Neuropeptide Y, Galanin, and Leptin Release in Obese Women and in Women With Anorexia Nervosa

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The study objective was to determine circulating levels of the appetite-controlling neuropeptides, neuropeptide Y (NPY), galanin, and leptin, in subjects with eating disorders. The study group consisted of 48 obese women aged 19 to 45 years, 15 women with anorexia nervosa aged 18 to 23 years, and 19 lean healthy women aged 18 to 42 years (control group). The obese women were divided into four groups: (A) body mass index (BMI) = 25 to 30 kg/m^2, n = 9 (overweight); (B) BMI = 31 to 40 kg/m^2, n = 23 (moderate obesity); (C) BMI greater than 40 kg/m^2, n = 9 (severe obesity); and (D) BMI = 31 to 40 kg/m^2, n = 7 (moderate obesity + non–insulin-dependent diabetes mellitus [NIDDM]). Plasma NPY, galanin, and leptin concentrations were measured in peripheral blood samples with radioimmunoassay methods. Plasma NPY levels in obese women (groups A, B, C, and D) were significantly higher as compared with the control group ($P < .01$, $P < .001$, $P < .001$, and $P < .001$, respectively). The highest plasma NPY concentrations were observed in obese women with NIDDM. Plasma galanin levels were significantly higher in groups B, C, and D ($P < .001$, $P < .001$, and $P < .001$, respectively). Plasma leptin concentrations were significantly higher in groups C and D as compared with the control group ($P < .001$ and $P < .001$, respectively). Plasma NPY and galanin concentrations in women with anorexia nervosa did not differ from the levels in the control group. However, plasma leptin concentrations were significantly lower in anorectic women than in the control group ($P < .01$). Our results indicate that inappropriate plasma concentrations of NPY, galanin, and leptin in obese women may be a consequence of their weight status, or could be one of many factors involved in the pathogenesis of obesity.

THE ROLE OF THE CENTRAL NERVOUS system in the regulation of appetite and feeding behavior in man and experimental animals has been investigated extensively, but many questions remain to be answered. Several anatomical regions of the brain appear to play an important role in the control of nutrient balance. Destruction of the ventromedial nuclei in the hypothalamus of rats produces hyperphagia, and destruction of the lateral hypothalamic nuclei results in decreased food intake and starvation, suggesting that these two areas of the brain are "satiety" and "feeding" centers, respectively.[1] Neuroendocrine control of feeding behavior involves both peripheral and central signals.[2,3]

It is commonly accepted that some signals such as (1) simple nutrients in the blood: glucose, fatty acids, or amino acids; (2) classic neurotransmitter molecules for rapid, short-term communication; (3) neuropeptides for slower, long-term transmission; and (4) hormones for the modulation of metabolic processes may affect eating behavior, energy balance, and body weight.[4] These signals arise from the adrenals, liver, pancreas, and gastrointestinal tract, and also from the central nervous system.

From the gastrointestinal tract, cholecystokinin and other peptides are released after a meal to coordinate digestion, absorption, and metabolism and to transmit information to the brain via the vagus nerve. The pancreatic peptide hormone insulin may influence satiety, metabolism, and utilization of food.[4] The adrenal steroids through mineralocorticoid receptors increase the ingestion and metabolism of fat, and through glucocorticoid receptors influence carbohydrate intake and metabolism.[4]

The neural center controlling food intake is mainly composed of catecholaminergic, serotoninergic, and peptidergic systems.[5-8] Several peptides such as neuropeptide Y (NPY) and galanin can modulate food intake. NPY and galanin may regulate appetite via both central and peripheral mechanisms.

NPY, a 36–amino acid peptide, is a member of the structurally related peptides of the pancreatic polypeptide family.[9] NPY is widely distributed in the central and peripheral nervous systems. Some experimental evidence suggests that NPY plays an important role in the regulation of feeding behavior in animals.[10,11] Centrally injected NPY elicited a marked feeding response in satiated animals[10,11] and enhanced ongoing nighttime feeding.[12] Morley[2] and Leibowitz[13] demonstrated that daily injections of NPY into the paraventricular nucleus (PVN) increased daily food intake and body weight gain. The passive immunoneutralization of endogenous NPY by anti-NPY antibodies decreased food intake in rats.[14] NPY-containing neurons have been identified in both the central and peripheral nervous systems.

In the periphery, NPY is found in the adrenal medulla and in the nerves of the autonomic nervous system, where it is often closely associated with or co-stored in catecholaminergic nerves.[15] NPY-immunoreactive (IR) nerve fibers are present in the gut of many species. NPY-IR fibers are present in the mucosa, submucosa, and smooth muscle layers and in the submucosal and myenteric ganglia.[15] NPY-containing nerves to the gut arise from both intrinsic and extrinsic (sympathetic) sources. The extrinsic fibers appear to be noradrenergic, whereas the intrinsic fibers are noncatecholaminergic.[15] In the gastrointestinal tract, NPY is predominantly localized to intrinsic neurons in the submucosal and myenteric nerve plexuses innervating the mucosa and smooth muscle layers.[16]

NPY-containing neurons are also found in the pancreas. Bennet et al[17] have demonstrated for the first time that NPY-encoding mRNA is present in human islets. Immunocyto-

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chemical studies have revealed that the NPY-like immunoreactivity in human islets is predominantly present in α cells. NPY is synthesized in human islets, and this peptide has the ability to modulate the release of insulin.[17]

In addition to the coexistence of NPY and the catecholamines, NPY has also been shown to coexist with a number of other neurotransmitters such as somatostatin, enkephalin, and gamma-aminobutyric acid (GABA).[18] The experimental data suggest that NPY is a physiological signal for the control of food intake in humans. The interaction between central and peripheral signals for the control of appetite is due to leptin.

Zhang et al[19] cloned and sequenced the mouse ob gene and its human homolog; 84% of the predicted amino acid sequence was identical in man and mouse. The ob gene encodes a fat cell–derived circulating satiety factor—a protein (leptin) that is involved in the regulation of food intake and energy expenditure.[20] Leptin, a hormone secreted by adipocytes, modulates the level of NPY and other peptides and plays a role in the regulation of food intake and energy expenditure.[20,21]

Galanin is a 29–amino acid peptide that was originally isolated from porcine intestine.[22] Galanin-like immunoreactivity has been described in a variety of mammalian species including rats, guinea pigs, rabbits, dogs, monkeys, and humans.[23] Specifically, galanin-like immunoreactivity has been noted in the central nervous system, the adrenal medulla, and the gastrointestinal, genitourinary, and respiratory tracts.[23,24] Within these tissues, galanin-like immunoreactivity is commonly localized in neural and neuroendocrine cells in conjunction with catecholamines, serotonin, GABA, or acetylcholine.[23,25,26] Galanin produced in the hypothalamus and anterior pituitary is highly stimulated by estrogens.[24,25,27-30]

Our previous studies showed that the neuropeptides and neurotransmitters modulating eating behavior play an important role in the neuroendocrine control of hormonal secretion in obese subjects and in patients with anorexia nervosa.[31-34] The aim of this study was to determine the circulating levels of NPY, galanin, and leptin in obesity and anorexia nervosa.

SUBJECTS AND METHODS

The subjects were 41 obese women aged 19 to 43 years, seven obese women with non–insulin-dependent diabetes mellitus (NIDDM) aged 36 to 45 years, 15 women with anorexia nervosa aged 18 to 23 years, and 19 lean women aged 18 to 42 years (control group). The obese women were subdivided into four groups (Table 1). Clinical data for the anorectic subjects are presented in Table 2.

All of the obese patients had normal blood pressure. All endocrine diseases known to cause obesity were excluded. On the basis of the waist to hip ratio (WHR), the obese women were classified as having gluteofemoral obesity. In all of the obese women, the WHR was less than 0.8. No pharmacological or dietetic treatment was introduced before the hormonal investigations. Obese women were in the stable phase of obesity.

Table 1. Clinical Data for the Obese and Healthy Subjects

Group	BMI Range	No. of Subjects	BMI (mean ± SEM)	Age, yr, (mean ± SEM)
A	25-30 kg/m², overweight	9	28.6 ± 0.4	39.6 ± 5.0
B	31-40 kg/m², moderate obesity	23	36.2 ± 2.6	35.4 ± 0.6
C	>40 kg/m², severe obesity	9	46.3 ± 3.2	34.3 ± 3.4
D	31-40 kg/m², moderate obesity + NIDDM	7	37.3 ± 2.6	43.8 ± 4.0
Control	22.0 ± 1.4	19	22.0 ± 0.3	28.7 ± 2.6

Table 2. Clinical Data for the Women With Anorexia Nervosa

Case No.	Age (yr)	Duration of Clinical Symptoms (yr)	Deficit of Body Weight (%)
1	20	2	45
2	21	2	47
3	19	2	38
4	18	2	34
5	22	3	30
6	23	2	35
7	18	2	42
8	19	2	41
9	20	2	42
10	21	3	40
11	20	2	30
12	21	1	35
13	20	1	37
14	21	2	39
15	20	3	40

The diagnosis of anorexia nervosa was established according to criteria described by Feighner et al[35] and Russell.[36] Women with anorexia nervosa were investigated during the weight loss phase of the disease, and at that time the weight deficiency was 30% to 47%. Bulimia was not observed in the anorectic women, and the duration of clinical symptoms of anorexia nervosa was 2 to 3 years.

Blood samples for NPY, galanin, and leptin assays were taken at 8 AM from fasting subjects. Plasma NPY, galanin, and leptin concentrations were measured by radioimmunoassay with commercial kits (Peninsula Laboratories, Belmont, CA). Sensitivity of the NPY assay was 2 pg/tube, and the interassay and intraassay coefficients of variation were 8.5% and 7.3%, respectively. Sensitivity of the galanin assay was 13 pg/tube, and the interassay and intraassay coefficients of variation were 7.3% and 6.1%, respectively. Sensitivity of the leptin assay was 0.5 ng/mL, and the interassay and intraassay coefficients of variation were 8.3% and 6.2%, respectively.

The statistical analysis was performed with an unpaired t test and ANOVA as appropriate. The data are presented as the mean ± SEM.

RESULTS

Plasma NPY concentrations in the control group, obese women, and obese women with NIDDM are presented in Fig 1. The arithmetical mean values for plasma NPY levels expressed as picograms per milliliter in groups A (8.1 ± 1.7), B (21.2 ± 5.1), C (29.7 ± 3.5), and D (41.0 ± 3.5) were significantly higher than in the control group (3.1 ± 0.3; $P < .01$, $P < .001$, $P < .001$, and $P < .001$, respectively). The highest plasma NPY concentrations were observed in obese women with NIDDM, but the arithmetical mean differences between groups B and D were not significant. Plasma galanin concentrations are shown in Fig 2. The arithmetical means for plasma galanin levels expressed as picograms per milliliter were significantly higher in groups B (54.0 ± 7.1), C (60.0 ± 7.0), and D (50.0 ± 8.0) compared with the control group (21.6 ± 7.0; $P < .001$, $P < .001$, and $P < .001$, respectively). We did not find significant differences in galanin levels between group A

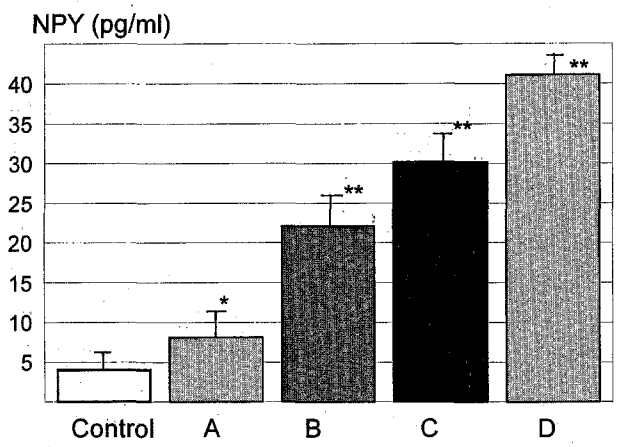

Fig 1. Plasma NPY concentrations in obese women. A, BMI 25 to 30 kg/m²; B, BMI 31 to 40 kg/m²; C, BMI >40 kg/m²; D, BMI 31 to 40 kg/m² + NIDDM. *P < .01, **P < .001.

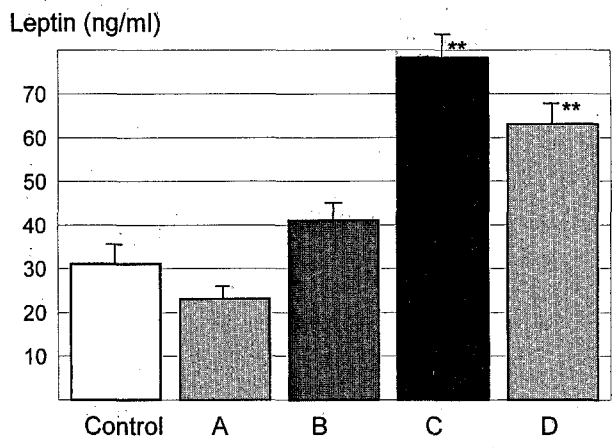

Fig 3. Plasma leptin concentrations in obese women. A, BMI 25 to 30 kg/m²; B, BMI 31 to 40 kg/m²; C, BMI >40 kg/m²; D, BMI 31 to 40 kg/m² + NIDDM. *P < .01, **P < .001.

(overweight group) and the control group. Plasma leptin concentrations are shown in Figs 3 and 4. Plasma leptin expressed as nanograms per milliliter were significantly higher in groups C (78.0 ± 6.0) and D (63.1 ± 4.1; $P < .001$ and $P < .001$, respectively) than in the control group (31.7 ± 2.4). Plasma NPY and galanin concentrations in anorectic women did not differ from those in the control group. However, the plasma leptin level in anorectic patients (18.8 ± 1.5) was significantly lower ($P < .01$) than in the control group.

DISCUSSION

Many of the peptides originally isolated from the gut are also found in the brain. This observation led to the concept of a brain-gut axis. Gut peptides are thought to be important in the regulation of satiety by the central nervous system. Furthermore, neurons of the central nervous system interact with those of the enteric nervous system to influence digestive processes. This interaction occurs via both afferent and efferent pathways and involves vagal and spinal neurons.[37] Neurons of the enteric nervous system exert local control over digestive processes including absorption, secretion, motility, immune function, and blood flow.[37]

The distribution of NPY in many sites of the gut suggests multiple functions for NPY, including a role in the regulation of intramural neuronal activity, smooth muscle tone, local blood flow, and epithelial transport. Our results show that plasma NPY concentrations were significantly higher in the overweight group and in both moderate and severe obesity. A correlation between BMI and plasma NPY was found. However, the highest plasma NPY concentrations were demonstrated in the group with moderate obesity and NIDDM. The source of NPY in peripheral plasma and the probable mechanism of its effect on food intake are an open question. It can be speculated that NPY originates from the pancreas and other sites of the gut, or from the adrenal medulla rather than from the brain. Some groups have reported that NPY, corticotropin-releasing hormone (CRH), and β-endorphin concentrations in the cerebrospinal fluid of obese patients are low.[38]

The orexigenic effect of NPY may be mediated by the activation or inhibition of other central or peripheral systems.

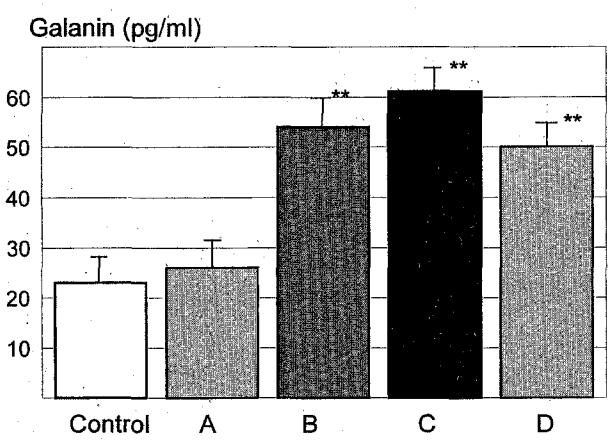

Fig 2. Plasma galanin concentrations in obese women. A, BMI 25 to 30 kg/m²; B, BMI 31 to 40 kg/m²; C, BMI >40 kg/m²; D, BMI 31 to 40 kg/m² + NIDDM. **P < .001.

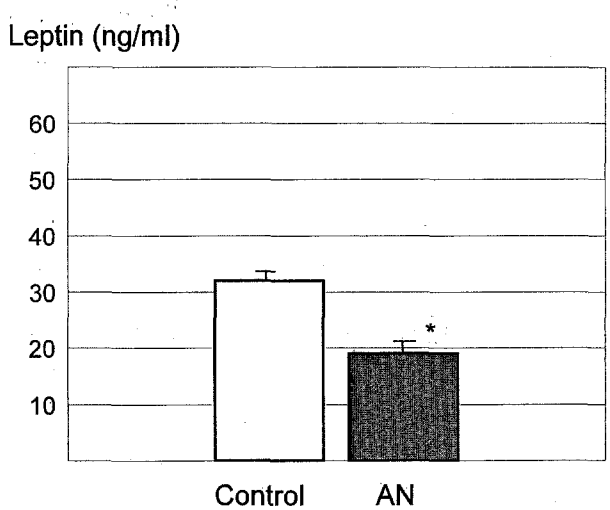

Fig 4. Plasma leptin concentrations in women with anorexia nervosa. *P < .01.

Experimental evidence indicates that NPY is the strongest orexigenic factor in the hypothalamic control of feeding behavior.[39] It is known that NPY is an important factor involved in the control of carbohydrate ingestion. In rats with streptozotocin-induced diabetes mellitus and in spontaneously diabetic Brattleboro rats, the NPY content in the hypothalamic PVN was significantly increased.[40,41] Increased NPY secretion from the PVN may lead to diabetic hyperphagia. In genetically obese Zucker rats, increased hypothalamic prepro-NPY mRNA and increased hypothalamic NPY content were found.[3,42] Long-term and continuous central injections of NPY in the Long-Evans rat reproduced the hyperphagia and obesity syndrome of the Zucker rat.[43] On the other hand, short- or long-term ingestion of high-carbohydrate diets induced changes in NPY in the arcuate nucleus (ARC) or PVN of the hypothalamus.[44] The action of NPY on food intake is mediated by NPY receptors. Injection of the NPY receptor antagonist PYX-2 into the PVN reduced the ingestion of carbohydrates.[39,45] These data suggest a physiologic function for NPY receptors in the PVN in the control of carbohydrate ingestion.

NPY itself induces peripheral hormonal and metabolic alterations via efferent routes. Long-term intracerebroventricular administration of NPY produced hyperphagia and increased basal insulinemia, as well as liver and adipose tissue lipogenic activity, and basal morning corticosteronemia. NPY stimulated glucose uptake and total lipoprotein lipase activity in white adipose tissue, and it resulted in an increase in the total activity of hepatic and white adipose tissue acetyl coenzyme A carboxylase.[46] NPY produced not only an increase of basal insulinemia and an increase of insulin-stimulated glucose uptake by adipose tissue but also a marked decrease in uptake by muscle.[47] Thus, NPY could be or primary importance in the establishment of obesity syndromes with incipient insulin resistance.[47]

IR NPY-like material was found in nerve fibers in pancreatic islets, and both direct and indirect actions of NPY on insulin secretion have been demonstrated.[48] The interaction between insulin and NPY is well established.

On the other hand, insulin may regulate NPY secretion.[39] Insulin administered centrally decreased prepro-NPY mRNA in the ARC and NPY in the PVN.[49] Insulin may regulate NPY secretion via the insulin-like growth factors IGF-1 and IGF-2.[50] Increased insulin secretion and insulin resistance are common findings in obesity. Our previous studies showed an exaggerated response of insulin to glucose injection and normal IGF-1 levels in obese women with gluteofemoral obesity.[51]

There were interactions between NPY and catecholamines as orexigenic signals.[11,13] These two signals stimulate carbohydrate intake. NPY coexists with catecholamines in the central and sympathetic nervous systems and in the adrenal medulla.[15,48] It is an open question as to whether an increased NPY release in our obese patients is a factor correcting the low sympathetic tonus. Activity of the sympathetic nervous system is low in obesity.[52]

The effects of catecholamines are mediated by α- and β-adrenoceptors. In adipose tissue, the important β effect of catecholamines is to stimulate lipolysis. Catecholamines promote depletion of triglyceride stores and, via increased thermogenesis, contribute to a release of the energy excess.[53] In obese Zucker rats, a significant decrease in the level of β-adrenoceptors and their mRNA was observed in both brown and white adipose tissue.[54] In obese diabetic (db/db) mice, reduced β_3-adrenoceptor mRNA expression was found.[55]

In the periphery, except for insulin, corticosteroids also have a reciprocal effect on energy storage.[56] NPY-containing neurons could cause hyperphagia, reduced energy expenditure, and obesity, and perhaps contribute to hyperinsulinemia, altered pituitary secretion, and stimulation of corticotropin and corticosterone release.[57] Hypothalamic NPY could also be partly regulated by central CRH.[58] Within the hypothalamic nuclei, neurotransmitters such as serotonin and neuropeptides such as CRH and NPY act to integrate these signals and mediate peripheral autonomic nervous system activity.[59]

The energy balance is controlled by a feedback loop in which the amount of stored energy is sensed by the hypothalamus, which adjusts food intake and energy expenditure to maintain a constant body weight. Neither the glucostatic theory nor theories based on the thermal regulation of food intake fully account for the precision with which energy balance is regulated in vivo.

Leptin, a hormone secreted by adipocytes, informs the brain of the amount of adipose tissue in the body.[21] There is some evidence that this factor acts as a satiety signal on the hypothalamic centers for food intake and energy expenditure.[21] The interaction between peripheral and central organs seems to represent an important feedback system for the tight control of body fat and body weight. The ob leptin receptor is expressed at a high level in the hypothalamus as compared with other tissues.[60] Leptin may modulate the activity of NPY and other peptides in the hypothalamus known to affect feeding behavior.[20,21] Defective leptin signaling due to either leptin deficiency in ob/ob mice or leptin resistance in db/db mice leads directly to hyperglycemia and the overexpression of hypothalamic NPY that is implicated in the pathogenesis of the obesity syndrome.[20] Our results showed that leptin concentrations were elevated in moderate and severe obesity. However, in anorectic patients, plasma leptin concentrations were decreased. These data about anorectic patients are consistent with the results of Grinspoon et al.[61]

We demonstrated a marked increase of NPY concentrations in obese patients. Considine et al[62] found that obese subjects had higher serum leptin concentrations that were correlated with body weight, suggesting that most obese persons are insensitive to endogenous leptin production. Both leptin and NPY may not only regulate appetite but may also affect metabolism through regulation of β_3-adrenoceptors and insulin secretion.[48,55] Chua et al[63] demonstrated that the phenotype of db/db mice, which includes severe, early-onset obesity, extreme insulin resistance, and strain-specific susceptibility to diabetes, was identical to that of ob/ob mice.

NPY and galanin produce hyperphagia, and reduce energy expenditure via central and peripheral mechanisms.[64] Galanin acts preferentially at the hypothalamic level on the ingestion of fat through neurons in the PVN, the medial preoptic area, and the median eminence.[64] Galanin is highly responsive to the stimulatory action of the estrogens, whereas NPY is highly responsive to corticosterone. The association between estrogen and galanin may be related to the deposition of fat after puberty, whereas the connection between NPY and corticosterone may

explain the development of obesity in conditions associated with hypercortisolemia and insulin resistance.

Recently, Invitti et al[65] reported that galanin plasma levels did not differ significantly between normal, anorectic, and obese women. Our results showed increased galanin concentrations in moderate and severe obesity. However, the individual values are comparable to those obtained by Invitti et al. The discrepancy probably results from the relatively higher and more scattered values in the control and anorectic subjects investigated by their group. Further study is required to clarify this matter. Galanin levels were markedly increased in obesity with NIDDM; nevertheless, they did not exceed the values for galanin observed in moderate obesity. The source of the increased galanin concentration in the peripheral plasma of obese patients is an open question.

The increased plasma galanin concentration in obese women is probably derived mostly from the gut rather than the brain. Galanin is also colocalized with catecholamines in sympathetic nerve endings that supply the islets of Langerhans, and there is convincing evidence that it is released locally in the islets and inhibits insulin secretion from the β cells.[65] Plasma galanin and NPY concentrations in women with anorexia nervosa did not differ from values in the control group.

An increase of NPY release in obesity with NIDDM indicates that NPY may be involved in the mechanism of hyperphagia and disturbed glucose tolerance in obese subjects. The vasoconstricting properties of NPY[66-68] may contribute to the mechanism of hypertension frequently present in obesity. Our obese patients had normal blood pressure, but it would be very interesting to measure NPY levels in hypertensive obese patients.

The open question remains as to whether the modification of circulating levels of NPY and galanin in obese subjects is a consequence of weight status or is one of many factors involved in the pathogenesis of obesity.

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