

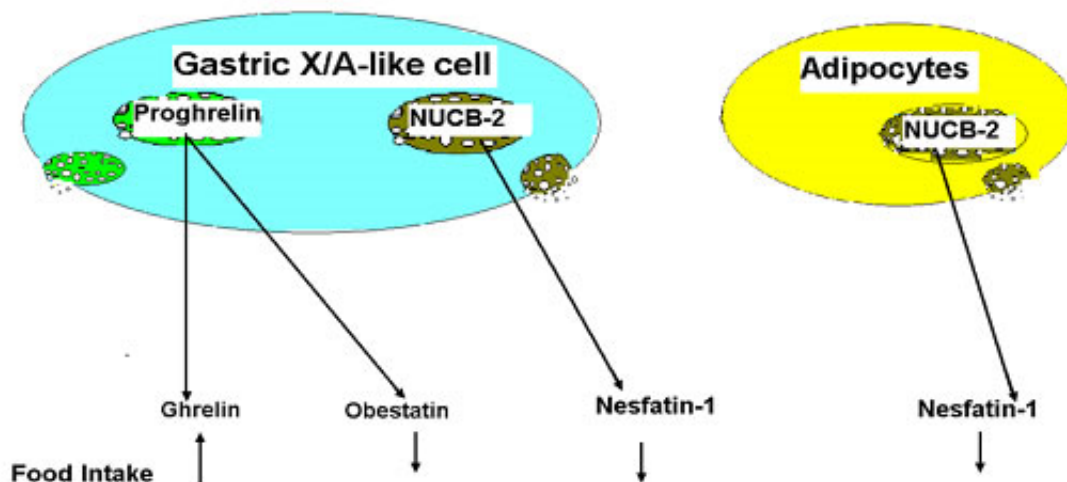
# Nesfatin-1

## Increased levels in Obesity

### Identification of Nesfatin-1 in Human and Murine Adipose Tissue: A Novel Depot-Specific Adipokine with Increased Levels in Obesity

Nesfatin-1 is a recently identified anorexigenic peptide derived from its precursor protein, nonesterified fatty acid/nucleobindin 2 (NUCB2). Although the hypothalamus is pivotal for the maintenance of energy homeostasis, adipose tissue plays an important role in the integration of metabolic activity and energy balance by communicating with peripheral organs and the brain via adipokines. Currently no data exist on nesfatin-1 expression, regulation, and secretion in adipose tissue. We therefore investigated NUCB2/nesfatin-1 gene and protein expression in human and murine adipose tissue depots. Additionally, the effects of insulin, dexamethasone, and inflammatory cytokines and the impact of food deprivation and obesity on nesfatin-1 expression were studied by quantitative RT-PCR and Western blotting. We present data showing NUCB2 mRNA ( $P < 0.001$ ), nesfatin-1 intracellular protein ( $P < 0.001$ ), and secretion ( $P < 0.01$ ) were significantly higher in sc adipose tissue compared with other depots. Also, nesfatin-1 protein expression was significantly increased in high-fat-fed mice ( $P < 0.01$ ) and reduced under food deprivation ( $P < 0.01$ ) compared with controls. Stimulation of sc adipose tissue explants with inflammatory cytokines (TNF $\alpha$  and IL-6), insulin, and dexamethasone resulted in a marked increase in intracellular nesfatin-1 levels. Furthermore, we present evidence that the secretion of nesfatin-1 into the culture media was dramatically increased during the differentiation of 3T3-L1 preadipocytes into adipocytes ( $P < 0.001$ ) and after treatments with TNF- $\alpha$ , IL-6, insulin, and dexamethasone ( $P < 0.01$ ). In addition, circulating nesfatin-1 levels were higher in high-fat-fed mice ( $P < 0.05$ ) and showed positive correlation with body mass index in human. We report that nesfatin-1 is a novel depot specific adipokine preferentially produced by sc tissue, with obesity- and food deprivation-regulated expression.

Ramanjaneya et al. *Endocrinology*. 2010 Apr 28. [Epub ahead of print]



Reference: Stengel A. et al.  
*Peptides*, 31 (2) 357-69, 2010

Reference:  
Ramanjaneya et al. *Endocrinology*  
ahead of print April 28, 2010

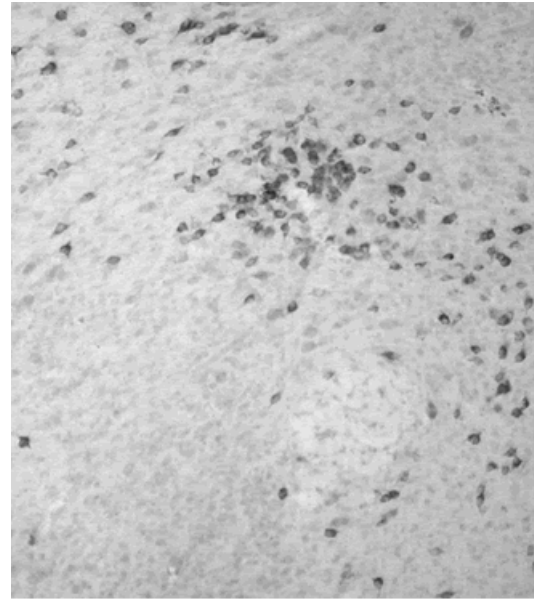


**PHOENIX PHARMACEUTICALS, INC.**  
330 BEACH ROAD, BURLINGAME CA, 94010, USA  
PHONE: (650) 558-8898 EMAIL: [info@phoenixpeptide.com](mailto:info@phoenixpeptide.com)  
[WWW.PHOENIXPEPTIDE.COM](http://WWW.PHOENIXPEPTIDE.COM)

**PHOENIX EUROPE GMBH**  
VIKTORIASTRASSE 3-5, D-76133 KARLSRUHE, GERMANY  
PHONE: +49-721-1611950 EMAIL: [germany@phoenixpeptide.com](mailto:germany@phoenixpeptide.com)  
[WWW.PHOENIXPEPTIDE.COM](http://WWW.PHOENIXPEPTIDE.COM)

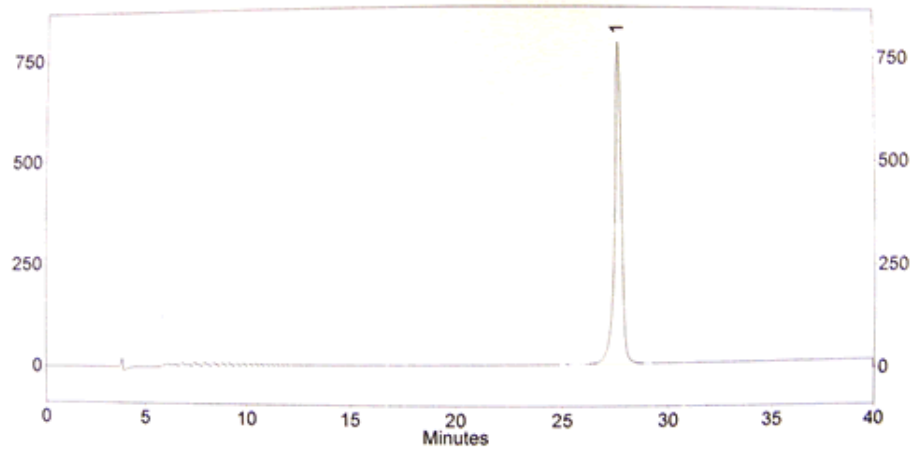


Photomicrograph of a section of rat hypothalamus labeled with (H) Rabbit Anti-Nesfatin-1 N-Terminal (Human) antiserum (H-003-97). Immunoreactive cells are noted in the paraventricular nucleus, supraoptic nucleus and zona incerta.

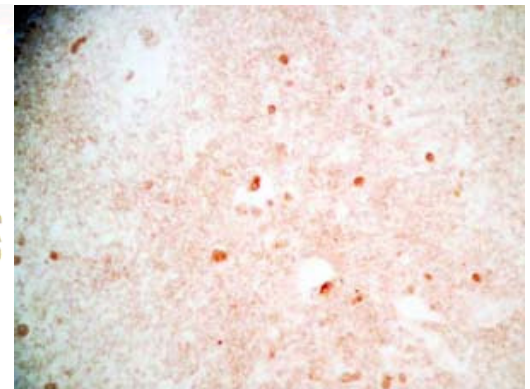
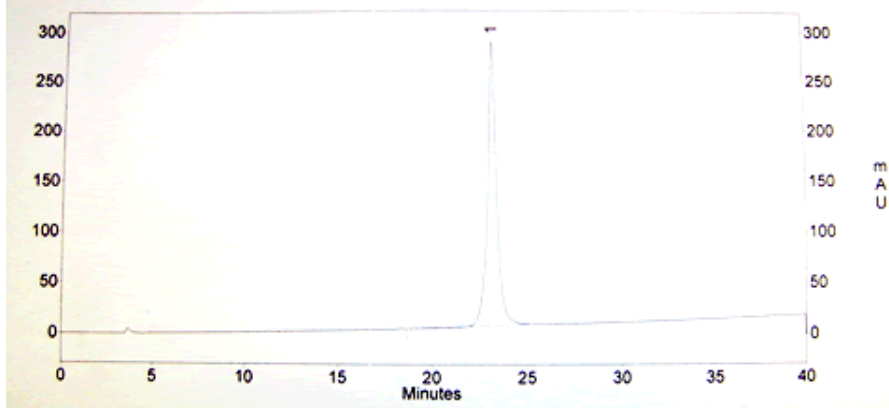


Photomicrograph of a section of rat hypothalamus labeled with Rabbit Anti-Nesfatin-1, N-Terminal (Human) antiserum (H-003-97). Numerous immunoreactive cells are noted in the lateral hypothalamus, dorsolateral to the fornix.

#### Purification of Rat Nesfatin-1 (82 amino acid peptide) by RP-HPLC



#### Purification of human Nesfatin-1 (82 amino acid peptide) by RP-HPLC



Human brain tissue was stained by Rabbit Anti-Nesfatin-1, C-Terminal (H) Antibody (Cat. No.: H-003-27)

|     |            |            |             |       |
|-----|------------|------------|-------------|-------|
| 1   | MRWRITILQY | CFLITCLLT  | ALEAVPIDID  | Human |
| 1   | MRWRTIQARY | CFLLVPCVLT | ALEAVPIDVD  | Rat   |
| 31  | KTQVNIHPV  | ESAKIEPPDT | GLYYDEYLKQ  | Human |
| 31  | KTQVHNVEPV | ESARIEPPDT | GLYYDEYLKQ  | Rat   |
| 61  | VIDVLETDKH | FREKLQKADI | EIKSGRLSK   | Human |
| 61  | VIEVLETDPH | FREKLQKADI | EIRSGRLSQ   | Rat   |
| 91  | ELDLVSHHVR | TKLDELKRQE | VGLRMLIKA   | Human |
| 91  | ELDLVSHKVR | TRLDELKRQE | VGLRMLIKA   | Rat   |
| 121 | KLDSLQDIGM | DHQAALKQFD | HLNHLNPKDF  | Human |
| 21  | KLDALQDTGM | NHLLLLKQFE | HLNHQNPDTF  | Rat   |
| 151 | ESTDLMLIK  | AATSDEHYD  | KTRHEEFKKY  | Human |
| 151 | ESKDLMLIK  | AATADLEQYD | RTRHEEFKKY  | Rat   |
| 181 | EMMKEHERRE | YLKTLNEEKR | KEESKFEEM   | Human |
| 181 | EMMKEHERRE | YLKTLSEEKR | KEEAQFAEM   | Rat   |
| 211 | KKKHENHPKV | NHPGSKDQLK | EVWEETDGLD  | Human |
| 211 | KRKHEDHPKV | NHPGSKDQLK | EVWEETDGLD  | Rat   |
| 241 | PNDFDPKTF  | KLHDVNSDGF | LDEQELEALF  | Human |
| 241 | PNDFDPKTF  | KLHDVNDGDF | LDEQELEALF  | Rat   |
| 271 | TKELEKVDYD | KNEEDDMVEM | EEERLRMREH  | Human |
| 271 | TKELDKVYNP | QNAEDDMIEM | EEERLRMREH  | Rat   |
| 301 | VMNEVDTNKD | RLVTLEEFK  | ATEKKEFLEP  | Human |
| 301 | VMNEIDNND  | RLVTLEEFK  | ATEKKEFLEP  | Rat   |
| 331 | DSWETLQQQ  | FFTEELKEY  | ENIIALQENE  | Human |
| 331 | DSWETLQQQ  | LFTEELKEY  | ESI IAIQESE | Rat   |
| 361 | LKKKADELQK | QKEELQRQHD | QLEAQKLEYH  | Human |
| 361 | LKKKADELQK | QKEELQRQHD | HLEAQKQEQY  | Rat   |
| 391 | QVIQQMEQKK | LQQGIPPSGP | AGELKFEPHI  | Human |
| 391 | QAVQQLEQKK | FQQGIAPSGP | AGELKFEPHT  | Rat   |

Oct. 09, 2006, Phoenix Pharmaceuticals, Inc.

### Alignment of rat and mouse Nesfatin-1, 2, 3, and NEFA/nucleobindin2 (NUCB2) amino acids sequence

|        |   |     |             |
|--------|---|-----|-------------|
| P81117 | MRWRITIQVYCFLLVPCMLTALEAVPIDVDKTKVHNTEPVENARIEPPDTGLYYDEYLKQ  | 60  | NUCB2_MOUSE |
| Q9J185 | MRWRTIQARYCFLLVPCVLTALAEAVPIDVDKTKVHNVEPVESARIEPPDTGLYYDEYLKQ | 60  | NUCB2_RAT   |
| P81117 | VIEVLETDPHFREKLQKADIEEIRSGRLSQELDLVSHKVRTRLDELKRQEVGLRMLIKA   | 120 | NUCB2_MOUSE |
| Q9J185 | VIEVLETDPHFREKLQKADIEEIRSGRLSQELDLVSHKVRTRLDELKRQEVGLRMLIKA   | 120 | NUCB2_RAT   |
| P81117 | KLDSLQDTGNHLLLLKQFEHLNHQNPNTFESRDLMLIKAATADLEQYDRTRHEEFKKY    | 180 | NUCB2_MOUSE |
| Q9J185 | KLDALQDTGNHLLLLKQFEHLNHQNPDTFESKDLMLIKAATADLEQYDRTRHEEFKKY    | 180 | NUCB2_RAT   |
| P81117 | EMMKEHERREYLKTLSEEKREKESKFEEMKRRKHEDHPKVNHPGSKDQLKEVWEETDGLD  | 240 | NUCB2_MOUSE |
| Q9J185 | EMMKEHERREYLKTLSEEKREKESKFEEMKRRKHEDHPKVNHPGSKDQLKEVWEETDGLD  | 240 | NUCB2_RAT   |
| P81117 | PNDFDPKTFKLDVNDGDFLDEQELEALFTRELEKVDYNPQNAEDDMIEMEEERLRMREH   | 300 | NUCB2_MOUSE |
| Q9J185 | PNDFDPKTFKLDVNDGDFLDEQELEALFTRELEKVDYNPQNAEDDMIEMEEERLRMREH   | 300 | NUCB2_RAT   |
| P81117 | VMNEIDNNDRLVTLEEFKRATEKKEFLEPPSWETLQQQLFTEDELKEYESIIAIQENE    | 360 | NUCB2_MOUSE |
| Q9J185 | VMNEIDNNDRLVTLEEFKRATEKKEFLEPPSWETLQQQLFTEDELKEYESIIAIQESE    | 360 | NUCB2_RAT   |
| P81117 | LKKKADELQKQKEDLQRQHDHLEAQKQEQYQAVQHLQKQLQQGIAPSGPAGELKFEPHT   | 420 | NUCB2_MOUSE |
| Q9J185 | LKKKADELQKQKEDLQRQHDHLEAQKQEQYQAVQHLQKQLQQGIAPSGPAGELKFEPHT   | 420 | NUCB2_RAT   |

- Nesfatin-1 [ NUCB2 (25-106)]
- Nesfatin-2 [ NUCB2 (109-187)]
- Nesfatin-3 [ NUCB2 (190-420)]

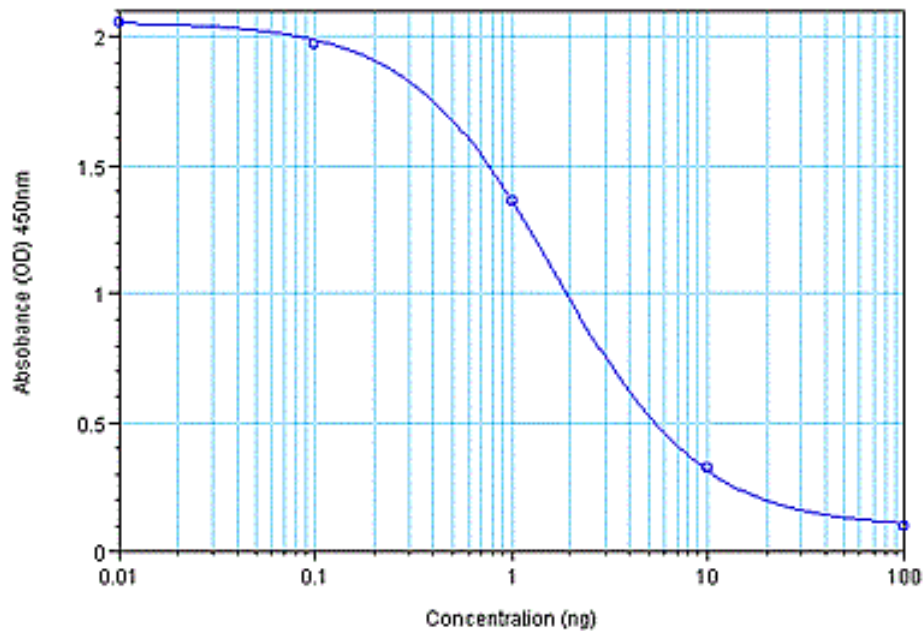
May 06, 2010 Phoenix Pharmaceuticals, Inc.

1 MRWRTILLQY CFLLITCLLT ALEAVPIDID  
 31 KTKVQNIHPV ESAKIEPPDT GLYYDEYLKQ  
 61 VIDVLETDKH FREKLQKADI EEIKSGRLSK  
 91 ELDLVSHHVR TKLDELKRQE VGRRLMLIKA  
 121 KLDSLQDIGM DHQALLKQFD HLNHLNPKDF  
 151 ESTDLDMLIK AATSDLEHYD KTRHEEFKKY  
 181 EMMKEHERRE YLKTLINEEKR KEEESKFEEM  
 211 KKKHENHPKV NHPGSKDQLK EVWEETDGLD  
 241 PNDFDPKTFE KLHDVNSDGF LDEQELEALF  
 271 TKELEKVYDP KNEEDDMVEM EEERLRMREH  
 301 VMNEVDTNKD RLVTTLEEFK ATEKKEFLEP  
 331 DSWETLDQQQ FFTEEELKEY ENIIALQENE  
 361 LKKKADELQK QKEELQRQHD QLEAQKLEYH  
 391 QVIQQMEQKK LQQGIPPSGP AGELKFEPHI

- Nesfatin-1 [NUCB2(25-106)]
- Nesfatin-2 [NUCB2 (109-187)]
- Nesfatin-3 [NUCB2 (190-420)]

Oct. 09, 2006, Phoenix Pharmaceuticals, Inc.

**Rat Nesfatin-1 (1-82) EIA Kit (EK-003-22)**  
**Linear Range: 1.26-17.7 ng/ml**

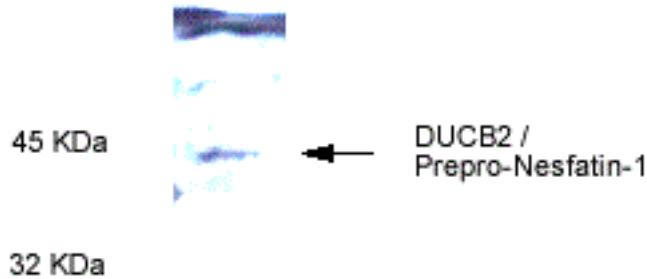


$y = \frac{(A-D)}{1+(x/C)^B} + D$

|  | A     | B     | C    | D     | R <sup>2</sup> |
|--|-------|-------|------|-------|----------------|
| Std (Standards; Concentration vs Mean Value) | 2.057 | 1.164 | 1.68 | 0.096 | 0.998          |



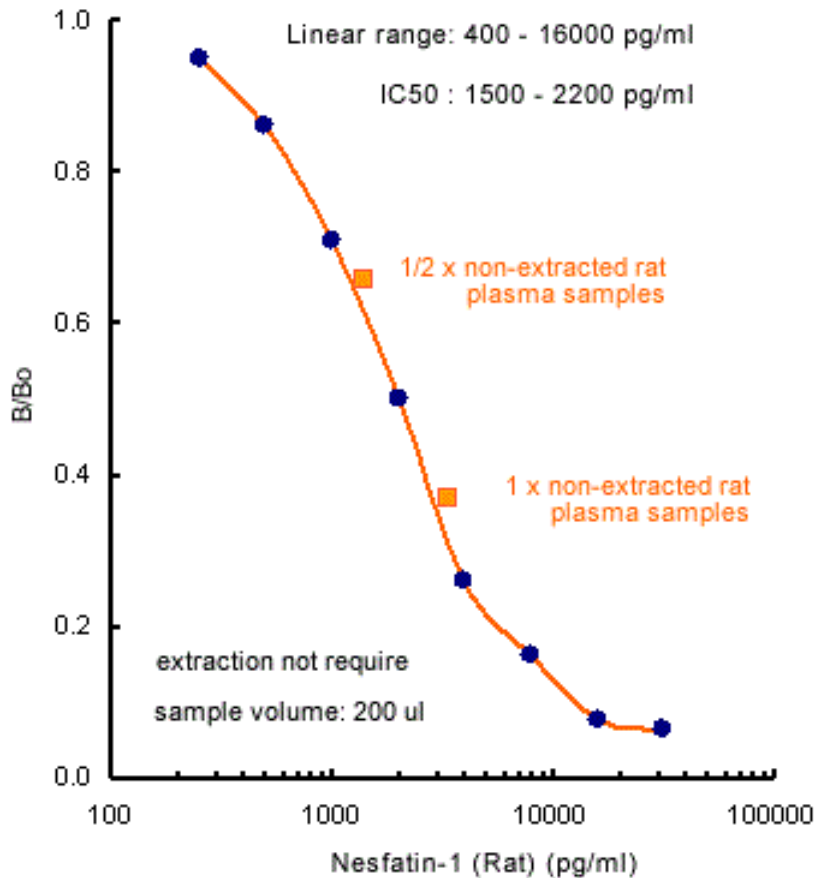
### Detection of NUCB2/Prepro Nesfatin-1 in Rat Brain by Nesfatin-1 (1-82) (Rat) Western Blot Kit (WBK-003-22)



Rat brain tissue homogenate was run on 15% SDS-PAGE gel

Rabbit Anti-Nesfatin-1 (1-82) (Rat) Antiserum (catalog No.: H-003-22, Lot No.: 2378-1)

### Rat Nesfatin-1 (1-82) RIA Kit (RK-003-22)

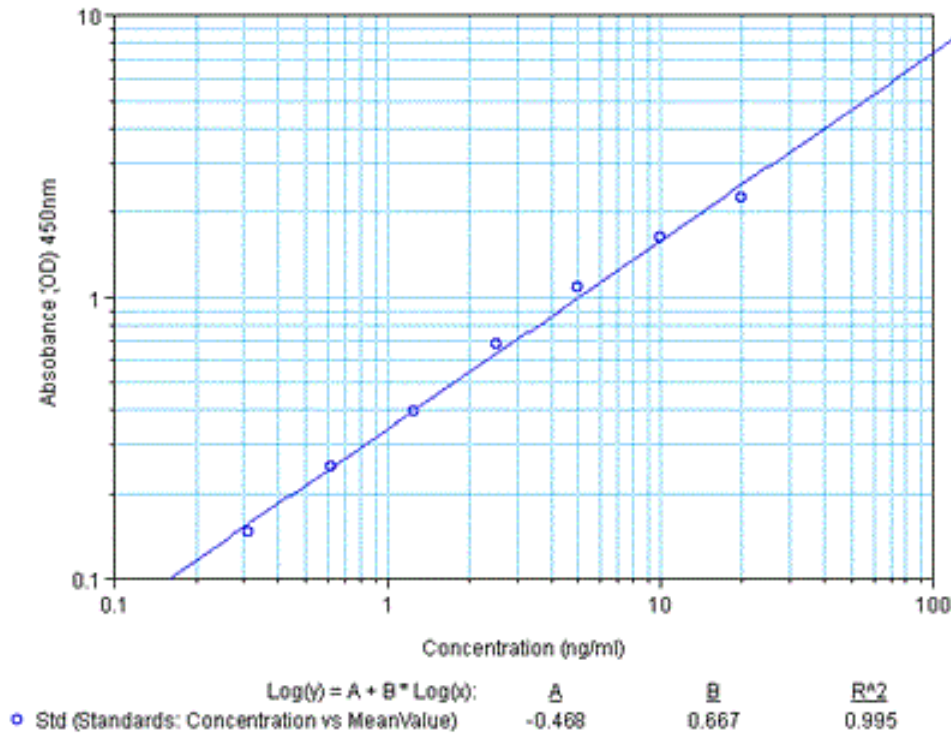


### Detection of NUCB2 in Rat Brain by Nesfatin-1 (1-82) (Rat) Western Blot Kit



- Rat brain tissue homogenate was run on 15% SDS-PAGE gel
- Rabbit anti-Nesfatin-1, C-Terminal Antiseum (catalog No.: H-003-96) at 1: 700

**Human Nesfatin-1 (1-82) / NUCB2 (25-106) ELISA Kit (EK-003-26)**  
**Standard Range: 0.78-50 (ng/ml)**

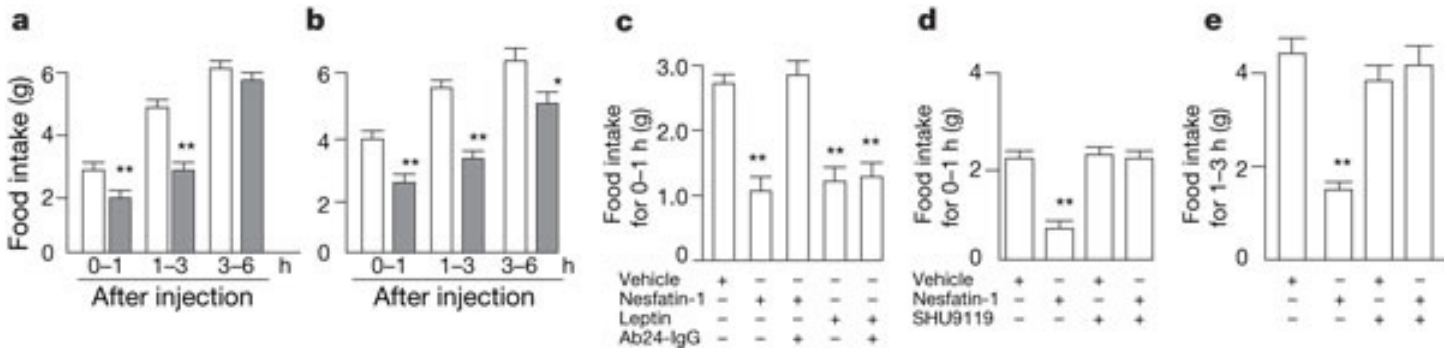


**Ghrelin, des-acyl ghrelin and nesfatin-1 in gastric X/A-like cells: role as regulators of food intake and body weight.**

Numerous peptides released from endocrine cells in the intestinal mucosa were established early on to be involved in the physiological regulation of food intake with a prominent role in termination of food ingestion when nutrients pass along the intestinal tract. Recently, peptides released from X/A-like endocrine cells of the gastric oxyntic mucosa were recognized as additional key players in the regulation of feeding and energy expenditure. Gastric X/A-like cells release the octanoylated peptide, ghrelin, the only known peripherally produced hormone stimulating food intake through interaction with growth hormone secretagogue 1a receptor (GHS-R1a). Additionally, non-octanoylated (des-acyl) ghrelin present in the circulation at higher levels than ghrelin is currently discussed as potential modulator of food intake by opposing ghrelin's action independent from GHS-R1a although the functional significance remains to be established. Obestatin, a ghrelin-associated peptide was initially reported as anorexigenic modulator of ghrelin's orexigenic action. However, subsequent reports did not support this contention. Interesting is the recent identification of nesfatin-1, a peptide derived from the nucleobindin2 gene prominently expressed in gastric X/A-like cells in different vesicles than ghrelin. Circulating nesfatin-1 levels vary with metabolic state and peripheral or central injection inhibits dark phase feeding in rodents. Overall, these data point to an important role of gastric X/A-like cells in food intake regulation through the expression of the orexigenic peptide ghrelin along with des-acyl ghrelin and nesfatin-1 capable of reducing food intake upon exogenous injection although their mechanisms of action and functional significance remain to be established.

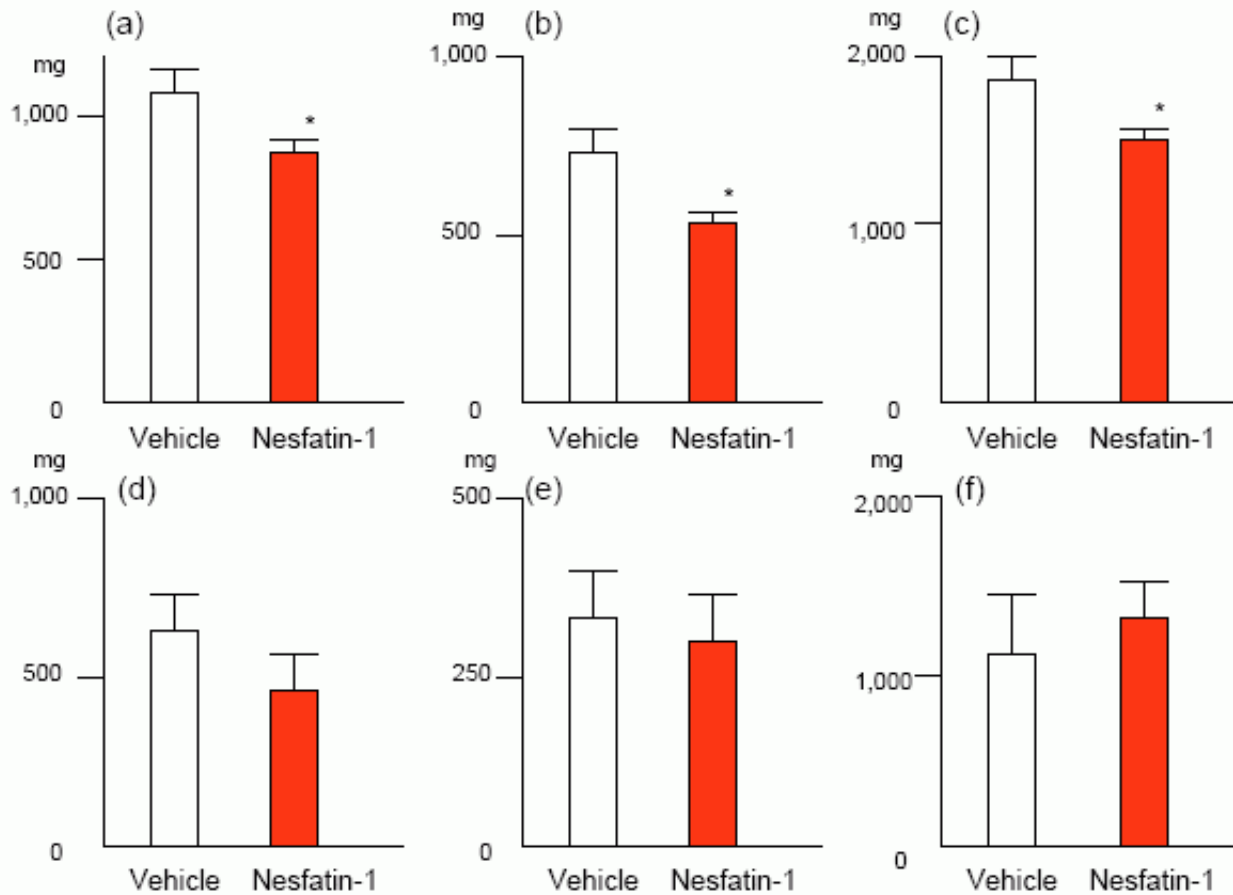
*Peptides. 2010 Feb;31(2):357-69.*

## Nesfatin-1-induced satiety associated with leptin or melanocortin signalling.



a, b, Food intake in lean (a) and Zucker (b) rats ( $n = 5$ ) after i.c.v. injection of 5 pmol nesfatin-1 (open bars, vehicle; filled bars, nesfatin-1). c, Effects of nesfatin Ab24 on leptin-induced anorexia ( $n = 6$ ). Vehicle, 5 pmol nesfatin-1 or 5 pmol leptin was centrally administered 15 min after i.c.v. injection of Ab24 IgG (8 mg) during the dark phase. d, e, Effects of SHU9119 on nesfatin-1-induced anorexia ( $n = 6$ ). Vehicle or 5 pmol nesfatin-1 was centrally administered 15 min after i.c.v. injection of 20 pmol SHU9119. Data are means  $\pm$  s.e.m. Asterisk,  $P < 0.05$ ; two asterisks,  $P < 0.01$  compared with vehicle. *Oh-I et al. Nature. 2006 Oct 12;443(7112):709-12.*

## 003-24 and 003-25 are two peptide fragments of human Nesfatin-1



**Figure 5** | Changes in the fat weights after continuous icv injection of nesfatin-1. Rats received chronic icv injection of vehicle or nesfatin-1 (daily 5 pmol) for 10 days. (a), Subcutaneous fat; (b), Epididymal fat; (c), Mesenteric fat; (d), Retroperitoneal fat; (e), Brown adipose tissue; (f), Gastrocnemius. \*,  $p < 0.01$  vs. vehicle injection. Each group contains 4–5 rats. Data are expressed as the mean  $\pm$  s.e.m. \*,  $p < 0.01$  vs. the vehicle-injection group.

## Nesfatin Products

| Catalog No.  | Name  | Size   |
|--------------|---|--------|
| FC5-G-003-22 | Nesfatin-1 (1-82) (Rat) - Cy5 Labeled Purified IgG                                    | 100 µl |
| FG-003-29A   | Nesfatin-1 (47-82) (Human) - FAM Labeled  | 1 nmol |
| B-G-003-24   | Nesfatin-1 (1-45) / Nesfatin-1 N-Terminal (Human) - Biotin Labeled Purified IgG       | 100 µl |
| H-003-27     | Nesfatin-3, C-Terminal (Human) - Antibody for Immunohistochemistry                    | 100 µl |
| B-G-003-22   | Nesfatin-1 (1-82) (Rat) - Biotin Labeled Purified IgG                                 | 100 µl |
| B-003-29     | Nesfatin-1 (47-82) (Human) - Biotin Labeled   | 10 µg  |
| MRK-003-22   | Nesfatin-1 (1-82) (Rat) - Magnetic Bead RIA kit                                       | 1 kit  |
| G-003-25     | Nesfatin-1 (46-82) (Human) - Purified IgG Antibody                                    | 200 µg |
| FG-003-26A   | Nesfatin-1 (1-82) (Human) - FAM Labeled   | 1 nmol |
| FG-003-24B   | Nesfatin-1 (1-45) / Nesfatin-1 N-Terminal (Human) - FITC Labeled                      | 1 nmol |
| FG-G-003-96B | Nesfatin-1 C-Terminal (Human) - FITC Labeled Purified IgG                             | 100 µl |
| FC5-003-22   | Nesfatin-1 (1-82) (Rat) - Cy5 Labeled   | 1 nmol |
| B-003-25     | Nesfatin-1 (46-82) (Human) - Biotin Labeled   | 10 µg  |
| FC5-G-003-96 | Nesfatin-1 C-Terminal (Human) - Cy5 Labeled Purified IgG                              | 100 µl |
| T-003-29     | Nesfatin-1 (47-82) (Human) - I-125 Labeled  | 10 µCi |
| FC3-G-003-24 | Nesfatin-1 (1-45) / Nesfatin-1 N-Terminal (Human) - Cy3 Labeled Purified IgG          | 100 µl |
| B-G-003-96   | Nesfatin-1 C-Terminal (Human) - Biotin Labeled Purified IgG                           | 100 µl |
| FC3-003-29   | Nesfatin-1 (47-82) (Human) - Cy3 Labeled  | 1 nmol |
| FG-003-29B   | Nesfatin-1 (47-82) (Human) - FITC Labeled   | 1 nmol |
| G-003-27     | Nesfatin-3, C-Terminal (Human) - Purified IgG Antibody                                | 100 µg |
| FG-003-26B   | Nesfatin-1 (1-82) (Human) - FITC Labeled  | 1 nmol |
| FG-003-25A   | Nesfatin-1 (46-82) (Human) - FAM Labeled  | 1 nmol |
| G-003-22     | Nesfatin-1 (1-82) (Rat) - Purified IgG Antibody                                       | 100 µg |
| FC5-003-26   | Nesfatin-1 (1-82) (Human) - Cy5 Labeled   | 1 nmol |
| FC3-003-24   | Nesfatin-1 (1-45) / Nesfatin-1 N-Terminal (Human) - Cy3 Labeled                       | 1 nmol |
| G-003-96A    | Nesfatin-1 C-Terminal (Human) - Purified IgG Antibody                                 | 100 µg |
| FG-G-003-22A | Nesfatin-1 (1-82) (Rat) - FAM Labeled Purified IgG                                    | 100 µl |
| B-003-22     | Nesfatin-1 (1-82) (Rat) - Biotin Labeled  | 10 µg  |
| 003-26       | Nesfatin-1 (1-82) (Human)   | 20 µg  |
| FC5-G-003-24 | Nesfatin-1 (1-45) / Nesfatin-1 N-Terminal (Human) - Cy5 Labeled Purified IgG          | 100 µl |
| 003-25       | Nesfatin-1 (46-82) (Human)  | 100 µg |
| FC3-G-003-22 | Nesfatin-1 (1-82) (Rat) - Cy3 Labeled Purified IgG                                    | 100 µl |
| FC5-003-29   | Nesfatin-1 (47-82) (Human) - Cy5 Labeled  | 1 nmol |
| H-003-24     | Nesfatin-1 (1-45) / Nesfatin-1 N-Terminal (Human) - Antibody for Immunohistochemistry | 100 µl |
| RK-003-22    | Nesfatin-1 (1-82) (Rat) - RIA Kit   | 1 kit  |
| T-003-22     | Nesfatin-1 (1-82) (Rat) - I-125 Labeled   | 10 µCi |
| T-003-24     | Nesfatin-1 (1-45) / Nesfatin-1 N-Terminal (Human) - I-125 Labeled                     | 10 µCi |
| WBK-003-22   | Nesfatin-1 (1-82) (Rat) - Western blot Kit  |        |