Emerging Clinical Applications of Leptin

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3. Hypothalamic Amenorrhea
4. Type 1 and Type 2 Diabetes
6. Obesity and Weight Maintenance
LEPTIN
IN GENERAL
Leptin

*Leptos* (Greek, thin)

16 kda Protein

167 amino acid

Mainly Produced by **white adipose tissue**

Expressed in placenta, ovaries, mammary epithelium, bone marrow, lymphoid tissue

Leptin itself was discovered in 1994 by Jeffrey Friedman at the Rockefeller University and Douglas L. Coleman

*Nature* 387, 206 - 209 (08 May 1997);
**ob gene** (*LEP* gene)

- Cloning *ob* protein (leptin)
  - *ob/ob* mouse:
    - inactivating mutation of *ob* gene
    - complete deficiency of leptin
    - obese, T2DM
  - 84% identical between human and mouse

*LEP* gene in Human
Chromosome 7
db gene (*LEPR* gene)

Coding leptin receptor
6 isoforms in mouse/rat
4 isoforms in human

Same Extracellular Domain
Only long form ObRb receptor isoform contains intracellular motifs

*db/db* mice:
early onset obesity, leptin insensitivity, Hyperphagia, morbid obesity, hypothalamic hypogonadism

- Ob-R mutations are extremely rare in humans

*Annu. Rev. Physiol. 62:413–437, 2000*
### Regulation factor of Leptin Secretion

<table>
<thead>
<tr>
<th>INCREASE LEPTIN</th>
<th>DECREASE LEPTIN</th>
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<tbody>
<tr>
<td>Adipose Tissue</td>
<td>Fasting</td>
</tr>
<tr>
<td>Overfeeding</td>
<td>Cold exposure</td>
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<tr>
<td>Obesity (except ob/ob)</td>
<td>Beta agonists</td>
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<tr>
<td>Insulin</td>
<td>Testosterone</td>
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<tr>
<td>Glucocorticoid</td>
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<tr>
<td>Acute Infection</td>
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<td>Proinflammatory cytokines</td>
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</tr>
</tbody>
</table>

*Physiology & Behavior 81:223–241, 2004*
Leptin Signaling

OBRb

STAT3

Cell membrane
Conserved cysteines
WSXWS motifs

Leptin

P JAK2
Box 1
JAK2

PTP1B

SHP2

GRB2

RAS/RAF

ERK1/2

p38 MAPK

Nucleus
Transcription of genes encoding
SOCS3, FOS, JUN and others
ROLE OF LEPTIN IN HUMAN
Leptin maintains energy homeostasis
- Adjusting Food intake and Energy expenditure
- Activate POMC and MSH / Inhibits the effect of NYP, AgRP
- Suppresses Appetite
- Mutation in POMC receptors, STAT3, ObRb results hyperphagia and obesity.

Rise in Leptin levels precedes the onset of puberty

Leptin antibody inhibits pubertal onset of rats

ob/ob mice: infertile, become fertile when treated with leptin

Leptin $\rightarrow$ Stimulation of LH secretion
Fasting $\rightarrow$ decrease in leptin $\rightarrow$ decrease goadotropin
Leptin’s Effect on LH Secretion is Indirect

GnRH neurons do not express ObRb
Leptin $\rightarrow$ AgRP/NPY and POMC neuron $\rightarrow$ GnRH neurons
Leptin $\rightarrow$ KiSS-1 neuron $\rightarrow$ GnRH neurons

Kiss-1, Kisspeptin and GPR54
mediator of Leptin & Reproduction
HypoGonadotrophin Hypogonadism

- **Congenital leptin deficiency:**
  inadequate secretion of GnRH,
  failure to reach puberty, lack of growth spurt,
  secondary sex characteristics, and menarch

- Leptin replacement can permit the appropriate onset of puberty

In Rodent:
Low Leptin $\rightarrow$ Low TRH $\rightarrow$ Low T3, T4
Leptin: Regulate TSH pulsatility and circadian rhythm

In Human:
Leptin’s role in regulating circulating levels of T3 and T4 may not be as important as it is in rodents

CRH is synthesized in the PVN.
Leptin stimulation of CRH release \textit{in vitro}.
Inverse relationship between circulating leptin and ACTH in healthy men.

\textbf{Leptin deficiency in humans, showed no major abnormalities in the adrenal axis}

\textit{Endocrinology} 1998;139(10):4264-8
\textit{Endocrinology} 1997;138(9):3859-63
Low Leptin $\rightarrow$ High infection rate

Immune cells express ObRb

Promotes production of pro-inflammatory cytokines (TNF-a, IL-6, IL,12)

Promotes lymphocyte survival (CD4+)


**Innate immunity**

- Activation of neutrophils
  - \( \rightarrow \) Chemotaxis
  - \( \rightarrow \) H\(_2\)O\(_2\)
  - \( \rightarrow \) O\(_2\)\(^{-} \)

- Cytokine induction by monocytes/macrophages
  - \( \rightarrow \) Acute-phase response
  - \( \rightarrow \) Inflammatory anorexia
  - \( \rightarrow \) Fever

- Activation of APCs
  - \( \rightarrow \) MHC molecules
  - \( \rightarrow \) Adhesion molecules
  - \( \rightarrow \) Phagocytosis

- Leukotriene B\(_4\)
- Cyclo-oxygenase 2

**Adaptive immunity**

- Thymic homeostasis
  - \( \uparrow \) Thymocyte number
  - \( \uparrow \) CD4\(^{+}\)CD8\(^{+}\) cells
  - \( \uparrow \) CD4\(^{+}\)CD8\(^{-}\) cells
  - \( \downarrow \) Apoptosis

- Proliferation
  - Naive T cells
  - \( \rightarrow \) IL-2

- \( \uparrow \) DTH
- \( \uparrow \) IgG\(_{2a}\) switch
- \( \uparrow \) CD8\(^{+}\) T-cell help
- \( \uparrow \) Macrophage activation

- Anti-apoptotic effects on T cells and on haematopoietic precursors
  - \( \uparrow \) BCL-2
  - \( \uparrow \) BCL-X\(_{L}\)

- \( \downarrow \) IgG\(_{1}\) switch

- \( \uparrow \) TH2-cell inhibition

**Leptin**

- TNF
- IL-2
Leptin Deficiency:
Insulin resistance, Diabetes, Hyperinsulinemia, Dyslipidemia

Administration of Leptin in ob/ob mice
improves glucose metabolism, reduces fat deposition, reduces body weight, improves insulin resistance, cholesterol, triglyceride, and LDL cholesterol level

Bone Metabolism

Direct Osteoblastic activity
Indirect effect via hypothalamus

- 10-week leptin replacement in women with hypothalamic amenorrhea yielded increased levels of Bone Formation Markers (bone-specific alkaline phosphatase and osteocalcin).
- 9 months of leptin administration; significant gains in bone mineral content and density.

Metabolism 60(9):1211-21, 2011
Effects on Beta Cell

- **Leptin**
  - SOCS3 activation
  - JAK-STAT activation
  - PTEN inhibition
  - PI3K-Akt activation
  - PI3K Activation
  - PDE3 Activation
  - Long-chain acyl-CoA Formation
  - Reduce Glucose Transport
  - Inhibit PLC-PKC Pathway
  - SREBP-1c Inhibition
  - FFA Oxidation
  - Inhibit iNOS Production
  - Bcl2 Activation

- **Effects on Beta Cell**
  - Inhibit Insulin Promoter Activity
  - Reduction of Insulin Gene Transcription
  - β-Cell Proliferation
  - Reducing cAMP
  - Activate K\textsubscript{ATP} Channels
  - Inhibition of Insulin Secretion
  - Reduce ATP-ADP Ratio
  - Block Lipid Accumulation
  - TG Depletion
  - Reduce NO
  - Suppression of β-Cell Apoptosis

**Improve Insulin Resistance**
**Decrease Needs of insulin secretion**

**Beta cell Protection**

Leptin is an anti-apoptotic molecule
Leptin is overexpressed in more than 80% cases of malignant tissues
Leptin and Leptin receptor are highly expressed in the breast cancer
Leptin and Ob-R Expression in human cancer tissues by ImmunoHistochemical Staining

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Subtype</th>
<th>Leptin</th>
<th>Ob-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-cell</td>
<td>Lymphoma</td>
<td>ND</td>
<td>39.8%</td>
</tr>
<tr>
<td>Bladder</td>
<td>Carcinoma</td>
<td>ND</td>
<td>↓↓</td>
</tr>
<tr>
<td>Brain</td>
<td>Glioma</td>
<td>55.2%</td>
<td>80.5%</td>
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<tr>
<td>Breast</td>
<td>Carcinoma</td>
<td>92%</td>
<td>83%</td>
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<tr>
<td>Colon</td>
<td>Carcinoma</td>
<td>73.5%</td>
<td>72.5%</td>
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<tr>
<td>Endometrioid</td>
<td>Adenocarcinomas</td>
<td>60%</td>
<td>31%</td>
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<tr>
<td>Esophagus</td>
<td>Carcinoma</td>
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<td>91%</td>
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<td>Kidney</td>
<td>Carcinoma</td>
<td>↑↑</td>
<td>38.6%</td>
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<td>Liver</td>
<td>Carcinoma</td>
<td>60.3%</td>
<td>53%</td>
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<tr>
<td>Lung</td>
<td>Adenocarcinoma</td>
<td>69.0%</td>
<td>ND</td>
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<tr>
<td>Ovary</td>
<td>Carcinoma</td>
<td>89.5%</td>
<td>59.2%</td>
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<tr>
<td>Prostate</td>
<td>Cancer</td>
<td>96.7%</td>
<td>63.3%</td>
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<td>Skin</td>
<td>Melanoma</td>
<td>90.9%</td>
<td>43.8%</td>
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<tr>
<td>Thyroid</td>
<td>Carcinoma</td>
<td>ND</td>
<td>80%</td>
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<tr>
<td>Thyroid</td>
<td>Carcinoma</td>
<td>37%</td>
<td>51%</td>
</tr>
</tbody>
</table>
• Raise Blood Pressure
• Platelet aggregation
• Endothelial Dysfunction
• Thrombosis
• Angiogenesis
CLINICAL APPLICATIONS OF LEPTIN IN HUMAN
Clinical Applications of Leptin

1. Congenital Leptin Deficiency
2. Diabetes & HyperTG in Lipodystrophy
3. Hypothalamic Amenorrhea
4. Type 1 Diabetes
5. Type 2 Diabetes
6. Weight Maintenance
7. Obesity
• Recombinant Human Methionyl Leptin (147 aa)
• Analog of Human Leptin (146 aa)
• r-metHuLeptin

• ATG (met) was added to the 5’end of the gene of human leptin
1. Congenital Leptin Deficiency
Mutation in the *LEP* gene

Obesity due to uncontrolled hyperphagia

Hypothalamic Hypogonadism

Pubertal Failure

Leptin Treatment

Congenital Leptin Deficiency

3 years old

9 years old

leptin

2. Lipodystrophy
Lipodystrophy

is a medical condition characterized by abnormal or degenerative conditions of the body's adipose tissue.

Lipoatrophy

is used when describing the loss of fat from one area (usually the face).
**Congenital lipodystrophy**
Congenital generalized lipodystrophy
Familial partial lipodystrophy

**Acquired lipodystrophy**
Acquired partial lipodystrophy (Barraquer-Simons syndrome)
Acquired generalized lipodystrophy
Centrifugal abdominal lipodystrophy
Localized lipodystrophy
**HIV-associated lipoatrophy**
**Insulin injection site**
HAART-induced Lipoatrophy in HIV (+) Patients
Highly active antiretroviral therapy (HAART)

Entry inhibitors (Fusion inhibitors): Maraviroc, enfuvirtide
CCR5 receptor antagonists:
Reverse transcriptase inhibitors:
Protease inhibitors
Integrase inhibitors (raltegravir)
Maturation inhibitors: Alpha interferon, bevirimat, Vivecon.

→ significantly reduces disease-associated mortality and morbidity in patients with HIV infection.
HAART induced Multiple metabolic abnormalities:
Hyperinsulinemia, insulin resistance, dyslipidemia, lipodystrophy, Hypoleptinemia
→ increasing the risk of cardiovascular disease.

Lipoatrophy: Generalized fat depletion, is present in up to 35% of HIV patients on HAART
HAART induced Lipoatrophy

Hyperinsulinemia
Diabetes
Dyslipidemia
Hypoleptinemia.

TREATMENT

1) Leptin Replacement
2) TZD (Pioglitazone)
Leptin Treatment in HAART induced Lipoatrophy

Rationale for Leptin Treatment

1) Hypoleptinemia is observed in HIV(+) Patients
2) Leptin improved Lipid profiles
3) Leptin could reduce blood glucose
4) Leptin improves insulin resistance
Leptin Treatment

Leptin improves **Insulin resistance**, increases **HDL cholesterol**, and reduces **trunkal fat mass**.

Leptin treatment improved **dyslipidemia**, **hepatic insulin sensitivity**, **Decreased lipolysis**, and **Visceral fat**

JCEM 2006, 2009
Leptin Treatment

Leptin Treatment Improves Insulin Resistance but also Reduces Total Body Fat in lipoatrophic HIV patients.
Rational for Pioglitazone Treatment

1) Pioglitazone improves Insulin Resistance
2) Pioglitazone lowers blood glucose
Pioglitazone Treatment

**Original article**

Effect of pioglitazone on HIV-1-related lipodystrophy: a randomized double-blind placebo-controlled trial (ANRS 113)

Laurence Siama1, Emilie Laroy2,3, Marc-Antoine Valantin4,5, Jean-Philippe Bustard6, Aziza Cherme7,8,9, Amal Boutekrit10, Demiana William-Faltao11, Eric Billand2, Jean-Michel Molina1, Jacqueline Capeau12, Dominique Costagliola13 and Willy Razonbaun14

**Text from image**

Improvement in Highly Active Antiretroviral Therapy–Induced Metabolic Syndrome by Treatment with Pioglitazone but Not with Rosiglitazone.

Pioglitazone improves insulin resistance & lipid profile.

Pioglitazone Improved Limb Fat Atrophy & lipid profile.
Pioglitazone Treatment improves Insulin Resistance and Increases Total Body Fat & Limb Fat Mass in HIV infected lipoatrophic patients.
Leptin + Pioglitazone ???

9 men (age 50, BMI 26kg/m^2) with HIV-1 infection and at least 6 mon. of HAART exposure, serum leptin concentrations < 4 ng/mL and clinical evidence of lipoatrophy were recruited.

Exclusion criteria: History of fasting hyperinsulinemia, hyperglycemia, impaired glucose tolerance, T2DM, and dyslipidemia before the initiation of HAART; abnormal hepatic and renal functions; active infection other than HIV; overt hypo- and hyperthyroidism; hypogonadism; hypercortisolism; treatment with steroids or GH, alcoholism and drug abuse.

Randomized in a double-blind fashion

Pioglitazone treatment (30 mg/day)

Receive either

metreleptin (n=5; 0.04 mg/kg/day, SQ) or placebo (n=4; same vol. as leptin) for 3 months.

Metreleptin increased **serum leptin concentrations** compared with placebo (P=0.02).

MetreLeptin significantly reduced **Fasting Serum Insulin Level**, **HOMA-IR** and **Postprandial Glucose levels**.

Metreleptin + Pioglitazone did **not affect BMI, body fat mass and distribution**, resting **BP and HR**, and lipid profile but increased serum adiponectin concentration.

Fig. 1 – Glucose (top) and insulin (bottom) concentrations and areas under the curve in response to a mixed meal before (white symbols) and after (black symbols) 3 months of placebo or metreleptin administration in HIV-infected lipoatrophic men treated with pioglitazone. Compared with placebo, metreleptin significantly attenuated postprandial glycemia ($P = .02$ and $P = .01$ for the on-treatment and intention-to-treat analyses, respectively), but did not affect postprandial insulinenia ($P = .57$ and $P = .82$, respectively).

Leptin + Pioglitazone Treatment in HAART induced Lipoatrophy

Leptin + Pioglitazone Treatment attenuates Fasting insulinemia and Insulin resistance and improves Postprandial Glycemia Without affecting Body Fat Mass in lipoatrophic HIV-positive men treated with pioglitazone.

Amylin Company Completes Biologics License Application for Metreleptin to Treat Diabetes and/or Hypertriglyceridemia in Patients With Rare Forms of Lipodystrophy

- If Approved, Metreleptin Would Represent First Therapy for Life-Threatening Metabolic Disease
3. Hypothalamic Amenorrhea
Cessation of menstrual cycles because of Dysfunction of the hypothalamic pituitary-gonadal axis, abnormalities in gonadotropin pulsatility, and subsequent Estrogen deficiency.
Causes: strenuous exercise, stress, and/or reduced food intake
State: chronic energy deficiency, Low leptin
Prevalence: accounts for more than 30% of cases of amenorrhea in women of reproductive age.
Clinical Problems: Infertility, Dysfunction of the thyroid, growth hormone, Bone loss (Osteoporosis) and propensity for fractures.
Lab. Findings of HA

- Low LH
- Subnormal/normal FSH
- Very low Estradiol
- Very low Serum Leptin
Current Available Treatments

- Wait and See !!!
- Weight Gain ???
- If Patient wants to be pregnant:
  - Hormone replacement (Moderate dose Estrogen + Progesterone)
- Calcium + Vitamin D for Osteopenia
- Estrogen replacement for Osteoporosis
- Bisphosphonate ???

LEPTIN
Leptin Treatment for HA

Recombinant Human Leptin in Women with Hypothalamic Amenorrhea

Corinne K. Welt, M.D., Jean L. Chan, M.D., John Bulfer, B.A., Robyn Murphy, M.S., Patricia Smith, B.S., Alex M. DePaoli, M.D., Aspasia Karafila, B.A., and Christos S. Mantzoros, M.D., D.O.,

Short Term
3 months

Leptin is an effective treatment for hypothalamic amenorrhea

Sharon H. Chou, John P. Chamberland, Xiaowen Liu, Giuseppe Matarrese, Chuanyun Gao, Rianna Stefanakis, Mary T. Brinkoetter, Huizhi Gong, Kalliopi Arampatzis, and Christos S. Mantzoros

*Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115; †Laboratorio di Immunologia, Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche and ‡Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II, Naples 80131, Italy; ‡Section of Endocrinology, Boston Veterans Administration Healthcare System, Harvard Medical School, Boston, MA 02130; and †Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115

Edited by Jeffrey M. Friedman, The Rockefeller University, New York, NY, and approved February 25, 2011 (received for review October 26, 2010)

PNAS 108(16):6585-6590, 2011

Long Term
1 year
### Leptin Increases LH & Estradiol (3 mon.)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (N=8)</th>
<th>Month 1 (N=8)</th>
<th>Month 2 (N=7)</th>
<th>Month 3 (N=5)</th>
<th>One-Month Follow-up (N=7)</th>
<th>Overall P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body composition</strong></td>
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</tr>
<tr>
<td>Body weight (kg)</td>
<td>54.7±4.5</td>
<td>54.1±4.3</td>
<td>54.0±3.6</td>
<td>52.2±3.5</td>
<td>53.6±3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass kg</td>
<td>12.5±2.8</td>
<td>11.8±2.2</td>
<td>11.1±2.0</td>
<td>9.6±1.7</td>
<td>10.2±1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%</td>
<td>22.4±3.7</td>
<td>21.4±3.2</td>
<td>20.4±2.9</td>
<td>18.1±2.9</td>
<td>18.6±2.9</td>
<td>&lt;0.001</td>
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<td><strong>Hormones</strong></td>
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<tr>
<td>Leptin (ng/ml)</td>
<td>3.4±1.5</td>
<td>9.7±4.1</td>
<td>20.6±15.7</td>
<td>37.4±30.1</td>
<td>9.4±10.1</td>
<td>&lt;0.001</td>
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<tr>
<td>LH (IU/liter)</td>
<td>3.1±3.6</td>
<td>5.1±4.5</td>
<td>5.7±2.7</td>
<td>6.7±4.2</td>
<td>2.2±2.0</td>
<td>&lt;0.001</td>
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<tr>
<td>FSH (IU/liter)</td>
<td>6.2±1.3</td>
<td>7.0±1.2</td>
<td>6.9±1.6</td>
<td>6.6±1.8</td>
<td>5.7±1.5</td>
<td>0.16</td>
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<tr>
<td>Estradiol (pg/ml)</td>
<td>26.9±7.7</td>
<td>44.1±25.7</td>
<td>54.4±20.9</td>
<td>71.2±22.8</td>
<td>28.4±9.6</td>
<td>&lt;0.001</td>
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<tr>
<td>Inhibin A (IU/ml)</td>
<td>0.89±0.4</td>
<td>1.15±0.5</td>
<td>1.66±1.2</td>
<td>1.85±1.8</td>
<td>0.88±0.5</td>
<td>0.37</td>
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<tr>
<td>Inhibin B (pg/ml)</td>
<td>99.7±46.8</td>
<td>136.3±54.3</td>
<td>126.1±35.1</td>
<td>145.0±55.5</td>
<td>110.7±53.2</td>
<td>0.54</td>
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<tr>
<td><strong>Free T₃ (pg/ml)</strong></td>
<td>1.90±0.2</td>
<td>1.99±0.3</td>
<td>2.23±0.4</td>
<td>2.59±0.4</td>
<td>2.23±0.4</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Free T₄ (ng/dl)</strong></td>
<td>1.08±0.1</td>
<td>1.08±0.1</td>
<td>1.17±0.2</td>
<td>1.28±0.1</td>
<td>1.12±0.2</td>
<td>&lt;0.001</td>
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<td>Thyrotropin (μIU/ml)</td>
<td>2.48±1.3</td>
<td>2.90±1.1</td>
<td>3.06±1.3</td>
<td>4.52±2.2</td>
<td>2.63±1.8</td>
<td>0.056</td>
</tr>
<tr>
<td>Cortisol (μg/dl)</td>
<td>17.2±3.3</td>
<td>19.3±4.2</td>
<td>20.1±4.7</td>
<td>19.9±4.4</td>
<td>15.3±4.6</td>
<td>0.07</td>
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<tr>
<td>Corticotropin (pg/ml)</td>
<td>18.1±7.2</td>
<td>20.3±7.1</td>
<td>19.2±7.9</td>
<td>20.2±6.0</td>
<td>14.6±7.6</td>
<td>0.40</td>
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<td>IGF-1 (ng/ml)</td>
<td>191.3±31.1</td>
<td>219.7±49.6</td>
<td>253.8±57.4</td>
<td>281.3±59.5</td>
<td>212.4±44.8</td>
<td>&lt;0.001</td>
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<tr>
<td>IGF-binding protein 3 (μg/ml)</td>
<td>4.46±0.6</td>
<td>4.44±0.8</td>
<td>4.80±0.9</td>
<td>5.20±0.3</td>
<td>4.51±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Bone markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone alkaline phosphatase (U/liter)</td>
<td>22.9±10.23</td>
<td>20.2±7.8</td>
<td>23.2±8.6</td>
<td>24.8±12.3</td>
<td>26.8±11.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>17.5±13.7</td>
<td>21.2±15.3</td>
<td>22.0±13.0</td>
<td>22.3±18.2</td>
<td>22.0±17.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary N-telopeptides:creatinine</td>
<td>49.1±33.6</td>
<td>59.3±25.0</td>
<td>54.0±26.8</td>
<td>58.7±25.1</td>
<td>64.6±58.2</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Leptin Increases Follicle size, Ovarian volume & Endometrial Thickness (3 mon.)
# Leptin increases E2 & Bone Formation Markers (1 year)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Group</th>
<th>Week 0 (baseline)</th>
<th>Week 12</th>
<th>Week 24</th>
<th>Week 36</th>
<th>Week 52 (follow-up)</th>
<th>P (trt)*</th>
<th>P (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hormones</strong></td>
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<tr>
<td>Leptin (ng/mL)</td>
<td>Metreleptin</td>
<td>4.55 ± 0.64</td>
<td>44.51 ± 8.74</td>
<td>57.26 ± 11.36</td>
<td>59.33 ± 14.15</td>
<td>8.64 ± 3.92</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>Placebo</td>
<td>4.10 ± 0.64</td>
<td>3.65 ± 0.61</td>
<td>3.51 ± 0.52</td>
<td>3.09 ± 0.51</td>
<td>2.64 ± 0.57</td>
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<tr>
<td>Free leptin (ng/mL)</td>
<td>Metreleptin</td>
<td>4.75 ± 1.24</td>
<td>23.87 ± 3.97</td>
<td>47.52 ± 11.98</td>
<td>49.04 ± 13.78</td>
<td>3.78 ± 1.00</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>Placebo</td>
<td>3.77 ± 0.62</td>
<td>3.35 ± 0.43</td>
<td>3.25 ± 0.41</td>
<td>2.75 ± 0.40</td>
<td>2.34 ± 0.30</td>
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<tr>
<td>Estradiol (pg/mL)</td>
<td>Metreleptin</td>
<td>23.0 ± 9.0</td>
<td>19.3 ± 4.5</td>
<td>27.2 ± 8.0</td>
<td>25.4 ± 7.8</td>
<td>22.0 ± 7.8</td>
<td>0.01</td>
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<tr>
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<td>Placebo</td>
<td>14.0 ± 1.7</td>
<td>13.9 ± 1.8</td>
<td>12.3 ± 1.4</td>
<td>11.8 ± 1.3</td>
<td>11.6 ± 1.8</td>
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<tr>
<td>Progesterone (ng/mL)</td>
<td>Metreleptin</td>
<td>4.5 ± 0.4</td>
<td>4.9 ± 0.3</td>
<td>14.5 ± 5.6</td>
<td>7.3 ± 3.5</td>
<td>7.8 ± 3.9</td>
<td>0.03</td>
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<tr>
<td></td>
<td>Placebo</td>
<td>4.6 ± 0.4</td>
<td>4.4 ± 0.4</td>
<td>4.4 ± 0.3</td>
<td>5.2 ± 0.7</td>
<td>3.7 ± 0.2</td>
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<tr>
<td>Cortisol (μg/dL)</td>
<td>Metreleptin</td>
<td>20.9 ± 1.1</td>
<td>18.2 ± 1.0</td>
<td>17.1 ± 1.1</td>
<td>12.8 ± 1.3</td>
<td>14.9 ± 1.6</td>
<td>0.02</td>
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<td>Placebo</td>
<td>20.0 ± 1.3</td>
<td>19.6 ± 1.4</td>
<td>20.2 ± 1.0</td>
<td>19.7 ± 0.9</td>
<td>17.7 ± 1.1</td>
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<tr>
<td>IGF1 (ng/mL)</td>
<td>Metreleptin</td>
<td>498.1 ± 66.7</td>
<td>462.2 ± 51.1</td>
<td>543.2 ± 49.7</td>
<td>491.0 ± 69.8</td>
<td>382.3 ± 71.7</td>
<td>0.23</td>
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<tr>
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<td>Placebo</td>
<td>422.1 ± 41.9</td>
<td>434.3 ± 37.2</td>
<td>404.4 ± 33.1</td>
<td>388.4 ± 40.9</td>
<td>331.6 ± 50.6</td>
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<tr>
<td>IGF1:IGFBP-3</td>
<td>Metreleptin</td>
<td>5.35 ± 0.49</td>
<td>5.14 ± 0.37</td>
<td>6.09 ± 0.40</td>
<td>5.46 ± 0.61</td>
<td>4.58 ± 0.72</td>
<td>0.32</td>
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<tr>
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<td>Placebo</td>
<td>5.05 ± 0.45</td>
<td>5.14 ± 0.38</td>
<td>4.82 ± 0.33</td>
<td>4.63 ± 0.42</td>
<td>3.91 ± 0.56</td>
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</table>

**Bone markers**

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Week 0 (baseline)</th>
<th>Week 12</th>
<th>Week 24</th>
<th>Week 36</th>
<th>Week 52 (follow-up)</th>
<th>P (trt)*</th>
<th>P (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASP (U/L)</td>
<td>Metreleptin</td>
<td>26.6 ± 5.7</td>
<td>28.2 ± 6.1</td>
<td>29.6 ± 7.3</td>
<td>30.7 ± 7.5</td>
<td>22.7 ± 4.5</td>
<td>0.09</td>
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<td>Placebo</td>
<td>16.7 ± 4.4</td>
<td>17.2 ± 4.3</td>
<td>13.4 ± 2.7</td>
<td>13.5 ± 2.4</td>
<td>14.2 ± 4.2</td>
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<tr>
<td>Osteocalcin (ng/mL)</td>
<td>Metreleptin</td>
<td>13.9 ± 2.6</td>
<td>20.9 ± 3.1</td>
<td>19.8 ± 3.1</td>
<td>20.9 ± 3.1</td>
<td>12.3 ± 2.1</td>
<td>0.0019</td>
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<td>Placebo</td>
<td>9.3 ± 0.8</td>
<td>8.5 ± 1.0</td>
<td>8.1 ± 0.9</td>
<td>7.7 ± 0.7</td>
<td>8.7 ± 1.1</td>
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<tr>
<td>Urinary NTX:creatinine</td>
<td>Metreleptin</td>
<td>49.4 ± 5.4</td>
<td>72.3 ± 9.0</td>
<td>56.1 ± 13.6</td>
<td>52.8 ± 13.4</td>
<td>45.9 ± 20.2</td>
<td>0.03</td>
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<tr>
<td></td>
<td>Placebo</td>
<td>30.8 ± 3.1</td>
<td>42.0 ± 10.0</td>
<td>56.9 ± 21.5</td>
<td>58.1 ± 12.0</td>
<td>38.2 ± 8.3</td>
<td></td>
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</tr>
</tbody>
</table>

PNAS 108(16):6585-6590, 2011
Restoration of Menstruation by Leptin (1 year)

A. Leptin conc.

B. Free Leptin conc.

C. Restoration of Mense (%)
Leptin Increases Spinal Bone Density

Metabolism 60:1211-1211, 2011
4. Type 1 Diabetes
What is the Fundamental Defect in Type 1 Diabetes? Sole Absolute Insulin Deficiency?
1) Glucagon Increases Hepatic Gluconeogenesis which presents in insulin deficiency

2) Hyperglucagononemia is present in every form of poorly controlled diabetes

3) Glucagon suppressors (Leptin & Somatostatin) suppress all catabolic manifestation shown in total insulin deficiency

4) Total beta cell destruction in glucagon receptor KO mice does not cause Diabetes

**Glucagon Suppression or inactivation Rather than Insulin monotherapy?**
Glucagon Receptor KO mice (Gcgr-/-)

STZ

Insulin for OGTT

Glucose for OGTT

No Insulin response with glucose tolerance

No Hyperglycemia in KO mice

Normal GTT without Insulin

Diabetes 60:391-397, 2011
Glucagon receptor KO mice show reduced CREB phosphorylation.
Glucagon receptor KO mice show reduced PEPCK mRNA expression in Liver
Leptin As a Glucagon Suppressor
Adenoviral Transfer of the Leptin Gene in NOD Mice

Hyperleptinemia reverses abnormalities of uncontrolled autoimmune diabetes in the absence of insulin

PNAS 14070-14075, 2008
Adenoviral Transfer of the Leptin Gene in Streptozotocin induced Diabetes

Hyperleptinemia reverses abnormalities of uncontrolled chemical diabetes in the absence of insulin.
Hyperleptinemia activates liver STAT-3 and down-regulates proteins of gluconeogenesis, while limiting postprandial hyperglycemia.

PNAS 14070-14075, 2008
Hyperleptinemia increases plasma IGF-1 and IGF-1 action on skeletal muscle, while restoring linear growth in severely insulin-deficient rats.

PNAS 14070-14075, 2008
Recombinant Leptin Infusion in Insulin Deficient Type 1 Diabetic Rat

A. Plasma Leptin

B. Blood Glucose

- Leptin Infusion
- Insulin
- Untreated mice fed ad libitum
- Untreated mice pair-fed with leptin treated group
Comparison of plasma glucose levels in type 1 diabetic NOD mice treated with twice daily injection of insulin alone at a total dose of 0.2 U/day (▲), twice daily insulin alone at a total dose of 0.02U/day fed ad libitum(○), twice daily insulin PF to the leptin-treated group (□), or a total daily dose of insulin of 0.02 U/day plus leptin injected at the doses and times indicated by arrows(●).
Why Insulin Monotherapy in T1DM cannot Restore Normal Glycemic Variability?
Pancreas specific ablation of the alpha-cell transcription factor, Arx, resulting in a complete loss of the glucagon-producing pancreatic alpha-cell.

Alpha Cell Lacking Mouse

D

STZ treated
Control
Arx deficient

Blood Glucose (mg/dl)

Day

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29

Where does insulin act first?

Pancreatic Alpha Cell
Reversal of Type 1 DM by Brown Adipose Tissue Transplant

C

Blood glucose mg/dl

Time (min)

0 10 20 30 40 50 60 70 80 90 100 110 120

Normal
Diabetic Untreated
Diabetic Pre-TP
1 month post-TP
6 months post-TP *

A

Plasma insulin ng/ml

0 0.5 1 1.5 2 2.5 3

Normal non-diabetic
Diabetic Untreated
Diabetic Pre-Transplant
BAT Transplant

0 min
15 min
30 min
1 hour
2 hours
Reversal of Type 1 DM by Brown Adipose Tissue Transplant

Diabetes 61:674-682, 2012
Leptin Treatment in Type 1 DM

1) T1DM: relative leptin deficiency
2) Leptin has antisteatotic effect
3) **Leptin suppress glucagon level**
4) Leptin reduce food intake
5) Leptin can restore insulin secretion
6) Leptin enhances insulin sensitivity
7) Leptin improves lipid metabolism
8) Leptin can cross talk with insulin signaling cascade.
9) **Leptin increases IGFBP2 which has antidiabetic effect**
Leptin therapy for type 1 diabetes?

- Food intake
- Adiposity
- Cholesterol
- Triglycerides
- Hypoglycemia?

↑ Blood pressure
↑ Inflammation
↑ Platelet aggregation
↑ Endothelial dysfunction
↑ Thrombosis
↑ Angiogenesis
↑ Immune response

PNAS 16;107(11):4793-4. 2010
Leptin Treatment in Type 1 Diabetes Mellitus

Effects of Metreleptin in Type 1 Diabetes Mellitus

This study is currently recruiting participants.
Verified August 2011 by University of Texas Southwestern Medical Center

First Received on December 29, 2010. Last Updated on August 2, 2011 History of Changes

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<tr>
<th>Sponsor</th>
<th>University of Texas Southwestern Medical Center</th>
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<tr>
<td>Collaborators</td>
<td>Juvenile Diabetes Research Foundation</td>
</tr>
<tr>
<td></td>
<td>Amylin Pharmaceuticals, Inc.</td>
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<tr>
<td>Information provided by</td>
<td>University of Texas Southwestern Medical Center</td>
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<tr>
<td>ClinicalTrials.gov Identifier</td>
<td>NCT01268644</td>
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</table>

Estimated Enrollment: 15
Study Start Date: September 2010
Estimated Study Completion Date: February 2013
Estimated Primary Completion Date: October 2012 (Final data collection date for primary outcome measure)
5. Type 2 Diabetes
In obese patients with diabetes, metreleptin administration for 16 weeks did not alter body weight or circulating inflammatory markers but reduced HbA1c marginally (8.01 → 7.96, P = 0.03).

Recombinant human leptin treatment does not improve insulin action in obese subjects with type 2 diabetes.
6. Obesity
Recombinant Leptin for Weight Loss in Obese and Lean Adults
A Randomized, Controlled, Dose-Escalation Trial

Steven B. Heymsfield, MD
Andrew S. Greenberg, MD
Ken Fujioka, MD
Russell M. Dixon, MD
Robert Kushner, MD
Thomas Hunt, MD
John A. Lubina, PhD
Janet Patane, MPH
Barbara Self, MPH
Pam Hunt, PhD
Mark McCamish, PhD, MD

Context  The protein hormone leptin is important to the homeostatic regulation of body weight. Treatment with exogenous leptin may affect weight loss.

Objective  To determine the relationship between increasing doses of exogenous leptin administration and weight loss in both lean and obese adults.

Design  A randomized, double-blind, placebo-controlled, multicenter, escalating dose cohort trial conducted from April 1997 to October 1998.

Setting  Four university nutrition and obesity clinics and 2 contract clinical research clinics.

Participants  Fifty-four lean (body mass index, 20.0-27.5 kg/m²; mean [SD] body weight, 72.0 [9.7] kg) and 73 obese (body mass index, 27.6-36.0 kg/m²; mean [SD] body weight, 89.8 [11.4] kg) predominantly white (80%) men (n = 67) and women (n = 60) with mean (SD) age of 39 (10.3) years.

Interventions  Recombinant methionyl human leptin self-administered by daily mouth injections, daily (24 injections) 0.20 mg, 0.40 mg, 0.80 mg, 1.60 mg, 3.20 mg, 6.40 mg, and 12.80 mg doses for 4 weeks.
Leptin Resistance and Leptin Sensitizer

**Pathophysiology/Complications**

**ORIGINAL ARTICLE**

**Leptin and Amylin Act in an Additive Manner to Activate Overlapping Signaling Pathways in Peripheral Tissues**

In vitro and ex vivo studies in humans

**C. Leptin/amylin synergism**

<table>
<thead>
<tr>
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<th>Amylin</th>
<th>Leptin</th>
<th>Amylin + Leptin</th>
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<tbody>
<tr>
<td>Body weight loss (%)</td>
<td><img src="Graph.png" alt="Graph showing body weight loss" /></td>
<td><img src="Graph.png" alt="Graph showing body weight loss" /></td>
<td><img src="Graph.png" alt="Graph showing body weight loss" /></td>
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</table>

Diabetes Care 34: 132-138, 2011
Expert Opin Biol Ther 11(12), 2011
Takeda, Amylin discontinue pramlintide/metreleptin development program for obesity

Aug. 5, 2011
1. Diabetes and HyperTG in HARRT induced LIPODYSTROPHY
   Significant decrease in Blood Glucose and TG, Under FDA review for clinical use
2. In Combination with Pramlintide for the Treatment of OBESITY.
3. TYPE 1 DIABETES: Phase 3 Clinical Trial ongoing, Reductions in A1C and TG (end in 2013)
4. With Insulin in TYPE 2 DIABETES
   improves blood glucose control and lipid panels in rodents.
5. WEIGHT MAINTENANCE after Wt reduction Surgery
   Ongoing
6. HYPOTHALAMIC AMENORRHEA
   increase LH and FSH, Restore LH pulse, Increase Estradiol, Restore Menstruation, increase Bone density
Thank You!