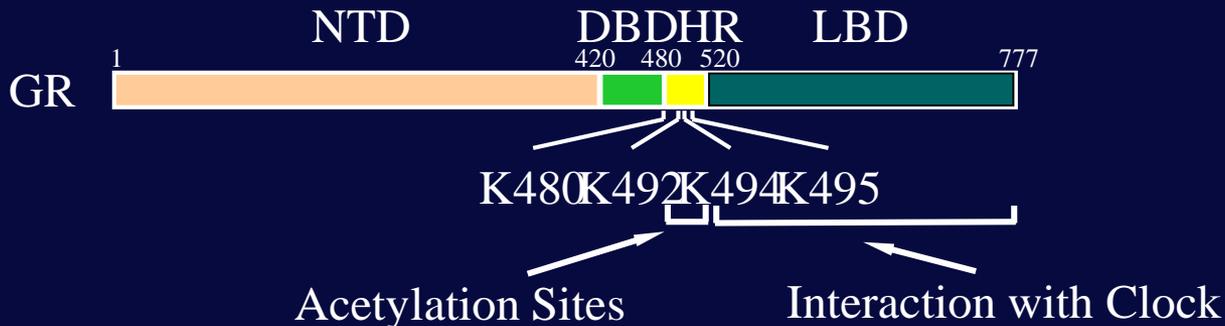
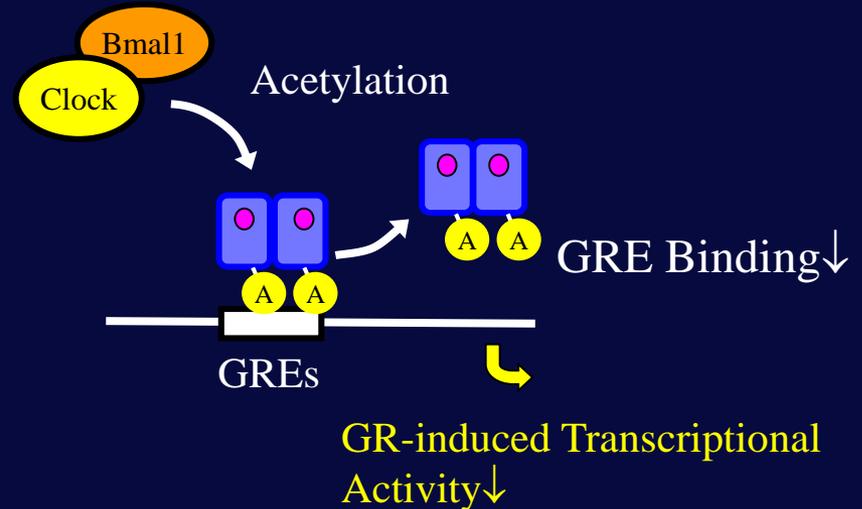


Results #1: Clock/Bmal1 Represses GR Transcriptional Activity through Acetylation

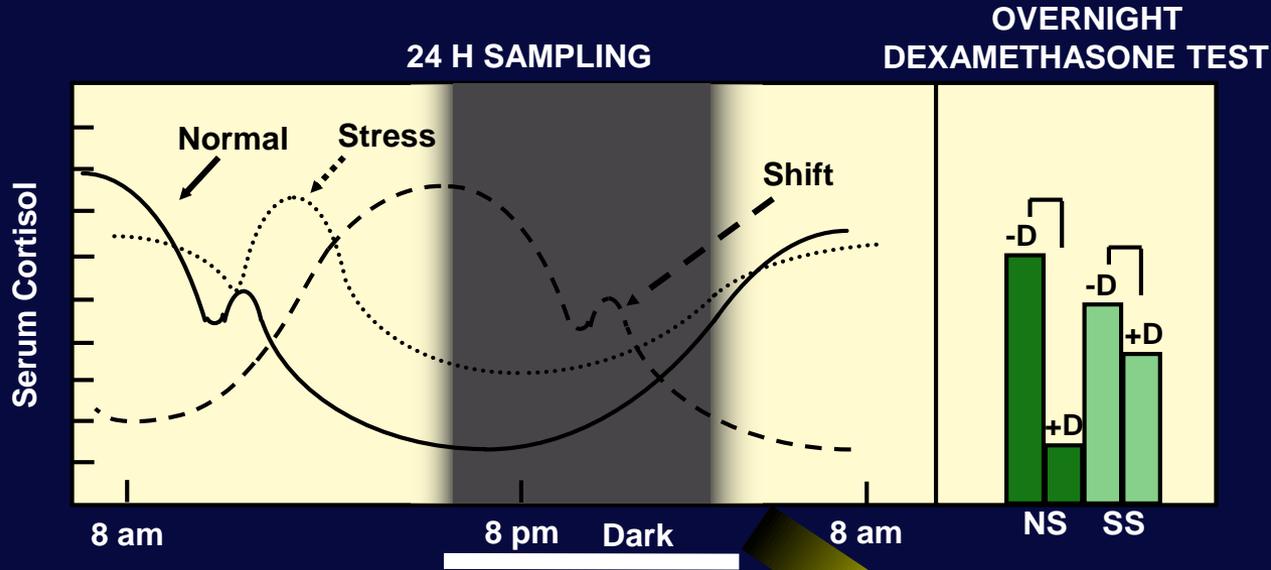
In the Absence of
Acetylation by CLOCK



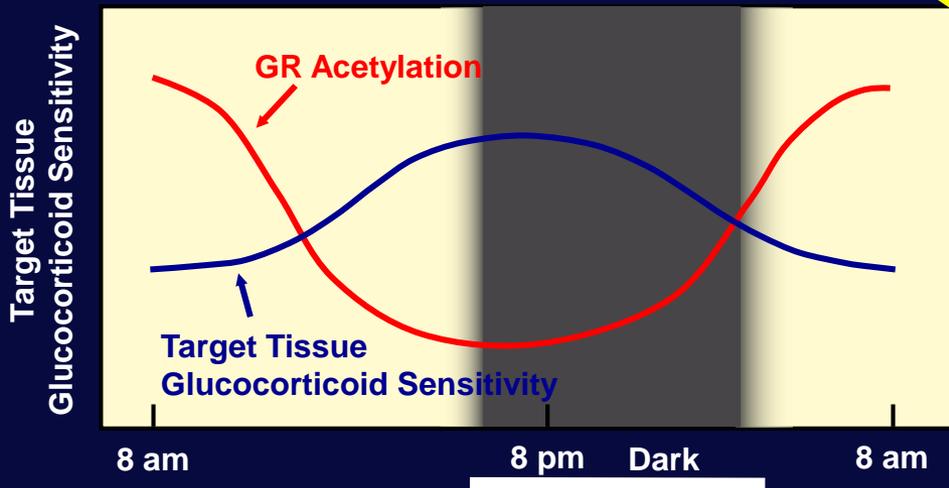
In the Presence of
Acetylation by CLOCK



Uncoupling between Circadian Rhythm of Serum Cortisol and Tissue Glucocorticoid Sensitivity



CIRCADIAN TISSUE GLUCOCORTICOID SENSITIVITY/GR ACETYLATION



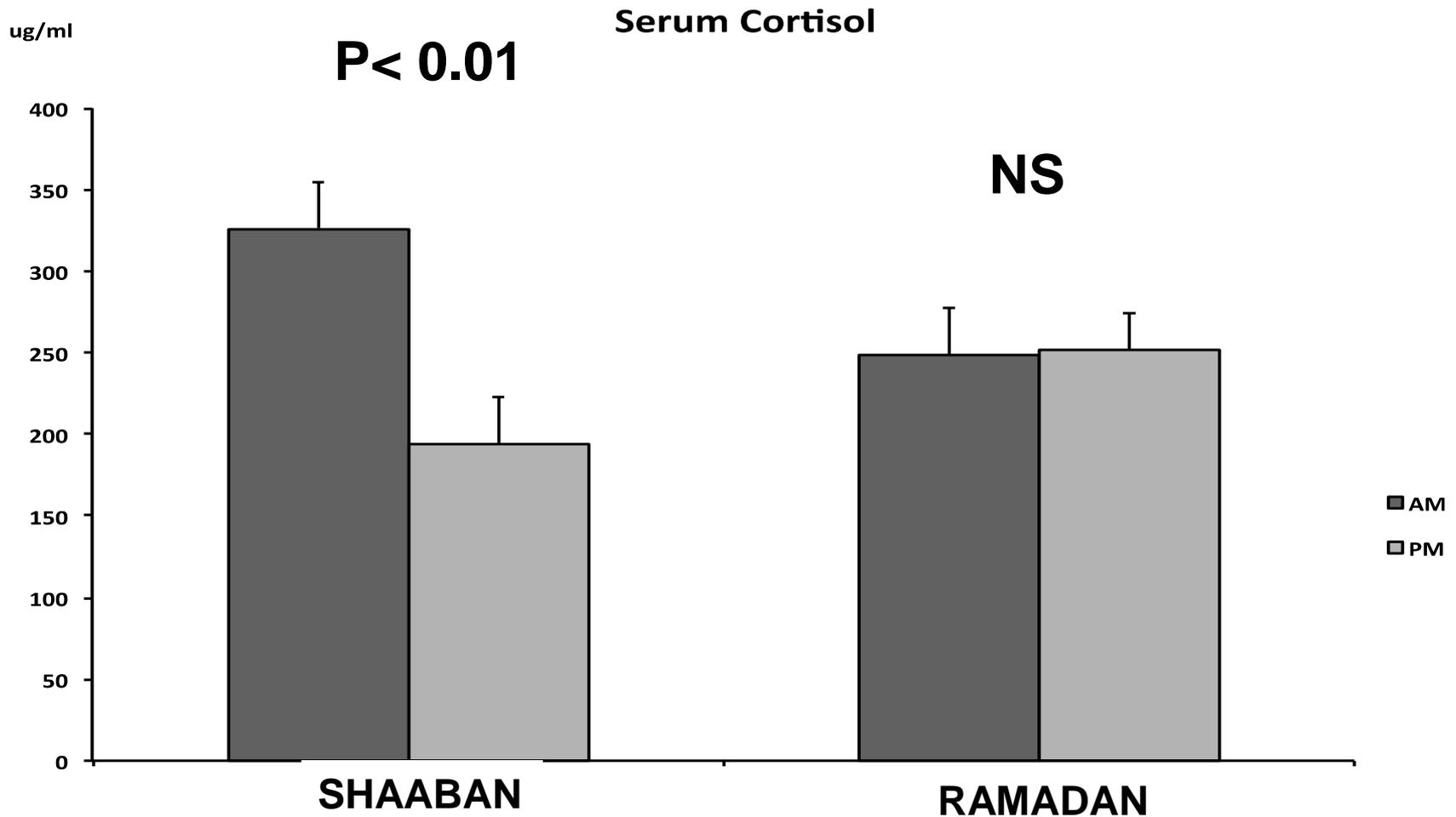
GR Acetylation
at Target Tissues

Functional
Glucocorticoid
Hypersensitivity

↓

Pathologic Consequences

Serum cortisol levels before and through fasting month:



MUSCLE MASS

**Best predictor of morbidity
and life expectancy**

DEFINITIONS

- **Osteosarcopenia vs. Paradoxical Obesity vs. Osteosarcopenic Obesity**

Decreased bone mass:

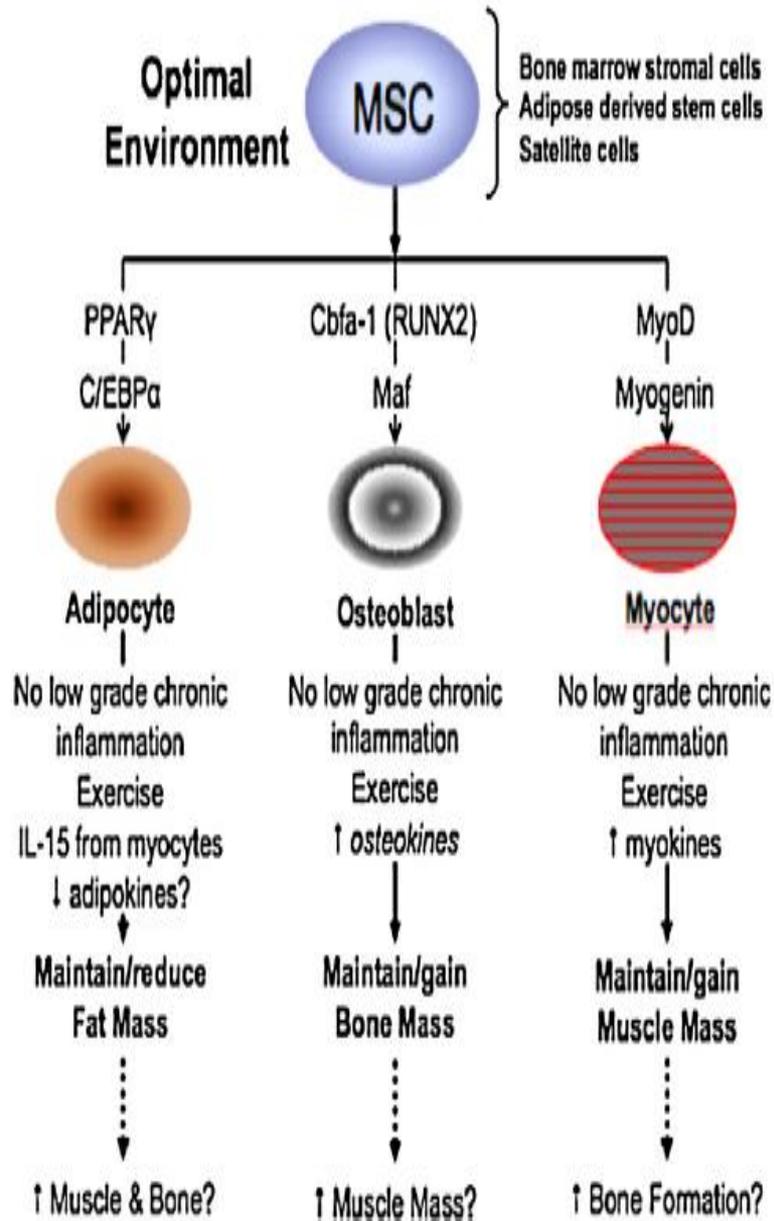
Osteopenia vs. Osteoporosis

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

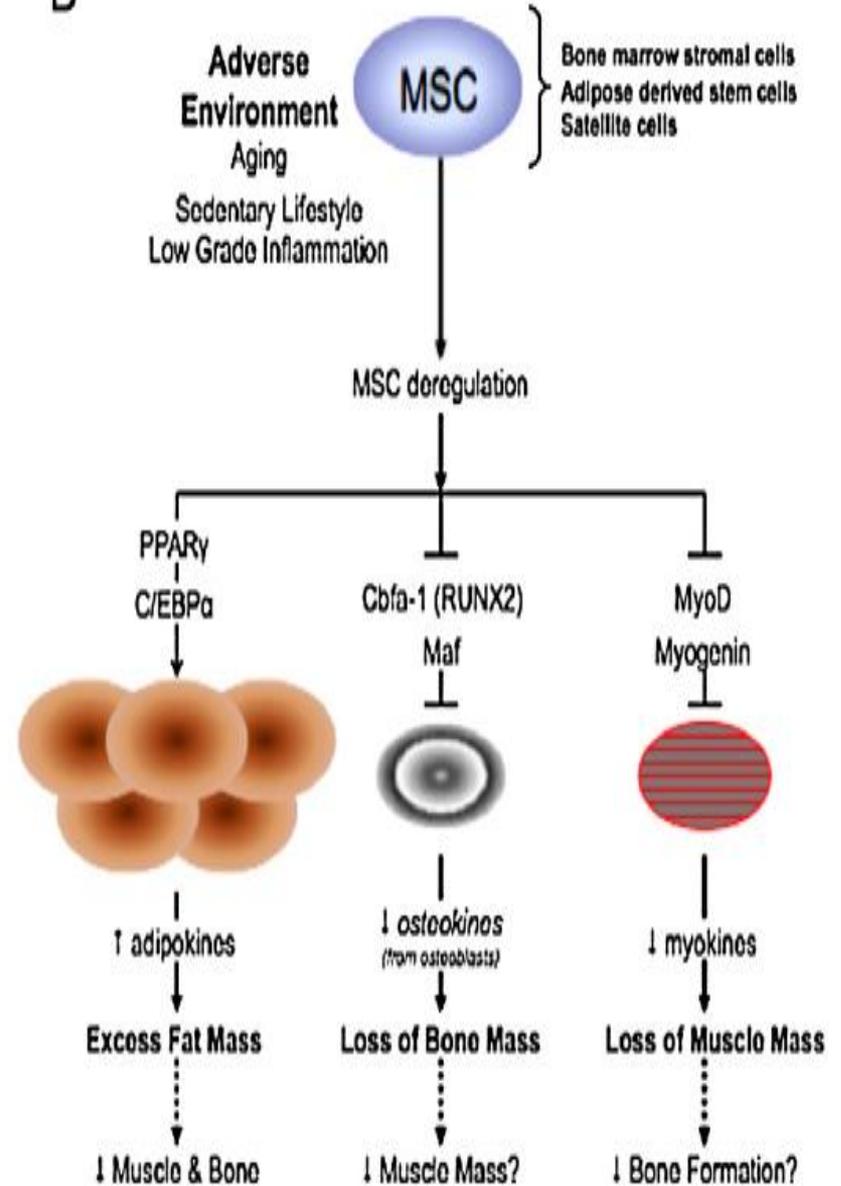
Decreased muscle mass:

Sarcopenia vs. Lipoatrophy + Sarcasthenia (frailty)

A



B



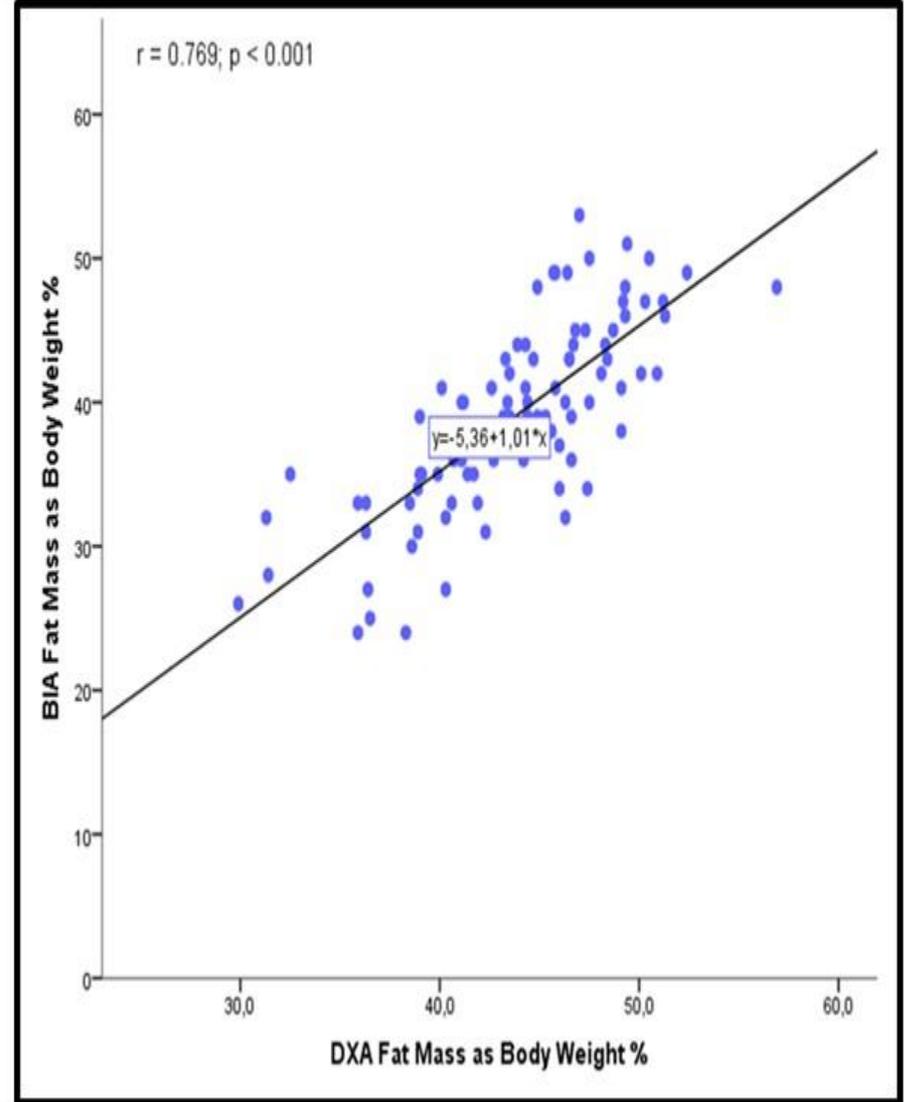
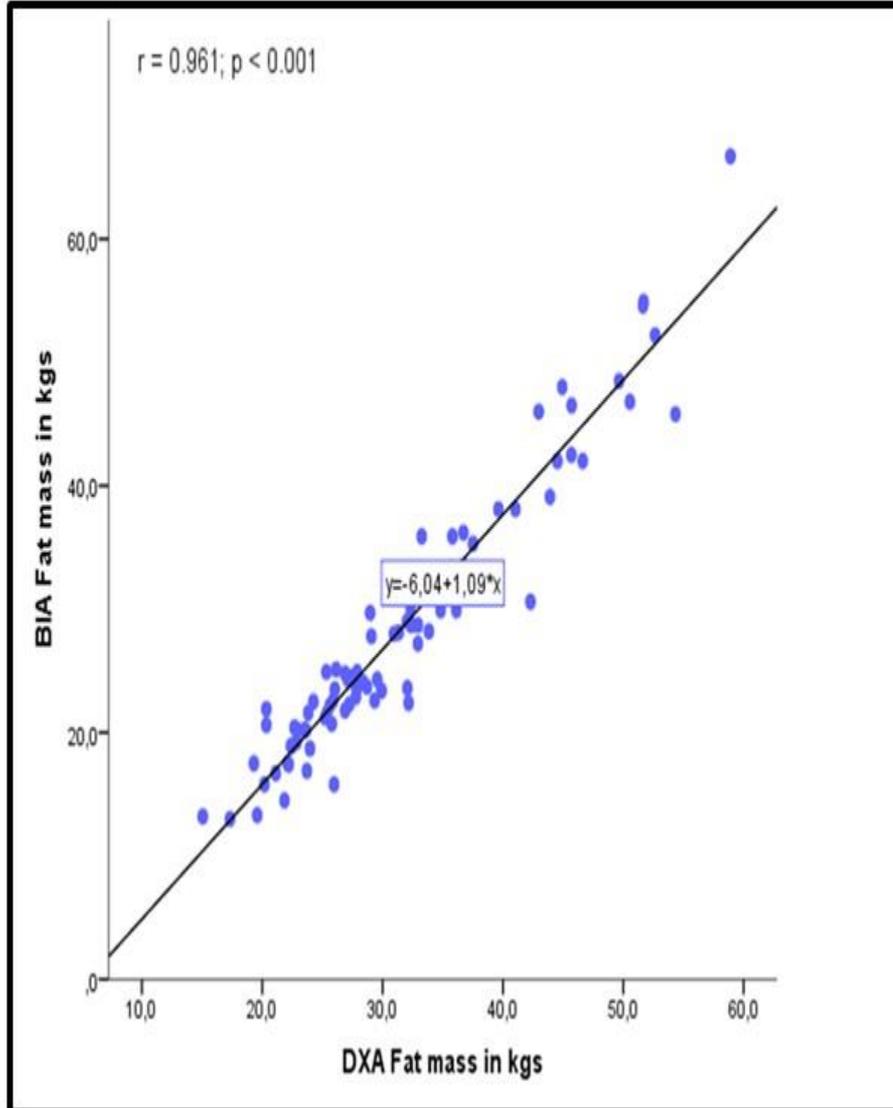
Weight +
Height

Hologic-DXA

BIA-ACC

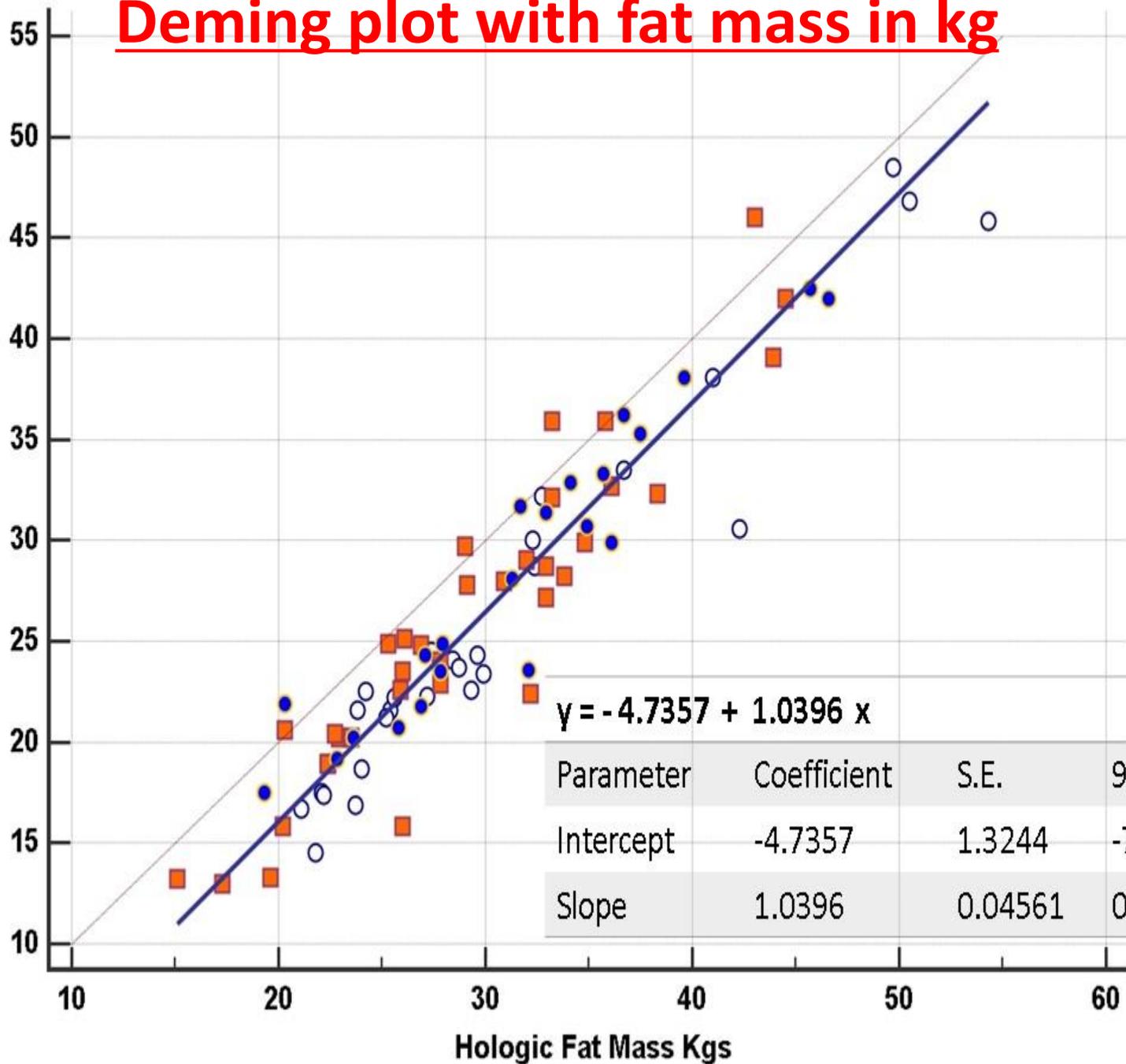


Pearson's correlation co-efficient for BIA and DXA Fat Mass in kg & as Body Weight %



Deming plot with fat mass in kg

BIA-ACC Fat Mass Kgs



$$y = -4.7357 + 1.0396x$$

Parameter	Coefficient	S.E.	95% CI
Intercept	-4.7357	1.3244	-7.3699 to -2.1015
Slope	1.0396	0.04561	0.9489 to 1.1303

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

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**
-

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.

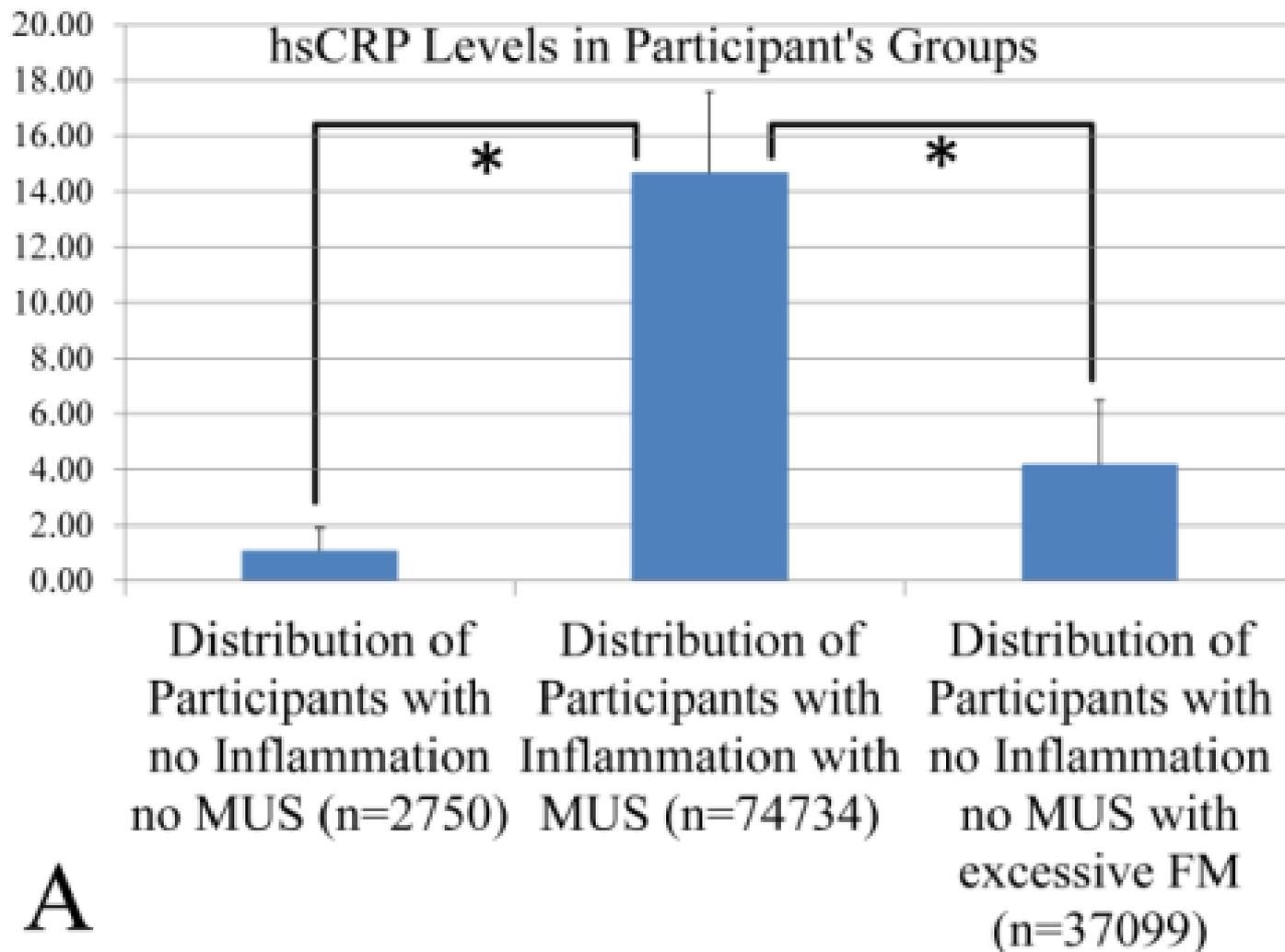
***Presence of MUS was defined as a positive answer to more than 3 of the above 14 questions.**

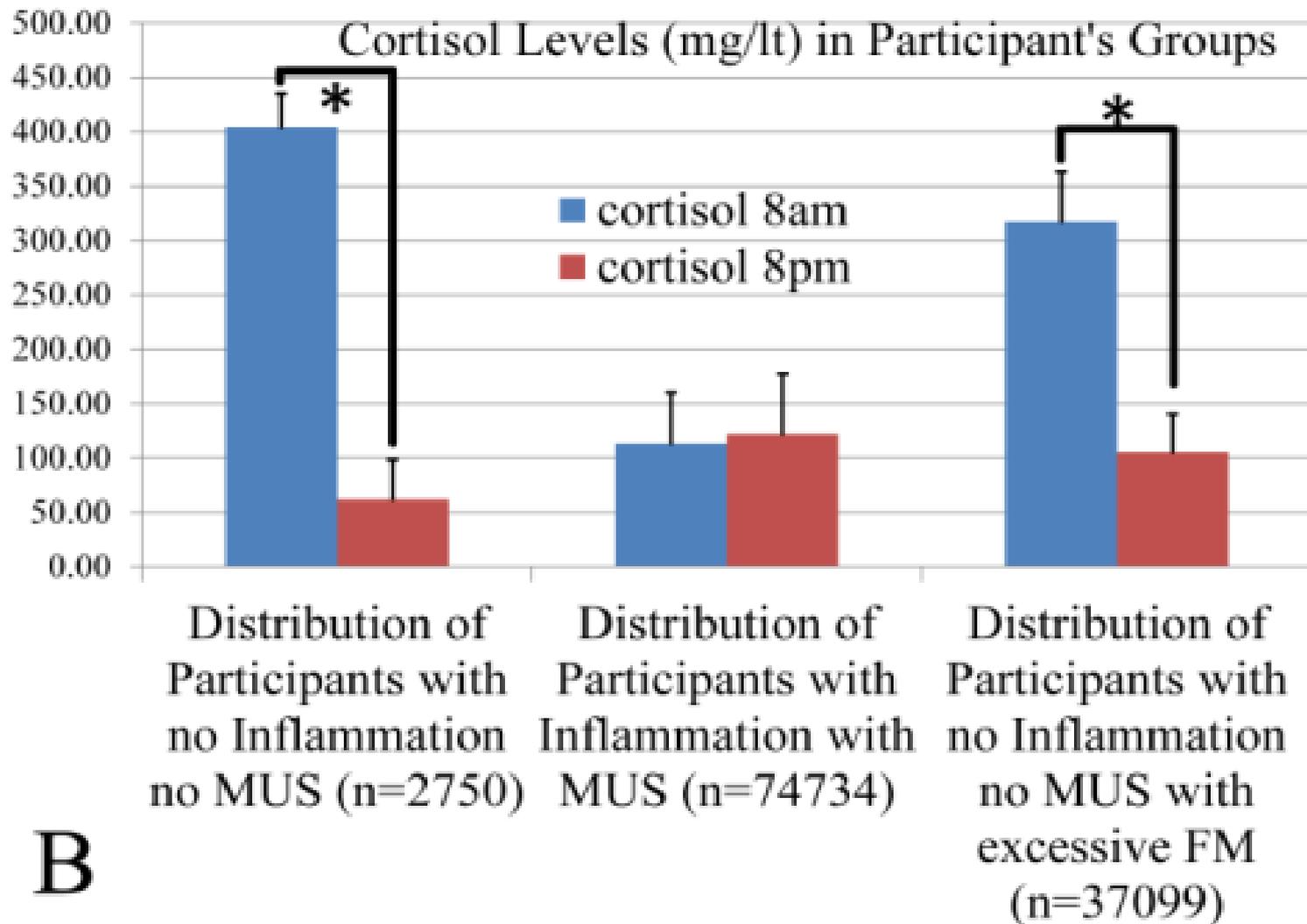
Evaluation included:

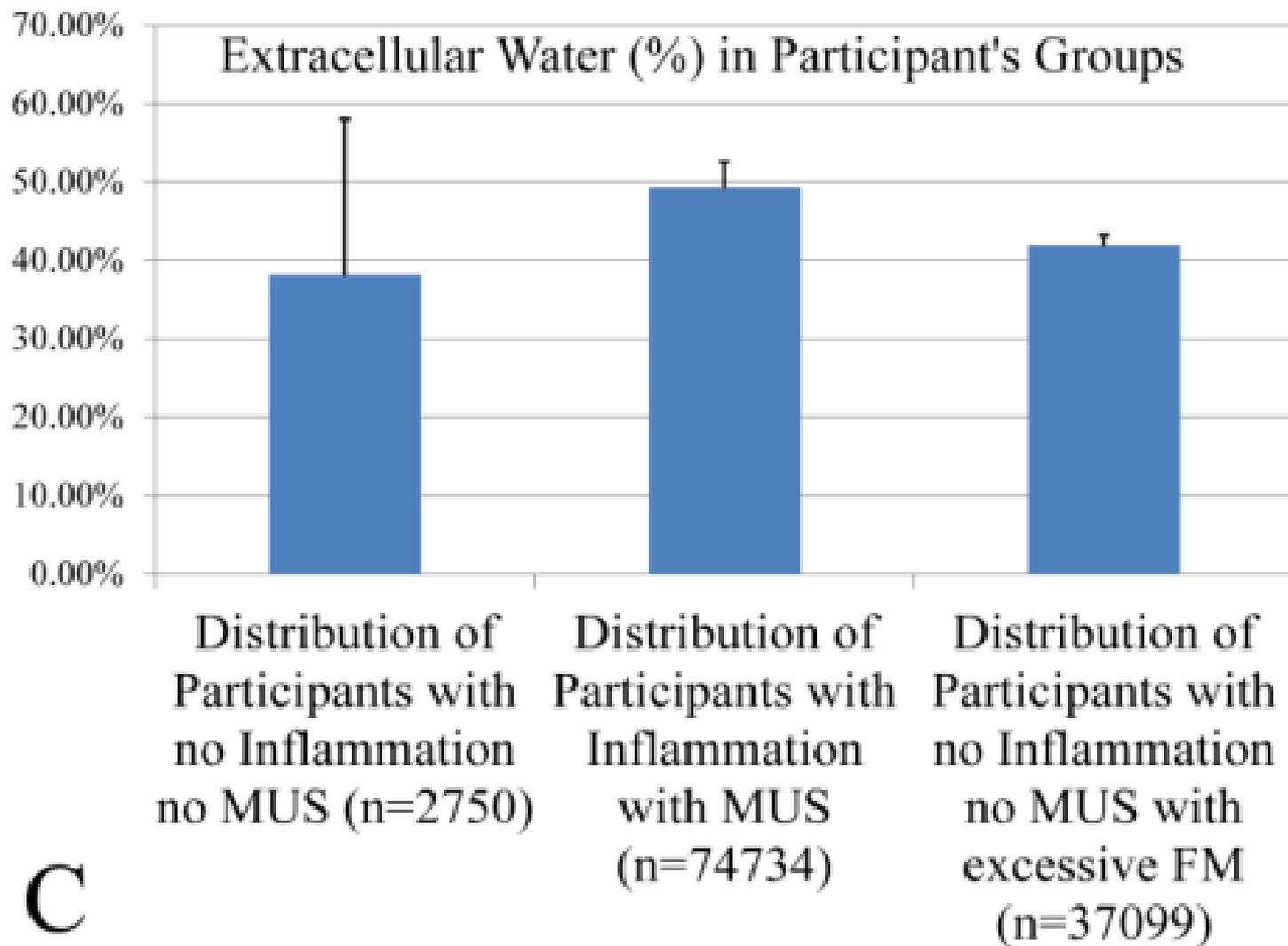
- **Advanced Body Composition Analysis by BIA-ACC**
 - **Indices of Inflammation: hsCRP, Interleukin-6**
 - **Indices of Stress: am and pm Salivary Cortisol, Delta-Cortisol (am-pm)**
-

Grouping:

- 10 416 **lean subjects** (8810 males) **with no MUS** (Group A),
 - 58 710 **lean subjects** (5581 males) **with MUS** (Group B), and
 - 30 445 **overweight/obese subjects** (15133 males) **with no MUS** (Group C).
-







Healthy Overweight/Obese Youth: Early Osteosarcopenic Obesity Features.

Stefanaki C, Peppas 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.

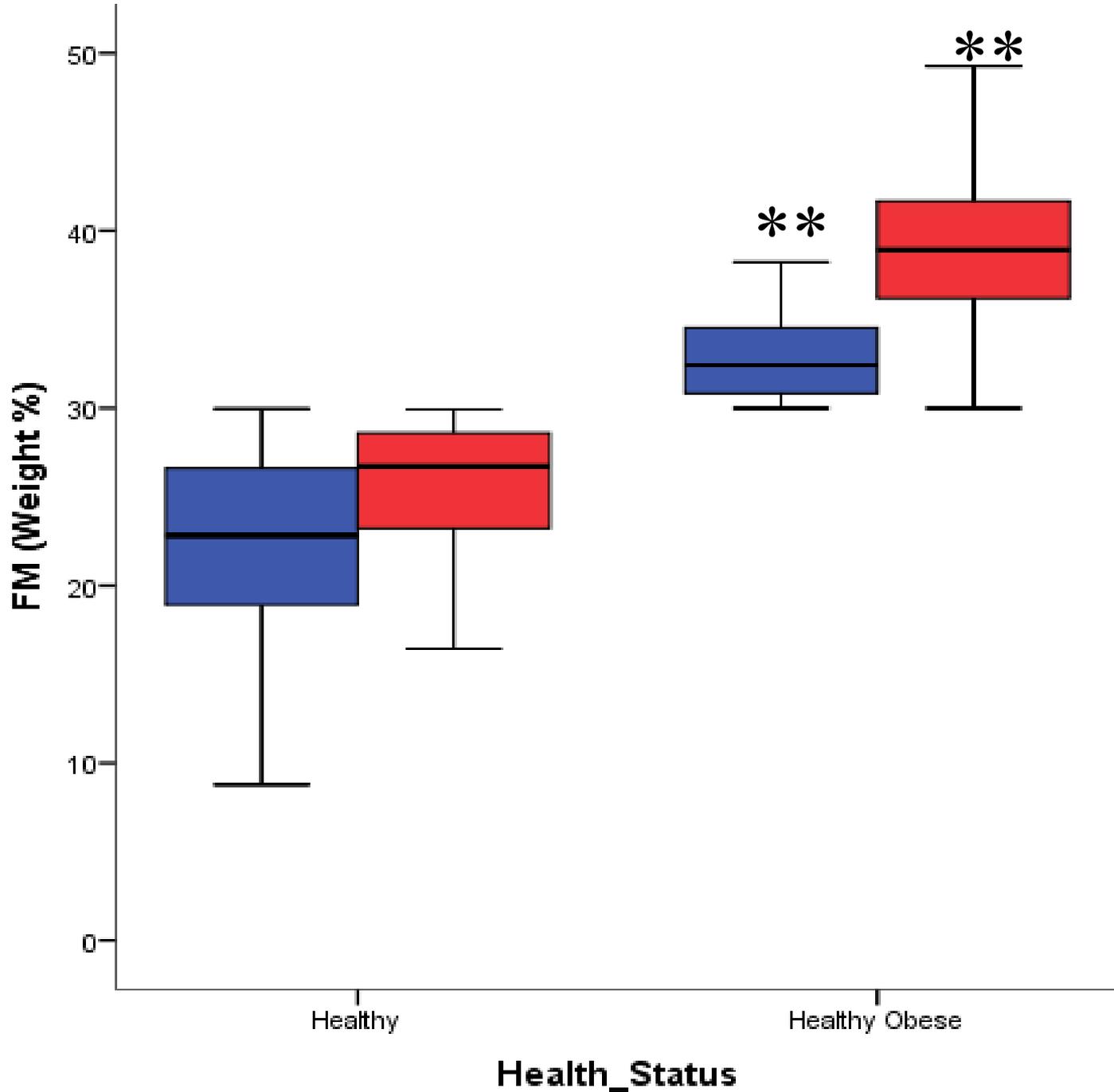
The healthy lean group included 1072 participants [900 males (84%) and 172 females (16%)].

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

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

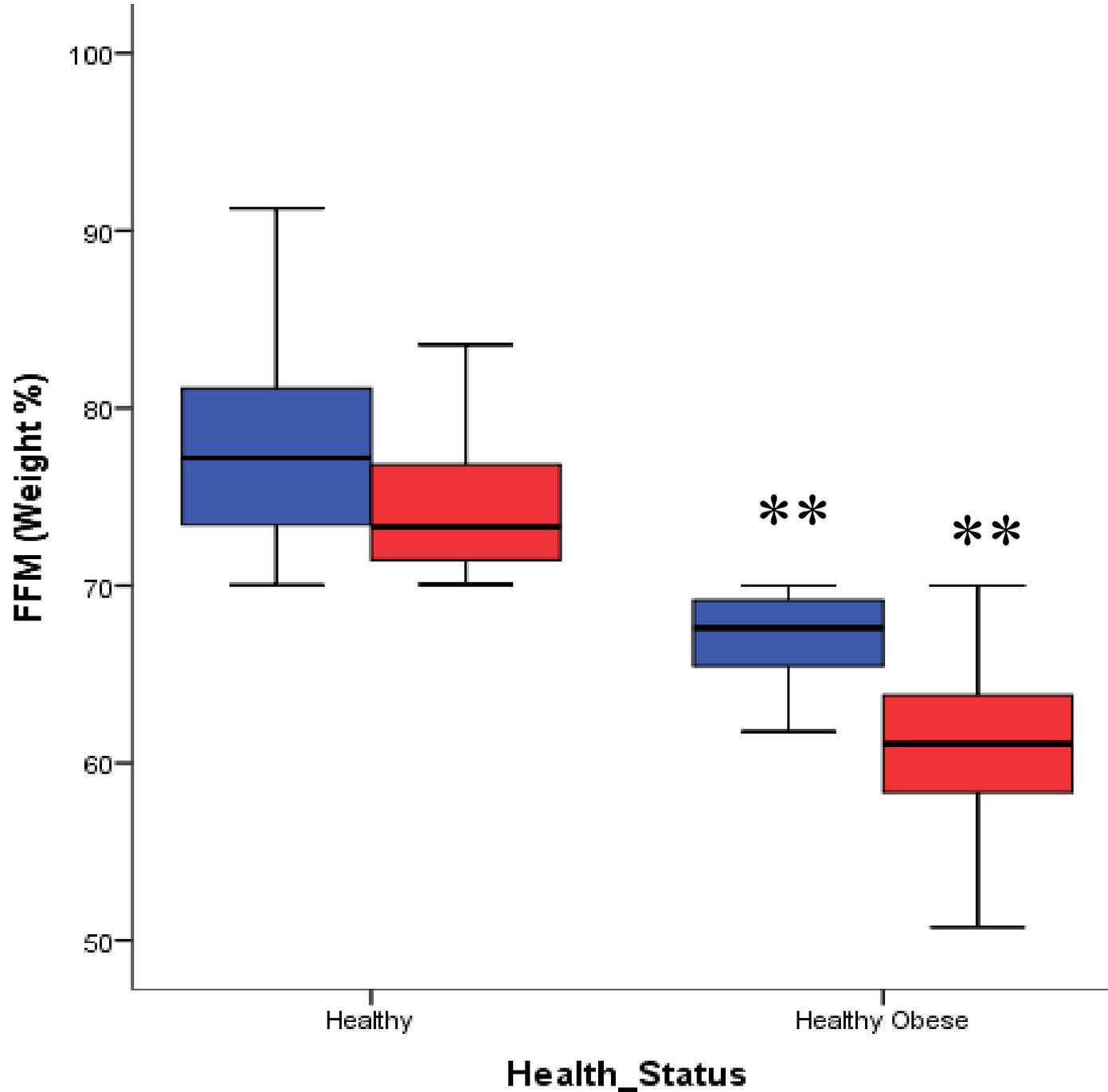
The healthy lean group included 1072 participants [900 males (84%) and 172 females (16%)].

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



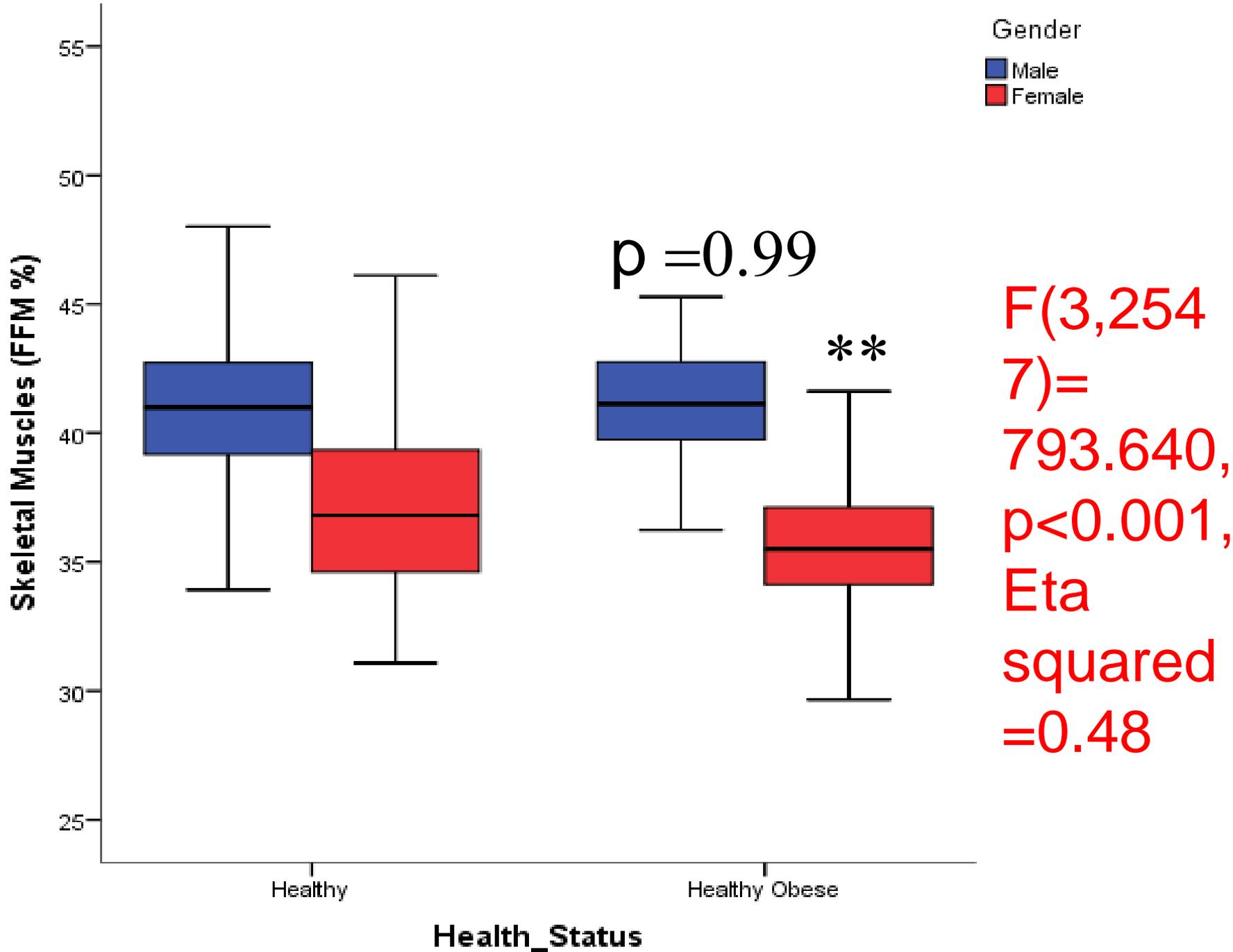
Gender
Male
Female

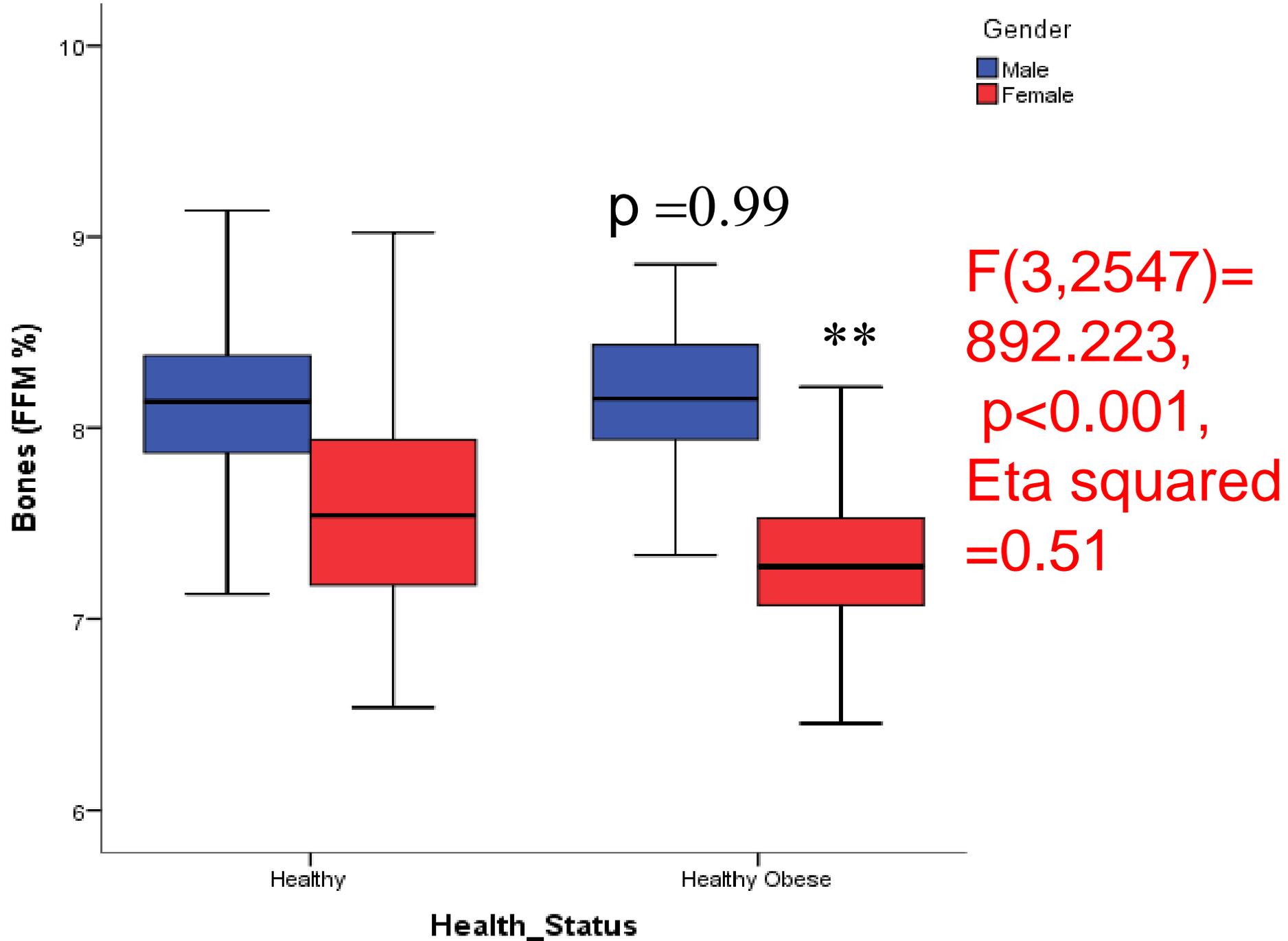
$F(3,2547)$
=
2824.545,
 $p < 0.001$,
Eta
squared
= 0.76

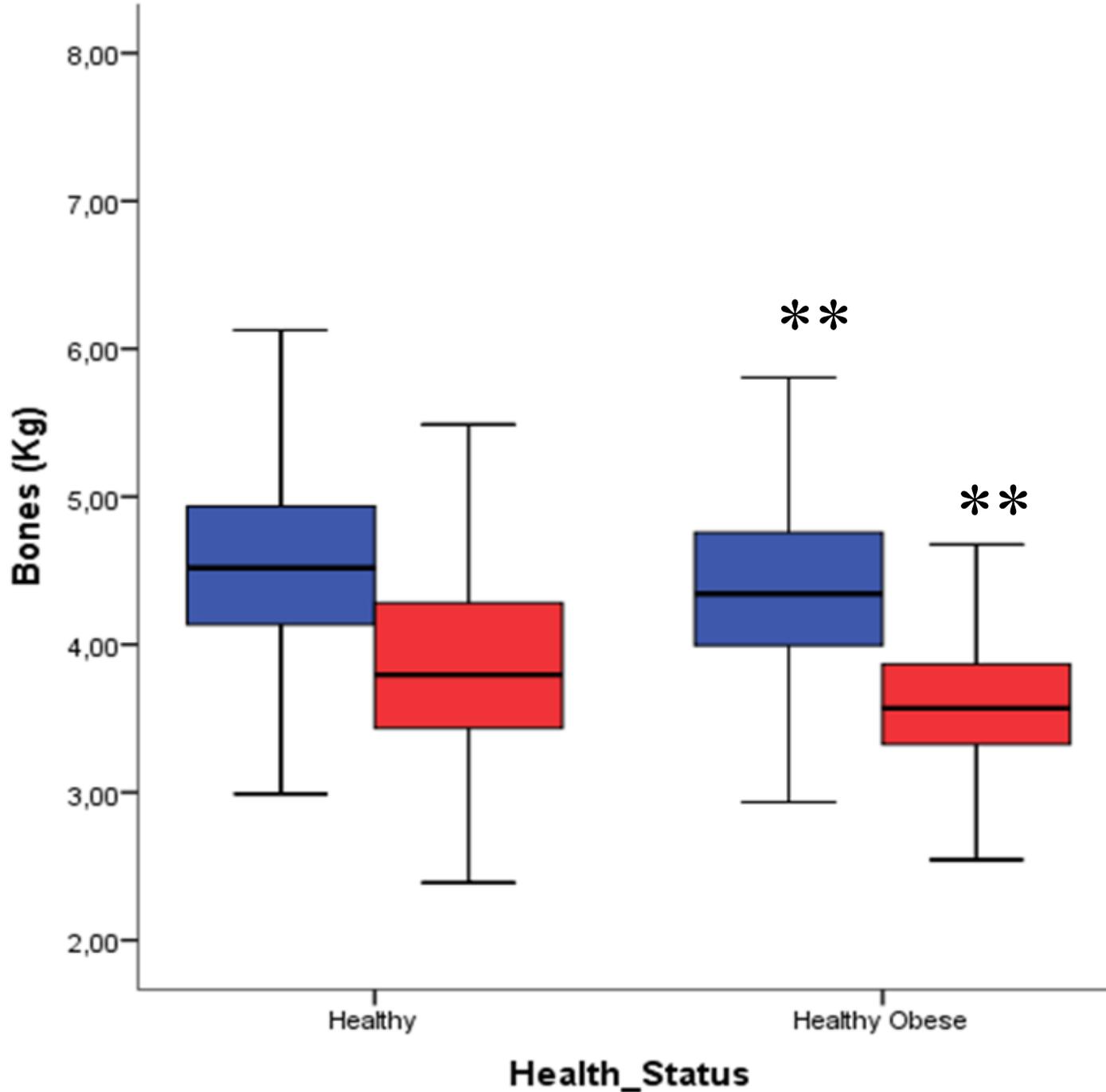


Gender
Male
Female

F(3,254
7)=
2824.54
5,
p<0.001,
Eta
squared
=0.76

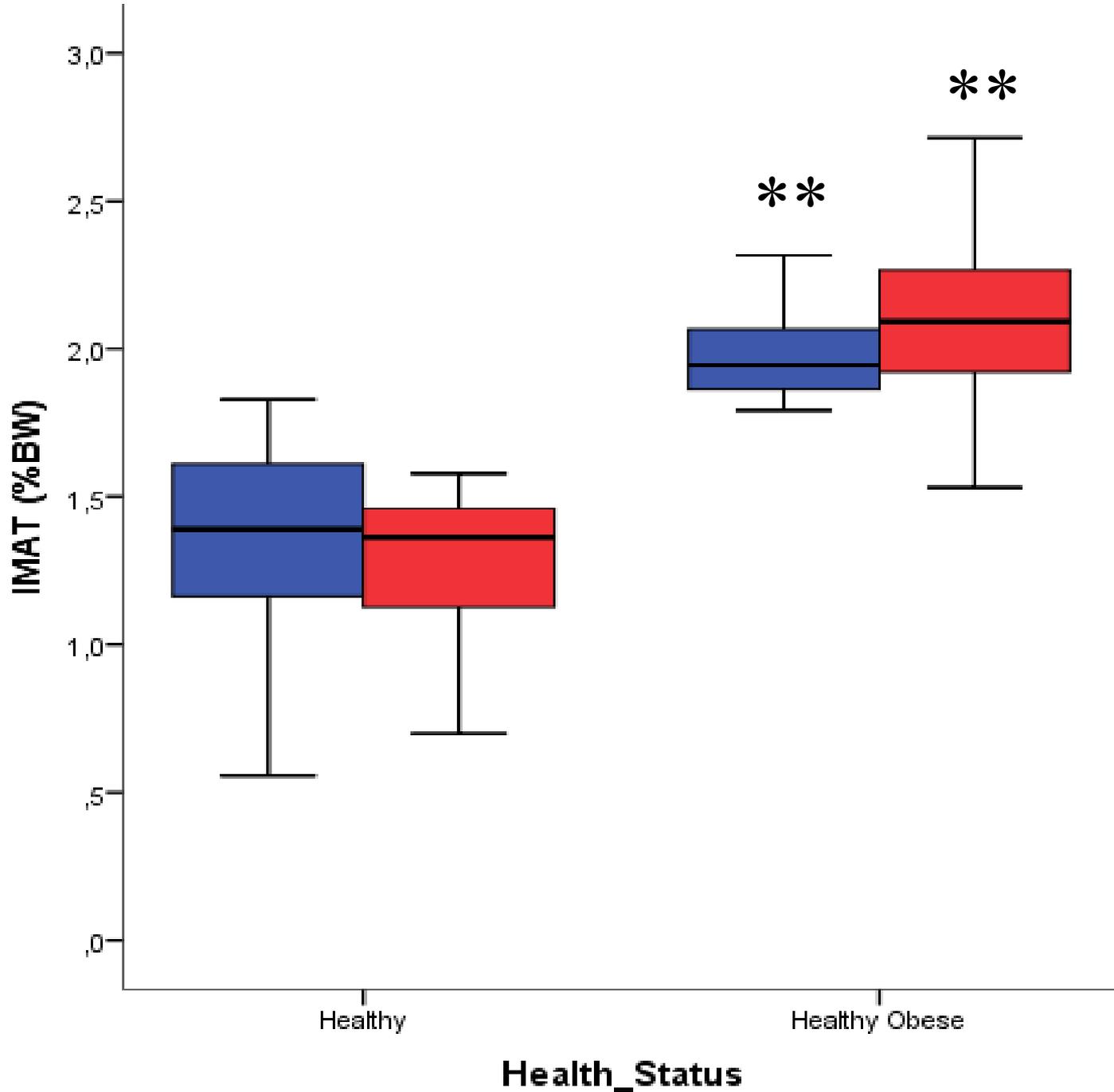






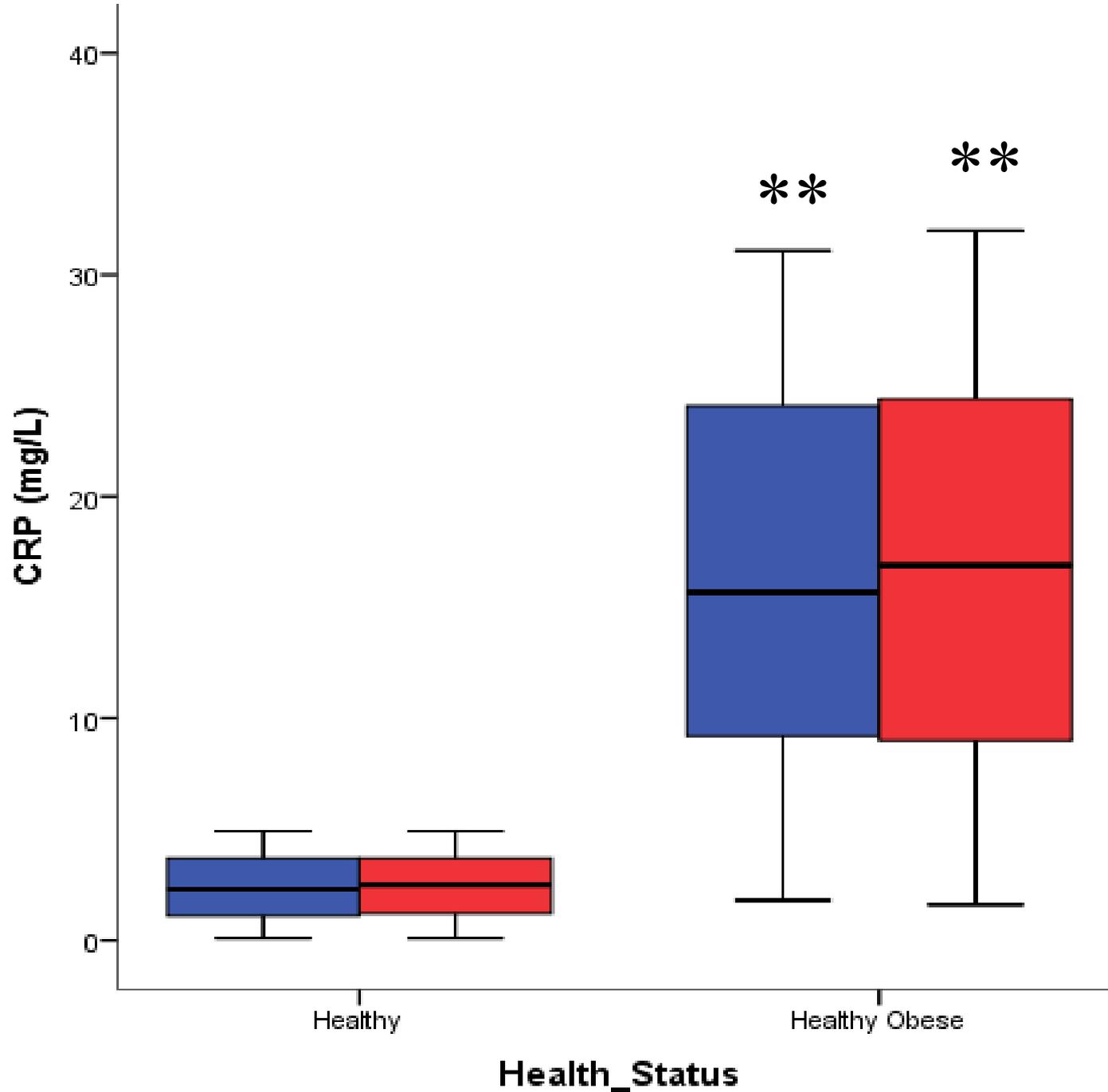
Gender
 Male
 Female

F(3,25
 47)=
 554.67
 9,
 p<0.00
 1,
 Eta
 square
 d=0.39

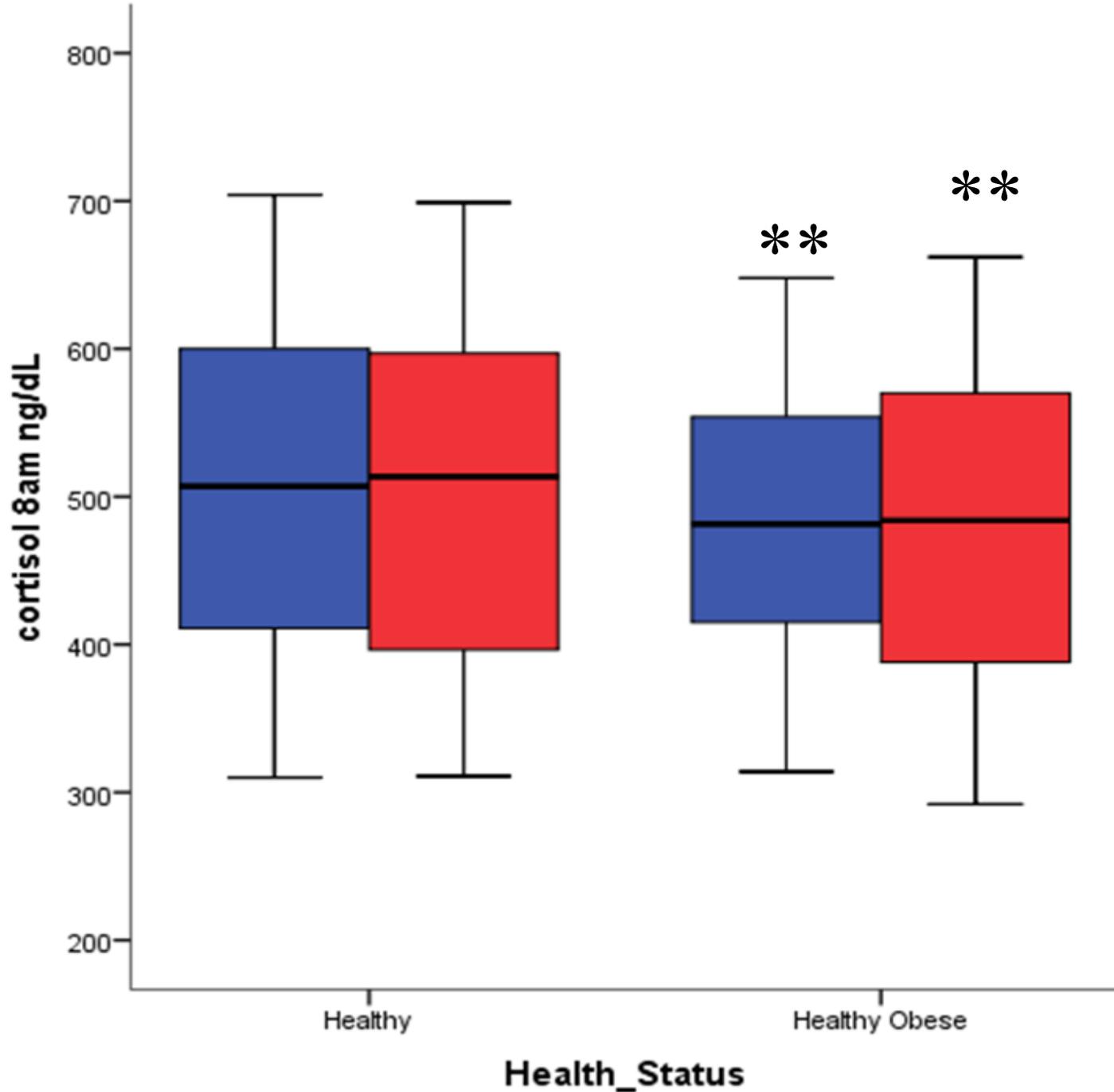


Gender
Male
Female

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

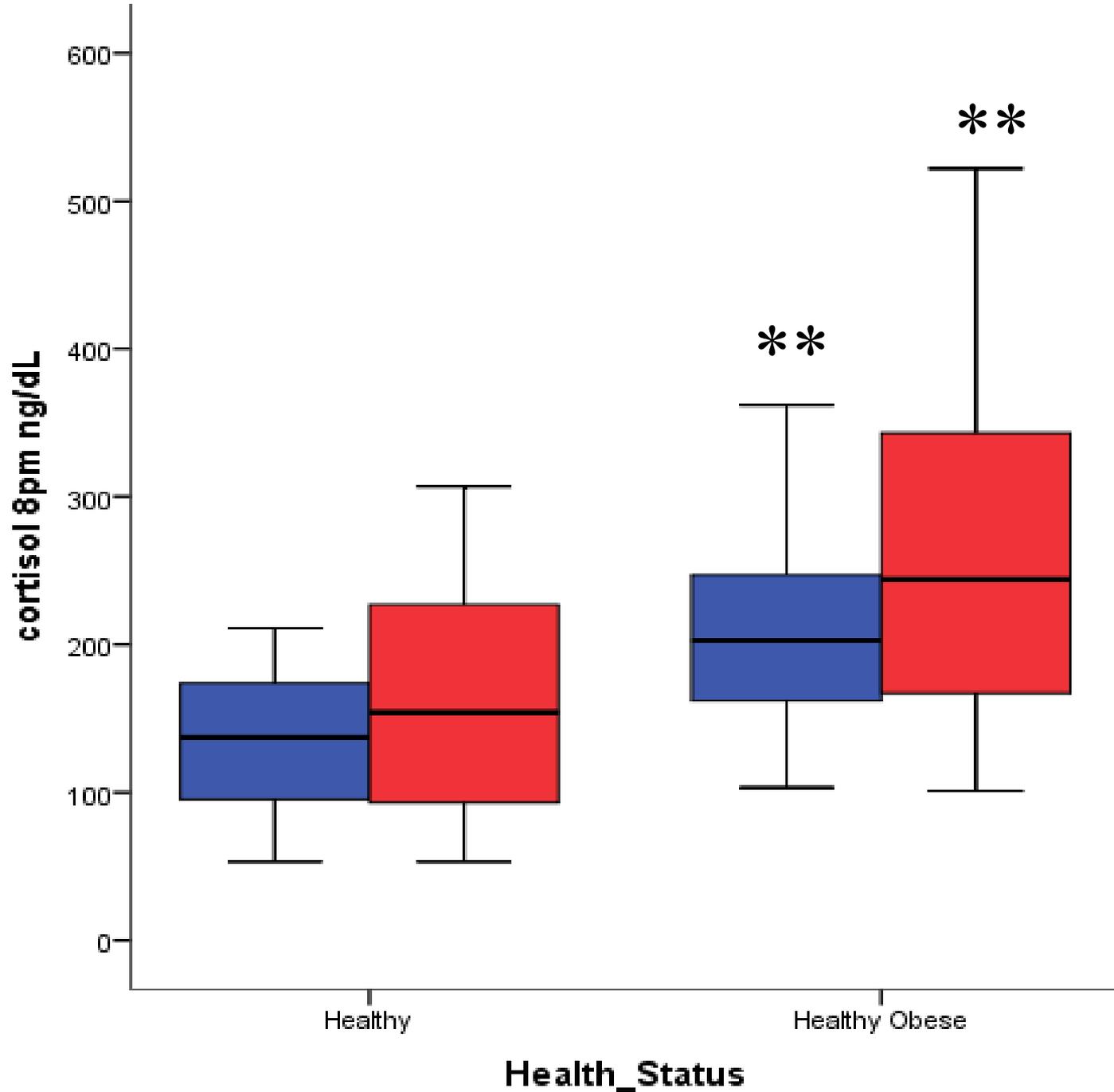


$F(3,2547)$
=
935.110,
 $p < 0.001$,
Eta
squared
= 0.52



Gender
Male
Female

$F(3,2547) = 10.901, p < 0.001, \text{Eta squared} = 0.012$



Gender
Male
Female

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

CONCLUSIONS

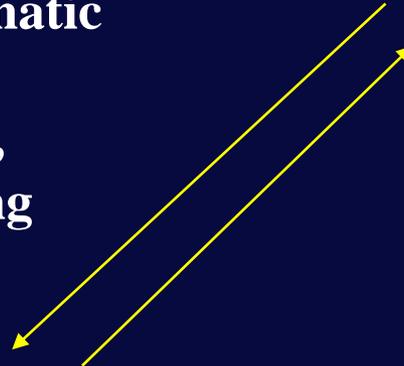
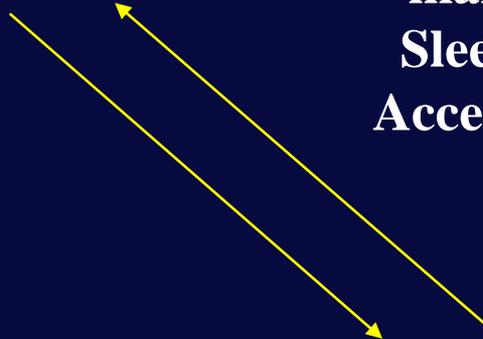
- **Osteosarcopenic phenotype is common and** exists even in the young, suggesting early start of prevention and treatment.
- “Healthy” overweight or obese populations demonstrate:
 1. **Decreased bone mass;**
 2. **Decreased muscle mass;**
 3. **Increased hsCRP concentrations;**
 4. **Flattening of 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.**

**Social
conditions:
Inequality/D
ignity, etc.**

**Stress+
Inflammation**

**Psychologic and somatic
manifestations,
Sleep disorders,
Accelerated Aging**

**“Chronic Stress and Inflammation
Syndrome”
(CSIS)**



ENVIRONMENTAL STRESSORS

Starvation
Dehydration
Injurious agents-inflammations
Adversaries-anticipation
Adversaries-avoidance
Injury-minimization
Social bonding disruption

Embryogenesis
Maternal Stress
Perinatal Stress

Species vs. Individual

Evolution

Genetics

CNS complexity

Development

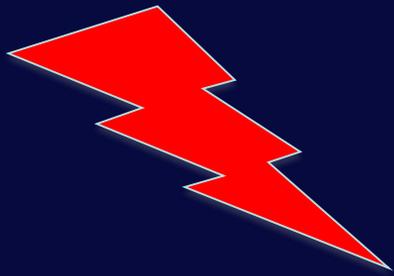
Epigenetics

CNS plasticity

Genotype

Epigenotype

Phenotype



Fetal Life

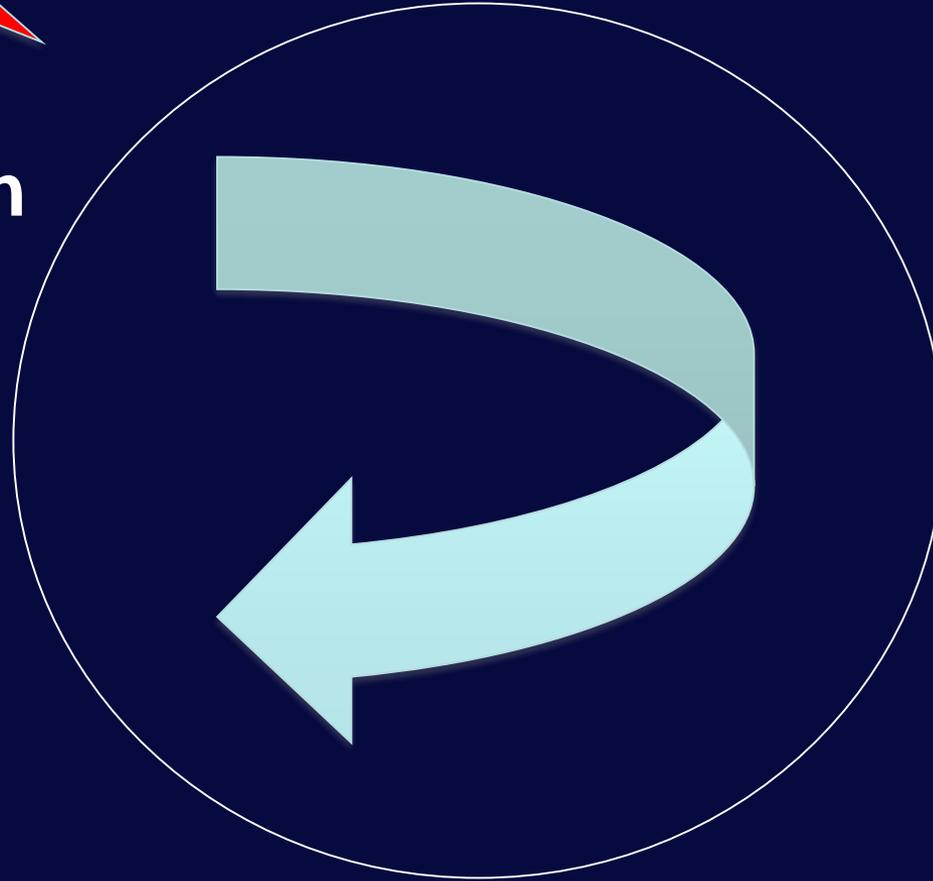
Conception

Childhood

Fertility

Puberty

Adulthood





ΕΠΙΚΤΗΤΟΥ
 ΕΓΧΕΙΡΙΔΙΟΝ.
 ΕΡΙΣΤΕΤΙ
 ENCHIRIDION.

ΚΕΦ. α.
CAP. I.

Πῶν ὄντων πῶς
 μὴ εἶναι ἐφ' ἡ-
 μῖν, πῶς ἡ σὺκ-
 ἐφ' ἡμῖν. * ἐφ'
 ἡμῖν μὴ ὑποβολήψις, ὄρ-
 μη, ὄρεξις, ἐκκλισις, κτθ'
 ἐνὶ λόγῳ, ὅσα ἡμέτερα
 ἔργα. ὁ σὺκ ἐφ' ἡμῖν δὲ, τὸ
 σῶμα, * ἡ κτῆσις, δόξα,
 δόξαί. κθ' ἐνὶ λόγῳ, ὅσα
 εἰς ἡμέτερα ἔργα.

Res quaedam in
 potestate no-
 stra sunt, quae-
 dam non sunt.
 In nostra potestate est opi-
 nio, appetitio, desiderium,
 averlatio; & ut uno com-
 plectar verbo, quaelibet
 nostrae actiones. Nostri
 arbitrii non sunt corpus,
 pecunia, gloria, imperia :
 ad summam, ea quae ipsi
 non agimus, omnia.

1 Tribuitur hoc Enchiridion Epi-
 ceto, quoniam ipse id non fecit
 scribit, sed Actianus, qui de uberioribus
 in id commentarium edidit, quo dis-
 putationes Epicteti plenius profequi-
 tur. Testatur id Simplicius in prefa-
 tione commentarii ad hunc libellum
 hisce verbis: τὸ βιβλίον τῶν τῶ
 Ἐπικτητοῦ ἐστὶν ἐπισημασμένον
 ἡμῖν, καὶ τῶν αὐτῶν ἐπισημασμένον ὁ
 Ἀρριανὸς, τὸ καὶ ἐπισημασμένον ὁ
 καὶ τῶν ἐφ' ἡμῖν ἐστὶν ἐπισημασμένον
 ὡς τὸ ἐπισημασμένον. Hoc est:

Idem Arrianus & hunc libellum, quod
 Enchiridion inscribitur composuit, de-
 ditque ex Epicteti discipulatus phi-
 losophia lecto maxime idoneo ac neces-
 sario, & animas vehementissime per-
 moventibus. Seneca.

2 Non solum pagio Graecis hoc
 nomine vocatur, sed etiam quid-
 quid ad manum est, & in usum
 promptum & obvium, ut instru-
 menta quaelibet ac vasa hygiea, &c.
 quae parata & in manu semper
 gestanda. Hesychius, Ἐγχειρίδιον,
 σκεῦος ἐργασίας σκεῦος, ἢ γυναικεί-
 ον.

□ **Νάφε και μέμνασο απιστείν'**
**"Be equanimous and remember
 not to believe easily"**