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PLASMA LEPTIN LEVELS ARE INCREASED IN SURVIVORS OF ACUTE SEPSIS: ASSOCIATED LOSS OF DIURNAL RHYTHM IN CORTISOL AND LEPTIN SECRETION

S.R. Bornstein*†, J. Licinio*, R. Tauchnitz†, L. Engelmann**, AB Negrão*, P. Gold*, G.P. Chrousos*
*Developmental Endocrinology Branch, NICHD, and Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892 USA, **Department of Internal Medicine I, University of Leipzig, †Department of Internal Medicine III, University of Leipzig 04103 Leipzig, Germany

Abstract: Recent animal and human studies have suggested that leptin secretion is closely linked to the functions of the hypothalamic-pituitary-adrenal (HPA) axis and the immune system, both of which are crucial in influencing the course and outcome of critical illness. Therefore, we measured basal plasma leptin levels and examined the circadian secretion of leptin, in parallel with the hormones of the HPA axis and a key cytokine, interleukin-6, in critically ill patients with acute sepsis. Sixteen critically ill patients from the University of Leipzig Intensive Care Unit were recruited for this study. All of these patients fulfilled the standard diagnostic criteria for sepsis. Plasma leptin levels were measured in all patients and controls at 09:00. In addition, in a subgroup of eight critically ill patients and all of the nine controls , plasma leptin, cortisol, ACTH and interleukin-6 concentrations were measured every 4 hours for 24 hours. Mean plasma leptin levels were three-fold higher (18.9 ± 4.5 ng/ml) in critically ill patients than controls (3.8 ± 1.0 ng/ml, $p<0.05$). Similarly, ACTH levels were lower (7.8 ± 3.4 pmol/l) in patients than in controls (17.1±1.5 pmol/l, $p<.001$), while plasma cortisol levels were increased (947.6 ±144 nmol/l) in patients compared to controls (361.1±29, $p< 0.001$). Morning plasma interleukin-6 levels were markedly elevated in all patients with sepsis (1238.0±543.1 pg/ml) *versus* controls (6.4 ±1.7, $p<0.001$). The controls exhibited a nyctohemeral fluctuation in plasma leptin levels with peak levels at 23:00; in contrast, septic patients, had no nocturnal rise of leptin. In healthy controls, plasma leptin and cortisol had reciprocal circadian rhythms with high nocturnal leptin levels and low nocturnal cortisol concentrations; in critically ill patients, this relation was abolished. Mean leptin levels were three-fold higher in patients who survived the septic episode (25.5±6.2, n=10) than in non-survivors (8.0 ±3.7, n=6, $p<0.01$). We conclude that in addition to its function as an anti-obesity factor, leptin may play a role in a severe stress state such as acute sepsis.

INTRODUCTION

Leptin is encoded by the OB gene. Its major source is the adipose tissue (1), and its circulating concentrations indirectly reflect body fat stores (2,3). Recently, congenital leptin deficiency was reported resulting in morbid obesity in humans(4). Leptin is secreted in a circadian fashion with a nocturnal rise in lean and obese patients and in patients with non-insulin dependent diabetes mellitus (5). Plasma leptin levels are highest between midnight and the early morning hours and lowest around noon to mid-afternoon.

It has been speculated that the nocturnal rise in serum leptin concentrations may be related to nighttime bed rest (inactivity) and the suppression of appetite during sleep. Lower concentrations of leptin during the day are related to increased activity and energy expenditure (6). However, the physiological or pathophysiological significance of circadian and pulsatile leptin secretion remains to be established. An inverse relation between leptin concentrations and the hypothalamic-pituitary-adrenal (HPA) axis has been found in rats and in humans, suggesting that leptin secretion and the human stress axis are closely linked (7-9).

The leptin-HPA axis interaction may be of clinical importance. Recent findings have led to the suggestion that leptin may be involved in the cytokine-induced anorexia and wasting syndrome of infection (10-14). However, no studies on leptin secretion in human patients with acute sepsis have been reported so far. We sought to characterize the dynamics of circulating leptin in patients with acute activation of the HPA axis and the inflammatory cytokine system in critical illness due to sepsis.

In the present study, mean morning and circadian plasma leptin levels were determined in parallel with concurrent plasma levels of ACTH, cortisol and/or interleukin-6 (IL-6) in critically ill patients fulfilling the criteria of sepsis syndrome (15).

MATERIALS AND METHODS

Study Subjects :

Sixteen critically ill patients from the Intensive Care Unit of the University of Leipzig were recruited for this study over a period of 6 months. All patients fulfilled the clinical criteria for sepsis syndrome (15). Clinical evidence of infection plus the presence of fever or hypothermia (temperature >38.3°C or <35.6°C), tachypnea (>20 breaths per minute) and tachycardia (>90 beats per minute) and at least one of the following manifestations of inadequate organ perfusion/function: hypoxemia (PaO_2 <75 mmHG); metabolic acidosis (pH < 7.30); oliguria (output < 30 ml for at least one hour); acute alteration in mental status.

Patients with previous history of diabetes mellitus, malignant disease, and of adrenal and pituitary diseases, hypophysectomized patients, previous history of prolonged use of exogenous corticosteroids, or current therapy with steroids were **excluded** from the study. The study was approved by the Ethical Committee of the University of Leipzig.

Nine healthy individuals, matched for gender and BMI, were used as controls. Leptin levels were determined at 09:00 in all 16 patients and in all controls. In addition, blood was subsequently drawn in a subgroup of 8 patients every 4 hours to examine the 24 hour profile of leptin, cortisol, and

ACTH. Interleukin-6 was measured in the same group at 09:00.

Assays

For all measurements, 8 ml venous blood was taken into a chilled syringe with EDTA and immediately centrifuged at 1600g for 15 min at 4°C. Plasma was stored at -70°C until analysis. Leptin was measured by a commercially available radioimmunoassay (RIA) (Linco Research Inc. St. Charles, MO). Controls were used in the low and high sections of the standard curve. Samples were run in duplicate and standards in triplicate. The intra- and inter- assay coefficients of variation were both below 5%. ACTH and cortisol levels were measured at the same time-points by specific RIAs (ACTH BRAHMS Diagnostica GMBH, Berlin, cortisol DPC, Los Angeles). IL-6 was measured by ELISA (Immunotech/Coulter company, Hamburg). The intra and inter-assay variation were under 8%.

Statistical Analysis

Data analyses were performed on an IBM computer using PRISM (San Diego, CA, 92121, USA). Difference of hormone levels for leptin, ACTH, cortisol, and IL-6 of healthy controls and critically ill patients for each time point were assessed by unpaired Student t-test. Data are expressed as mean ±SE. Data of survivor and non-survivor subgroups are presented in a scatter diagram in which each patient's values can be shown. Statistical difference between controls and the two groups was calculated by analysis of variance (ANOVA) for repeated measures with *post hoc* testing with Dunnett's test where appropriate. Comparison of hormone levels after 5 am were compared with the 5 am level using ANOVA for repeated measures and Dunnett's test where appropriate. Percentage change in hormone levels was calculated using the formula: variability at time t=(hormone level at time t / 24h individual average level) X 100.

RESULTS

Mean Body Mass Index (BMI) and gender were similar in critically ill patients (BMI=25.6 ±0.65, 7 males/9 females) and healthy controls (BMI=23.07±1.15, 4 males/5 females). Mean age of the patients (59. 62±4.62) was higher than the mean age of healthy controls (29.44± 2.5). However, there was no correlation of age and leptin levels in either group (r=0.49). Average leptin levels were significantly higher in acutely ill patients (18.9 ± 4.5 ng/ml) compared to healthy controls (3.8 ±1.0 ng/ml, p< 0.01) (FIG 1a). ACTH levels were lower 7.8±3.4 pmol/l than in normal controls (17.1±1.5 pmol/l, p<0.01) (FIG. 1b). Cortisol levels were also significantly increased (947.6 ±144 nmol/l) in critical patients with acute sepsis versus controls (361.1 ±29, p< 0.001) (FIG 1c). All patients with acute sepsis showed a strong increase in their morning plasma concentrations of IL-6 (1238.0±543.1 pg/ml), whereas normal values were low (6.4±1.7 pg/ml, p<0.001).

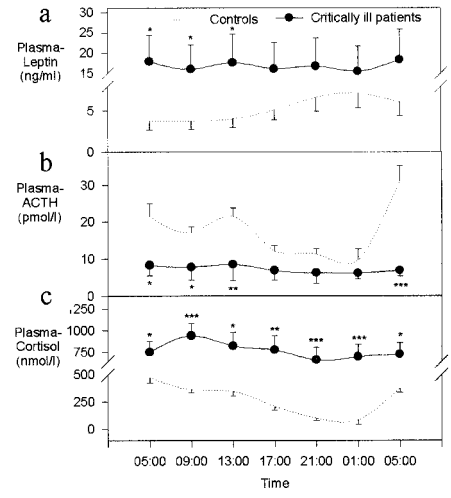

Figure 1: Twenty-four-hour profile of plasma leptin (a), ACTH (b), and cortisol (c) in controls and critically ill patients with acute sepsis (mean ± SE). Leptin and cortisol levels are increased in critically ill patients, while ACTH levels are decreased. In all critically ill patients with acute sepsis, the circadian rhythm of leptin, cortisol, and ACTH is blunted

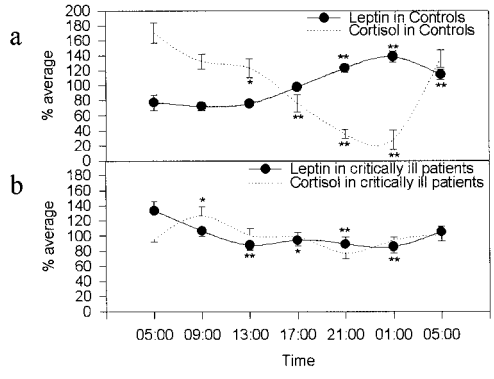

Figure 2: Simultaneous circadian profile in leptin and cortisol levels in healthy controls (a) and critically ill patients (b). Each measurement is expressed as percent of individual 24 hour averages. In controls, there is a clear reciprocal relation between leptin and cortisol levels (a). In critically ill patients, the reciprocal relation between leptin and cortisol is abolished(b).

In all critically ill patients, there was a complete absence of a nocturnal leptin rise on all three days of investigation. In fact, leptin levels were lower during the night than during the day (FIG 1a). In contrast, all healthy controls demonstrated a significant nocturnal leptin increase with levels twice as high during the night as during the day. Therefore, nocturnal leptin profile appeared to be inverted in the critically ill patients as compared to the controls. The circadian rhythm

sepsis, average cortisol levels were twice as high as in normal individuals, while ACTH levels were reduced. Such a dissociation between ACTH and cortisol levels has been previously described in critically ill patients with severe sepsis (21). Inflammatory cytokines, and possibly other mediators, as well as neural input to the adrenal are markedly increased in sepsis and able to stimulate glucocorticold secretion from the adrenals directly (22,23).

It has been demonstrated that glucocorticoids acutely increase leptin expression and levels in animals and humans (24-27). It is, thus, possible that the elevated cortisol increases the levels of leptin in our patients. Second, cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 1 (IL-1) and IL-6, as well as the endotoxin LPS, stimulate leptin in rodents (10,11). Recently, interleukin 1 α was reported to increase serum leptin concentrations in humans (13). Also, a strong positive association between leptin and TNF α was observed in healthy controls and diabetic patients (14) as well as a positive correlation of BMI and IL-6 plasma levels (28). In critical illness due to acute sepsis, these inflammatory cytokines are markedly elevated (29).

High concentrations of IL-6 are found early in the course of the acute-phase response, well before the induction of acute-phase proteins such as C-reactive protein (30). An increase in the level of serum IL-6 is strongly associated with an increase in the levels of TNF-α and IL-1 in septic shock (31), and this is compatible with the fact that both TNF-α and IL-1 stimulate production of IL-6. IL-6 concentration is associated with a subject's susceptibility to endotoxin indicating the clinical relevance of this cytokine for monitoring acute inflammation (31). Because of these previous reports, we measured IL-6 levels in our patients with acute sepsis and found a marked elevation of its plasma concentrations. This suggests that inflammatory cytokines may also contribute to the changes of leptin levels in patients with sepsis.

Leptin, which is thought to be ancestrally related to the cytokines, may contribute to the anorexia and wasting syndrome induced by inflammatory cytokines in acute sepsis (11). By regulating corticotropin-releasing-hormone (32-34), a central nervous system neuropeptide that suppresses food intake, leptin may further contribute to anorexia in these patients. Therefore, given the prominent role that anorexia plays in the wasting syndrome of critical illness, leptin antagonists might be useful in blocking excessive catabolism in this state. Leptin inhibits the rise in corticosterone following starvation and restraint stress (7,34) and directly suppresses the biosynthesis in the adrenals (35). These data suggest that elevated leptin might contribute to the frequently unexplained "functional" adrenocortical insufficiency, that occurs in the context of acute sepsis (36).

Cortisol levels have been related to the mortality of critically ill patients (37,38). In our study, there was no difference in cortisol levels between survivors or non-survivors. On the other hand, average leptin levels in the survivors were threefold higher than in the non-survivors, while average IL-6 were ten-fold lower in the survivors than the non-survivors. Therefore, low leptin and high IL-6 levels indicate an unfavorable prognosis in patients with sepsis syndrome. of both ACTH and cortisol was blunted in critically ill patients with sepsis (FIG 1bc). There was a clear reciprocal relation between cortisol and leptin levels in healthy controls with high nocturnal leptin and low nocturnal cortisol concentration (FIG 2a). In contrast, in critically ill patients, the reciprocal relation of cortisol and leptin rhythmicity was abolished (FIG. 2b).

Mean leptin levels were higher in survivors (25.5±6.2 ng/ml; n=10) than in non-survivors of acute sepsis (8.0±3.75 ng/ml n=6, $p<0.01$) (FIG 3). ACTH and cortisol levels were not substantially different between survivors and non-survivors.

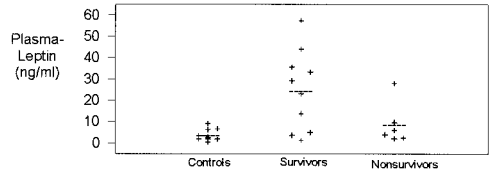

Figure 3: Scatter diagram of 09:00 leptin levels of survivors (n=10) and non-survivors of acute sepsis (n=6)

DISCUSSION

Leptin communicates the metabolic status of the peripheral adipocyte to the brain. In normal lean or obese individuals, leptin secretion shows a circadian profile with a two-fold nocturnal rise in its concentrations (5). We report here that leptin levels are increased in critically ill patients, while its circadian rhythmicity is significantly altered. Changes in diurnal leptin levels suggest an increase and/or decrease respectively in its production rate and/or metabolic clearance rate.

It has been postulated that the nighttime rise of leptin suppresses appetite during sleep, and that the lack of a nocturnal rise in serum leptin could contribute to the development of obesity (5,6). Patients with starvation due to anorexia nervosa have a decrease of leptin levels (16). All of our patients with acute sepsis had the anorexia and weight loss phenomena that accompany critical illness, however, their leptin levels were increased. This increase could not be explained by acute changes in feeding or diet composition since neither of these affect leptin levels (17). Therefore, the primary role of leptin in critically ill patients does not appear to be the prevention of obesity, but rather it may represent an acute stress-mediated response which participates in the sickness syndrome.

What is the mechanism of the increase in leptin levels and the blunted leptin rhythmicity in severely ill patients with acute sepsis? Two recent findings may provide an explanation. First, there is a clear reciprocal relation between the activity of the HPA axis and the leptin system in rodents and in humans (7,8). The activation of the HPA-axis induced by the stress of critical illness in sepsis results in a marked rise of circulating glucocorticoids, considered to be an important component of the host defense mechanism (18-20). In our patients with acute

Finally, it has been reported that leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells and can enhance the production and phagocytic activity of macrophages (39). Hemopoietic analysis of db/db mice revealed an inherent deficit in lymphopoiesis. Impaired lymphopoiesis in the leptin receptor defective animals is further illustrated by the reduced levels of lymphocytes in the peripheral blood and the inability of these cells to recover following irradiation (40) . This may suggest that leptin itself is involved in the host defense of acute inflammation: a decrease in leptin levels might therefore be expected to impair the body's capability to fight the acute disease.

We conclude that leptin is not only an adipostatic hormone whose function is to prevent obesity, but also a stress-related hormone, which might be important for survival. The potential diagnostic, prognostic, and therapeutic roles of leptin in sepsis should be further investigated.

1. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.** 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**:425-432.
2. **Rink TJ.** In search of a satiety factor. *Nature* **372**:406-407. 1996.
3. **Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV.** 1996 Leptin: the tale of an obesity gene. *Diabetes* **45**:1455-1462..
4. **Montague CT, Farooqi IS, Whitehead JP, et al.** 1997 Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387**:903-908.
5. **Sinha MK, Ohannesian JP, Heiman ML, et al.** 1996 Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. *J Clin Invest* **97**:1344-1347.
6. **Matkovic V, Ilich JZ, Badenhop NE, et al.** 1997 Gain in body fat is inversely related to the nocturnal rise in serum leptin level in young females. *J Clin Endocrinol Metab* **82**:1368-1372
7. **Ahima, RS, Prabakaran D, Mantzoros C, et al.** 1996 Role of leptin in the neuroendocrine response to fasting. *Nature* **382**:250-252.
8. **Licinio, J. Mantzoros C, Negrao AB, *et al.*** 1997 Human Leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nature Med* **3**:575-579.
9. **Bornstein SR.** 1997 Is leptin a stress-related peptide? *Nature Med* **3**:937.
10. **Grunfeld C, Zhao C, Fuller J, et al.** 1996 Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. *J Clin Invest* **97**:2151-2157
11. **Sarraf, P, Frederich, RC, Turner, EM, et al.** 1997 Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J Exp Med* **185**:171-175.
12. **Schwartz MW, Seeley RJ.** 1997 Neuroendocrine responses to starvation and weight loss. *N Engl J Med* **336**:1802-1811.
13. **Janik JE, Curti BD, Considine RV *et al.*** 1997 Interleukin 1α increases serum leptin concentrations in humans. *J Clin Endocrinol Metab.* **82**:3084-3086.
14. **Mantzoros CS, Moschos S, Avramopoulos I, *et al.*** 1997 Leptin concentrations in relation to body mass index and the tumor necrosis factor-alpha system in humans. *J Clin Endocrinol Metab* **82**:3408-3413.
15. **Bone RC, Fisher CJ, Clemmer TP, Slotman GJ, Metz CA, Balk RA.** 1987 A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. *N Engl J Med* **317**:653-658
16. **Mantzoros C, Flier JS, Lesem MD, Brewerton TD, Jimerson DC.** 1997 Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. *J Clin Endocrinol Metab* **82**:1845-1851.
17. **Schrauwen P; van Marken Lichtenbelt WD; Westerterp KR; Saris WH.** 1997 Effect of diet composition on leptin concentration in lean subjects. *Metabolism* **46**:420-4.
18. **Rothwell PM, Udwadia ZF, Lawler PG**: 1991 Cortisol response to corticotropin and survival in septic shock. *Lancet* **337**:582-583.
19. **Sibbald WJ, Short A, Cohen MP, Wilson RF.** 1977 Variation in adrenocortical responsiveness during severe bacterial infections. Unrecognized adrenocortical insufficiency in severe bacterial infections. *Ann Surg* **186**:29-33.
20. **Span LFR, Hermus ARM, Bartelink AK, et al.** 1992 Adrenocortical function: an indicator of severity of disease and survival in chronic critically ill patients. *Intensive Care Med* **18**:93-96.
21. **Vermes I, Beishuizen A, Hampsink RM, Haanen C.** 1995 Dissociation of plasma adrenocorticotropin and cortisol levels in critically ill patients: possible role of endothelin and atrial natriuretic hormone. *J Clin Endocrinol Metab* **80**:1238-1242.
22. **Path G, Bornstein SR, Ehrhart-Bornstein M, Scherbaum WA.** 1997 Interleukin-6 and the interleukin-6 receptor in the human adrenal gland: expression and effects on steroidogenesis. *J Clin Endocrinol Metab* **82**:2343-2349.
23. **Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP.** 1997 Intra-adrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev in press.*
24. **De Vos P, Saladin R, Auwerx J, Staels B.** 1995 Induction of ob gene expression by corticosteroids is accomplished by body weight loss and reduced food intake. *J Biol Chem* **270**:15958-15961.
25. **Murakami T, Iida M, Shima K.** 1995 Dexamethasone regulates obese expression in isolated rat adipocytes. *Biochem Biophys Res Commun* **214**:1260-1267.
26. **Slieker LJ, Sloop KW, Surface PL, et al.** 1996 Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. *J Biol Chem* **271**:5301-5404.
27. **Papaspyrou-Rao S, Schneider SH, Petersen RN, Fried, SK.** 1997 Dexamethasone increase leptin expression in humans *in Vivo*. *J Clin Endocrinol Metab* **82**:1635-1637.
28. **Vgontzas AN, Papanicolaou DA, Bixler AK, *et al.*** 1997 Elevation of plasma cytokines in disorders of excessive daytime sleepiness: Role of sleep disturbance and obesity. *J Clin Endocrinol Metab* **82**:1313-1316.
29. **Bendtzen, K.** 1988 Interleukin 1, IL-6 and tumor necrosis factor in infection, inflammation and immunity. *Immunol Lett* **19**:183-192.
30. **Ohzato H, Yoshizaki K, Nishimoto H, et al.** 1992 Interleukin-6 as a new indicator of inflammatory status: Detection of serum levels of interleukin-6 and C-reactive protein after surgery. *Surgery* **111**:201-209.
31. **Yoshimoto T, Nakanishi K, Hirose S, et al.** 1992 High serum IL-6 level reflects susceptible status of the host to endotoxin and IL-1/Tumor Necrosis Factor. *J Immunol* **148**:3596-3603.
32. **Raber J, Chen S, Mucke L, Feng L.** 1997 Corticotropin-releasing factor and adrenocorticotrophic hormone as potential central mediators of OB effects. *J Biol Chem* **272**:15057-15060.
33. **Schwartz MW, Seeley RJ, Campfield A, Burn P, Baskin DG.** 1996 Identification of targets of leptin action in rat hypothalamus. *J Clin Invest*:**98**:1101-1106.
34. **Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS.** 1997 Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology*: **138**:3859-3863.
35. **Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M, Scherbaum WA.** 1997 Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland; Leptin inhibits cortisol release directly. *Diabetes* **46**:1235-1238.
36. **Aygen, B, Inan, M, Doganay M, Kelestimur,** F. 1997 Adrenal function in patients with sepsis. *Exp Clin Endocrinol Diabetes* **105**:182-186.
37. **Finlay WEI, McKee JI.** 1982 Serum cortisol levels in severely stressed patients. *Lancet* 1414-1415.
38. **McKee JI, Finlay WEI.** 1983 Cortisol replacement in severely stressed patients. *Lancet* I:484.
39. **Gainsford T, Willson TA, Metcalf D, et al.** 1996 Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proc Natl Acad Sci* **93**:14564-14568.
40. **Bennett BD, Solar GP, Yuan JQ *et al.*** 1996 A role for leptin and its cognate receptor in hematopoiesis. *Cur Biol* **6**:1170-1180.

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