New biochemical markers in chronic heart failure

E.M. El-Bindary [1] and A.Z. Darwish [2]

الواصمات الكيميائية الحيوية الجديدة في الفشل القلبي المزمن

إيناس البنداري وأحمد درويش زغلول

الخلاصة: قمنا باستقصاء مستويات عامل النخر الورمي ألفا، والليبتين، والإنسولين، في البلازما وعلاقتها بمَنْسَب كتلة الجسم في ثمانين مريضاً من الذكور الذين يشكون من الفشل القلبي المزمن (متوسط العمر: 47 ± 4 سنوات) بالمستشفى الجامعي بطنطا، وتبيَّن ارتفاع مستويات ليبتين البلازما وعامل النخر الورمي ألفا والإنسولين، بصورة ملموسة، وانخفاض مَنْسَب كتلة الجسم بصورة ملموسة كذلك في الصنفَيْن 3 و4 من المرضى طبقاً لتصنيف رابطة طب القلب بنيويورك. كما تبيَّن وجود ترابُط إيجابي مشترك بين عامل النخر الورمي ألفا والليبتين والإنسولين؛ ووجود ترابُط سلبي مشترك بين كتلة عامل النخر الورمي ألفا ومَنْسَب كتلة الجسم، وبين الليبتين ومنسب كتلة الجسم في المرحلتين الثالثة والرابعة من الفشل القلبي. ونخلص من ذلك إلى أن التنشيط العصبي الصمّاوي للسيتوكين قد يمثّل جزءاً من المراحل المتقدمة للفشل القلبي. كما أنه قد يكون مسؤولاً عن تفاقم الدنف، إضافة إلى إمكانية استخدامه كواصمة لتحديد درجة وخامة المرض.

ABSTRACT We investigated the plasma levels of tumour necrosis factor-alpha (TNF-α), leptin and insulin, and their relation to body mass index (BMI) in 80 male patients who presented with chronic heart failure (mean age: 47 ± 4 years) at Tanta University Hospital. Plasma leptin, TNF-α and insulin were significantly increased and BMI significantly decreased in New York Heart Association classes III and IV patients. TNF-α, leptin and insulin were positively correlated, and TNF-α and BMI and leptin and BMI were negatively correlated in stages III and IV of heart failure. We conclude that cytokine neuroendocrine activation may form part of advanced stage heart failure. It may also be responsible for worsening cachexia, and can be used as a marker to determine disease severity.

Nouveaux marqueurs biochimiques dans l'insuffisance cardiaque chronique

RESUME Nous avons examiné les niveaux plasmatiques du facteur nécrosant des tumeurs alpha (TNF-α), de la leptine et de l'insuline, et leur relation avec l'indice de masse corporelle chez 80 patients de sexe masculin qui sont venus consulter pour une insuffisance cardiaque chronique (âge moyen : 47 ± 4 ans) à l'hôpital universitaire de Tanta. La leptine, le TNF-α et l'insuline plasmatiques étaient en augmentation significative et l'indice de masse corporelle diminuait considérablement chez les patients des classes III et IV définies par la « New York Heart Association ». Il y avait une corrélation positive entre le TNF-α, la leptine et l'insuline et une corrélation négative entre le TNF-α et l'indice de masse corporelle et entre la leptine et l'indice de masse corporelle aux stades III et IV de l'insuffisance cardiaque. Nous concluons que l'activation neuroendocrinienne par les cytokines peut faire partie de l'insuffisance cardiaque au stade avancé. Elle peut être également responsable de l'aggravation de la cachexie, et peut être utilisée comme marqueur pour déterminer la sévérité de la maladie.

[1]*Department of Physiology;* [2]*Department of Cardiology, Tanta Faculty of Medicine, University of Tanta, Tanta, Egypt.*

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Introduction

A highly conserved protein, leptin, has been discovered to play a role in regulating energy balance in humans and rodents. A strong association between plasma concentration of tumour necrosis factor-α (TNF-α) and the presence of cachexia has been noted. Chronic heart failure constitutes a complex syndrome associated with disturbance in several metabolic and endocrine functions. There is accumulated evidence that the weight loss and cachexia seen in advanced stages of chronic heart failure constitute a sign of poor prognosis in these patients. Increased plasma leptin is possibly implicated in the wasting associated with the late stage of heart failure.

Contradictory views regarding the level of leptin in patients with congestive heart failure (CHF) are described in the literature. Leyva et al. demonstrated hyperleptinaemia in chronic heart failure patients, with leptin levels elevated by 81.3% compared to a normal control group [*1*]. Bottner et al. reported that patients with severe heart failure had a higher serum leptin level compared to those with less advanced heart failure [*2*]. On the other hand, Anker et al. observed that serum leptin levels were decreased by less than half in patients with a severe grade of CHF [*3*]. Similarly, Murdoch et al. reported that leptin tends to decrease in cachectic patient with more advanced stage disease [*4*]. However, Toth et al. reported normal leptin levels in heart failure patients [*5*].

Chronic heart failure is also characterized by a hyperinsulinaemic state [*6*], in which there is a striking loss of both muscle and adipose tissue leading to overt cardiac cachexia in these patients [*7*].

Recent attention has focused on cytokine activity in heart failure. A strong correlation between plasma concentration of TNF-α and the presence of cachexia has been noted [*8*]. It is important to investigate whether leptin is implicated in the pathogenic process of the disease or more likely affected as a part of the disease. This study was designed to assess the changes of plasma leptin and plasma TNF-α in patients with CHF, and to evaluate their relationship with the severity of disease. The study also sought to evaluate the relationship between plasma leptin, TNF-α and plasma insulin levels.

Methods

From the Cardiac Unit of Tanta University Hospital, 80 male patients presenting with CHF (mean age: 47 ± 4 years) were studied. The severity of CHF was classified according to the New York Heart Association classification [*9*] (Table 1). Their clinical and haemodynamic conditions were stable. Patients with significant concomitant diseases such as infection, pulmonary disease, malignancy or collagen vascular disease were not included. A control group comprised of 20 healthy individuals matched for age and sex, without a history of cardiac disease or any family history of coronary artery disease (CAD) was also studied.

All patients underwent full clinical and physical examination. Haemodynamic studies using cardiac catheterization were performed in the 80 patients for the assessment of CAD and measurement of pulmonary artery wedge pressure (PAWP). Echocardiographic studies were performed on all patients and controls for measurement of left ventricular ejection fraction (LVEF%) and cardiac output. Cardiac index was calculated as the ratio of cardiac output to the body surface area.

Table 1 **New York Heart Association functional classifications for patients with heart disease**

Class	Functional classification
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
IV	Patient with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

Fasting blood samples were collected from controls and patients using disposable plastic syringes containing EDTA and placed in ice until separation of the plasma, which was stored until assay was performed and thawed only once. Plasma leptin was measured using commercially available kits [Medipan Diagnostica, Selchow, Germany]. Plasma insulin was measured by radioimmunoassay, also using commercially available kits [*10*]. Plasma TNF-α was measured using enzyme immunoassay kits [Diaclone, Besançon, France]. In assessing body mass index (BMI), individuals with BMI > 27 kg/m^2 were considered obese [*11*]. Data were tabulated and statistically analysed using the independent sample *t*-test and correlation coefficient *r*-test [*12*].

Results

There was a significant increase in all parameters studied in patients classed as stages III and IV heart disease ($P < 0.001$), and an insignificant change in patients classed as stages I and II when these results were compared to controls ($P > 0.05$) (Table 2). In stage III, there was an increase in plasma concentrations of fasting insulin, plasma leptin and TNF-α of 71.6%, 49.4% and 30.74% respectively, and a decrease in BMI of 20.84%, compared to controls. In stage IV, plasma levels of fasting insulin, leptin and TNF-α were higher than those of the control group by 88.4%, 81.2% and 161.3% respectively, while BMI was 31.3% lower.

These results suggest a positive correlation between plasma levels of TNF-α and leptin, between TNF-α and insulin, and also between plasma leptin and insulin in patients with stage IV heart failure (Figures 1–3). There is a negative correlation between both plasma TNF-α, leptin and BMI in the late two stages of chronic heart failure (Figures 4,5).

Although there was a gradual increase in plasma levels of TNF-α and leptin with increasing NYHA class, there were signifi-

Table 2 **The level of fasting plasma insulin, plasma leptin, and plasma TNF-α, and BMI, LVEF%, PAWP and CI in male control subjects and in patients of four stages of chronic heart failure (according to NYHA)**

Variable	Controls ($n = 20$)	Stage I ($n = 20$)			Stage II ($n = 20$)			Stage III ($n = 20$)			Stage IV ($n = 20$)		
		Mean ± *s*	*t*	% change[a]	Mean ± *s*	*t*	% change[a]	Mean ± *s*	*t*	% change[a]	Mean ± *s*	*t*	% change[a]
Fasting insulin (IU/mL)	10.14 ± 3.80	11.78 ± 3.50	0.91	16.20	15.00 ± 3.60	2.71	47.90	17.40 ± 3.90	6.01[b]	71.60	19.10 ± 4.40	7.05[b]	88.40
Plasma leptin (ng/mL)	12.25 ± 3.90	12.35 ± 4.20	0.05	0.80	14.90 ± 4.00	1.33	21.60	18.30 ± 5.40	4.71[b]	49.40	22.20 ± 6.80	5.32[b]	81.20
Plasma TNF-α (pg/dL)	29.60 ± 7.50	30.50 ± 8.00	0.42	3.04	35.00 ± 70.00	2.32	18.24	38.70 ± 7.60	3.82[b]	30.74	77.35 ± 24.50	16.51[b]	161.30
BMI (kg/m²)	25.90 ± 3.10	25.70 ± 3.60	0.18	0.80	23.60 ± 3.50	1.91	8.90	20.50 ± 2.80	5.21[b]	20.84	17.80 ± 2.20	7.91[b]	31.30
LVEF%	41.80 ± 2.40	37.85 ± 4.44	–	–	31.50 ± 2.18	–	–	27.55 ± 3.72	–	–	20.80 ± 1.96	–	–
PAWP (mm Hg)	–	10.75 ± 1.29	–	–	11.25 ± 1.12	–	–	24.37 ± 1.29	–	–	27.12 ± 2.19	–	–
CI (L/min./m²)	–	3.44 ± 0.57	–	–	3.02 ± 0.58	–	–	2.31 ± 0.42	–	–	1.86 ± 0.19	–	–

[a] *There was a percentage increase in fasting insulin, plasma leptin and plasma TNF-α. There was a percentage decrease in BMI.*
[b] *Significant difference at P < 0.001 compared with the control group.*
TNF-α = tumour necrosis factor-alpha.
BMI = body mass index.
PAWP = pulmonary artery wedge pressure.
NYHA = New York Heart Association.
LVEF% = left ventricular ejection fraction.
CI = cardiac index.
s = standard deviation.

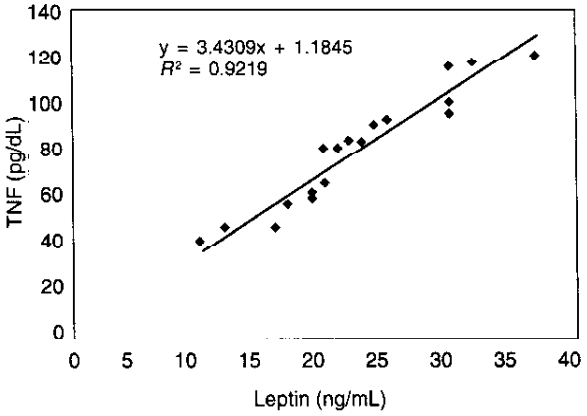

Figure 1 **Correlation between plasma TNF-α and leptin levels in NYHA class IV heart failure**

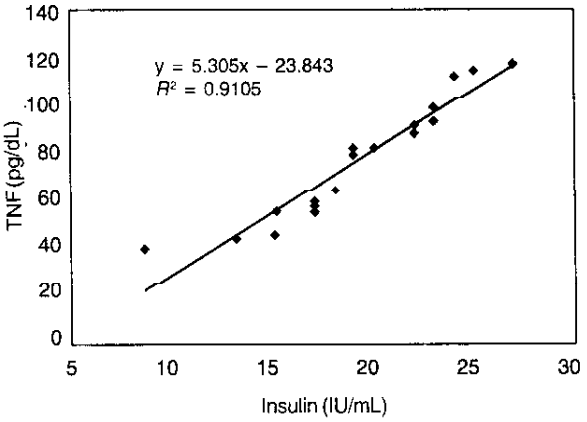

Figure 2 **Correlation between plasma TNF-α and insulin levels in NYHA class IV heart failure**

cant elevations of both in NYHA classes III and IV, and a significant inverse correlation between both leptin and TNF-α, with those of LVEF% (Figures 6,7).

Discussion

It is clear from our results that in late stages of CHF, there is a significant elevation of TNF-α (Table 2). These results are in ac-

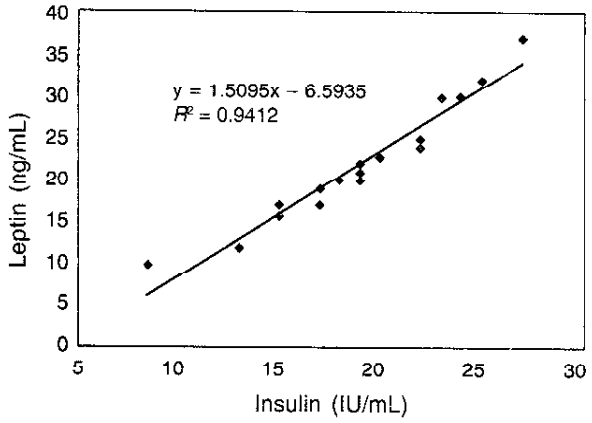

Figure 3 **Correlation between plasma leptin and insulin levels in NYHA class IV heart failure**

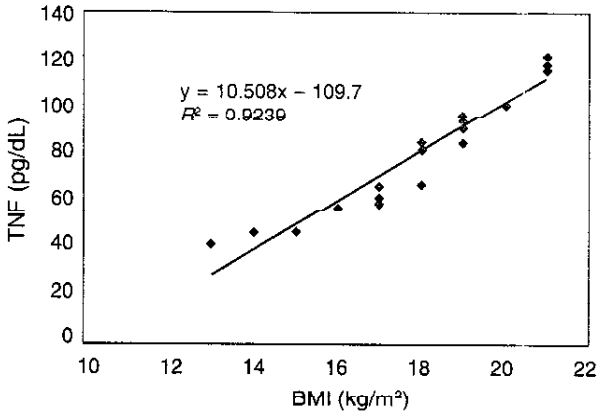

Figure 4 **Correlation between plasma level of TNF-α and BMI in NYHA class IV heart failure**

cordance with those previously reported by Torre-Amione et al., who observed that the level of circulating TNF-α is elevated in patients with advanced CHF [*13*]. Mann and Young reported that TNF-α may contribute to the progression of the cardiac decompensation that occurs in advanced cases of CHF [*14*]. Different but still unknown mechanisms may be responsible for the increased release of these proinflammatory

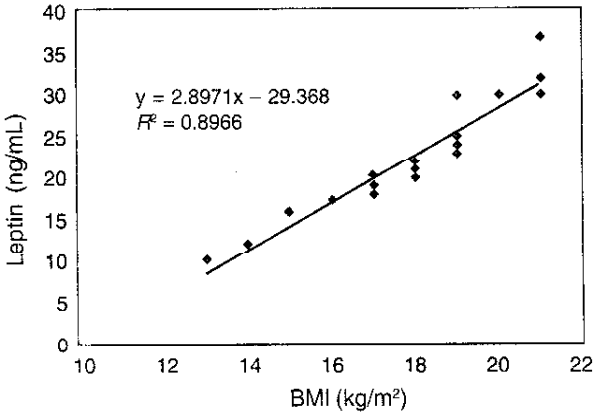

Figure 5 **Correlation between plasma level of leptin and BMI in NYHA class IV heart failure**

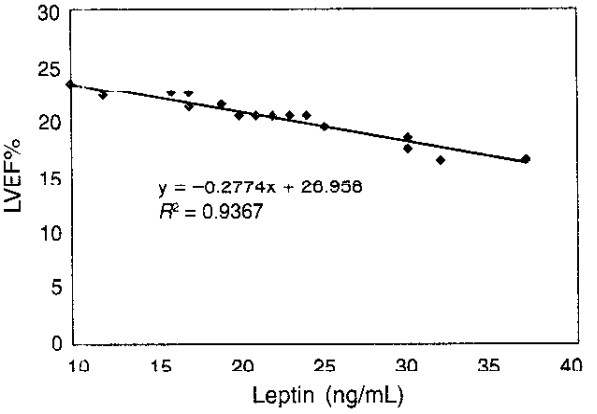

Figure 6 **Correlation between plasma level of leptin and LVEF% in NYHA class IV heart failure**

cytokines in late stages of heart failure. The heart itself may be a source of TNF-α [*15*].

Kapadia et al. [*16*] demonstrated that the heart is capable of synthesizing biologically active TNF-α, as evidenced by increased TNF-α mRNA and protein in conditions of failure of the human heart. TNF-α produces a negative inotropic effect on the cardiac tissues as well as on

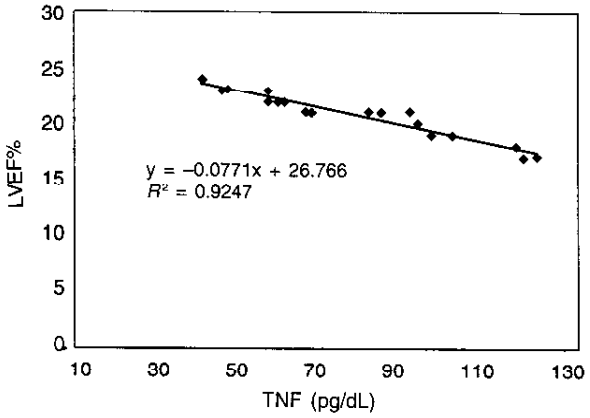

Figure 7 **Correlation between plasma level of TNF-α and LVEF% in NYHA class IV heart failure**

cardiac muscle cells [*17*]. This raises the interesting possibility that over-expression of myocardial TNF-α may be one of the important maladaptive mechanism in cases of advanced heart failure. Anker et al. [*8*] focused on the strong association between the plasma concentration of TNF-α and the presence of cachexia in late stage heart failure.

The results of our study reveal a negative correlation between TNF-α and BMI in patients with stage III and stage IV (Figure 4), and a positive correlation between TNF-α and plasma leptin concentration (Figure 1). Few reports on the mechanism by which TNF-α affects BMI and plasma leptin are available. TNF-α may act directly on the adipocyte to increase the production of the lipostatic factor leptin [*18*]. Zumbach et al. showed that the level of serum leptin is under the control of TNF-α [*19*]. These results are similar to those previously reported by Leyva et al. [*1*], who observed an increase of plasma leptin in late stages of heart failure. Leptin is an endogenous appetite suppressant and may increase energy production by non-shivering thermogenesis [*5*]. This may explain the decrease of BMI and cahexia that occurs in late stages of CHF [*7*].

It is evident from our results that there is a significant elevation of the insulin level in late stages of CHF (Table 2). The observed increase in insulin level could be secondary to increased TNF-α in these patients in late stages of the disease. TNF-α promotes significant insulin resistance [*20*]. Hostens et al. also observed that exposure of the α-cells of the pancreas to cytokines released from infiltrating immunocytes (TNF-α) causes elevation of the proinsulin level [*21*]. Figure 2 demonstrates a positive correlation observed between TNF-α and insulin levels in stages III and IV of heart failure.

Another mechanism for the observed hyperinsulinaemia in late stages of CHF is the increase in plasma leptin. Figure 3 shows a positive correlation between plasma leptin and insulin level in late-stage disease. These results are similar to those previously reported by Haffner et al. [*22*]

and Saad et al. [*23*], who found a positive association between plasma leptin and insulin concentration, independent of adiposity. The increased plasma leptin and associated increase in insulin could be another factor for cachexia and decreased BMI observed in this work. Leyva et al. [*1*] found a positive correlation between plasma leptin and insulin suggesting that the insulin leptin axis may be related to increased energy expenditure in patients with advanced-stage CHF.

We can conclude that the increase in plasma TNF-α, leptin and insulin levels, and the positive correlation between them in late stages of heart failure may constitute a part of the many vicious circles of advanced stage CHF, and may provide new information about the interplay between cells of the immune system, adipocyte and endocrine system. It is possible that cytokine neuroendocrine activation is responsible for worsening heart failure, and may be a marker for detecting the severity of disease.

References

1. Leyva F et al. Hyperleptinaemia in chronic heart failure. Relationships with insulin. *European heart journal*, 1998, 19:1547–51.
2. Bottner A et al. Hyperleptinaemia does not correlate with plasma catecholamine levels in chronic heart failure. *European heart journal*. 1999, 20:1051–2.
3. Anker SD et al. Wasting as an independent risk factor for mortality in chronic heart failure. *Lancet*, 1997, 349:1050–3.
4. Murdoch DR et al. Inappropriately low plasma leptin concentration in the cachexia associated with chronic heart failure. *Heart (British Cardiac Society)*, 1999. 82:352–6.
5. Toth, MJ et al. Plasma leptin concentrations and energy expenditure in heart failure patients. *Metabolism: clinical and experimental*, 1997, 46:450–3.
6. Swan JW et al. Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure. *Journal of the American College of Cardiology*, 1997, 30:527–32.
7. Cleland JG, Clark AL. Leptin and cardiac cachexia: marker or mediator? *European heart journal*, 1998, 19:1421–2.
8. Anker SD et al. Tumor necrosis factor and steroid metabolism in chronic heart failure: possible relation to muscle wasting. *Journal of the American College of Cardiology*, 1997, 30:997–1001.
9. Goldman L et al. Comparative reproducibility and validity of systems for assessing cardiovascular functional class: advantages of a new specific activity scale. *Circulation*, 1981, 64:1227–34.
10. Kahn SE et al. Quantification of the relationship between insulin sensitivity and â-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes*, 1993, 42:1663–72.
11. Ronnemaa T et al. Relation between plasma leptin levels and measures of body fat in identical twins discordant for obesity. *Annals of internal medicine*, 1997, 126:26–31.
12. Armitage P, Berry G. *Statistical methods in medical research*. 2nd ed. Oxford. Blackwell, Scientific Publications, 1987:93–140.
13. Torre-Amione G et al. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. *Circulation*, 1996, 93:704–11.

14. Mann DL, Young JB. Basic mechanisms in congestive heart failure. Recognizing the role of proinflammatory cytokines. *Chest*, 1994, 105:897–904.

15. Lommi J et al. Haemodynamic, neuroendocrine and metabolic correlates of circulating cytokine concentrations in congestive heart failure. *European heart journal*, 1997, 18:1620–6.

16. Kapadia S et al. Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. *Journal of clinical investigation*, 1995, 96:1042–52.

17. Yokoyama T et al. Cellular basis for the negative inotropic effects of tumour necrosis factor-alpha in the adult mammalian heart. *Journal of clinical investigation*, 1993, 92:2303–12.

18. Finck BN, Johnson RW. Tumor necrosis factor (TNF)-alpha induces leptin production through the P55 TNF receptor. *American journal of physiology. Regulatory, integrative and comparative physiology*, 2000, 278:R537–43.

19. Zumbach MS et al. Tumor necrosis factor increases serum leptin levels in humans. *Journal of clinical endocrinology and metabolism*, 1997, 82:4080–2.

20. Hrnciar J et al. Vzt'ah cytokinov (TNF-alfa, IL-1 a 6) a homocysteinu k androidnej obezite a k fenomenom syndromu inzulinovej rezistencie. [Relation between cytokines (TNF-alpha, IL-1 and 6) and homocytsteine in android obesity and the phenomenon of insulin resistance syndromes.] *Vnitrni lekarstvi*, 1999, 45:11–6.

21. Hostens K et al. Exposure of human islets to cytokines can result in disproportionately elevated proinsulin release. *Journal of clinical investigation*, 1999, 104:67–72.

22. Haffner SJ et al. Leptin concentrations and insulin sensitivity in normoglycemic men. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity*, 1997, 21:393–9.

23. Saad MF et al. Sexual dimorphism in plasma leptin concentration. *Journal of clinical endocrinology and metabolism*, 1997, 82:579–84.