LEPTIN AND REPRODUCTION

François Pralong
Division of Endocrinology
Lausanne University Hospital

nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

Volume 372 No. 6505 1 December 1994 £4.00 FF44 DM13 Lire13000

Mouse weighed down by genetics

The *Lep*^{Ob} Mouse

- **Genetically obese**
- **Multiple metabolic and endocrine abnormalities**
  - ***hyperglycemia and insulin resistance***
  - ***defects in thyrotrope and corticotrope axes***

The *Lep*^{Ob} Mouse

Hypogonadotropic hypogonadism

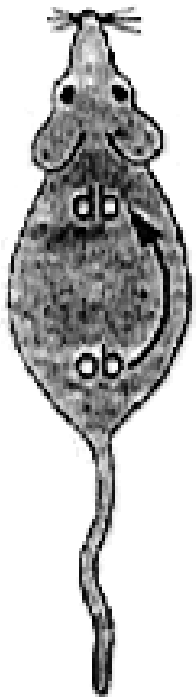
- **Normal response to pulsatile GnRH**
- **Ovaries from *Lep*^{ob} mice transplanted into wild type recipients function normally**

The Leptin-Leptin Receptor System

Parabiosis experiments

receptor
hypothalamus

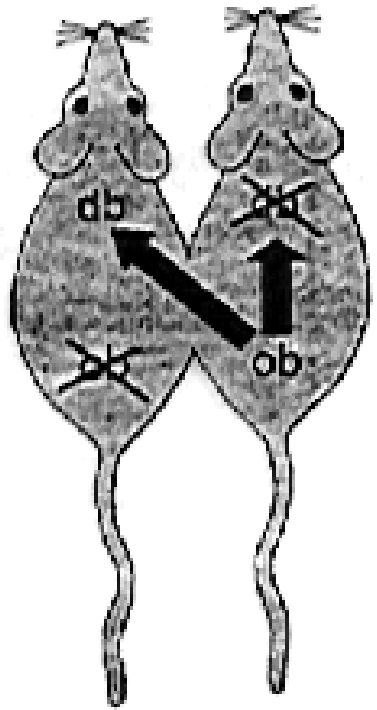
leptin
fat cells



stops
eating

decreased
body
weight

Lep^{ob} *Lepr*^{db}



continues
to eat

remains
fat

Coleman et al., Diabetologia 14, 1978

The Leptin Gene

A positional cloning approach in the *Lep^{ob}* mouse allows to identify the locus of the gene encoding for the *ob* protein

Genes comprised in a 650 kb interval were further identified by exon trapping. Each trapped exon was sequenced and searched in Genebank

One of the trapped exons hybridized to a Northern blot of mouse WAT

Zhang *et al.*, Nature 372, 1994

The Leptin Gene

Expression of *ob* gene limited to WAT

Encodes for a 167 amino acids, with a cytokine-like tertiary structure

C to T missense mutation in *Lep^{ob}* mice results in an Arg105X mutation in the *ob* protein

Levels of *ob* gene expression are markedly increased in WAT of *Lep^{ob}* mice, suggesting that the truncated protein is biologically inactive

Zhang *et al.*, Nature 372, 1994

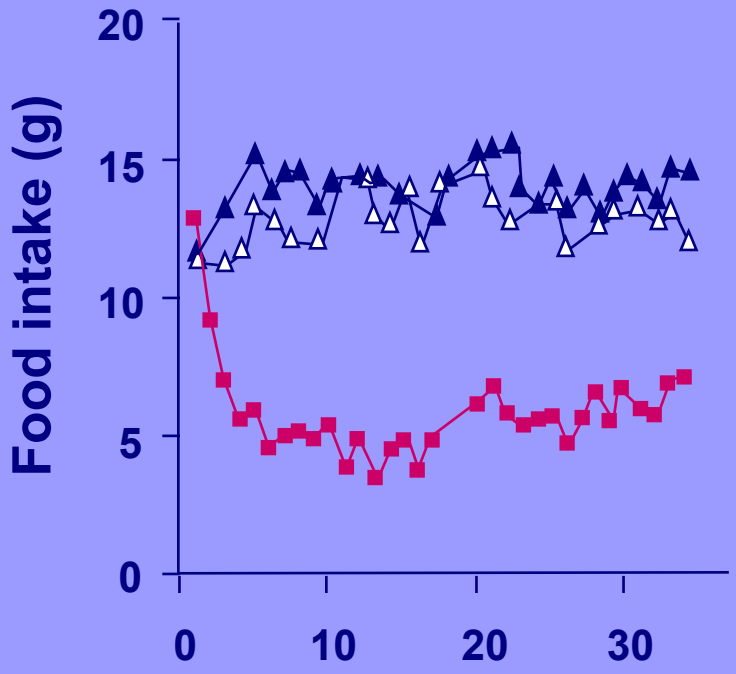
Expression Cloning of the Leptin Receptor :OB-R

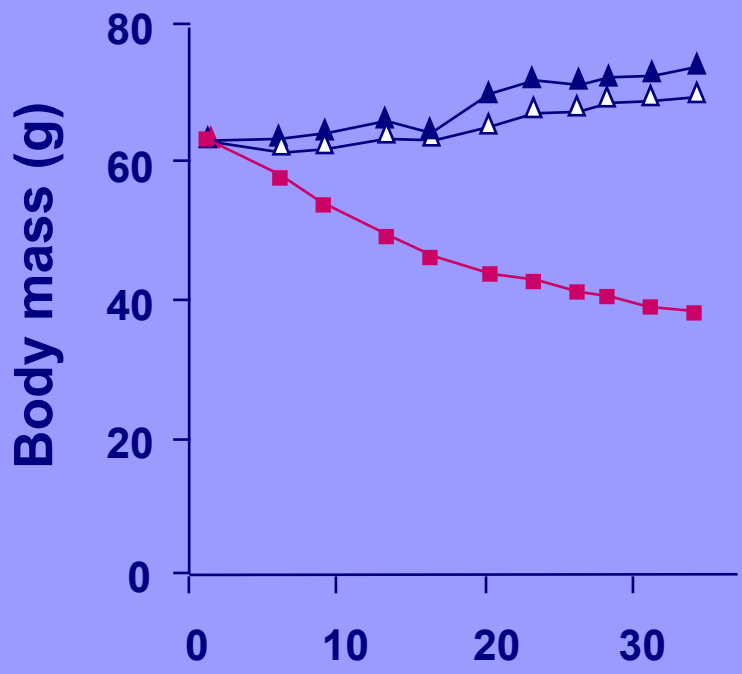
Screening of a wide variety of mammalian cell lines and tissues for leptin binding, using 125I-leptin and AP-OB fusion proteins

Leptin binding identified in mouse choroid plexus

Tartaglia et al., Cell 83, 1995

Leptin Decreases Food Intake in *Lep*^{ob} Mice





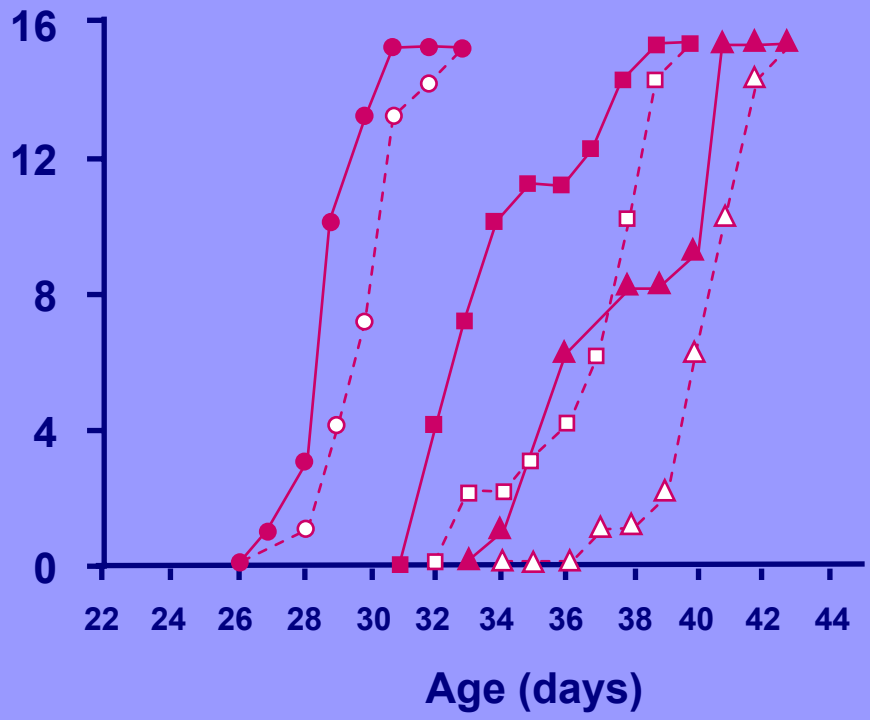
Halaas at al., Science 269, 1995

Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinent leptin

Leptin treatment rescues the sterility of genetically obese ob/ob males

Mounzih et al., Endocrinology 138, 1997

Leptin Accelerates Pubertal Development of Normal Mice



Ahima et al., JCI 99, 1997

Leptin Counteracts the Deleterious Effects of Poor Metabolic Conditions on the Gonadotrope Axis

Exp 1

	Day at VO
Ad lib	32.0+/-1.1
Leptin	33.6+/-1.3
Pair-fed	>38

Exp 2

	Day at VO
Ad lib	35.0+/-0.9
Leptin	41.8+/-1.0
Vehicle	>43

Cheung et al., Endocrinology 138, 1997

Physiological Roles of Leptin in Rodents

Afferent satiety hormone

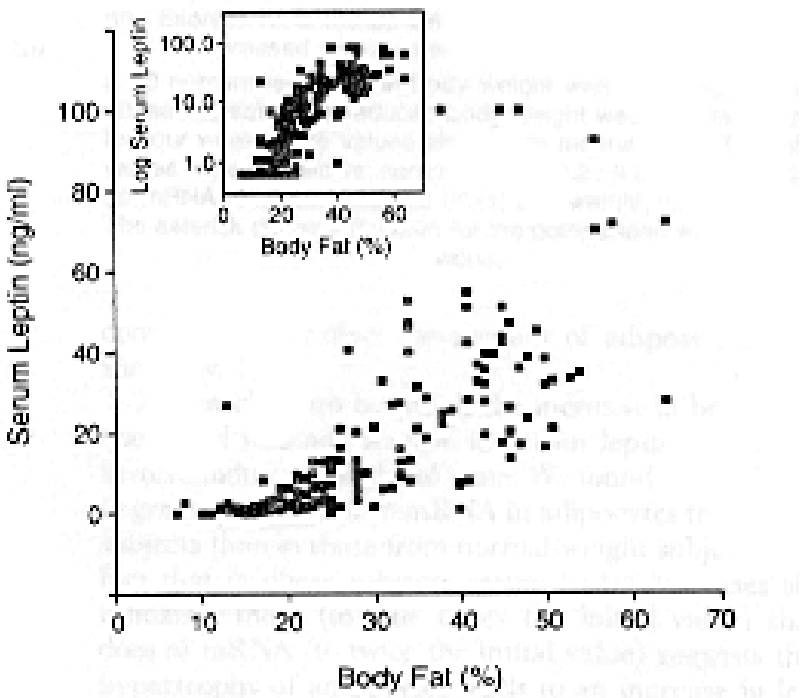
- **inhibits food intake**
- **stimulates energy expenditure**

Central maturation of the reproductive system

- **rescues the fertility of *Lep^{ob}* mice**
- **participates to the biological clock of puberty**

Correlations in Human Reproductive Physiology

Is Human Obesity Caused by Leptin Deficiency ?



Large, population-based screenings show that circulating leptin levels are appropriately high in the humans

Considine *et al.*, NEJM 334, 1996

Congenital leptin deficiency is associated with severe early-onset obesity in humans

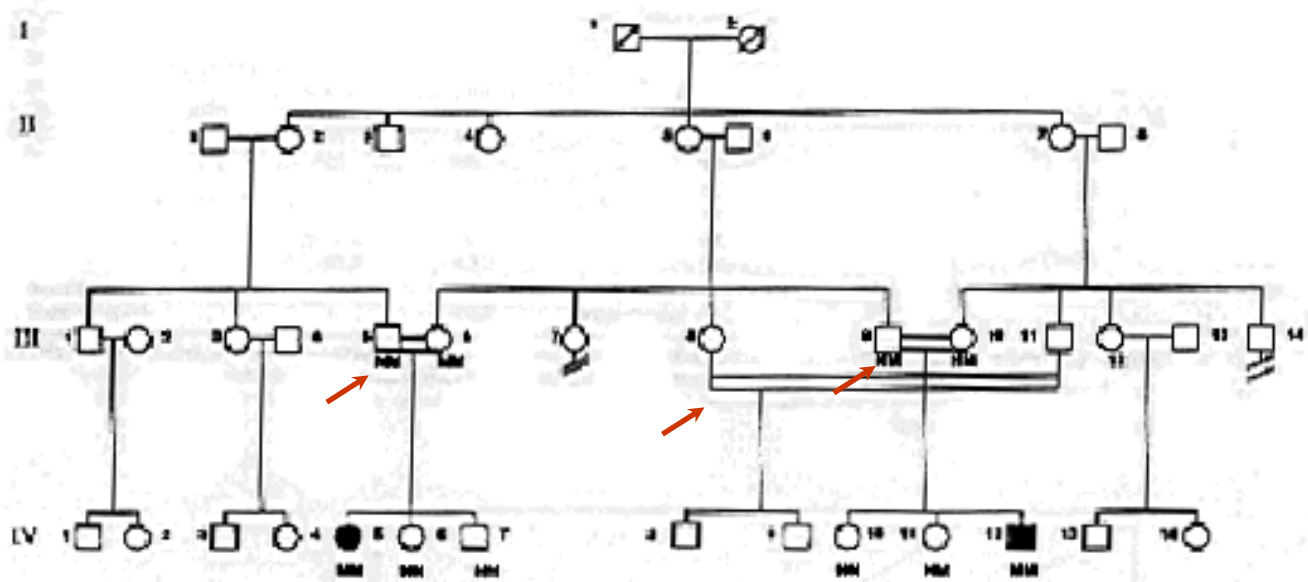
Montague et al., Nature 387, 1997

A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction

Clément et al., Nature 329, 1998

Leptin Gene Mutation in Humans

Study of two first degree cousins, members of a highly consanguineous family, presenting with marked, early onset hyperphagia



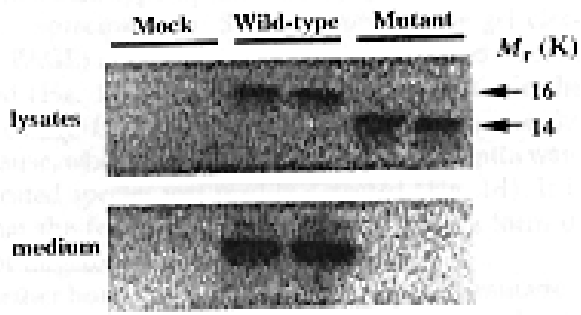
Montague *et al.*, Nature 387, 1997

Identification of A. Leptin Gene Point Mutations in Humans

Single G deletion at codon 133

- disruption of reading frame
- 14 aberrant aa after Gly 132
- premature stop codon

Impaired secretion of mutant protein

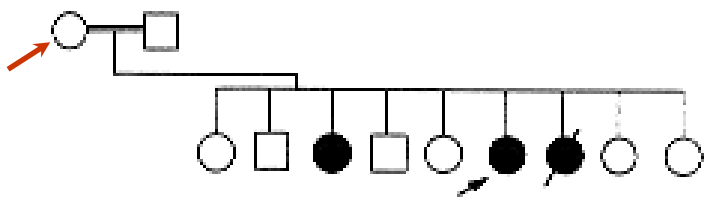


Montague *et al.*, Nature 387, 1997

A Mutation in the Human OB-R Gene Causes Obesity

Study of a family with strong prevalence of morbid obesity occurring early in life

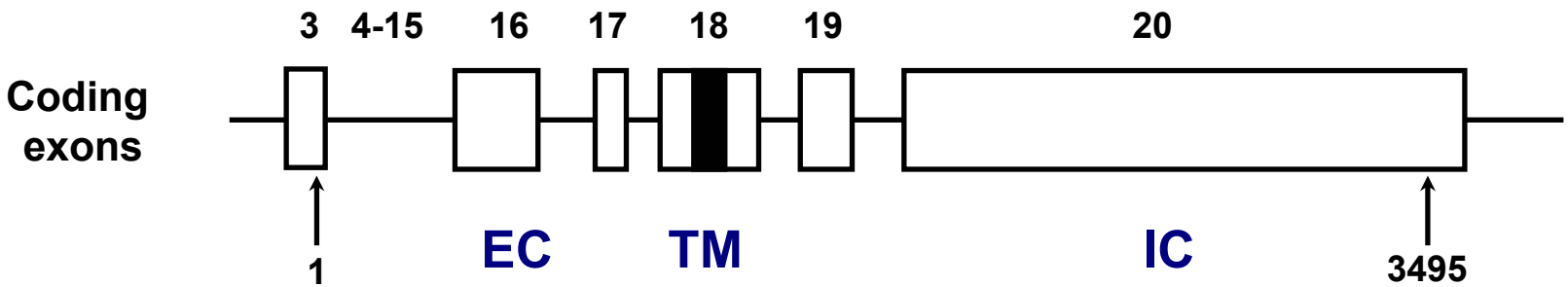
Affected patients with markedly elevated leptin levels



Clément *et al*., Nature 392, 1998

A Mutation in the Human OB-R Gene Causes Obesity

G to A substitution in splice donor site of exon 16



Resulting transcript skips exon 16

Clément *et al.*, Nature 392, 1998

A Mutation in the Human OB-R Gene Causes Obesity

Resulting protein contains 831 aa, comprising part of the extracellular domain, but lacking the transmembrane and intracellular signaling portion

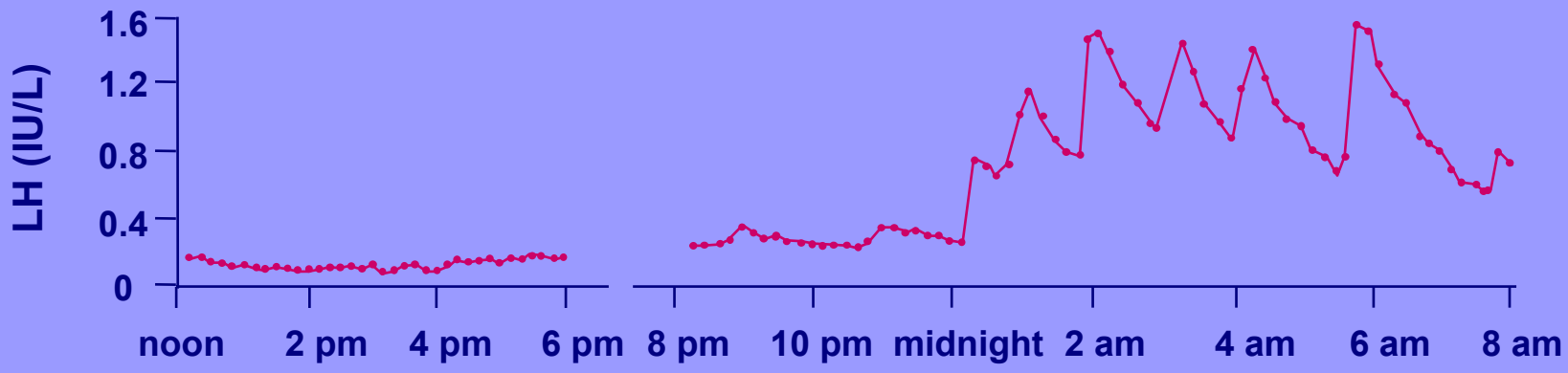
Mutant protein has similar leptin binding capability than the endogenous circulating OB-R form

Clément *et al*., Nature 392, 1998

Summary of the Phenotype of Human Leptin-Leptin receptor Mutations

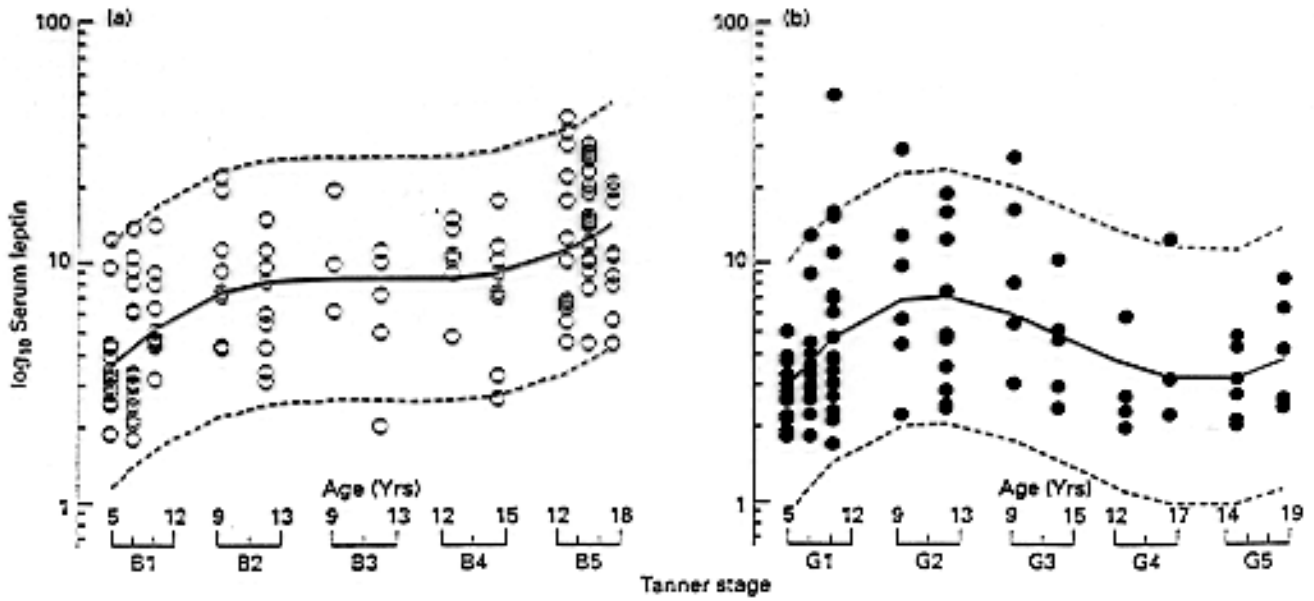
	OB 1	OB 2	OB 3	OB 4	OB-R 1	OB-R 2
	Montague *et al*	Montague *et al*	Strobel *et al*	Strobel *et al*	Clément *et al*	Clément *et al*
Age at diag	**8 y**	**2 y**	**34 y**	**22 y**	**19 y**	**19 y**
Sex	**F**	**M**	**F**	**M**	**F**	**F**
Mutation	G deletion at codon 133 (frameshift)	G deletion at codon 133 (frameshift)	R105W	R105W	G to A in splice donor site, exon 16	G to A in splice donor site, exon 16
Clinical feat	**Pre-pubertal**	**Pre-pubertal**	**Primary amenorrhoea**	**Delayed puberty impuberism**	**Primary amenorrhoea**	**Primary amenorrhoea**
LH (IU/L)	**<0.2**	**<0.2**	**NA**	**4.4**	**<0.2**	**<0.8**
FSH (IU/L)	**0.8**	**0.2**	**NA**	**9.0**	**<0.1**	**1.2**
E2 (pmol/L)	**<20**	**-**	**NA**	**-**	**17**	**13**
T (nmol/L)	**-**	**<0.2**	**-**	**5**	**-**	**-**

Correction of Hypogonadotropic Hypogonadism by Leptin Treatment in Human Leptin Deficiency



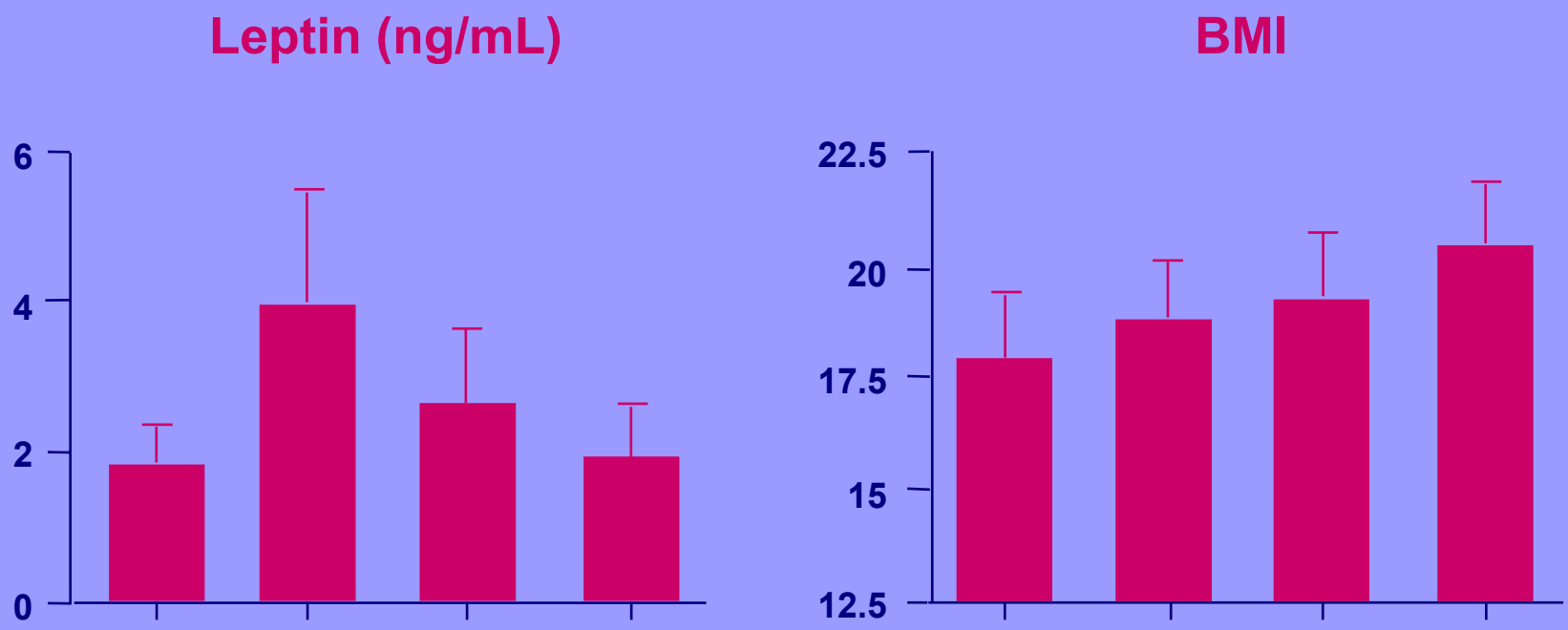
Farooqi et al., NEJM 341, 1999

Rising Serum Leptin Levels Before Puberty



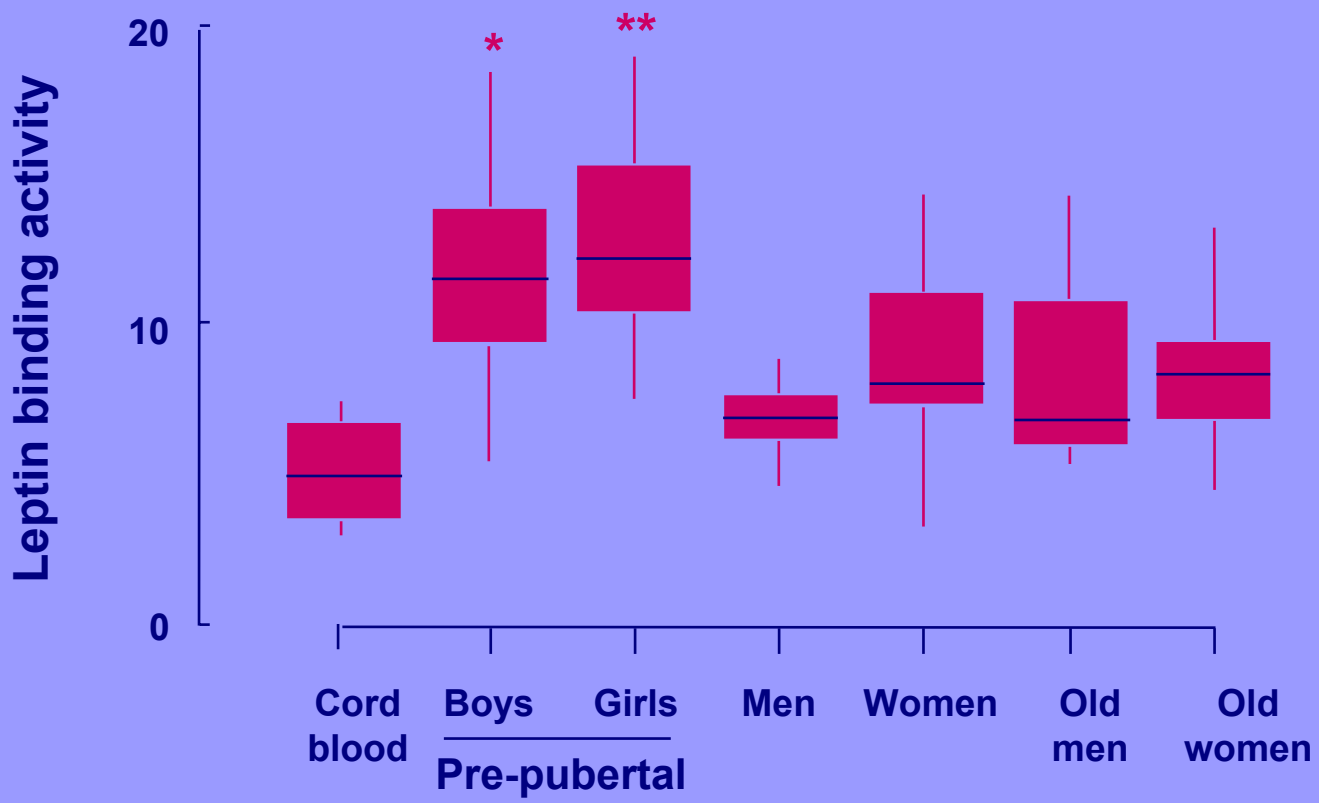
Clayton et al., Clin Endocrinol 46, 1997

Rising Serum Leptin Levels Before Puberty



Mantzoros et al., JCEM 82, 1997

Elevated Serum Leptin Binding Protein Levels Before Puberty

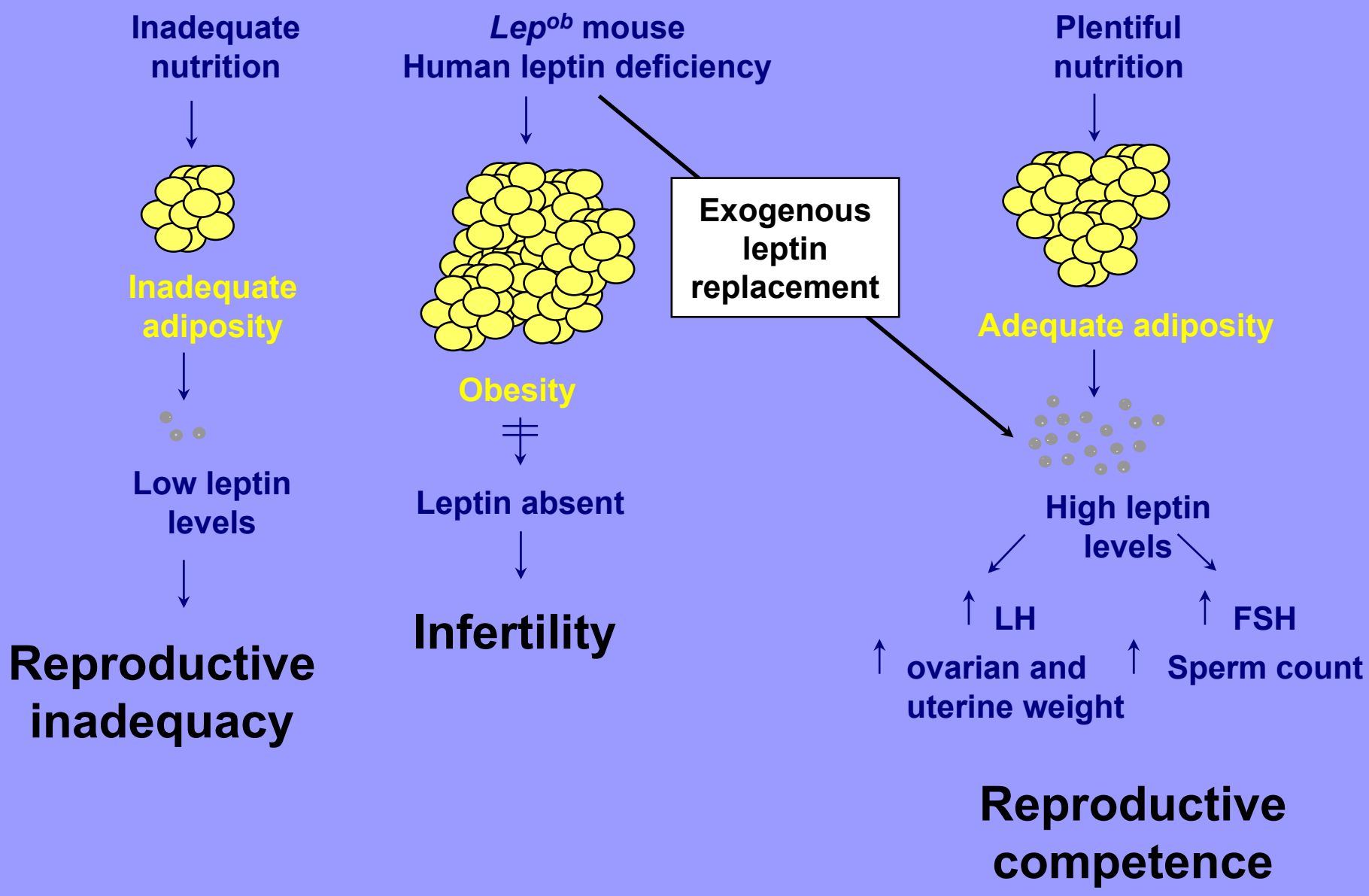


Quinton et al., JCEM 84, 1999

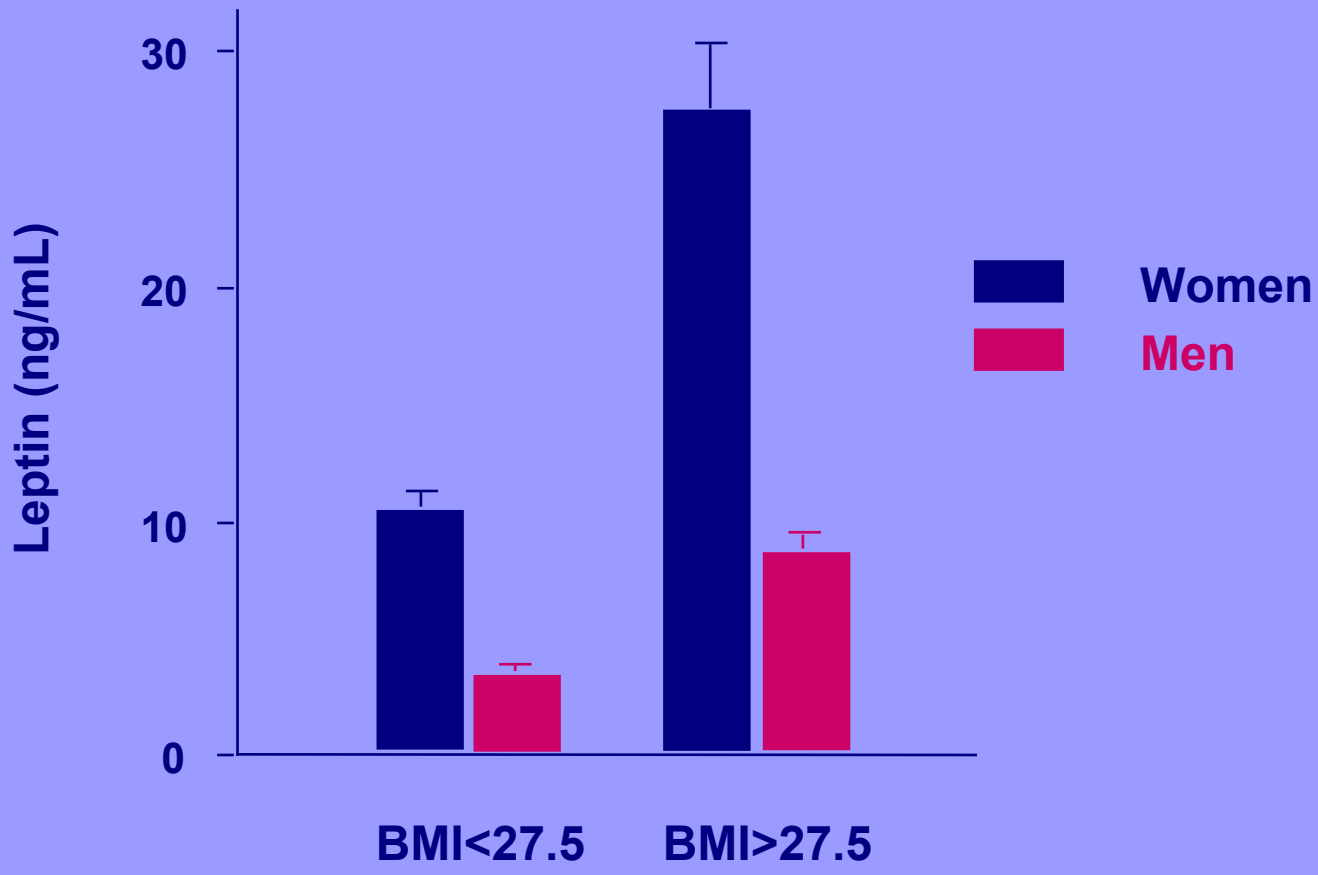
Leptin Meets the Criteria for a Blood-borne Metabolic Signal Timing Puberty

- **The circulating leptin levels are different in the sexually immature and mature individuals**
- **When administered, leptin leads to a change in the hypothalamic secretion of GnRH**

The Critical Fat Mass Hypothesis Revisited

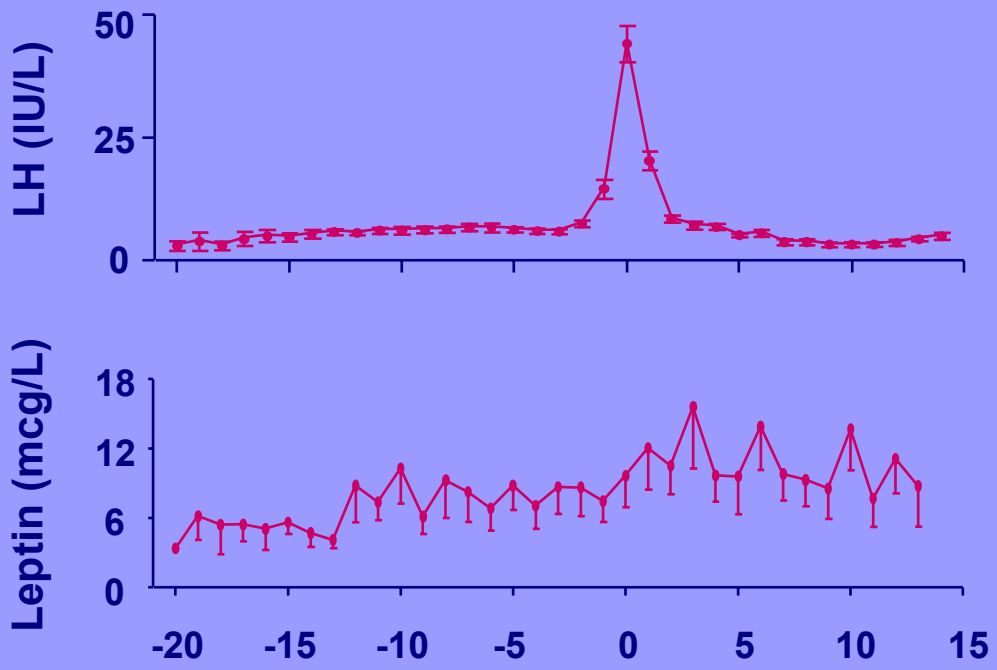


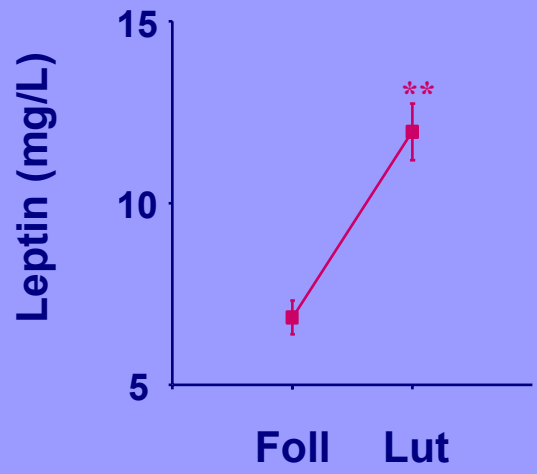
Sexual Dimorphism of Circulating Leptin Levels



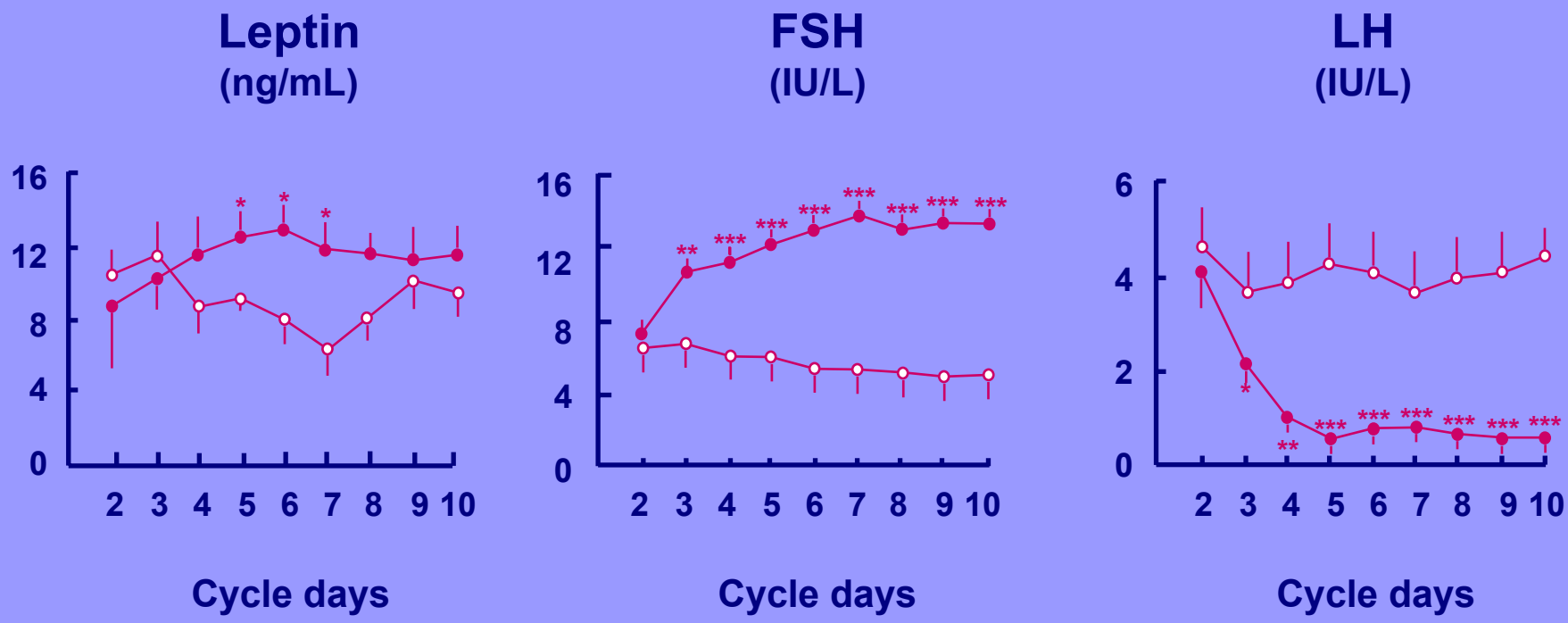
Ostlund et al., JCEM 81, 1996

Leptin Levels Rise during the Luteal Phase





Leptin Secretion During Ovarian Stimulation

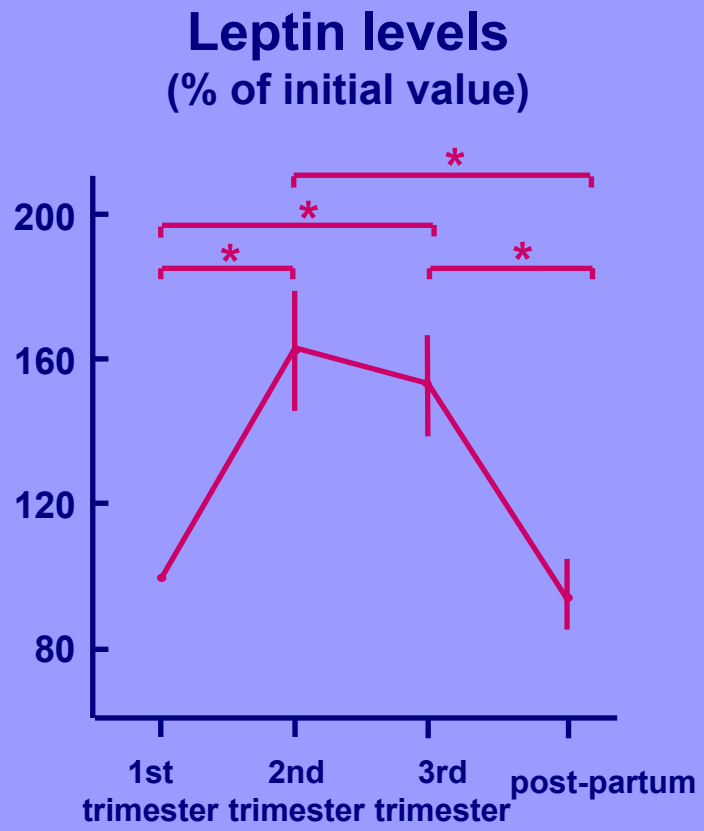


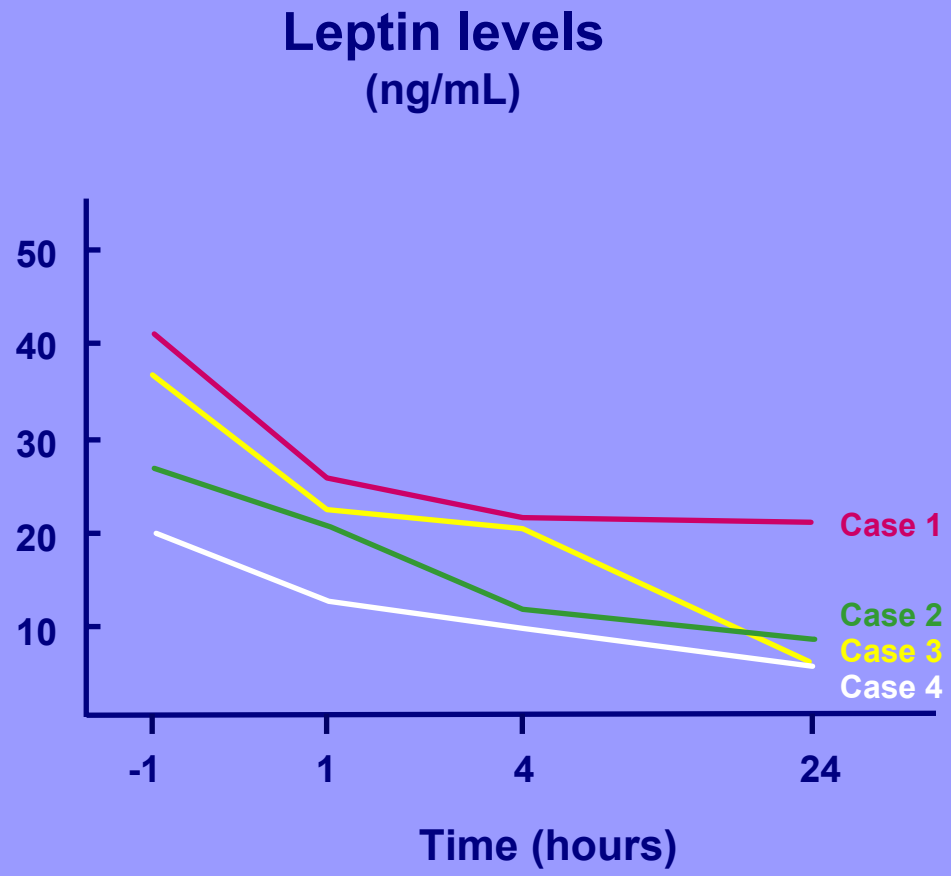
Messinis et al., Human Reprod 13, 1998

Leptin Levels and OB-R Expression Relevant to Female Reproduction

- **OB-R and leptin are expressed in the ovary; leptin modulates steroid synthesis *in vitro***
- **OB-R is expressed in the placenta**
- **High leptin concentrations in pregnancy; rapid decline after birth in mothers and neonates**

Elevated Leptin Levels During Pregnancy





Masuzaki et al, Nat Med 3, 1997

Summary I

Leptin regulates body fat stores via:

- **inhibition of feeding behavior**
- **stimulation of sympathetic nervous system and metabolism**

Summary II

- **Leptin probably signals to the brain at what time the body is ready for sexual maturation**

- **Leptin stimulates the production of reproductive hormones, either via a direct stimulation of the GNRH/LH-FSH axis or through the modulation of other afferent neurotransmitters (*i.e.* NPY)**

Summary III

- **During late pregnancy, when adequate maternal and fetal fat stores are vital, leptin might signal the correct expansion of fat stores to the brain**

- **The uncoupling of eating behavior observed during pregnancy would make sense to prepare additional energy stores before the stress of birth**

Summary IV

The significance of the high expression of OB-R and leptin in the human ovary remains to be elucidated:

- **Pathophysiological role in PCOD?**
- **Physiological role in steroid synthesis, in follicular development?**

Conclusion

Leptin, once called the « Voice of Adipose Tissue », is expressed in many reproductive organs:

- **the hypothalamus**
- **the ovary**
- **the placenta**
- **the pituitary gland**

Strong evidence suggests that it is a hormone of reproduction in the human.

However, its precise role other than to participate in the timing of puberty remains to be elucidated.

Future perspectives

- **Clinical usefulness as a diagnostic tool.**
- **Potential use as a therapeutic agent, providing more insight is gained into its function to modulate ovarian function directly.**