



University of Modena and Reggio Emilia, Italy

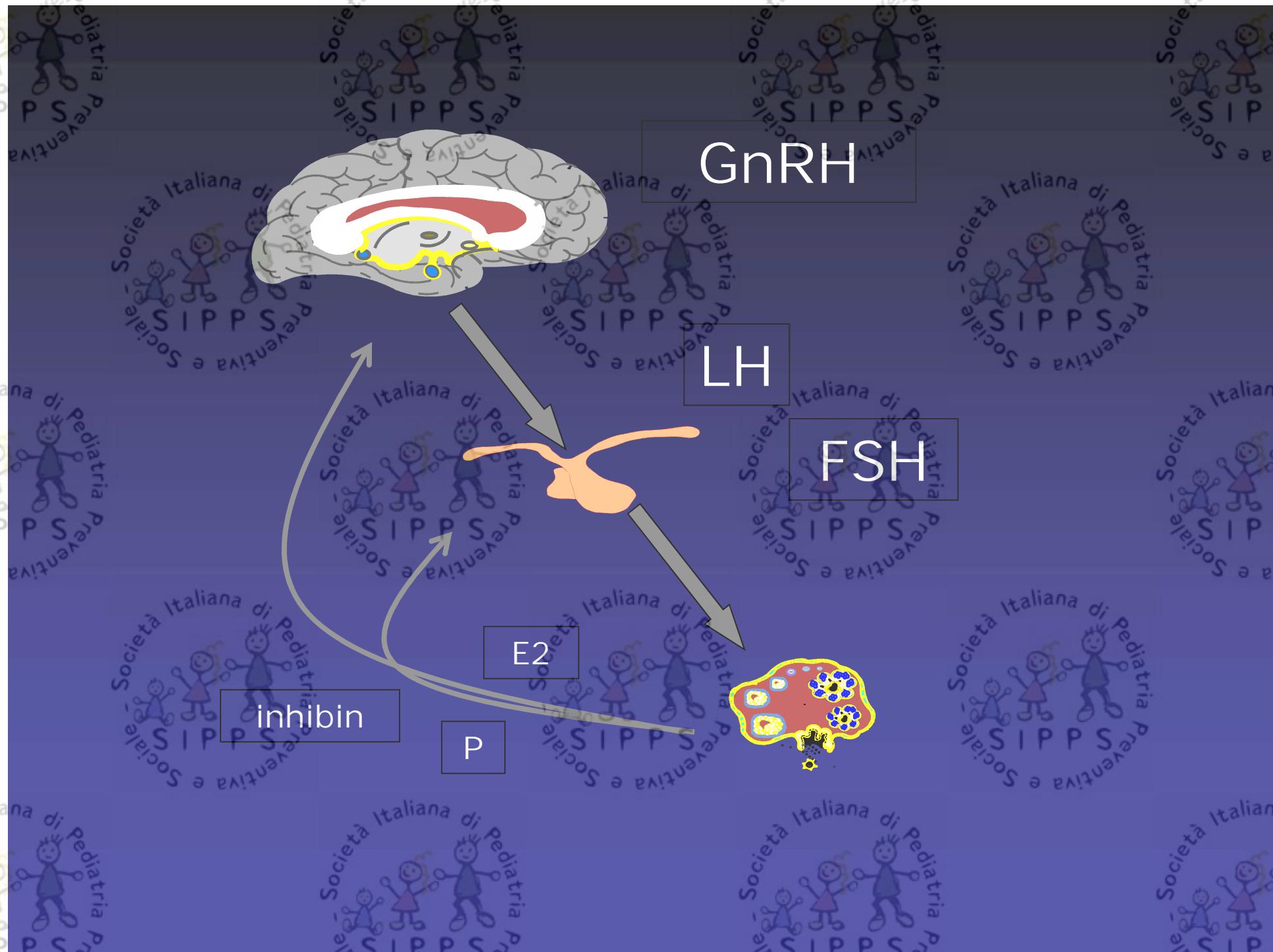


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Gynecological Endocrinology Center

**Effetti delle basse dosi di estrogeni nei quadri di
amenorrea ipotalamica**



GnRH driving clock

**Hyperandrogenism
Thyroid diseases
Adrenal diseases
Hyper PRL**

**Metabolic diseases
Obesity
Weight-loss
Undernutrition
Systemic diseases**

FSH is less affected than LH

**nurotransmitters
neuromodulators
neuropeptides**

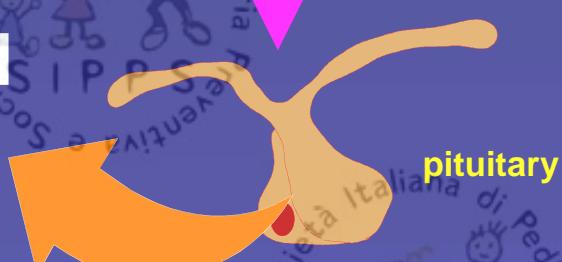


**GnRH
secreting neurons
intrinsic secretion**

hypothalamus

LH

FSH



?? differential control ??

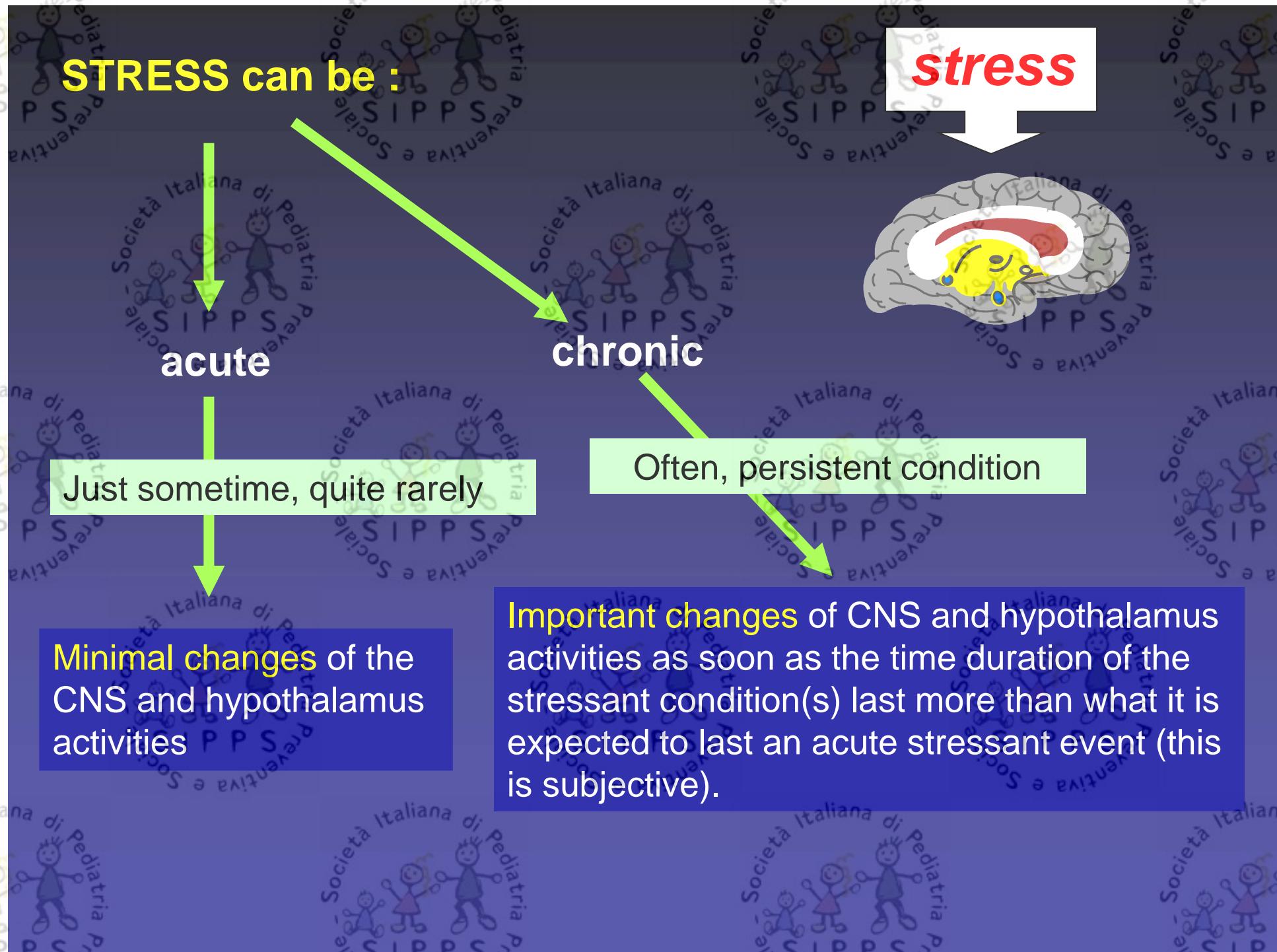
Hypothalamic amenorrhea

- No systemic causal factors
- No CNS disease/lesion (tumors, trauma)
- No endocrine diseases.

Patients affected by HA are usually characterized by two possible situations:

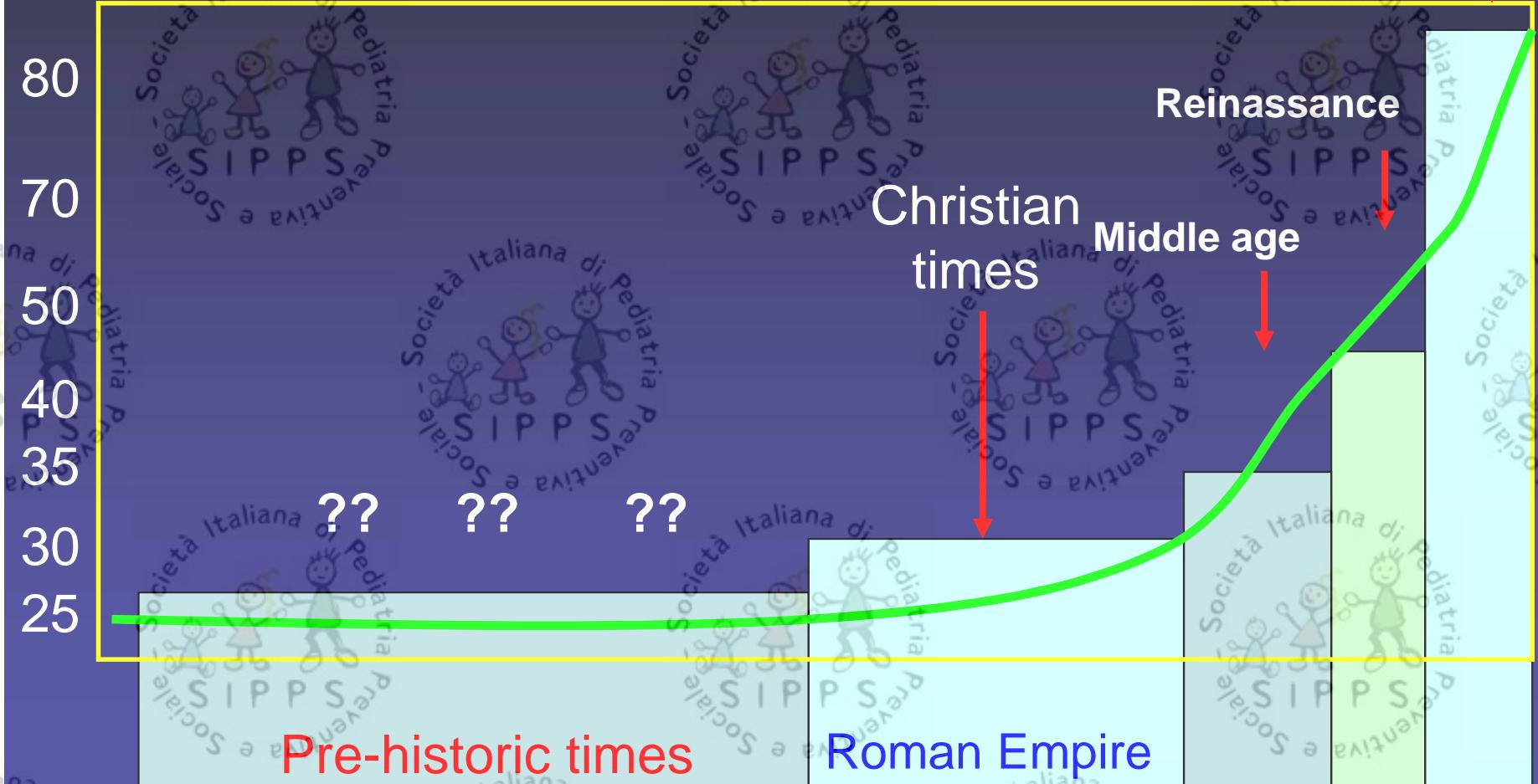
HYPOGONADOTROPINISM (LH \leq 3 mIU/ml)

NORMOGONADOTROPINISM (LH $>$ 3 mIU/ml)



Life was quite difficult in ancient times !

Yrs of age (life expectancy)



The main problems were:

- To find food and/or to survive up to the moment food was “in hands”
- In case no food was available the search for food obliged also **long migrations**. A lot of energies were lost during such migrations and for the “human body” those were really stressant days !

Search for food created the conditions for

- Severe energy consumption
- Severe “dieting”
- Psychological stress (fear to be eaten by wild animals or to die for accident or for the fasting)
- Reduction of rest and sleep intervals increased the psychological stress

Weight control & dieting

Fitness and training

Excess in working activity

Familiar problems

Psychological weakness

All these factors are able to interfere with a lot of CNS activity and with many of the homeostatic controls of hypothalamus resulting in the induction of a temptative solution through

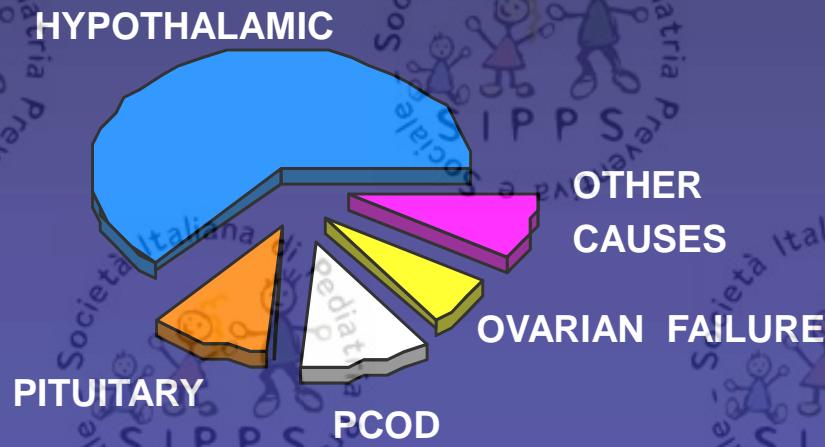
STRESS

NOTE:

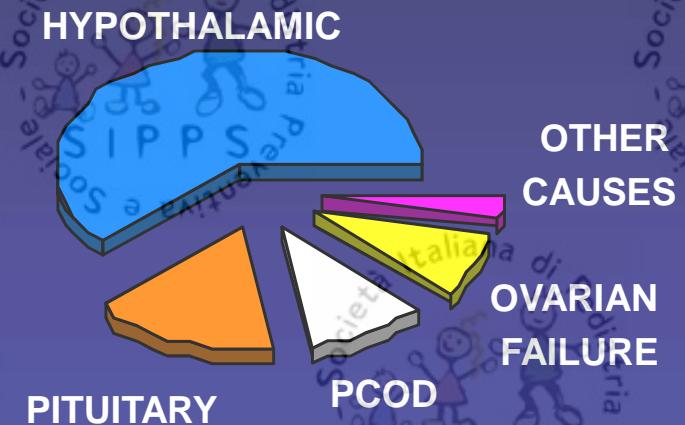
STRESS means a specific adaptive response to adverse external stimuli. Such response usually activates endogenous biochemical synthesis and secretions of neurotransmitters, neuromodulators and neuropeptides and, obviously, there is the activation/modulation of the many pathways they control and/or modulate.

ETIOLOGY OF MENSTRUAL DISORDERS IN FERTILE WOMEN

AGE 12-19

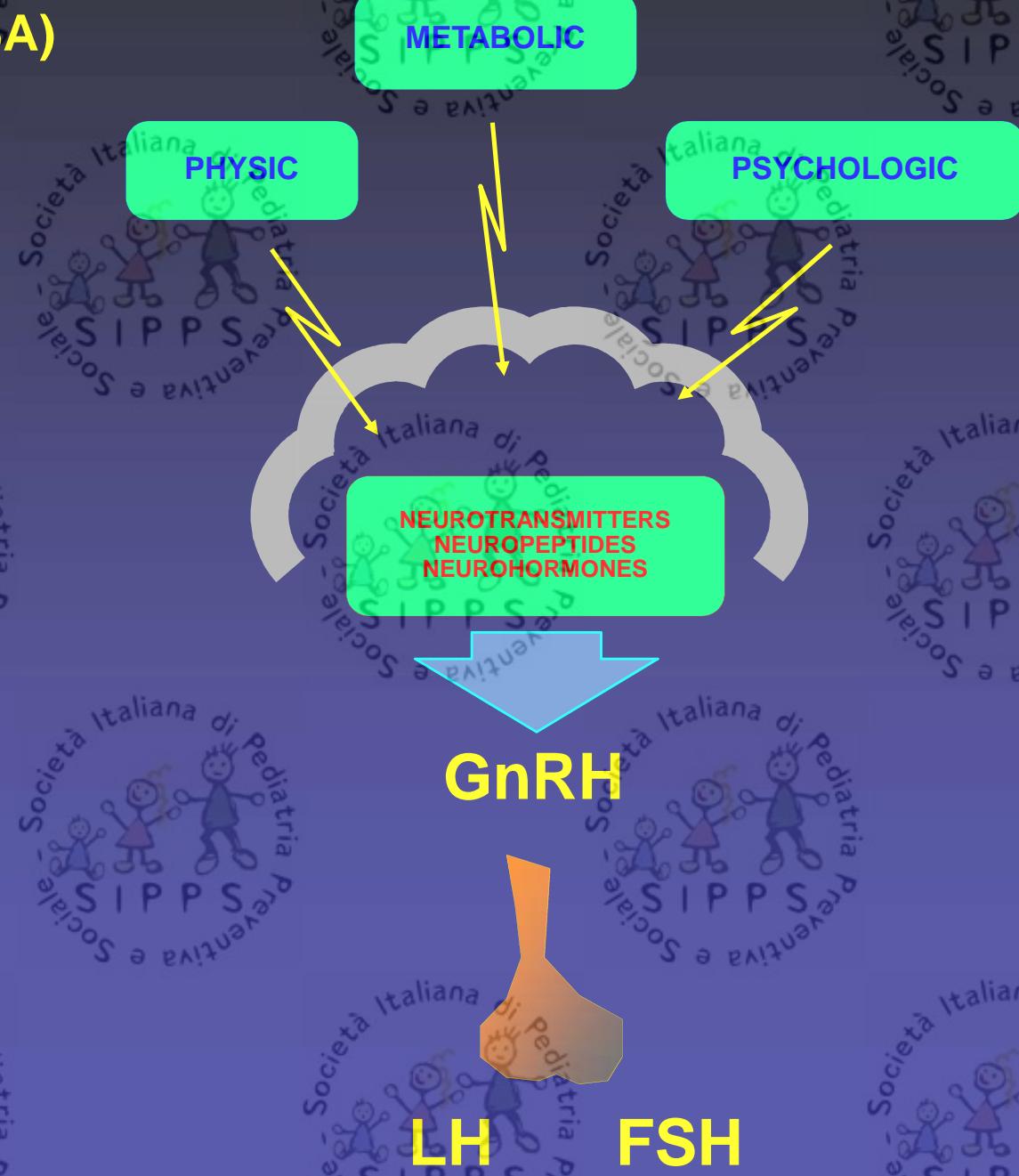


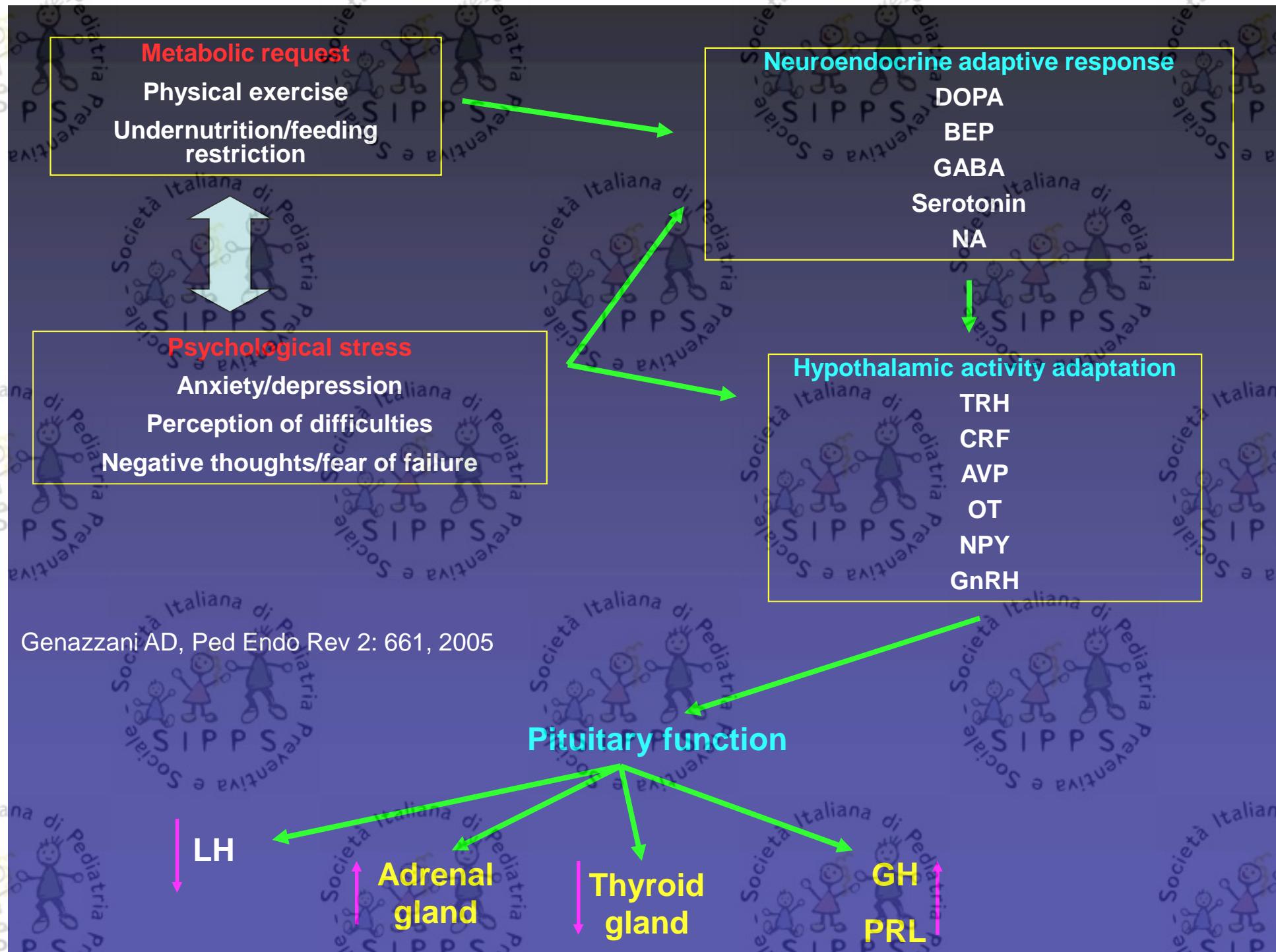
AGE 20-39



Hypothalamic amenorrhea (up to ANOREXIA NERVOSA)

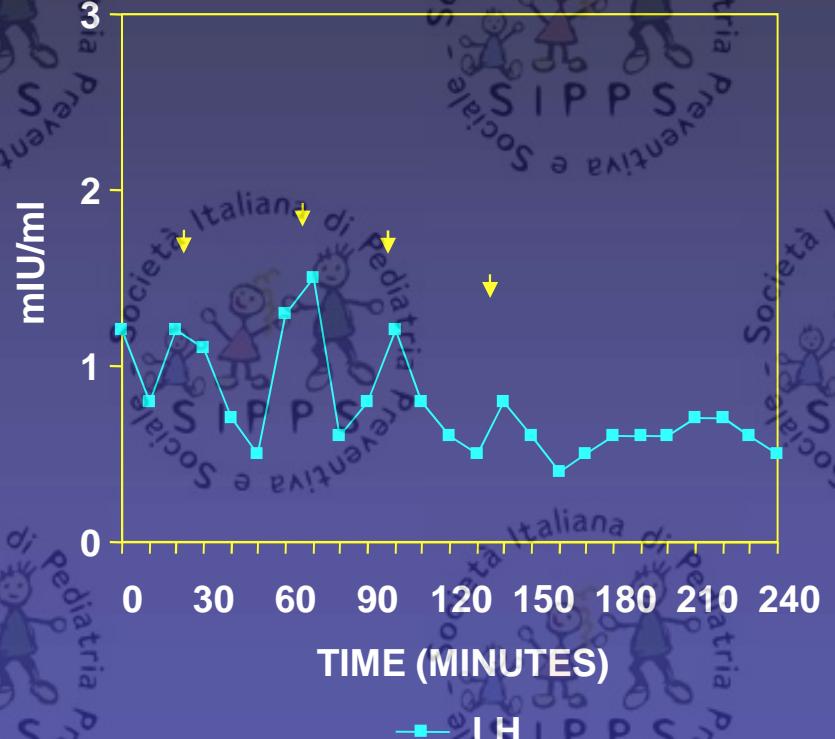
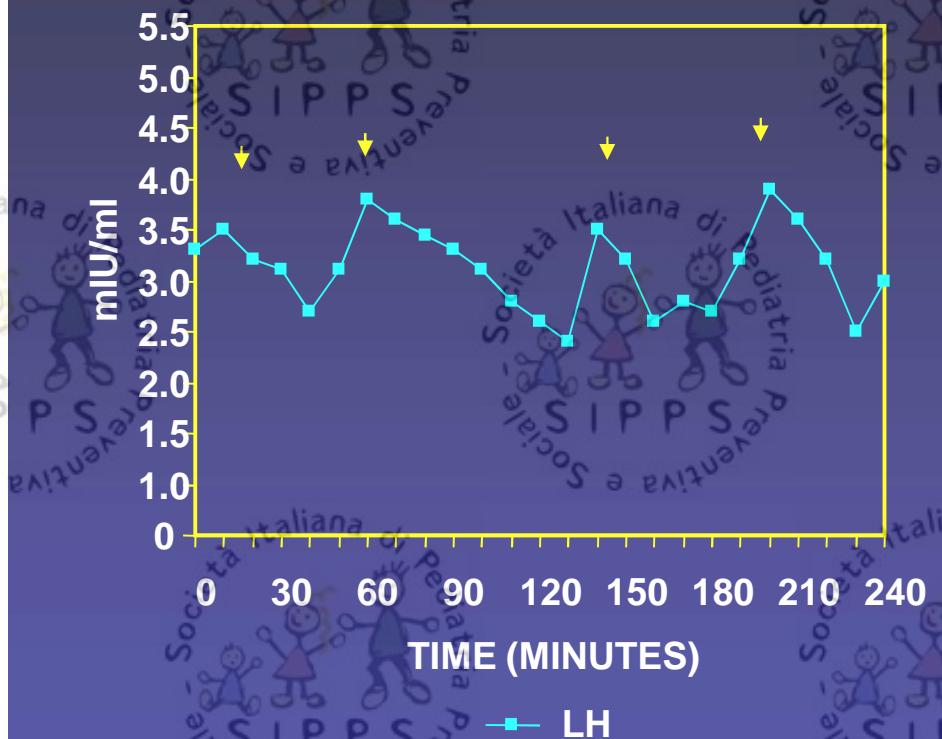
- = ▲ anemia
- = ▲ leucopenia
- = ▲ GOT, GPT
- = ▲ glicemia
- = ▲ total cholesterol
- = ▲ amilase
- = ▲ β -carotene
- = ▲ CCK
- = ▲ cortisol
- = ▲ LH
- = FSH
- = PRL
- = fT3
- = TSH
- = fT4
- = aldosterone
- = insulin



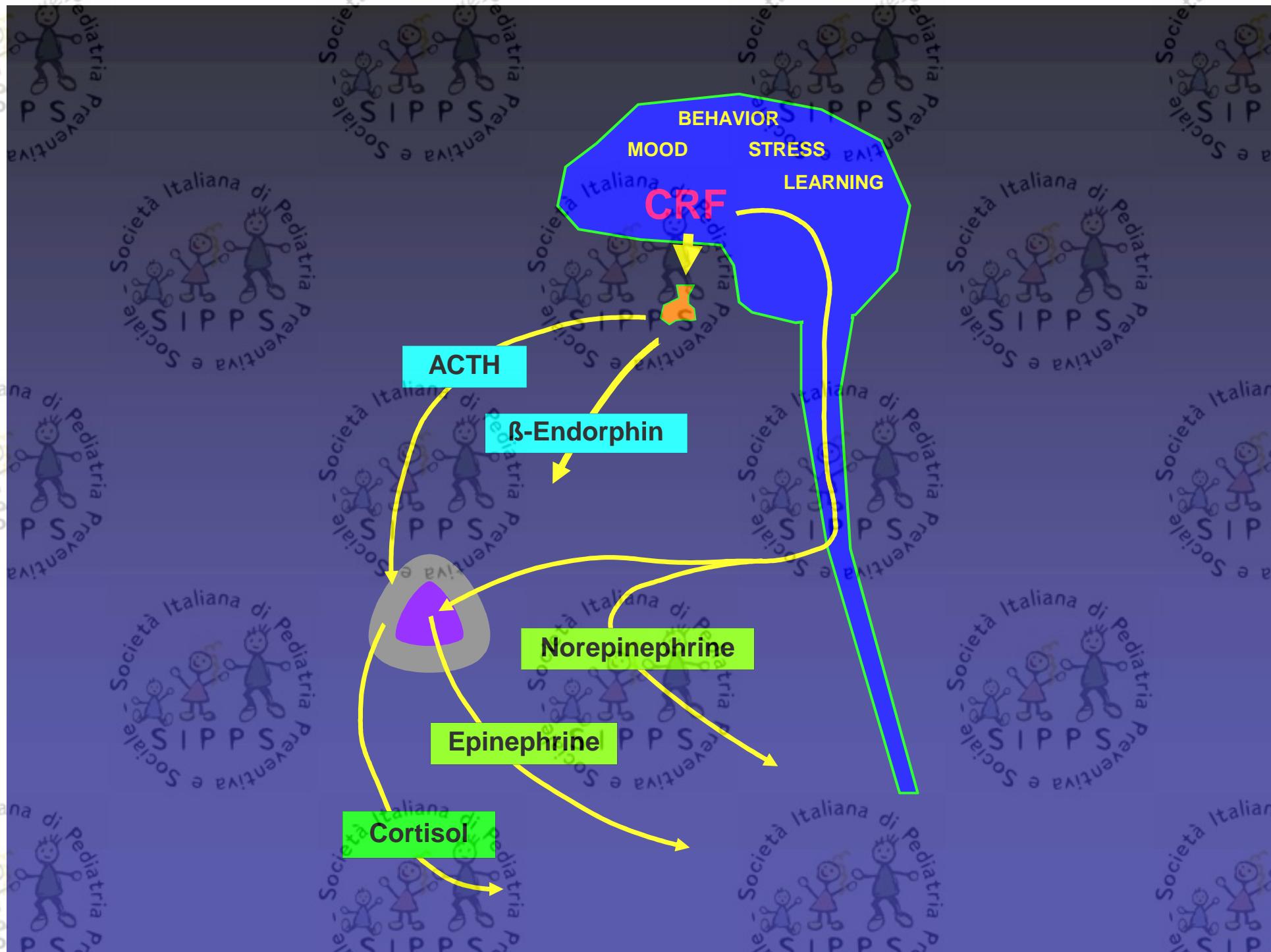


HYPOTHALAMIC AMENORRHEA

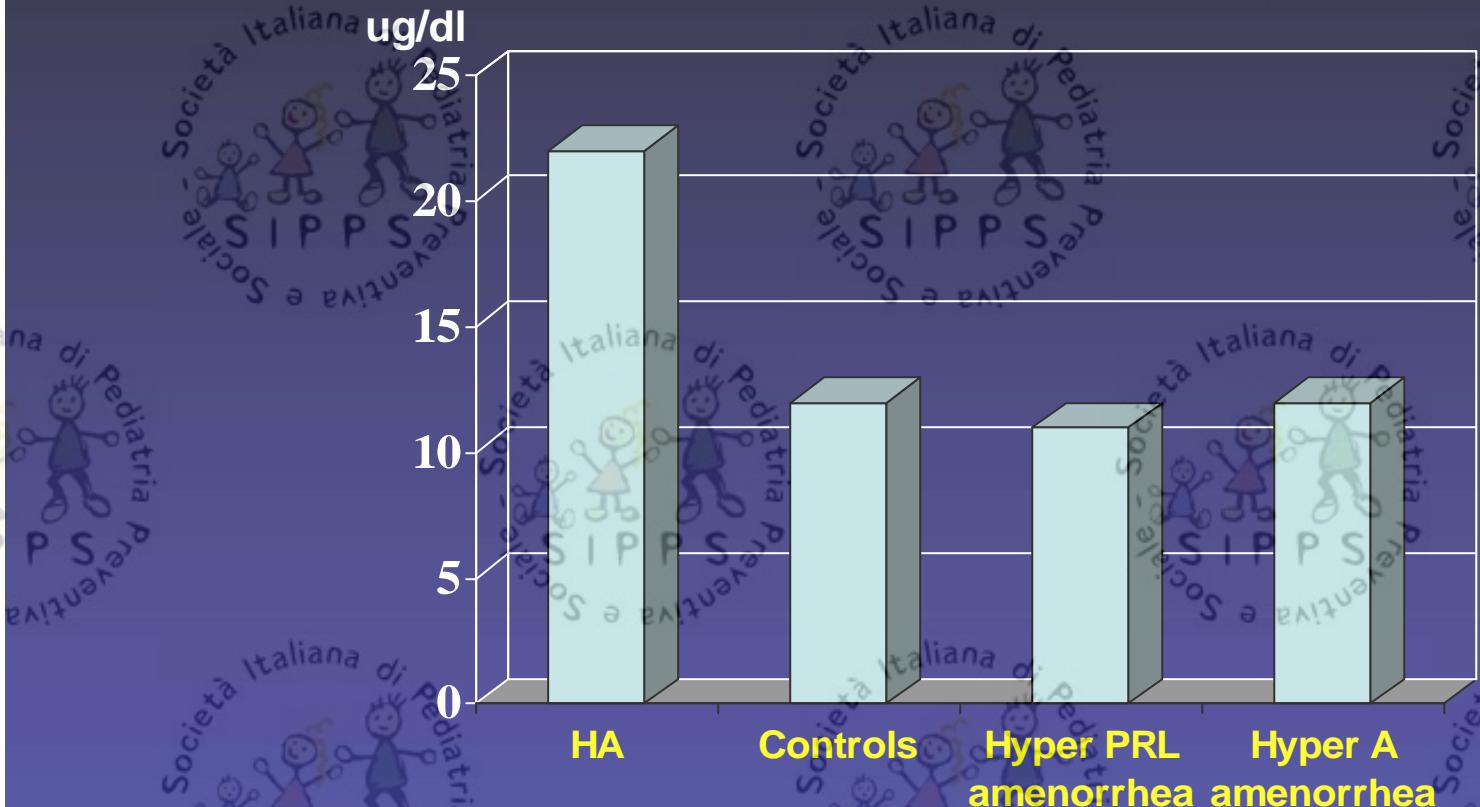
LH pulsatility study



Genazzani AD et al., Fertil Steril 54: 222, 1990



Hypercortisolemia and amenorrhea



Elevation of cortisol levels coincides with the occurrence of the “true” hypothalamic amenorrhea.

Genazzani AD, Ped Endo Rev 2: 661, 2005



TABLE II.—LIFE EVENTS DISTRIBUTION IN SECONDARY AMENORRHOEA ACCORDING TO ENDOCRINE DIAGNOSTIC SUBGROUPS

	Hypothalamic hypogonadotr. (N = 67)	Hypothalamic normogonadotr. (N = 33)	Hyperandrogenic (N = 31)	Control (N = 64)
Absent	27 (40.2%)	23 (69.6%)	22 (73.4%)	43 (67.1%)
Present	40 (59.8%)	10 (30.4%)	8 (26.6%)	21 (32.9%)

Overall chi-squared = 14.3; $p = 0.002$.

Hypothalamic amenorrhea

Whatever we might do has to act on:

- CNS peptides (opioids, CRF)
- Neurotransmitters, neuromodulators (DA, GABA, serotonin, etc.)
- Various hormones (cortisol, PRL, thyroid hormones)
- Feeding and/or energy balance (proteins, glucose, training)

Amenorrhea therapeutical strategies

Acetyl-L-Carnitine

Naltrexone cloridrate

Cabergoline - Bromocriptine

Pivagabine

Cyproheptadine

Estriol or hyperlow estrogen doses

Quality of food (more than quantity)

GnRH (pulses) - Gonadotropins

Oral contraceptives

Hypothalamic amenorrhea

Therapeutic strategies

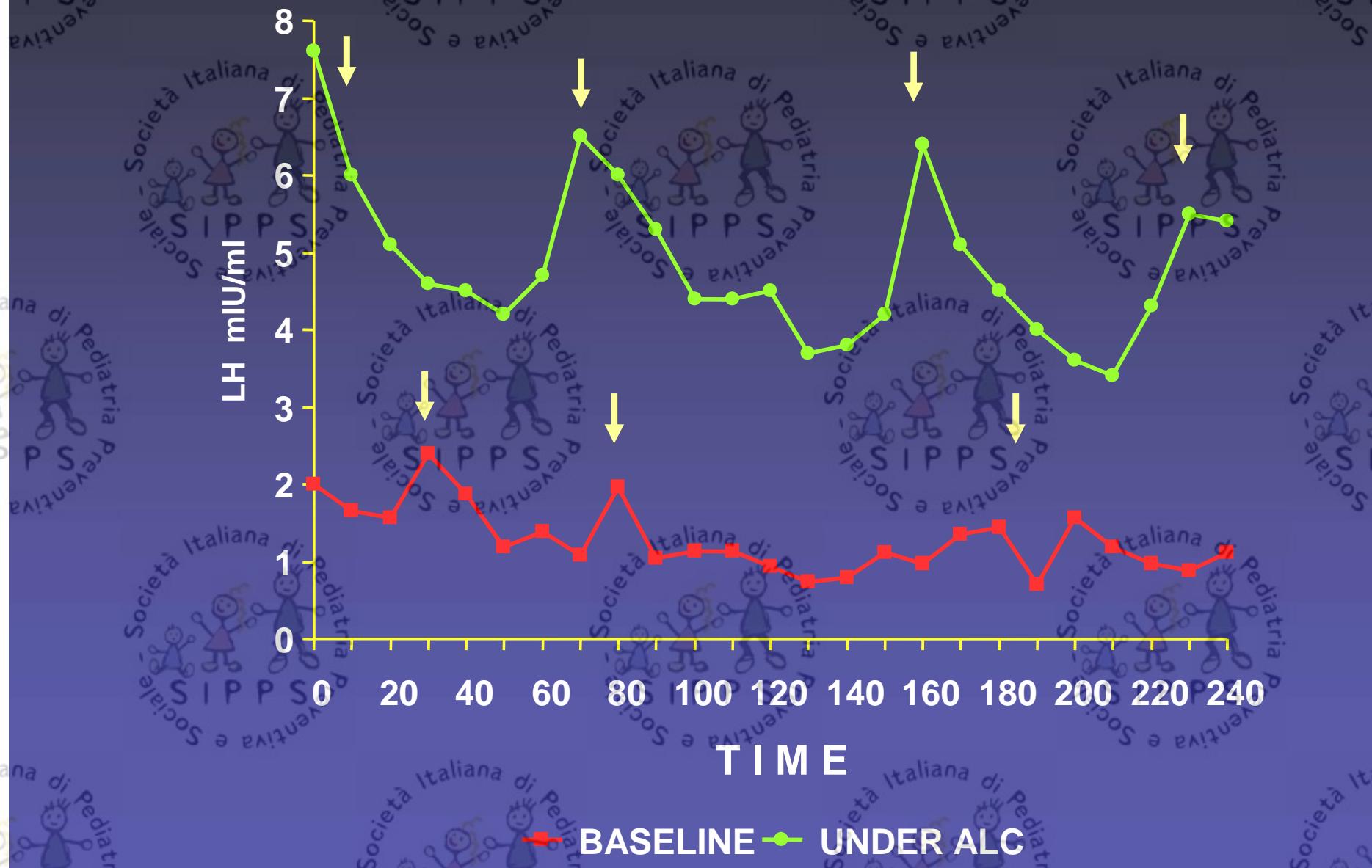
CNS peptides (opioids, CRF)

The aim is to reduce the negative modulation / action
on the hypothalamic functions.

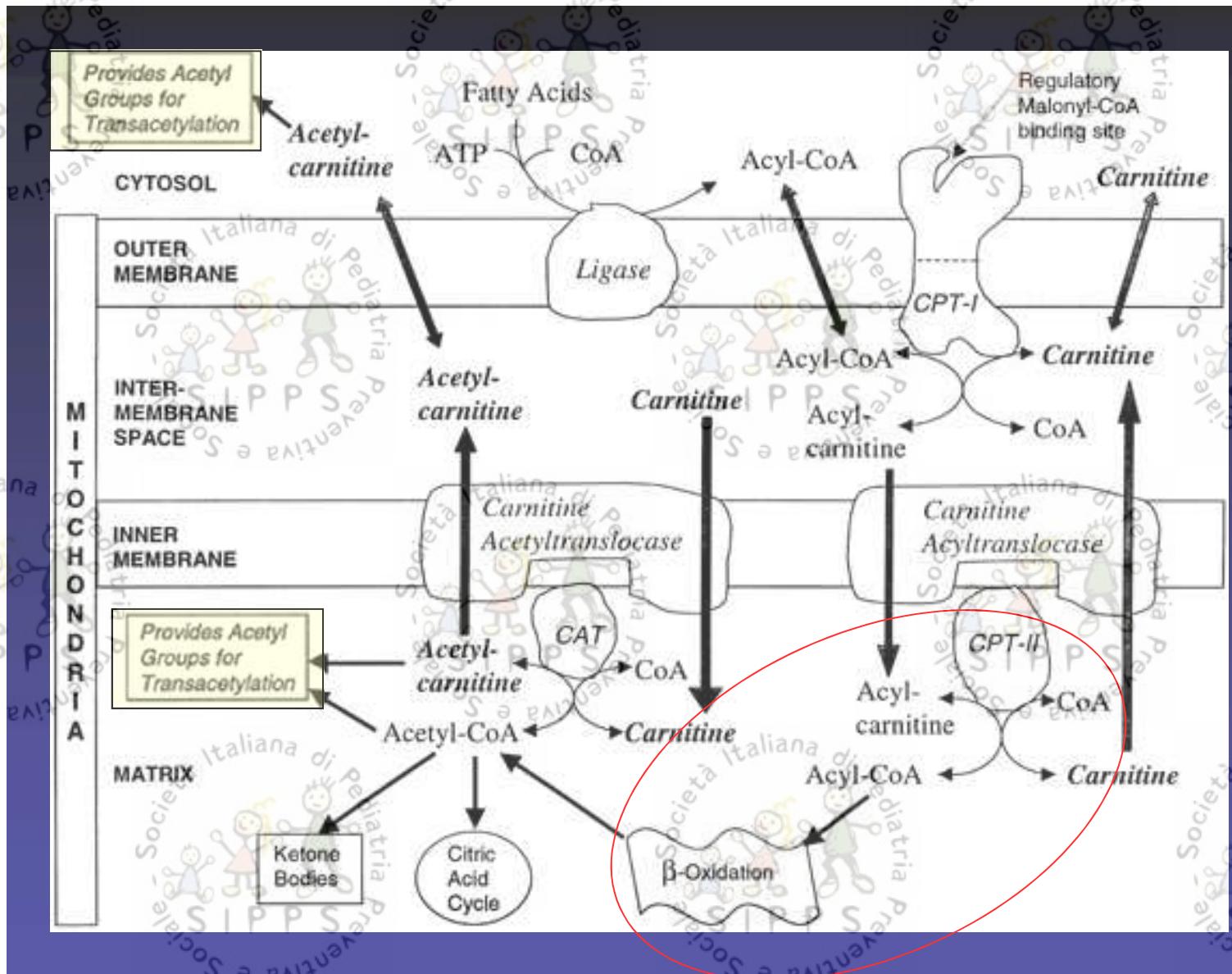
L-acetyl-carnitine (LAC) (1-2 g/die)

Naltrexone cloridrate (50 mg/die)

Hypothalamic amenorrhea



Genazzani AD et al., Acta Obstet Gynec Scand 70: 487, 1991



Carnitine has a pivotal role in the transport of activated fatty acids for oxidation so that to produce energy, especially in dieting or impaired energy metabolism.

Acetyl groups derived from ALC are used inside the brain for inactivation of active amines and neuropeptides (such as opioids)

Schematic of the role of mitochondrial carnitine and Acetyl-L-Carnitine in shuttling free and acyl carnitines across the inner mitochondrial membrane. CPT, carnitine palmitoyl transferase; CAT, carnitine acetyltransferase.

Hypothalamic amenorrhea

Therapeutic strategies

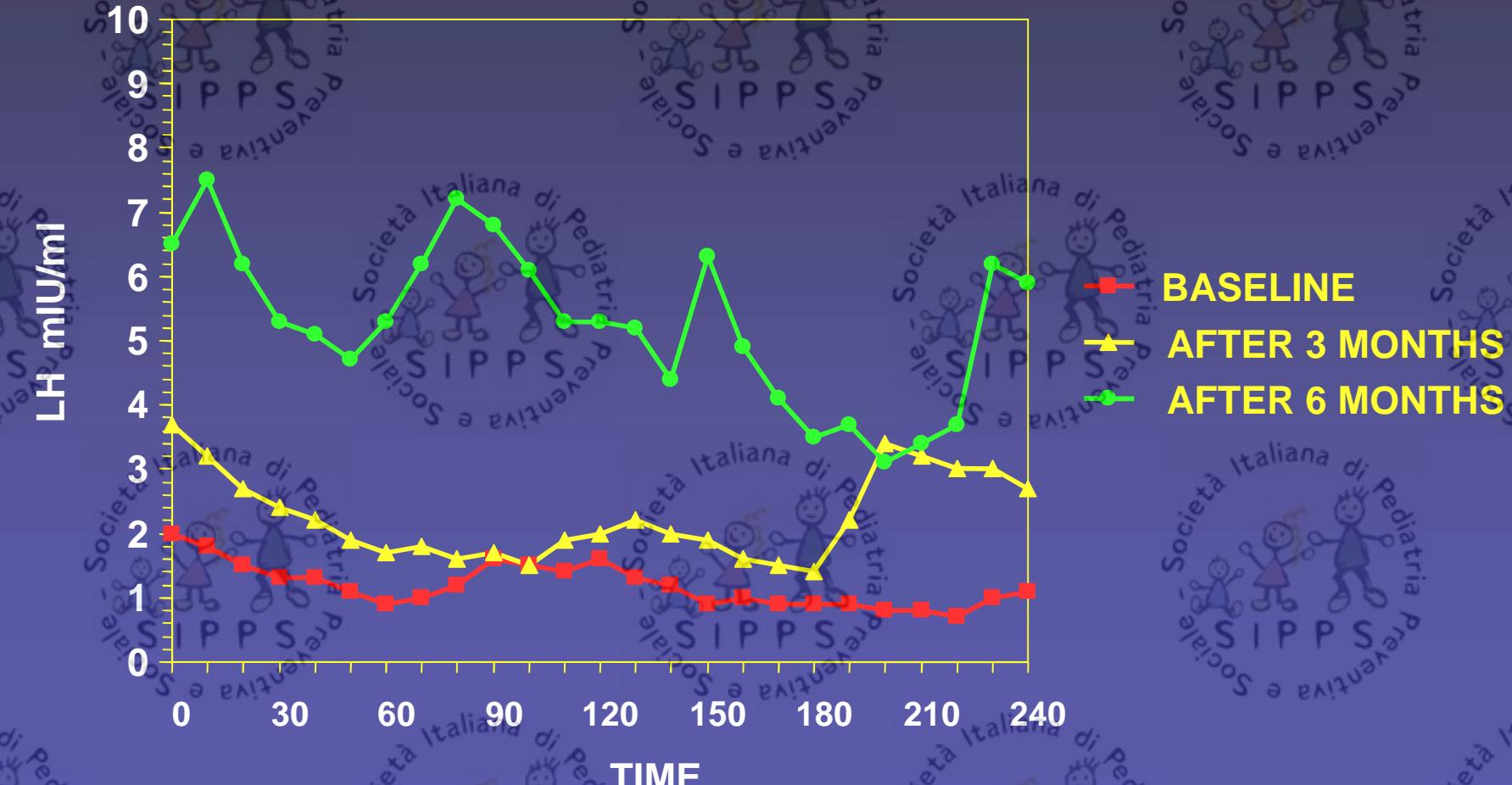
CNS peptides (opioids, CRF)

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L-acetyl-carnitine (LAC) (1-2 g/die)

Naltrexone cloridrate (50 mg/die)

HYPOTHALAMIC AMENORRHEA under naltrexone 50 mg/die



Genazzani AD et al., Fertil Steril 64: 951, 1995

Hypothalamic amenorrhea

Therapeutic strategies

Neurotransmitters, neuromodulators (DA, GABA, serotonin, etc.) and other hormones (PRL, cortisol)

The aim is to reduce the negative modulation / action on the hypothalamic functions.

On PRL

Cabergoline 0.5 mg/twice weekly

Bromocriptine 2.5 – 5 mg/die

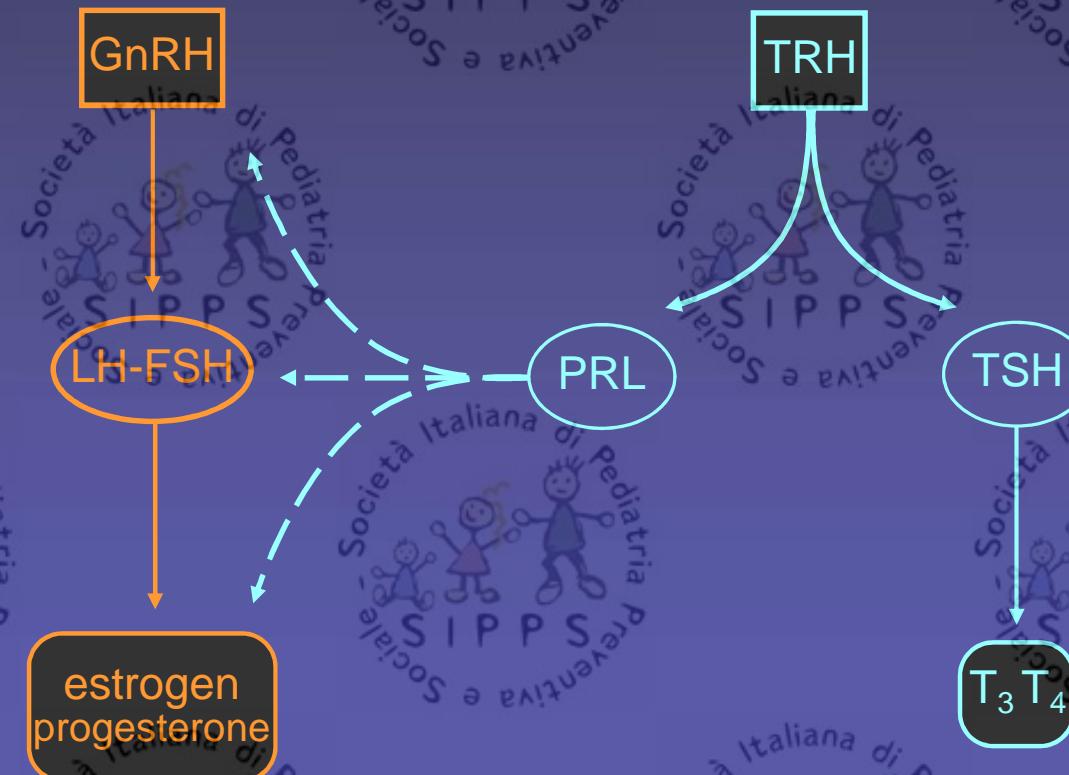
On GABAergic pathway

Pivagabine 1.8 g/die

On serotonergic pathway

Cyproheptadine 4-8 mg/die

INTERACTION BETWEEN HYPOTHALAMIC-PITUITARY AXES



Hypothalamic amenorrhea

Therapeutic strategies

Neurotransmitters, neuromodulators (DA, GABA, serotonin, etc.) and other hormones (PRL, cortisol)

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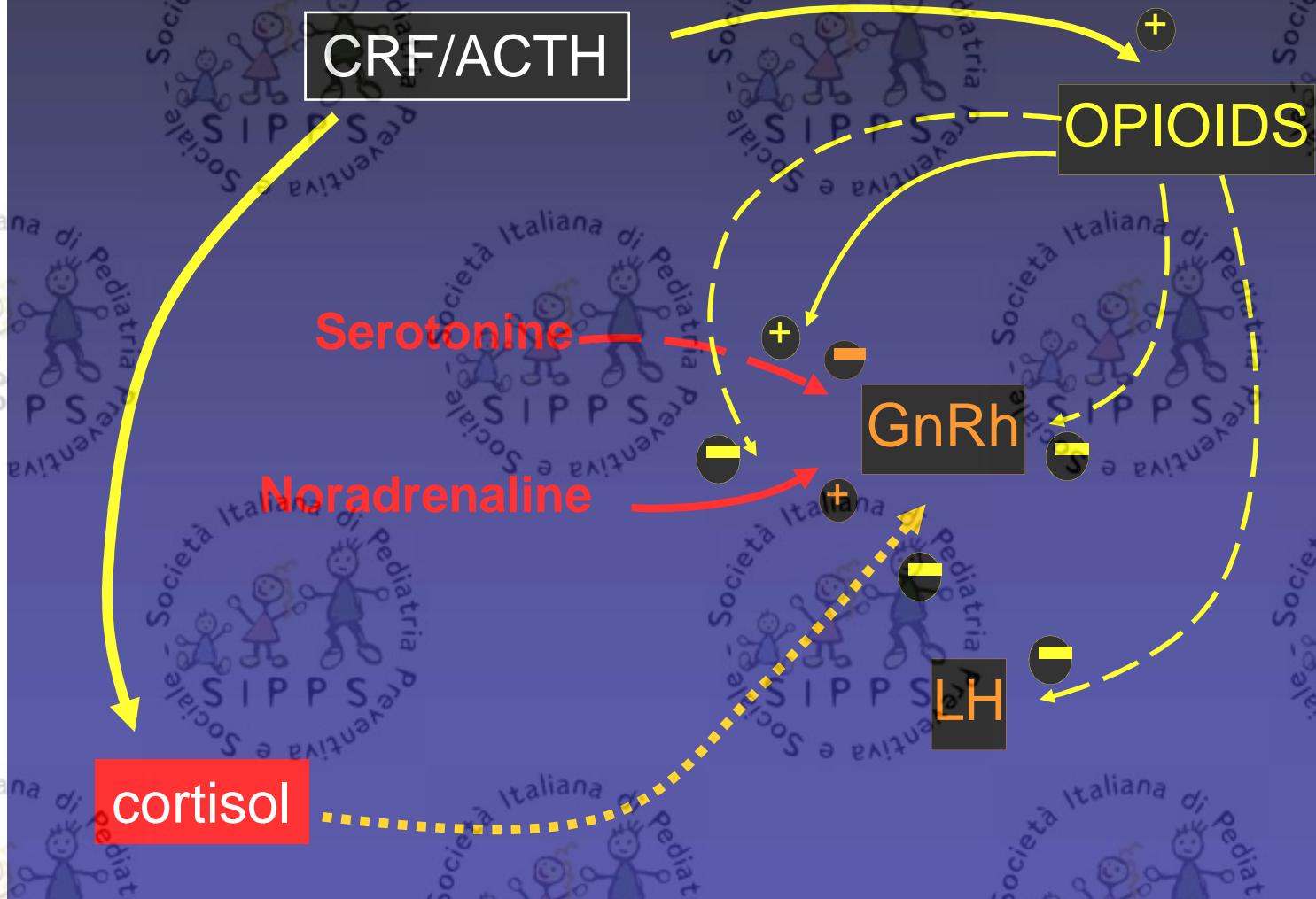
On GABAergic pathway

Pivagabine 1.8 g/die

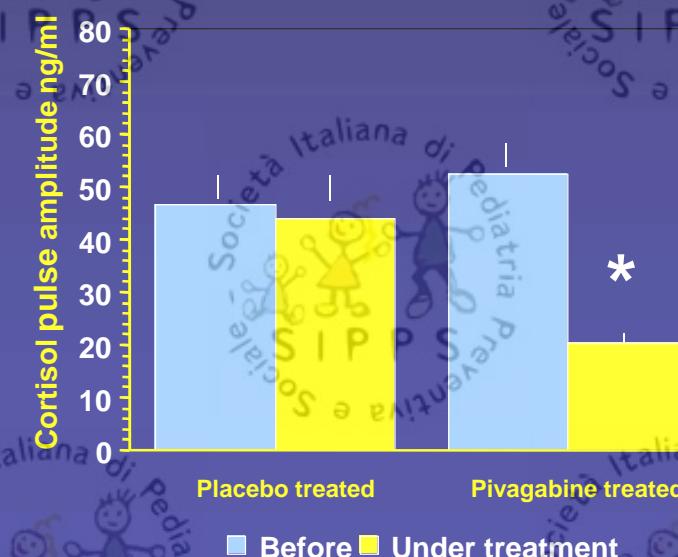
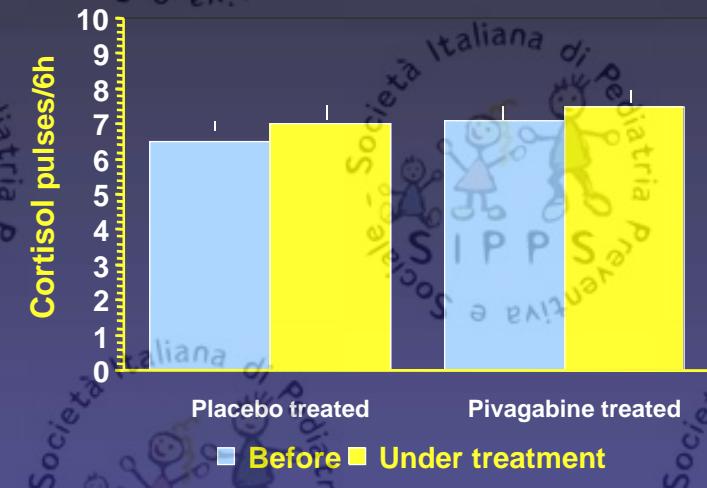
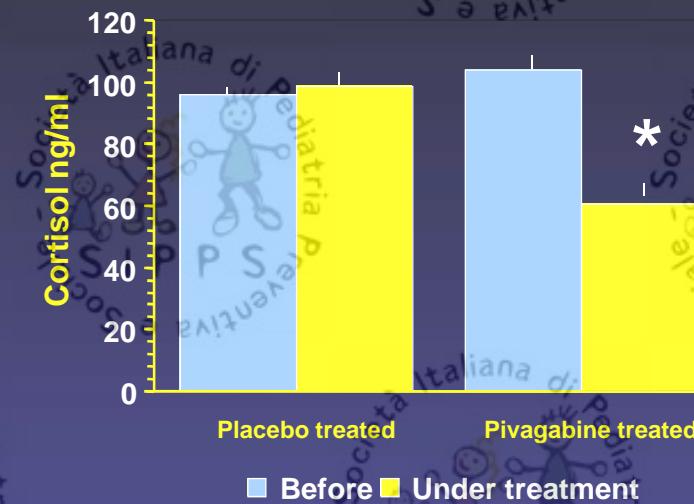
On serotonergic pathway

Cyproheptadine 4-8 mg/die

HPA, OPIOIDS AND LH : mechanism of action



Hypothalamic amenorrhea Cortisol under pivagabine administration



Genazzani AD et al., J Endocrinol Invest 23: 526, 2000

IL RATIONALE E LE OPZIONI TERAPEUTICHE

Fattori stressanti ripetitivi e/o cronici sono alla base dell'onset dell'amenorrea ipotalamica. Questo blocco è ipoteticamente reversibile con l'uso di vari tipi di farmaci

Le azioni terapeutiche per il trattamento dell'amenorrea ipotalamica indotta dallo stress devono agire su uno o più dei parametri citati e qui elencati:

-]) Neurotrasmettitori, neuro-modulatori (DA, GABA, serotonina, etc.)
-]) Vari ormoni (PRL, ormoni tiroidei)
-]) Alimentazione e/o bilancio energetico (proteine, glucosio, esercizio fisico)

**...ma dobbiamo considerare anche l'ipotesi del
*priming***

con estrogeni in basse dosi

andiamo con ordine

Hypothalamic amenorrhea

Therapeutic strategies

Inducing a specific response from selected tissues

The aim is to affect hypothalamus and/or pituitary
(i.e. gonadotrope cells) function with estrogens.

On GnRH and/or LH-FSH receptor expression

Estriol 2 mg/die

Hyper low E2 doses

Estriolo

Estriolo è 1000 volte meno potente in termini biologici dell'estradiolo

Il meccanismo su cui la sua efficacia si basa è prettamente legato ad effetti endo cellulari

... weak estrogen administration in patients who are not GnRH deficient increase both basal gonadotropin plasma levels and the amount of gonadotropin secreted after a GnRH bolus; it induced both a greater synthesis and storage of LH inside the gonadotropes

Genazzani AR et al., Fertil Steril 1978;30:654-60

Estriol administration modulates luteinizing hormone secretion in women with functional hypothalamic amenorrhea

TABLE 1

FHA (n = 12)	LH	FSH	PRL	E ₂	A	17-OHP	T	TSH	fT ₃	fT ₄	Insulin	Cortisol	BMI
	mIU/mL	mIU/mL	ng/mL	pg/mL	ng/dL	ng/mL	ng/dL	μIU/mL	pg/mL	pg/mL	μIU/mL	μg/L	
Baseline	8.2 ± 0.1	3.2 ± 0.3	18.6 ± 0.6	22.6 ± 2.8	157.2 ± 16.1 ^a	9.68 ± 0.1	38.4 ± 1.0	1.8 ± 0.1	2.3 ± 0.1	9.7 ± 0.8	5.1 ± 0.5	18.3 ± 0.9	20.9 ± 0.2
Under estriol	3.5 ± 0.3 ^b	4.2 ± 0.4 ^b	10.4 ± 0.6	28.6 ± 3.8	195.2 ± 14.3 ^a	0.84 ± 0.1	48.2 ± 3.5	2.0 ± 0.2	2.4 ± 0.2	9.0 ± 0.7	5.6 ± 0.4	19.0 ± 0.9	21.3 ± 0.4

Note: Values are mean ± standard error of the mean. 17-OHP = 17 α -hydroxyprogesterone; A = androstenedione; BMI = body mass index; E₂ = estradiol; FHA = functional hypothalamic amenorrhea; FSH = follicle-stimulating hormone; fT₃ = free triiodothyronine; fT₄ = free thyroxine; LH = luteinizing hormone; PRL = prolactin; T = testosterone; TSH = thyroid-stimulating hormone.

^a P < .01.

^b Genazzani. Estriol modulation of LH secretion in FHA. *Fertil Steril* 2012.

TABLE 2

FHA (n = 12)	Spontaneous LH pulses				GnRH-induced LH pulse			
	Raw data		ISR computation		Raw data		ISR computation	
	LH (mIU/mL)	No. of pulses/150 min	Pulse amplitude (mIU/mL)	Pulse duration (min)	Pulse amplitude (mIU/mL)	Pulse duration (min)	Pulse amplitude (mIU/mL)	Pulse duration (min)
Baseline	0.43 ± 0.2	1.1 ± 0.2	0.31 ± 0.09	60 ± 8	0.3 ± 0.01	20.3 ± 0.8	5.8 ± 1.5	48.3 ± 5.8
Under estriol	0.99 ± 0.3 ^a	1.2 ± 0.2	1.4 ± 0.4 ^a	72 ± 9	0.4 ± 0.07 ^b	22.0 ± 0.8	15.2 ± 3.2 ^a	9.2 ± 1.3 ^b

Note: Values are mean ± standard error of the mean. FHA = functional hypothalamic amenorrhea; GnRH = gonadotropin-releasing hormone; ISR = instantaneous secretory rates; LH = luteinizing hormone.

^a P < .01 versus baseline.

^b P < .05 versus baseline.

Genazzani. Estriol modulation of LH secretion in FHA. *Fertil Steril* 2012.

Spontaneous LH pulses

GnRH induced LH pulses

Genazzani AD et al., *Fertil Steril* 2012

Short term (10 days) estriol administration modulates luteinizing hormone secretion in FHA

Table 1 Hormonal characteristics of patients under study before and after 10 d of estriol (2 mg/d) administration (mean \pm SEM).

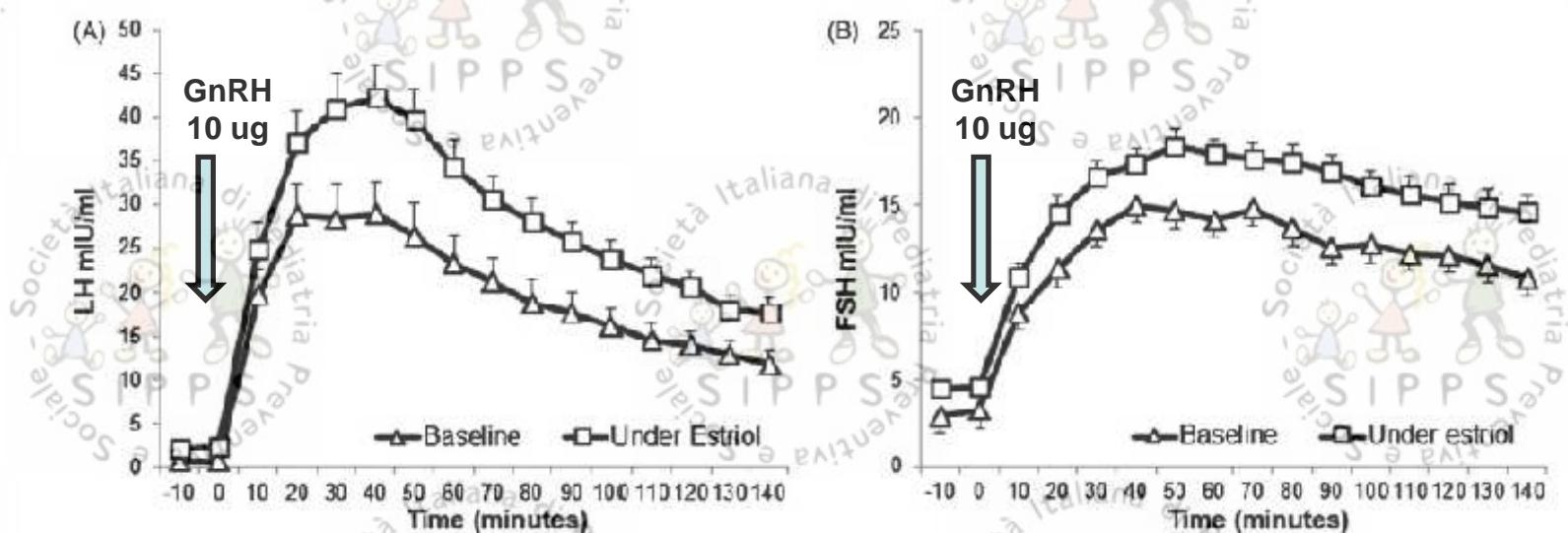
Patients (n = 12)	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	E2 (pg/ml)	P (ng/ml)	17-OHP (ng/ml)	A (ng/ml)	Cortisol (μ g/dl)	Insulin (mU/ml)	TSH (μ IU/ml)	FT ₃ (pg/ml)	FT ₄ (ng/dl)
Baseline	0.6 \pm 0.2	2.9 \pm 0.4	5.5 \pm 0.7	7.1 \pm 0.9	0.4 \pm 0.05	0.6 \pm 0.1	1.7 \pm 0.1	20.2 \pm 0.4	6.6 \pm 0.9	1.9 \pm 0.3	2.6 \pm 0.1	1.0 \pm 0.04
Under estriol	2.1 \pm 0.3***	4.4 \pm 0.4**	4.5 \pm 0.4	26.5 \pm 3.7***	0.4 \pm 0.05	0.5 \pm 0.1	3.2 \pm 0.3*	18.1 \pm 0.4	5.8 \pm 0.4	1.3 \pm 0.28	2.7 \pm 0.2	1.1 \pm 0.06

*** $p < 0.007$;

** $p < 0.003$;

^ $p < 0.02$;

* $p < 0.01$.



Genazzani AD et al., Gynecol Endocrinol 2016

Estriol administration in FHA

.. these changes occur as the result of various events such as

- increased sensitivity/number of GnRH receptors on the anterior pituitary cells
Genazzani AR et al., Fertil Steril 1978;30:654–60
Bohnet HG et al., Fertil Steril 1980;34:346–50
- increased amount of GnRH per each single hypothalamic secretory burst
Genazzani AD, J Endocrinol Invest 2011;34:287–91
- higher LH synthesis by the gonadotropes
Diaz MC et al., J Endocrinol Invest 1989;12:1–7
- higher synthesis and expression of GnRH receptors, higher synthesis and storage of LH inside the gonadotropes, all these being the mechanism of the **positive feedback**
Bohnet HG et al., Fertil Steril 1980;34:346–50
Diaz MC et al., J Endocrinol Invest 1989;12:1–7

Estriol administration 2 mg/die does not change estradiol plasma concentrations greatly

Dall'osservazione degli effetti dell'estriolo è nata l'idea di provare l'uso di basse, bassissime dosi di estradiolo

Attualmente non ci sono preparazioni di estradiolo a basse dosi

Le uniche utilizzabili sono frazionamenti di terapia utili nel trattamento della menopausa

- Estradiolo (Dermestrill 50 µg transdermico) bisettimanale
- Estradiolo valerato (Progynova 2 mg)

Unica preparazione a bassissima
concentrazione di estradiolo attualmente
disponibile è

Guna-beta-estradiol (d6)

OMEOCODIFA: 801846003

*ma quanto “low” deve essere una
low dose per essere efficace ...?*

DEFINIZIONI

g (gram) = 1

$$10^{-1} = 0.1$$

$$10^{-2} = 0.01$$

mg (milligram) = 10^{-3} = 0.001

μ g (microgram) = 10^{-6} = 0.000001

ng (nanogram) = 10^{-9} = 0.00000001

pcg (picogram) = 10^{-12} = 0.000000000001

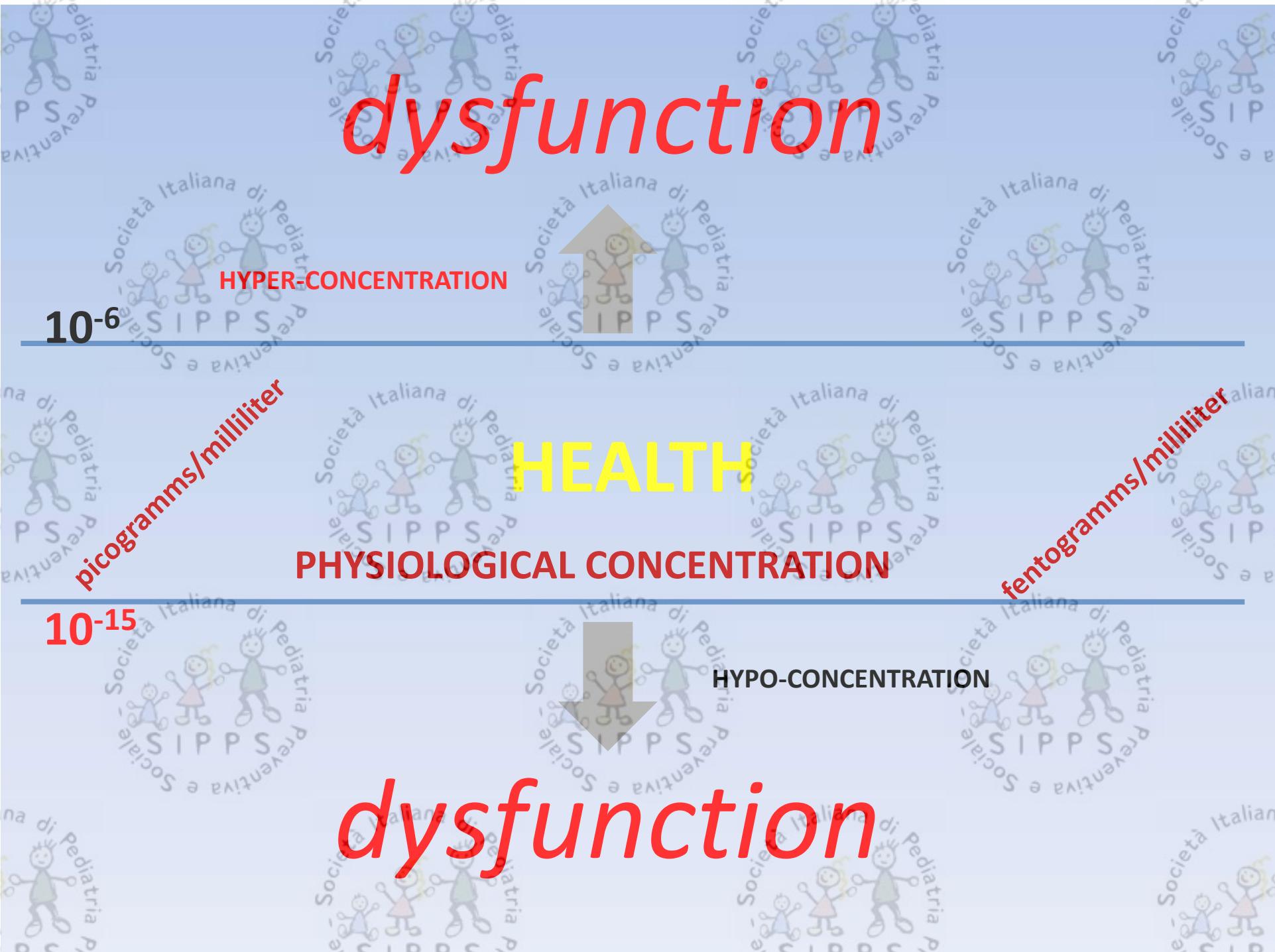
fg (fentogram) = 10^{-15} = 0.000000000000001

Estradiolo valerato

Estradiolo
transdermico

Beta-estradiolo D6

Concentrazione di «lavoro» dei feedback,
in particolare di quello positivo degli estrogeni



Protocollo clinico:

17 pazienti con amenorrea ipotalamica da stress (no cause endocrine di altra natura), BMI 19,5, no terapie ormonali in corso (contraccettivi, per tiroide, per PRL)

Le pazienti NON volevano terapie con ormoni «classici» (contraccettivi)

Valutazioni ormonali:

- Basali ormonali e test al Naloxone (4 mg in bolo) (come da procedura diagnostica presso il Centro di Ginecologia Endocrinologica, Università di Modena e Reggio Emilia)
- 8 settimane di trattamento con 25 gocce x 2 al di Beta-estradiolo D6 (1 ora dopo colazione e 1 ora dopo cena), **nessuna imposizione alimentare**
- Ripetizione dei basali e del Naloxone test durante il trattamento

Pharmacological and Integrative Treatment of Stress-Induced Hypothalamic Amenorrhea

Alessandro D. Genazzani, Giulia Despini, Elisa Chierchia,
Camilla Benedetti, and Alessia Prati

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A.R. Genazzani, B.C. Tarlatzis (eds.), *Frontiers in Gynecological Endocrinology: Volume 3: Ovarian Function and Reproduction. From Needs to Possibilities.*
ISGE Series, DOI 10.1007/978-3-319-23865-4_9

Hyperlow E2 administration (Beta-estradiol D6)

25 drops x 2 times every day, sublingual

FHA n=17	LH mIU/ml	FSH mIU/ml	E2 pg/ml	P ng/ml	PRL ng/ml	DHEA ng/ml	A ng/ml	cortisol µg/100 ml	Insulin ~g/ml	TSH µUI/ml	fT3 pg/ml	fT4 ng/100 ml	BMI
Basal	1.8±0.3	3.1±0.3	26.0±4	0.4±0.05	13.5±2.5	2.1±0.2	0.8±0.1	21.5±1.8	2.6±0.3	2.5±0.3	2.2±0.4	1.2±0.2	20.8±0.3
After 12 weeks	3.5±0.6 *	5.0±0.5 *	32.5±9.5	0.5±0.1	15.5±1.8	2.0±0.3	1.1±0.2	19.5±2.5	3.2±0.2	2.3±0.2	2.4±0.2	1.3±0.2	21.1±0.3

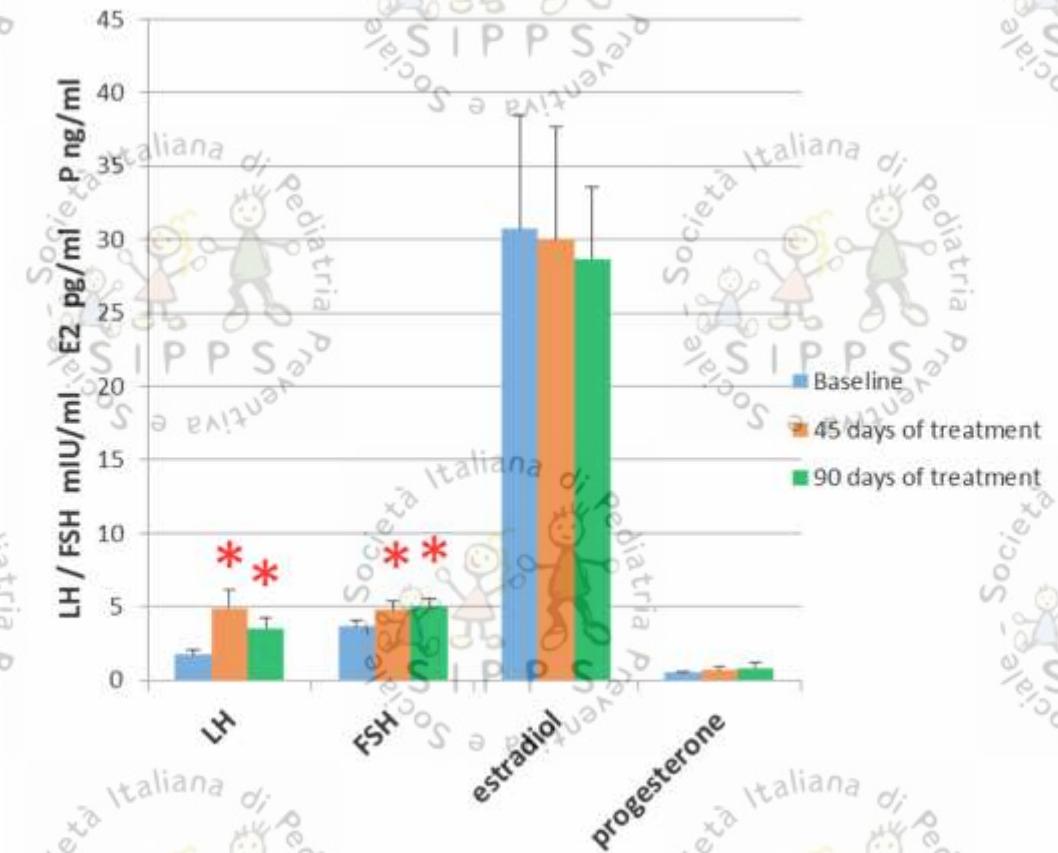
* p<0.05

Genazzani AD et al., 2016

Hyperlow E2 administration (Beta-estradiol D6)

25 drops x 2 times every day (20 pg/ml), sublingual, 12 weeks

Hypothalamic amenorrhea (n=17)



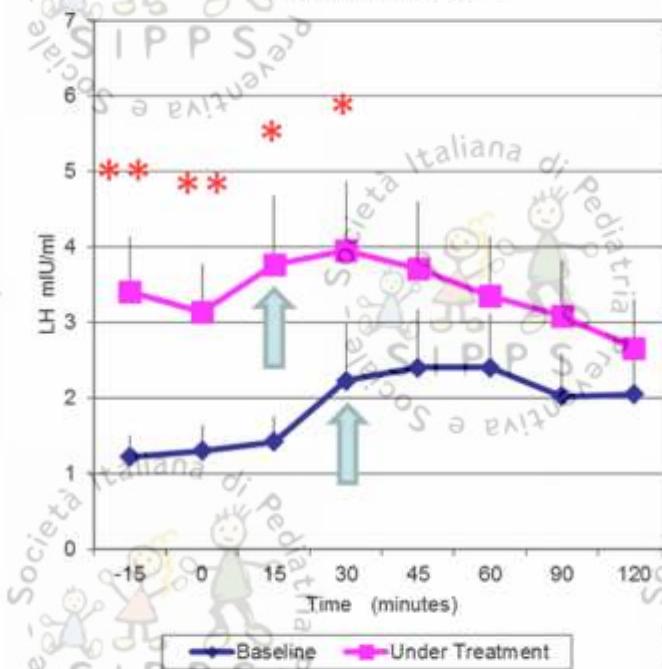
Genazzani AD et al., 2016

Hyperlow E2 administration (Beta-estradiol D6)

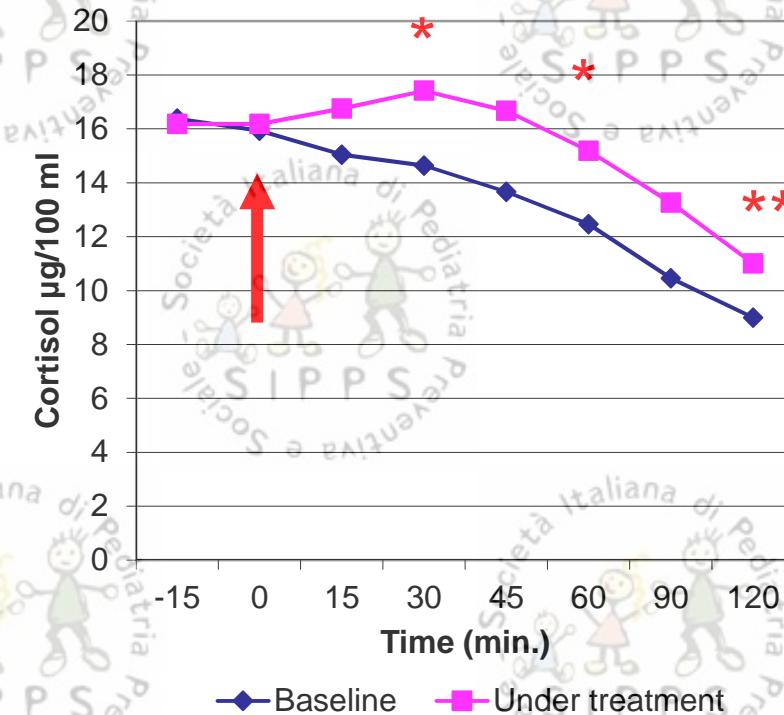
25 drops x 2 times every day, sublingual, 12 weeks

Hypothalamic amenorrhea (n=17)

Naloxone test



Hypothalamic amenorrhea Naloxone test (4mg)



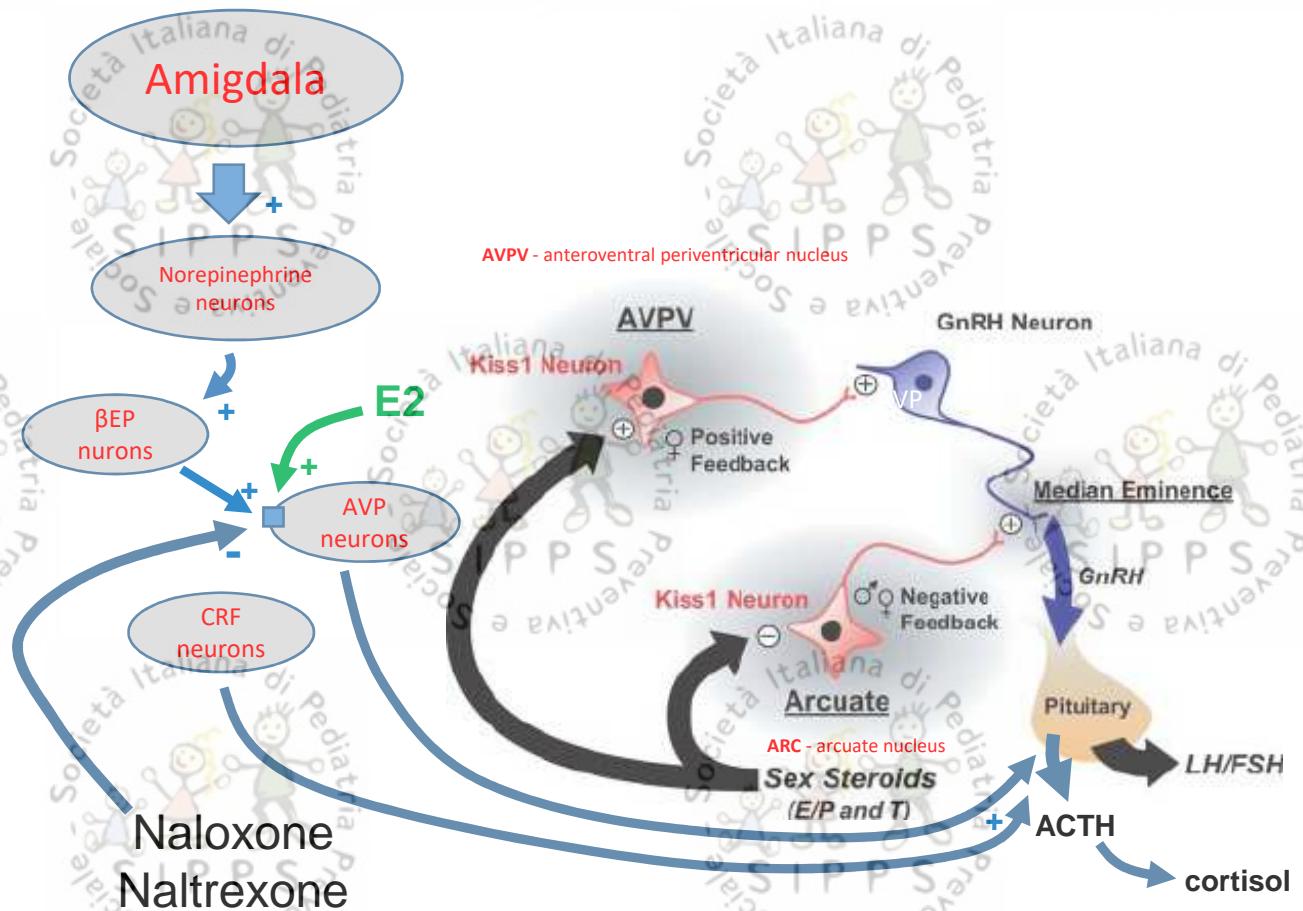
Anticipation of LH response to Naloxone

Genazzani AD et al., 2016

Restoration of cortisol response to naloxone

Genazzani AD et al., 2019

Kisspeptin and GnRH regulation β EP, CRF and ACTH regulation



Positive and Negative feed-backs of gonadal steroids is played on Kiss neurons

AVP = arginine vasopressin

CONCLUSIONI

L'utilizzo di E2 low dose induce un effetto specifico a livello ipotalamico ed ipofisario

- Sfruttando i meccanismi del feedback positivo si determina una aumentata sensibilità al GnRH e una maggior espressione dei recettori del GnRH, permettendo così un aumento della sintesi e secrezione di LH ed FSH.
- L'azione di E2 low dose induce il recupero della risposta del LH e del cortisolo alla infusione di Naloxone.

Si prospetta una strategia terapeutica «nuova» che sfrutta un meccanismo biologico, ovvero il feedback positivo

Di fatto c'è la potenziale capacità di riattivare l'asse HPG con l'uso di **«low dose» di E2** (GUNA-Beta-estradiol D6) contribuendo così al recupero delle funzioni riproduttive in caso di amenorrea ipotalamica funzionale (FHA).



Grazie per l'attenzione