LEPTIN AND REPRODUCTION

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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

Hypogonadotropin 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

- Leptin (ob) stops eating
- Leptin (ob) decreases body weight
- Leptin receptor in hypothalamus
- Fat cells remain fat

Coleman et al., Diabetologia 14, 1978
The Leptin Gene

A positional cloning approach in the $\text{Lep}^{ob}$ 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 \textit{ob} gene limited to WAT

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

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

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

Zhang \textit{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

<table>
<thead>
<tr>
<th>Exp 1</th>
<th></th>
<th>Exp 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day at VO</td>
<td></td>
<td>Day at VO</td>
</tr>
<tr>
<td>Ad lib</td>
<td>32.0 +/- 1.1</td>
<td>Ad lib</td>
<td>35.0 +/- 0.9</td>
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<tr>
<td>Leptin</td>
<td>33.6 +/- 1.3</td>
<td>Leptin</td>
<td>41.8 +/- 1.0</td>
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<tr>
<td>Pair-fed</td>
<td>&gt;38</td>
<td>Vehicle</td>
<td>&gt;43</td>
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</table>

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

<table>
<thead>
<tr>
<th>Age at diag</th>
<th>Sex</th>
<th>Mutation</th>
<th>Clinical feat</th>
<th>LH (IU/L)</th>
<th>FSH (IU/L)</th>
<th>E2 (pmol/L)</th>
<th>T (nmol/L)</th>
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<tbody>
<tr>
<td>OB 1</td>
<td>Montague et al</td>
<td>8 y</td>
<td>F</td>
<td>G deletion at codon 133 (frameshift)</td>
<td>&lt;0.2</td>
<td>0.8</td>
<td>&lt;20</td>
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<td>OB 2</td>
<td>Montague et al</td>
<td>2 y</td>
<td>M</td>
<td>G deletion at codon 133 (frameshift)</td>
<td>&lt;0.2</td>
<td>0.2</td>
<td>-</td>
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<tr>
<td>OB 3</td>
<td>Strobel et al</td>
<td>34 y</td>
<td>F</td>
<td>R105W</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>OB 4</td>
<td>Strobel et al</td>
<td>22 y</td>
<td>M</td>
<td>R105W</td>
<td>4.4</td>
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<td>Clément et al</td>
<td>19 y</td>
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<td>G to A in splice donor site, exon 16</td>
<td>&lt;0.2</td>
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<tr>
<td>OB-R 2</td>
<td>Clément et al</td>
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<td>&lt;0.8</td>
<td>1.2</td>
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</tbody>
</table>
Correction of Hypogonadotrophic 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

Inadequate nutrition → Inadequate adiposity → Low leptin levels → Reproductive inadequacy

Lep<sup>ob</sup> mouse Human leptin deficiency

Plentiful nutrition → Adequate adiposity → High leptin levels → Reproductive competence

Exogenous leptin replacement

Obesity → Leptin absent → Infertility

LH, FSH, ovarian and uterine weight, Sperm count

Reproductive inadequacy

Reproductive competence
Sexual Dimorphism of Circulating Leptin Levels

Ostlund et al., JCEM 81, 1996
Leptin Levels Rise during the Luteal Phase
Leptin Secretion During Ovarian Stimulation

Leptin (ng/mL)  FSH (IU/L)  LH (IU/L)

Cycle days  Cycle days  Cycle days

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 \textit{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

Leptin levels (% of initial value)

Leptin levels (ng/mL)

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.