doi:10.1006/cyto.2002.1051, available online at http://www.idealibrary.com on IDEAL®

LEPTIN ROLE IN ADVANCED LUNG CANCER. A MEDIATOR OF THE ACUTE PHASE RESPONSE OR A MARKER OF THE STATUS OF NUTRITION?

María Remedios Alemán,[1] Francisco Santolaria,[1] Norberto Batista,[2] María J. de la Vega,[3] Emilio González-Reimers,[1] Antonio Milena[3] Marta Llanos,[2] Juan Luis Gómez-Sirvent[1]

Leptin is an anorexia inductor peptide produced by adipocytes and related to fat mass. Leptin is also produced by fat under proinflammatory cytokine action. Our objective is to study serum leptin levels in relation to nutritional status and acute phase response in advanced-stage non-small cell lung cancer.

Seventy-six patients newly diagnosed of non surgical non-small cell lung cancer before chemotherapy treatment and 30 healthy controls were included. BMI, serum leptin and cholesterol levels and lymphocyte count were decreased in lung cancer patients. Cytokine IL-6, TNF-α, sTNF-RII, sIL-2R, IL-12, IL-10 and IFN-γ, and other acute phase reactants as α1 antitrypsin, ferritin, CRP and platelets were all raised in patients, whereas the IL-2 was decreased. We found a direct relationship between leptin and other indicators of the status of nutrition, especially total fat mass. We also found a close relationship between the status of nutrition and the performance status (Karnofsky index). However, serum leptin and nutritional status were inversely correlated with acute phase proteins and proinflammatory cytokines, suggesting a stress-type malnutrition. Although serum leptin levels, nutritional status and Karnofsky index are related to survival, at multivariate analysis they all were displaced by the acute phase reaction markers.

These results suggest that cancer anorexia and cachexia are not due to a dysregulation of leptin production. Circulating leptin concentrations are not elevated in weight-losing cancer patients and are inversely related to the intensity of the inflammatory response. In advanced lung cancer patients serum leptin concentrations only depend on the total amount of fat.

Leptin is a peptide member of the cytokine receptor family, which is produced primarily by fat cells. It regulates fat mass by decreasing food intake (it decreases the content of neuropeptide Y at the hypothalamus) and increasing resting energy expenditure.[1] Serum leptin concentrations are highly correlated with body fat content,[2] and its production by adipocytes rapidly declines during starvation. Besides this, leptin is also produced by fat under proinflammatory cytokine action, its increase having been reported in the acute phase reaction of sepsis. Leptin increases by the action of endotoxin or cytokines, and has been involved in the anorexia of infection.[3–6]

Weight loss is frequently observed in advanced cancer.[7,8] Decreased food intake, hypermetabolism, and acute phase response with metabolic disturbances, partly due to host-derived and tumour substances, including various cytokines, are considered important wasting factors.[9–12] Since anorexia and hypermetabolism frequently play a role in the development of cancer cachexia, it has been hypothesized that increased leptin secretion could be involved in its pathogenesis. There is evidence that ob gene expression may be up-regulated by pro-inflammatory cytokines such as TNFα[13] and IL-1,[14] which are also involved in the pathophysiology of cancer cachexia. However, Simons *et al.*[15] reported low or undetectable circulating leptin concentrations in patients with lung cancer and

From the [1]Servicio de Medicina Interna, [2]Sección de Oncología Médica, [3]Servicio de Laboratorio, Hospital Universitario de Canarias, Tenerife, Spain

Correspondence to: María Remedios Alemán, Servicio de Medicina Interna, Hospital Universitario de Canarias, C/ Ofra s/n, Universidad de La Laguna, 38320 Tenerife, Spain. Tel: (922) 678684; Fax: (922) 653808; E-mail mraleman@comtf.es

Received 3 August 2001; received in revised form 17 May 2002; accepted for publication 10 June 2002

1043–4666/02/$-see front matter © 2002 Elsevier Science Ltd. All rights reserved.

KEY WORDS: lung cancer/malnutrition/acute phase reaction/cytokine/leptin

weight loss, although these investigators did not examine the relationship with the inflammatory response. Wallace *et al.*[16] found that circulating leptin concentrations in gastrointestinal cancer patients were not related to inflammatory response, suggesting that cancer cachexia is not due to a dysregulation of leptin production. Moreover, Lopez Soriano *et al.*[17] found that anorexia was not related to leptin changes in experimental cancer.

Our objective is to analyse the relation of serum leptin levels with the nutritional status and the inflammatory response (acute phase reaction) in patients with advanced non-small cell lung cancer.

RESULTS

The distribution by stage was: 6 III_A non surgical, 30 III_B and 40 IV. The histological distribution was 37 (48.7%) adenocarcinoma, 22 (28.9%) epidermoid and 17 (22.4%) large cell carcinoma. Regarding status performance assessment, 19 (25%) had a Karnofsky of 90%, 36 (47%) a Karnofsky of 80%, and 21 (27,6%) a Karnofsky of 70%. Serum LDH levels were also raised in patients compared with controls ($P<0.001$).

Thirty three patients (43.4%) reported anorexia, 30 (23%) had lost at least 5% of the body weight (27% of them had lost 10% or more) and in 12 (15.8%) patients the BMI was under 20 kg/m^2. Serum leptin, cholesterol and total lymphocyte count were all lower in patients regarding controls ($P<0,001$). However, we did not find significant differences regarding serum albumin levels (Table 1).

Proinflammatory cytokines IL-6, TNF-α, sTNF-RII, sIL-2R and IL-12, antiinflammatory cytokine IL-10, other acute phase reactants as α1 antitrypsin, ferritin, CRP and platelets and immune IFN-γ were all significantly raised in lung cancer patients compared with controls whereas IL-2 was decreased (Table 1).

Leptin, nutritional status and acute phase reaction

High serum leptin levels were related with a better status of nutrition, assessed by serum albumin levels, body fat mass, BMI, serum leptin levels and subjective nutritional score. Moreover, we observed a very close, and highly significant, correlation between serum leptin levels and fat mass assessed by bioimpedance (Spearman, rho=0.800, $P<0.001$) (Table 2). We also observed a close relationship, between the nutritional status with the performance status (Karnofsky index): the worse the nutritional status the worse the performance status (serum albumin: $P=0.017$, fat mass: $P=0.015$, serum leptin: $P=0.015$, BMI: $P=0.019$ and SNS: $P=0.021$).

We found a negative relation between serum leptin levels and PCR, α1antitrypsin, platelet count, serum ferritin and the proinflammatory cytokines IL-6, sTNF-RII and sIL-2R. So, the status of nutrition was worse and the leptin levels were lower in parallel with a more intense acute phase response (Table 2). On the

TABLE 1. Nutritional status, leptin, LDH, acute phase response and cytokines levels in patients and controls

	Patients X ± EE(x)	Controls X ± EE(x)	*P* (UMW)
General characteristics			
LDH (U/L)	417.66 ± 22.83	305.93 ± 8.14	0.000
Nutritional status			
Leptin (ng/ml)	7.11 ± 0.91	18.50 ± 4.17	0.000
Cholesterol (mg/dl)	193.57 ± 5.92	211.40 ± 5.83	0.000
Albumin (g/dl)	3.94 ± 0.07	4.08 ± 0.04	0.435
Acute phase response			
Platelets (cell/mm^3)	281.84 ± 10.77	217.97 ± 8.10	0.001
CRP* (mg/dl)	5.08 ± 0.53	0.87 ± 0.06	0.001
α1 antitrypsin (mg/dl)	219.56 ± 8.26	168.79 ± 3.86	0.001
ferritin (μg/dl)	352.56 ± 32.14	155.23 ± 19.88	0.001
Cytokines			
IL-6 (pg/ml)	34.06 ± 21.44	5.25 ± 0.08	0.002
TNF-α (pg/ml)	15.02 ± 8.17	5.89 ± 0.32	0.006
s-TNFRII (ng/ml)	3.44 ± 0.20	1.99 ± 0.07	0.001
s-IL-2R (UI/ml)	906.27 ± 64.64	507.34 ± 26.13	0.001
IL-12 (pg/ml)	37.77 ± 12.31	13.61 ± 4.05	0.000
IL-10 (pg/ml)	18.61 ± 1.68	7.68 ± 1.14	0.000
IFN-γ (UI/ml)	1.61 ± 0.22	0.25 ± 0.03	0.001
IL-2 (UI/ml)	159.19 ± 20.26	267.61 ± 26.53	0.001

*CRP: C reactive protein.

TABLE 2. Relation between leptin and Karnofsky index and nutritional status and acute phase response

	Leptin	
	Spearman rho	*P*
Karnofsky index	0.364	0.001
Nutritional status		
BMI* (kg/m^2)	0.643	0.001
SNS**	− 0.572	0.001
Cholesterol (mg/dl)	0.439	0.001
Albumin (g/dl)	0.357	0.002
fat mass (kg)	0.800	0.001
Acute phase response		
α1 antitrypsin (mg/dl)	− 0.290	0.012
ferritin (μg/dl)	− 0.380	0.001
Platelets (cell/mm^3)	− 0.289	0.012
CRP*** (mg/dl) − 0.462	0.001	
Cytokines		
IL-6 (pg/ml)	− 0.414	0.001
TNF-α (pg/ml)	− 0.151	0.197
s-TNFRII (ng/ml)	− 0.335	0.003
s-IL-2R (UI/ml)	− 0.503	0.001
IL-12 (pg/ml)	− 0.199	0.088
IL-10 (pg/ml)	0.091	0.436
IL-2 (UI/ml)	0.333	0.003
IFN-γ (UI/ml)	0.129	0.270

*BMI: body mass index; **SNS: subjective nutritional score; ***CRP: C reactive protein.

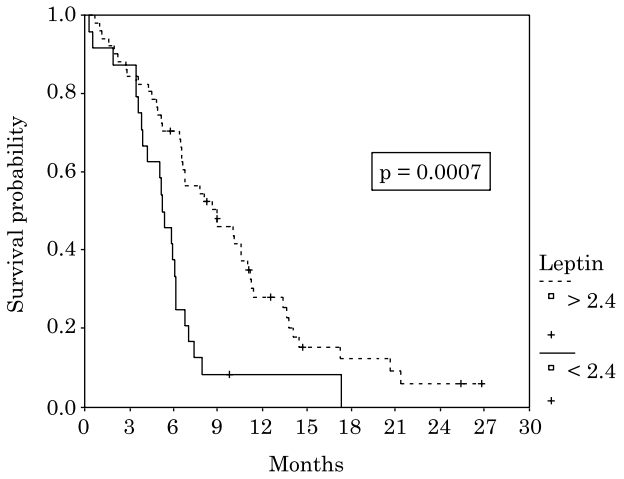

Figure 1. Decreased serum leptin levels are related to shorter survival.

However at multivariate analysis acute phase reaction (proinflammatory cytokines) provide a better information about prognosis.

contrary, we found a direct relation between serum leptin levels and the proimmune lymphocyte cytokines IFN-γ and IL-2.

Survival analysis

The median survival of the whole group was 198 days. Patients with higher serum LDH levels, lower Karnofsky index and a more impaired nutritional status (lower BMI, serum albumin and leptin levels (Fig. 1), and worse subjective nutritional score) had a shorter survival, analysed by Kaplan and Meyer curves (Table 3). Patients with an enhanced acute phase response also showed an impaired prognosis. Excluding TNFα, all proinflammatory cytokines IL-6, sTNF-RII, sIL-2R and IL-12, were related to a worse prognosis. On the contrary, proimmune cytokines IFN-γ, IL-2, were related to a better prognosis. Multivariate analysis (Cox regression analysis with covariates) showed that raised serum levels of sTNF-RII, α1 antitrypsin, LDH and platelets were all survival factors with independent prognostic value, whereas all the nutritional variables, including serum leptin levels, and Karnofsky index were displaced by the acute phase reactants.

DISCUSSION

Patients with advanced non-small cell lung cancer frequently suffer anorexia, weight loss and malnutrition leading to a low BMI, low fat mass and serum leptin levels, all of them factors associated with a worse prognosis. The Karnofsky index is another well known prognostic factor in patients with cancer. Moreover, we observed a close relationship between the performance status and the nutritional status,

TABLE 3. Survival in advanced non-small lung cancer patients. Univariate and multivariate analysis

	Univariate analysis				Multivariate analysis (Cox regression)		
	Log rank test	*P*	Median survival	CI 95%	R.R.	C.I. 95%	*P*
General characteristics							
LDH>400 (UI/L)	16.01	0.0001	137	97–177	2.18	3.67–1.30	0.0033
Karnofsky <90%	11.91	0.0006	184	152–216			
Nutritional status							
Leptin <2.4 (μg/L)	11.43	0.0007	157	126–223			
SNS* >2	11.74	0.0006	162	135–189			
BMI** <22 (kg/m^2)	4.29	0.0384	162	129–195			
Cholesterol <200 (mg/dl)	9.52	0.0020	184	160–208			
Acute phase response							
Platelets >320 × 10^3/mm^3	14.14	0.0002	156	149–163	2.20	4.45–1.08	0.0292
CRP*** >6	13.44	0.0002	157	123–191			
α1 antitrypsin >170 (mg/dl)	18.80	0.0000	176	144–208	3.54	7.32–1.71	0.0007
Ferritin >440 (μg/dl)	7.87	0.0050	157	153–161			
Cytokines							
IL-6 >5 (pg/ml)	16.01	0.0001	176	151–201			
rs-TNF-α >3.6 (ng/ml)	20.76	0.0000	156	125–187	2.47	4.95–1.23	0.0114
rs-IL-2 > 750 (UI/ml)	12.51	0.0004	176	147–205			
IL-12 > 4 (pg/ml)	5.28	0.0216	156	135–176			
IFN-γ <1.5 (UI/ml)	5.81	0.0159	192	170–214			
IL-2 <130 (UI/ml)	3.98	0.0459	184	161–206			
TNF-α >5 (pg/ml)	0.56	0.4554	193	176–210			
IL-10 >9.5 (pg/ml)	1.65	0.1996	193	176–210			

*SNS subjective nutritional score; **BMI: body mass index; ***CRP: C reactive protein.
R.R.: relative risk; C.I. 95%: confidence interval.
Cut-off points were selected according to those which provided a better significance regarding survival with the Kaplan and Meier analysis.

probably reflecting the effect of muscle and other protein loss on performance.

It is unclear whether leptin acts as an acute phase reactant, leading to anorexia and malnutrition, or if it is only a simple marker of fat mass in cancer associated malnutrition. Different studies have shown that leptin concentrations are increased during cytokine-induced inflammatory response in sepsis patients,[3–6] suggesting that raised leptin levels may be related to anorexia. However in many common diseases leading to cachexia, in which there is also an inflammatory status caused by raised proinflammatory cytokines, serum leptin levels are decreased. This is the case of wasting associated to chronic obstructive pulmonary disease (COPD) in which, despite an increase of TNFα, there are low leptin levels which keep a relation with decreased fat mass,[18,19] or chronic heart failure cachexia, in which serum TNFα and IL-1 levels are increased but leptin ones are decreased.[20,21] Also, in chronic inflammatory bowel disease and in wasting AIDS low or normal serum leptin levels have been reported despite an increase of sTNF-RII.[22,23]

Our results are in accordance with last studios. Serum leptin levels were lower in patients than in controls and, more decreased in the malnourished patients (as expected the relation was especially close with fat mass), despite an increase of proinflammatory cytokines and acute phase reactants. In advanced lung cancer patients we found that acute phase reactants as CRP, ferritin and α1 antitrypsin, the proinflammatory cytokines IL-6, TNF-α, sTNF-RII, sIL-2R, IL-12, the antiinflammatory IL-10 and the proimmune IFN-γ were all raised, together with, an impaired nutritional status and low serum leptin levels. On the contrary, IL-2, a proimmune cytokine, was decreased, perhaps pointing to a depressed T cell immune response. Moreover, the increased acute phase response and cytokine levels were related to an impaired status of nutrition including decreased serum leptin levels. So, the increase of acute phase reactants related to lower serum leptin levels and worse nutritional and performance status, suggest a stress-type malnutrition.

Our results do not support the hypothesis that high serum leptin levels, produced by a intense acute phase reaction, could be involved in anorexia and cachexia associated to cancer. Moreover, serum leptin levels were not higher in patients with anorexia, and weight loss was associated with low serum leptin concentrations. These results are similar to the reports of Simons *et al.* (1997)[15] and Brown *et al.* (2001)[24] in lung cancer, and Wallace *et al.* (1998)[16] in gastrointestinal cancer and Mantovani *et al.* (2000)[25] in pancreas cancer, in which leptin concentrations were not elevated in weight-loosing cancer patients.

Although the median survival of the whole group is only 198 days, by survival analysis we found that the status of nutrition, including leptin, the performance status and the acute phase reaction, were related to prognosis. However, in the multivariate analysis the performance status was displaced by the status of nutrition and this one by the acute phase reaction, indicating that all of them are related, providing similar information about survival, and reinforcing the importance of acute phase reaction and stress malnutrition in the evolution of advanced lung cancer.

As conclusion, serum leptin levels are not raised in patients with advanced non-small cell lung cancer, and its production is not induced by the inflammatory response. Moreover, concentrations of leptin seem appropriate for the amount of body fat. Therefore, it would appear that cancer anorexia and cachexia are due to stress malnutrition and they are not caused by a dysregulation of leptin production. Perhaps in advanced malnutrition serum leptin levels are more dependent on to fat mass than on cytokine effect.

MATERIALS AND METHODS

Patients

Between January 1997 and November 1999 we studied 76 patients (67 males and 9 females), with a median age of 62.5 years (range 36–75) recently diagnosed of non-small cell lung cancer, in advanced (non-surgical) stage, and before chemotherapy treatment. Patients were not included if brain metastasis were present, or if a poor performance status (Karnofsky index less than 70%), or if any other chronic disease producing malnutrition was present. The control group was composed of 30 healthy subjects (26 males and 4 females) with a median age of 58.5 years (range 38–75). All patients had been diagnosed by histological or cytological means. Status performance was assessed by the Karfnosky scale[26]. The TNM classification[27] was assessed by chest and upper abdominal CT scan, and a bone radionuclide scan, and was used for sstaging. Serum lactic dehydrogenase (LDH) levels were also used as an index of tumour bulk.

Nutritional assessment

Weight and height were recorded at admittance, with further calculation of body mass index (BMI) as weight/height^2. Anorexia and weight loss were also recorded with further calculation of weight lost percentage as: weight lost/(current weight+lost weight) × 100.

Subjective nutritional assessment included examination of the muscle masses of the upper and lower limbs and of the temporal muscle, defining two degrees of atrophy (severe, moderate), and absence of atrophy. We assigned 2.1 and 0 points to each category, respectively. Bichat's fat and subcutaneous fat atrophy, recorded by physical examination were classified in the same way. Thus, we have defined a subjective nutritional score (SNS) based on the sum of the assigned points, the poorest value being 10, and 0 the best one, as previously reported.[28] The fat mass and fat-free mass

were assessed by bioelectric impedance. We also determined serum of cholesterol, lymphocytes and serum albumin levels.

Acute phase proteins, cytokines and leptin assessment

Blood samples were obtained at 0800h in fasting conditions and serum was frozen at $-40°C$ for further determination. Assays performed were: serum leptin levels by Inmunoradiometric Assay (IRMA), CRP by Fluorescence Polarization Immunoassay (FPIA) (Diagnostic Division Abbott), IL-6, TNF-α and sIL-2R by chemiluminescent immunometric assay (Diagnostic Products Corporation), IFN-γ, IL-2 and IL-12 by immunoenzymatic assay (Immunotech), IL-10 by enzyme immunometric assay in a microplate format (Diagnostic Products Corporation), sTNF-R by Enzyme Amplified Sensitivity Immunoassay (EASIA) performed on microtiter plate (Biosource). All the assays were of high standard quality with very low cross reactivity, i.e. very high specificity. We also determined cholesterol, albumin, ferritin and α1 antitrypsin. The same analysis were performed to 30 healthy controls.

Statistical analysis

As most variables, especially leptin, cytokines and CRP, did not fit the normal distribution, as assessed by the Kolmogorov-Smirnov test, we have performed non parametric tests as Mann Withney U, Kruskal-Wallis and Spearman correlation.

Patients were followed until death. Survival curves were plotted by the method of Kaplan and Meier and log rank test were performed for differences in survival. Multivariate analysis (Cox regression with covariate survival analysis) was performed in order to discern which parameters yield independent predictive value on survival. For the multivariate analysis the cut-off points were selected according to those which showed a better significance regarding survival with the Kaplan and Meier analysis.

REFERENCES

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. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292–295.

3. Carlson GL, Saeed M, Little RA, Irving MH (1999) Serum leptin concentrations and their relation to metabolic abnormalities in human sepsis. Am J Physiol 276:658–662.

4. Arnalich F, López J, Codoceo R, Jiménez M, Madero R, Montiel C (1999) Relationship of plasma leptin to plasma cytokines and human survivalin sepsis and septic shock. J Infect Dis 180: 908–911.

5. Papathanassoglou ED, Moynihan JA, Ackerman MH, Mantzoros CS (2001) Serum leptin levels are higher but are not independently associated with severity or mortality in the multiple organ dysfunction/systemic inflammatory response syndrome: a matched case control and a longitudinal study. Clin Endocrinol (Oxf) 54:225–233.

6. Moses AG, Dowidar N, Holloway B, Waddell I, Fearon KC, Ross JA (2001) Leptin and its relation to weight loss, ob gene expression and the acute-phase response in surgical patients. Br J Surg 88:588–593.

7. De Wys WD, Begg C, Lavin PT, Çbband PR, Bennet JM, Bertino JR, Cohen MH, Douglass HO, Engstrom PF, Exdinli EZ, Horton J, Johnson GJ, Moertel CG, Oken MM, Perlia C, Rosebaum C, Sivertein MN, Skeel RT, Sponzo RW, Tormey DC (1980) Prognostic effect of weight loss prior to chemotherapy in cancer patients. Am J Med 69:491–497.

8. Daly JM, Cech AC (1996) Nutrition and neoplasia. In Fischer JE (ed.) Nutrition and Metabolism in the Surgical Patient. Little Brown, Boston, pp. 601–621.

9. Martín F, Santolaria F, Batista N, Milena A, González-Reimers E, Brito MJ, Oramas J (1999) Cytokine levels (IL-6 and IFN-γ), acute phase response and nutritional status as pronostic factors in lung cancer. Cytokine 11:80–86.

10. Staal-van den Brekel AJ, Dentener MA, Schols AMWJ, Buurman WA, Wouters EFA (1995) Increased resting energy expenditure and weight loss are related to a systemic inflammatory response in lung cancer patients. J Clin Oncol 13:2600–2605.

11. McMillan DC, Scott HR, Watson WS, Preston T, Milroy R, McArdle CS (1998) Longitudinal study of body cell mass depletion ant the inflammatory response in cancer patients. Ntr Cancer 31:101–105.

12. McMillan DC, Preston T, Watson WS, Simpson JM, Shenkin A, Burns HJG, McArdle CS (1994) The relationship between weight, reduction of body cell mass and the inflammatory response in cancer patients. Br J Surg 81:1011–1014.

13. Zumbach MS, Boehme MWJ, Wahl P, Stremmel W, Ziegler R, Nawroth PP (1997) Tumor necrosis factor increases serum leptin levels in humans. J Clin Endocrinol Metab 82:4080–4082.

14. Janik JE, Curti BD, Considine RV, Rager HC, Powers GC, Alvord WG, Smith JW, Gause BL, Kopp WC (1997) Interleukin 1α increases serum leptin concentrations in humans. J Clin Endocrinol Metab 82:3084–3086.

15. Simons JP, Annenmie MWJ, Schols L, Campfield A, Wouters EFM, Saris WH (1997) Plasma concentration of total leptin and human lung-cancer-associated cachexia. Clin Sci 93:273–277.

16. Wallace AM, Naveed S, McMillan DC (1998) Effect of weight loss and the inflammatory response on Leptin concentrations in Gastrointestinal cancer patients. Clinical Cancer Research 4: 2977–2979.

17. Lopez-Soriano J, Carbo N, Tessitore L, Lopez-Soriano FJ, Argiles JM (1999) Leptin and tumor growth in rats. Int J Cancer May 31;81(5):726–729.

18. Takabatake N, Nakamura H, Abe S, Hino T, Saito H, Yuki H, Kato S, Tomoike H (1999) Circulating leptin in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 159:1215–1219.

19. Schols AM, Creutzberg EC, Buurman WA, Campfield LA, Saris WH, Wouters EF (1999) Plasma leptin is related to proinflammatory status and dietary intake in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 160:1220–1226.

20. Murdoch DR, Rooney E, Dargie HJ, Shapiro D, Morton JJ, McMurray JJ (1999) Inappropriately low plasma leptin concentration in the cachexia associated with chronic heart failure. Heart 82:352–356.

21. Filippatos GS, Tsilias K, Venetsanou K, Karambinos E, Manolatos D, Kranidis A, Antonellis J, Kardaras F, Anthopoulos L, Baltopoulos G (2000) Leptin serum levels in cachectic heart failure patients. Relationship with tumour necrosis factor-alpha system. Int J Cardiol 76:117–122.

22. Yarasheski KE, Zachwieja JJ, Horgan MM, Powderly WG, Santiago JV, Landt M (1997) Serum leptin concentrations in human immunodeficiency virus-infected men with low adiposity. Metabolism 46:303–305.

23. Ballinger A, Kelly P, Hallyburton E, Besser R, Farthing M. (1998) Plasma leptin in chronic inflammatory bowel disease and HIV: implications for the pathogenesis of anorexia and weight loss. Clin Sci (Colch) 94:479–483.

24. Brown DR, Berkowitz DE, Breslow MJ (2001) Weight loss is not associated with hyperleptinemia in humans with pancreatic cancer. J Clin Endocrinol Metab 86:162–166.

25. Mantovani G, Maccio A, Mura L, Massa E, Mudu MC, Mulas C, Lusso MR, Madeddu C, Dessi A (2000) Serum levels of

leptin and proinflammatory cytokines in patients with advanced-stage cancer at different sites. J Mol Med 78: 554–561.

26. Mor V, Laliberti L, Morris JN, Wiemann M (1984) The Karnofsky performance status scale. Cancer 53:2002–2007.

27. Mountain CF (1997) Revisions in the International System for Staging Lung Cancer. Chest 111:1710–1717.

28. Tormo A, Santolaria F, González Reimers E, Oramas J, Rodríguez Rguez E, Rodríguez Moreno F, Martínez Riera A, Alonso MM, Raya JM (1994) Short-term prognostic value of subjective nutritional assessment in general medical patients. Journal of Nutritional Medicine 4:287–295.