Do catecholamines influence the level of plasma leptin in patients with phaeochromocytoma?

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ABSTRACT

The relationship between plasma leptin and catecholamine concentrations during chronic and acute catecholamine excess is studied. Patients with phaeochromocytoma, divided according to gender, were examined under basal conditions (n=18) and at selected time-points during surgical removal of the tumour (n=12). Appropriate controls were used (n=23) for the basal study. Plasma leptin was determined by radioimmunoassay (RIA) and plasma noradrenaline (NA) and adrenaline (A) by high-performance liquid chromatography (HPLC). Statistical evaluation employed Student's t-test, Wicoxon test and Spearman's correlation coefficient. Gender-related differences in plasma leptin in normal subjects was confirmed, and these were maintained in the patients. Phaeochromocytoma patients had normal plasma leptin levels in the basal state and decreased levels following the massive catecholamine surge provoked by surgery. Plasma leptin concentration did not correlate with plasma NA or A in either group studied. In the patients with phaeochromocytoma, acute but not chronic catecholamine excess affected plasma leptin, suggesting a role for sympathetic activity in modulating leptin release.

KEY WORDS: Catecholamines. Leptin. Pheochromocytoma. Sympathetic activity.

Introduction

Discovery of leptin in 1994 by Zhang *et al*.[1] has focused attention on the role of proteins secreted by adipose tissue. Leptin is the 16 kDa secretory protein product of the *ob* gene, which is synthesised primarily by adipocytes and demonstrates hormonal properties. It regulates body weight and energy balance, and its receptor (OB-Rb) is expressed primarily in the hypothalamus but also in the human vasculature. Leptin provides a signal of nutritional status to the brain and peripheral organs.

ob gene expression correlates significantly with both body fat percentage and body mass index (BMI). Mechanisms that control human leptin secretion are poorly understood; however, factors such as insulin, glycoproteins, a high-fat diet and sex hormones[2-6] are proposed as regulators of leptin synthesis. In addition, leptin modulates other physiological actions such as lipid metabolism, haematopoiesis, pancreatic cell function and thermogenesis.

Interaction between leptin and sympathetic activity also should be considered, as it is well documented that sympathetic activity plays a major role in the regulation of human fat metabolism. β_3-adrenergic receptors, mostly expressed in adipose tissue, promote lipolysis as well as thermogenesis, and animal studies demonstrate that leptin stimulates adrenergic fibres innervating brown adipose tissue.[7,8]

Possible modulation of sympathetic activity by leptin suggests that it has a role in the development of arterial hypertension. However, it is reported that noradrenaline decreases plasma leptin,[9] probably through activation of protein kinase A, which modulates leptin gene expression in the murine adipocyte cell line 3T3-L1.[10,11]

Clinical data on the influence of catecholamnines on plasma leptin concentration are scarce. Phaeochromocytoma – an adrenal medullary tumour and model of excessive catecholamine release – provides a good opportunity to study the relationship between catecholamines and leptin. Sakane *et al*.[12] observed a decreased serum leptin level in a single patient with phaeochromocytoma, while Böttner *et al*.,[13] who studied a group of patients, did not comment on the influence of chronic catecholamine excess on circulating leptin level in phaeochromocytoma.

Here, we investigate the relationship between catecholamines and plasma leptin level in patients with phaeochromocytoma, both in chronic hypercatecholaminaemia and during acute catecholamine surge associated with surgical manipulation of the tumour.

Materials and methods

A group of 22 patients (14 women, 8 men) with phaeochromocytoma were studied under basal conditions and/or during surgical manipulation/removal of the tumour. A control group consisted of 23 healthy volunteers (13 women, 10 men).

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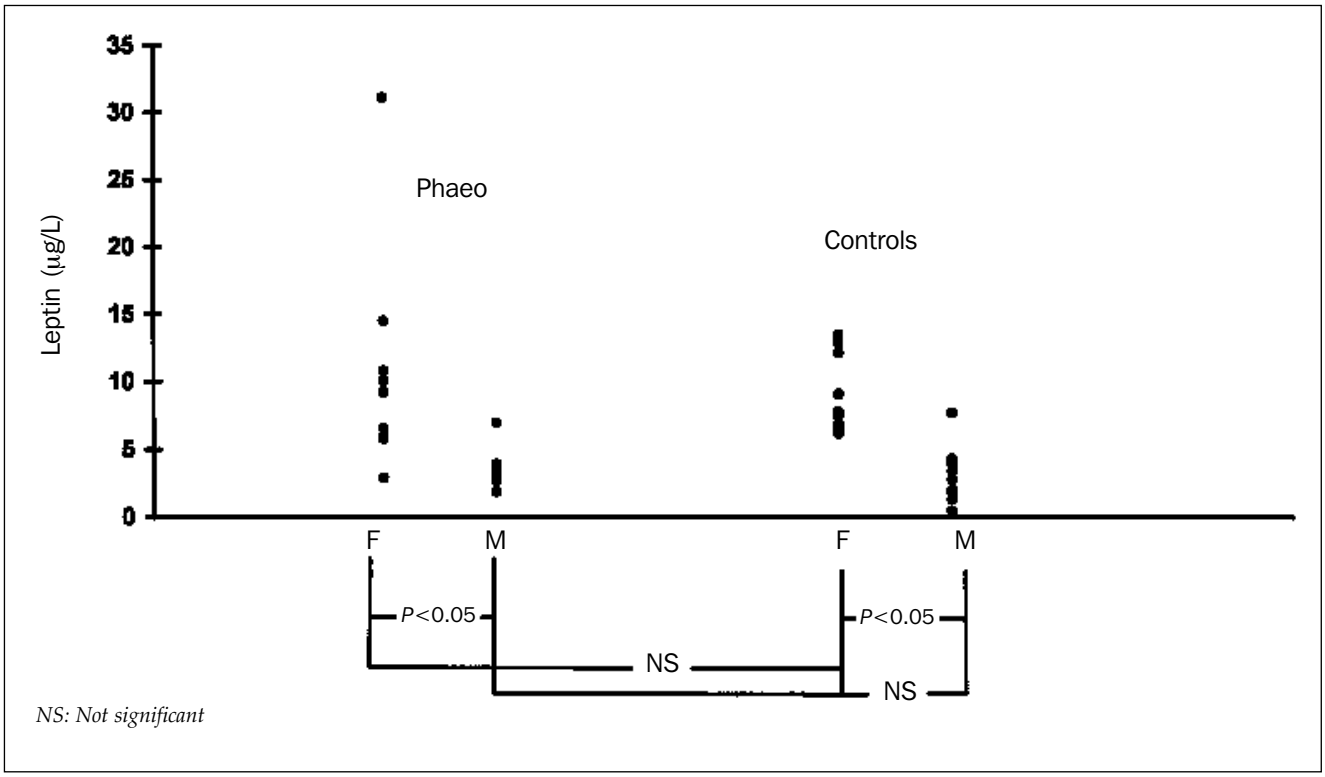
Fig. 1. Plasma leptin concentration in patients with phaeochromocytoma and in healthy controls.



Diagnosis of phaeochromocytoma was based on the usual clinical, biochemical and radiological criteria.[14] All patients underwent surgical removal of the tumour and diagnosis was confirmed histologically.

Eight patients excreted increased amounts of both adrenaline (A) and noradrenaline (NA), while exclusive NA excretion was found in another eight patients. In the remaining six cases excessive excretion of catecholamine metabolites only was detected.

In 18 of the 22 patients (10 women [age: 39.2±9.5 yrs] and eight men [age: 44.1±16.4 yrs]) and in the control group (13 women [age 34.1±11.9 yrs] and 10 men [age: 37.5±9.9 yrs]) studies were performed in the basal state (no previous drug therapy, normal sodium intake). Blood samples for measurement of leptin, NA, and A were taken from an antecubital vein, after 30 min recumbent rest, via a previously inserted cannula.

In 12 of the 22 patients (age: 48.7±11.3 years [7 women, 5 men]), studies were performed three times during surgical intervention: after laparotomy (phase I), during manipulation of the tumour (phase II) and 15 min after excision of the tumour (phase III). Surgical removal of phaeochromocytoma was carried out under combined anaesthesia with a mixture of nitrogen suboxide and oxygen, and an analgesic muscle relaxant. All patients undergoing surgery had previously been given α-adrenergic blocking drugs and, in some cases, β-blockers. Elevation of blood pressure during surgery was treated with sodium nitroprusside or nitroglycerin.

Plasma leptin level was measured by radioimmunoassay (RIA), using Linco Research Inc kits. Intra- and inter-assay variation was 8.3% and 6.2%, respectively, for the mean leptin value (4.9 μg/L), and 4.7% and 3.0%, respectively, for the mean leptin concentration (15.7 μg/L).

Plasma NA and A concentrations were measured by high-performance liquid chromatography (HPLC), using Bio-Rad equipment with an electrochemical detector. Intra- and inter-assay variabilities were 3.4% and 2.8% (NA) and 5.3% and 4.7% (A), respectively. Body mass index (BMI) was calculated by dividing body weight (kg) by height^2 (m^2).

Statistical analysis was performed using Student's *t*-test or the Wilcoxon test, depending on distribution of data. Parameters studied were expressed as median values when distribution differed from normal, and as mean ± standard deviation (SD) for normally distributed values. Correlation coefficients were calculated according to Spearman. $P < 0.05$ was considered statistically significant.

Results

Demographic characteristics, BMI and basal leptin, NA and A levels are summarised in Table 1. As expected, plasma NA and A concentrations were significantly higher in the phaeochromocytoma patients than in the control group. Gender difference in age, BMI or catecholamine concentration was not observed in either group.

Basal plasma leptin concentration in phaeochromocytoma patients did not differ from that found in the control group. In both groups, plasma leptin was significantly higher in women than in men (Figure 1).

Concentrations of leptin (for males and females, separately), NA and A (expressed as median values) among phaeochromocytoma patients at the three selected time points during surgery are presented in Figure 2. As expected, plasma NA and A levels increased significantly during mechanical compression of the tumour (phase II) and decreased after its removal (phase III). Plasma leptin

Table 1. BMI and plasma leptin, noradrenaline (NA) and adrenaline (A) concentrations in phaeochromocytoma (Phaeo) patients and in healthy controls.

	BMI (kg/m²)	Leptin (μg/L)	NA (pg/mL)	A (pg/mL)
Phaeo female *n*=8	23±1.9	2.9-3.1 Med. 9.25	249.8-5280.4 Med. 2348	12.8-2768.5 Med. 99.3
Phaeo male *n*=8	22.7±1.7	1.9-7.0 Med. 9.25	249.8-5280.4 Med. 2099.6	14.9-1216.0 Med. 79.9
Phaeo: female *vs* male	NS	$P<0.01$	NS	NS
Control female *n*=13	21.1±2.3	6.2-13.5 Med. 7.8	249.8-5280.4 Med. 280.6	18.8-47.1 Med. 35.0
Control male *n*=10	22.8±3.0	1.0-7.0 Med. 2.5	249.8-5280.4 Med. 206.0	27.0-63.0 Med. 44.0
Phaeo female *vs* male	NS	$P<0.05$	NS	NS
Phaeo female *vs* control male	NS	NS	$P<0.01$	$P<0.02$
Phaeo male *vs* control male	NS	NS	$P<0.01$	NS

NS: Not significant
Med: Median value

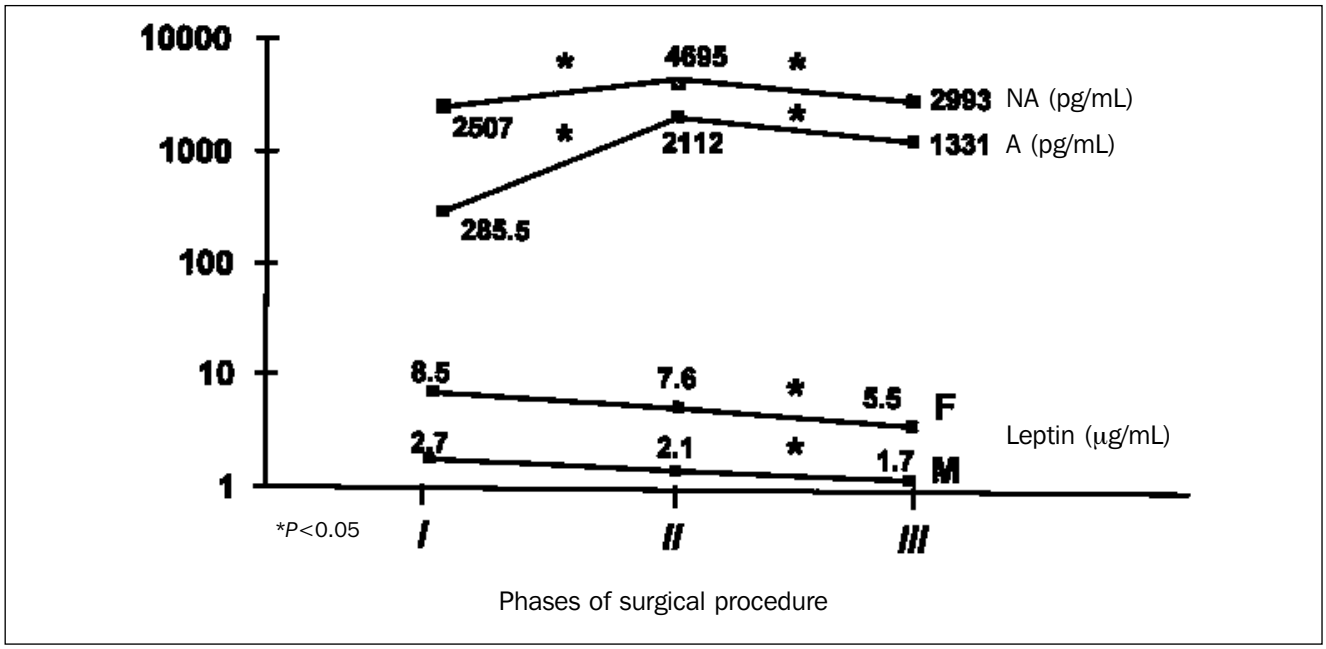


Fig. 2. Plasma leptin, noradrenaline (NA) and adrenaline (A) concentration expressed as a median value in patients with phaeochromocytoma during surgical removal of the tumour. Phase I: before manipulation of the tumour; phase II: during manipulation of the tumour; phase III: after removal of the tumour.

concentration remained unchanged during phases I and II, but decreased significantly during phase III, in both males ($P=0.002$) and females ($P=0.034$).

Plasma leptin concentration did not correlate with that of NA or A, either in basal conditions or during surgery, or in the healthy controls.

Discussion

The present study indicated that patients with phaeochromocytoma exhibit normal plasma leptin levels. It is of note, however, that gender differences in plasma leptin concentration[2,4,5] seen in controls were also present in the phaeochromocytoma group. Our finding indicates that chronic excess of circulating catecholamines is not associated with either increased or suppressed plasma leptin. This supports the conclusions of the study by Böttner *et al.*,[13] although the report of a single case[12] suggested suppression of leptin in phaeochromocytoma.

It should be noted, however, that during chronic catecholamine excess – characteristic of most patients with phaeochromocytoma – down-regulation of adrenergic receptors may take place,[15] and such adaptive changes might interfere with the possible modulation of leptin by catecholamines.

Presence of β_3-receptors expressed in brown adipose tissue is thought to play a key role in the sympathetic modulation of leptin.[3,16] It seems plausible, therefore, that β_3-receptors might undergo down-regulation in the face of chronic catecholamine excess in phaeochromocytoma, which, in turn, could prevent the inhibitory action of NA on leptin.

Although chronic catecholamine excess did not influence leptin level in the present study, a significant decrease in leptin concentration was observed following the massive catecholamine surge provoked by surgical manipulation of

the tumour.

Mechanisms involved in the regulation of leptin level are not fully understood. This regulation could have a molecular basis, as leptin gene transcription and translation occurs in adipose tissue; however, direct modulation of leptin release by catecholamines cannot be excluded. Kosaki *et al*.[11] demonstrated that administration of both catecholamines and synthetic β-agonists reduces leptin messenger RNA (mRNA) expression both *in vivo* and *in vitro* – an effect diminished by propranolol.

In human studies, sympathetic activity (particularly β-receptor activity) appears to be implicated in leptin release from the adipocytes via a negative feedback mechanism.[9] Administration of β-agonists reduces plasma leptin concentration, supporting the role of β-receptors in the modulation of leptin release.[10]

We believe that decrease in leptin level following massive catecholamine surge may be linked, although Banks[17] suggests that adrenaline could increase transport across the blood-brain barrier, and that this might explain the transient fall in leptin during surgery. Repeat determination of leptin level in six patients two weeks after surgery revealed normal basal levels (data not published).

Conclusions

Chronic elevation of circulating catecholamines in patients with phaeochromocytoma does not affect plasma leptin concentration, irrespective of gender. Acute catecholamine surges that occur during surgical manipulation of the tumour can influence plasma leptin level, in both men and women, suggesting a role for sympathetic activity in modulating leptin release. Further studies to fully elucidate the complex relationship between sympathetic activity and leptin, including studies of receptor sensitivity, are required.

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