Leptin, Insulin Sensitivity and TNF-α as Parameters for Metabolic Changes in Chronic Heart Failure with and without Cardiac Cachexia

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Abstract: The development of cachexia is a particular predictor of adverse prognosis in chronic heart failure (CHF). Less is known about anabolic metabolism in CHF. Leptin – the hormone product of obesity gene- has been shown to inhibit food intake, increase energy expenditure and fat oxidation. Insulin sensitivity and secretion is related to leptin. Leptin has been reported also to stimulate proliferation of CD4 T cells and increases cytokine production. The study aimed to investigate leptin, insulin sensitivity and tumor necrosis factor-α (TNF-α) in chronic heart failure with and without cachexia. We studied 51 male patients with CHF, mean New York Heart Association Functional Class (NYHA, 2.52±0.81) and 23 male healthy control subjects, of matched age. Of the CHF Patients, 24 were cachectic (cCHF) with non-edematous weight loss >7.5% over at least 6 months and 27 non cachectic. Serum insulin, leptin and TNF-α were measured using commercially available ELISA kit. Insulin sensitivity was assessed by intravenous glucose tolerance test. Compared with the healthy control subjects, patients had elevated levels of leptin, fasting insulin and TNF-α (P<0.001), but reduced insulin sensitivity (p<0.001).The cCHF subgroup compared with ncCHF subgroup showed reduced leptin and fasting insulin levels (P<0.001 & P<0.01 respectively) and elevated TNF-α levels (P<0.001). In both patients and control subjects there was a positive correlation between leptin and fasting insulin levels (r=0.59, P<0.001 & r=0.54, P<0.05 respectively). The relative risk of incidence of cCHF in NYHA functional class (I&II) versus NYHA functional class (III &IV) was 0.427 (P<0.05). In conclusion CHF is hyperleptinaemic state and is associated with decreased insulin sensitivity and elevated serum insulin levels. The state of cardiac cachexia is associated with higher TNF-α levels and more worse NYHA functional class. Leptin and TNF-α may be a valid targets for novel therapeutic interventions in patients with CHF.
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Key Words: Leptin, Insulin Sensitivity, Chronic Heart Failure (CHF), Cachexia

Introduction:

Chronic heart failure (CHF) is a heterogeneous syndrome with an overall adverse prognosis. Two particular predictors of adverse prognosis are neurohormonal abnormalities and the development of cachexia ***(Araujo et al., 2009)***. The syndrome of cardiac cachexia has been recognized for many centuries, but little is known about the mechanism of transition from heart failure to cardiac cachexia ***(Akashi et al., 2005)***. Several endocrine systems may be involved in this process, which is characterized by an imbalance between catabolic and anabolic mechanisms ***(Schulze et al., 2003)*.**

An increased resting metabolic rate which is regulated primarily by thyroid hormones and catecholamines has been reported in CHF patients ***(Berry and Clark 2000)*.** Cortisol is also increased in untreated severe congestive heart failure patients ***(Wieselthaler et al., 2007)*.**

Less is known about anabolic metabolism in chronic heart failure. Regulation of growth hormone (GH) receptor expression and hence tissue GH sensitivity may be important for conflicting results found in treatment studies with recombinant growth hormone in CHF ***(Doehner et al., 2001)*.** Leptin, the hormone product of the obesity (ob) gene, is synthesized exclusively in adipose tissue ***(Sierra-Johnson et al., 2008)*** and its expression and release is stimulated by insulin ***(Walkeret al., 2005)***. Leptin strongly predicts growth hormone binding protein (GHBP) which corresponds to the extracellular domain of GH receptor. Leptin could be the signaling link between adipose tissue and GHBP/GH receptor expression in CHF ***(Doehner et al., 2001)***. Leptin also has been shown to inhibit food intake possibly by decreasing the expression of neuropeptide Y **(Ghamari-Langroudi et al., 2001)** and to increase energy expenditure and fat oxidation ***(Araujo et al., 2009)***. Furthermore, insulin sensitivity and secretion have also been reported to influence plasma GHBP activity and is related to leptin ***(Fernandez-Real et al., 2000)***.

Cytokine activation is a potential mechanism for the development of cachexia in CHF ***(Schulze et al., 2003)***. There is a significant relation between interleukin-6 and tumor necrosis factor alpha and plasma levels of nor-epinephrine, supporting the concept of a cytokine cascade, which may be related to neurohormonal activation ***(Konstantino et al., 2007)***.

Aim of the Work: This search aimed to study serum leptin and TNF-α changes, as well as insulin sensitivity in patients with CHF with and without cardiac cachexia.

Subjects and Methods:

The present study comprised 51 male patients with CHF, mean age (59.87 ± 6.91 years), 23 of them secondary to ischemic heart disease and 28 subjects with idiopathic dilated cardiomyopathy, as well as 23 healthy male subjects with mean age (55.92 ± 6.33 years) as a control group. We studied only male subjects to prevent results from being biased by gender-based differences in the regulation of leptin in human ***(Doehner et al., 2001; Guerra et al., 2008)***. They were selected from attendants of Outpatient Clinics of Specialized Medical Hospital, Mansoura University.

All participants provided written informed consent after receiving oral and written information concerning the study. All patients and controls were subjected to: through history taking, clinical examination (includes: pulse, blood pressure, temperature, cardiac, chest and abdominal examination), complete blood picture, liver functions test, serum creatinine, creatinine clearance as well as abdominal ultrasound. Subjects with serum creatinine >1.2 mg/dl, abnormal creatinine clearance, abdominal ultrasound or laboratory evidence of chronic hepatic disease, chronic lung disease, myocardial infarction in the preceding 3 month, signs of acute infection or other primary cachectic states such as cancer were excluded. At the time of the study patients were free of peripheral edema and did not have significantly raised jugular venous pressure or hepatomegaly. Cardiac cachexia was defined clinically as documented nonintensional, nonedematous weight loss of > or = to 5 kg (all >7.5% of their previous normal weight) over a period of at least 6 months. A second criterion of body mass index (BMI) (weight/height²) of <24 kg/m² was fulfilled for all our cachectic patients.

Control subjects and patients fulfilling the selection criteria were subjected to echocardiographic examination using ESAOTE XP-10 (ESAOTE Biomedica Coroporation) equipped with 2.5-5 MHz transducer. The examination included two dimensional, 2-D derived M-mode, continuous wave, pulsed wave and colored doppler study. Standard left parasternal, apical, right parasternal, subcostal and suprasternal views were obtained in a successive pattern. LV function was assessed by:

- Ejection fraction: the left ventricular ejection fraction was calculated as an indicator of pump function.
- Shortening fraction which is the most widely used M-mode index of left ventricular function. It is the percent change in left ventricular diameter that occurs with systole, it is calculated using the following equation: SF= 100X (LVDD – LVSD)/LVDD.
- E point–septal separation: it is less than 4 mm in normal adults and is inversely related to ejection fraction. It has an advantage over shortening fraction in the presence of abnormal septal motion ***(Snider et al., 1997)***.

Patients with echocardiographic evidence of left ventricular functional impairment and the healthy control subjects were subjected to:

- Fasting serum insulin measurement by enzyme immunoassay using Medgenix-Ins-EASIA kit (BioSource, Belgium) ***(Frier et al., 1981)***.
- Insulin sensitivity and glucose dynamic study by intravenous glucose tolerance test (ivGTT) ***(Chen et al., 1985; Thorburn and Proietto 1999)***.
- Serum leptin assay using ELISA kit (Diagnostic Biochem, Canda) ***(Ahamadi et al., 2008)***.
- Serum TNF-α levels using ELISA kit (Quantikine ELISA kits, R&D Systems, Minneapolis, USA) ***(Testa et al., 1996)***.

Statistical Methods:

Statistical analysis was done by using SPSS program "statistical package for social science" version 10, 1999. The data were parametric by using Kolinogrov-Smirnov test. The parametric data was presented in the form of mean and standard deviation. Student t test was used for comparison of quantitative data of two groups. Non parametric Kendall's correlation was used to test for linear relationship between different quantitative variables. Regression analysis was used to test for leptin and TNF-α on other variables. Chi-square and relative risk was used to test association between cardiac cachexia and NYHA functional class. Significance was considered when P value less than 0.05.

Results:

Patients showed statistically significant lower body mass index (BMI) (24.17 ± 3.59 vs 27.65 ± 1.59 kg/m², P<0.01), lower EF (0.331 ± 0.085 vs 0.699 ± 0.033, P<0.001), lower serum Na (135 ± 2.92 vs 141.1 ± 1.19 mEq/L, P<0.001), lower insulin sensitivity (5.35 ± 1.06 vs 7.81 ± 1.8 $min^{-1}.uU.ml^{-1}.10^4$, P<0.001), higher fasting insulin levels (14.33 ± 4.4 vs 7.41 ± 1.63 uU/ml, P<0.001), higher leptin levels (10.15 ± 3.72 vs 4.66 ± 1.3 ng/ml, P<0.001), higher TNF-α levels (51.93 ± 14.57 vs 25.34 ± 7.4 pg/ml, P<0.001) compared with control subjects (tables 1 & 2).

There was no statistically significant difference between the (27) ncCHF patients and the (23) control subjects as regard, body weight and BMI, however ncCHF group showed statistically significant lower EF (0.382 ± 0.07 vs 0.699 ± 0.033, P<0.001). While the (24) cCHF patients showed statistically significant lower body weight (58.64 ± 3.69 vs 79.6 ± 3.49 kg, P<0.001), lower BMI (20.51 ± 0.62 vs 27.65 ± 1.59 kg/m², P<0.001) and lower EF (0.27 ± 0.056 vs 0.699 ± 0.033, P<0.001) compared with control subjects. However, comparison of cCHF group with ncCHF group revealed that cCHF patients had statistically significant lower body weight (58.64 ± 3.69 vs 77.5 ± 4.96 kg, P<0.001), lower BMI (20.51 ± 0.62 vs 27.18 ± 1.6 kg/m², P<0.001) and lower EF (0.27 ± 0.056 vs 0.382 ± 0.07, P<0.001) (table 3).

There was no statistically significant difference between the (27) ncCHF patients and the (23) control subjects as regard, fasting plasma glucose levels. However, ncCHF group showed statistically significant higher fasting insulin (16.45 ± 2.92 vs 7.41 ± 1.63 uU/ml, P<0.01), higher leptin levels (12.47 ± 2.95 vs 4.66 ± 1.3 ng/ml, P<0.001), higher TNF-α levels (42.45 ± 6.78 vs 25.34 ± 7.4 pg/ml, P<0.001) and lower serum Na (137.93 ± 1.09 vs 141.1 ± 1.19 mEq/L, P<0.001) compared with control subjects (table 4).

The (24) cCHF patients showed statistically significant lower insulin sensitivity (5.27 ± 1.06 vs 7.81 ± 1.8 $min^{-1}.uU.ml^{-1}.10^4$, P<0.001), higher fasting plasma glucose levels (93.5 ± 3.16 vs 87.7 ± 4.63 mg/dl, P<0.01), higher fasting insulin (11.76 ± 4.6 vs 7.41 ± 1.63 uU/ml, P<0.01), higher leptin levels (7.34 ± 2.39 vs 4.66 ± 1.3 ng/ml, P<0.001) and higher TNF-α levels (63.44 ± 13.16 vs 25.34 ± 7.4 pg/ml, P<0.001) compared with the control subjects (table 4).Comparison of cCHF group with ncCHF group revealed that cCHF patients had statistically significant lower serum Na levels (133.5 ± 2.56 vs 137.93 ± 1.09 mEq/L, P<0.001), lower fasting insulin levels (11.76 ± 4.6 vs 16.45 ± 2.92 uU/ml, P=0.005), lower leptin levels (7.34 ± 2.39 vs 12.47 ± 2.95 ng/ml, P=0.001), and lower insulin sensitivity (5.27 ± 10.6 vs 5.42 ± 1.09 $min^{-1}.uUml^{-1}.10^4$). But statistically significant higher fasting plasma glucose levels (93.5 ± 3.16 vs 86.84 ± 4.6 mg/dl, P<0.001) and higher TNF levels (63.44 ± 13.16 vs 42.45 ± 6.78 pg/ml, P<0.001) (table 4) (figure1).

Correlation analysis of leptin in control group showed positive correlation of leptin with BMI (r=0.52, P<0.05), positive correlation with fasting insulin (r=0.54, P<0.05), near negative correlation with insulin sensitivity (r=-0.39, P=0.065). There was also positive correlation of leptin with body weight, BMI, and fasting insulin (r=0.4, P<0.01 & r=0.4, P<0.01 & r=0.59, p<0.001 respectively) and negative correlation with insulin sensitivity (r= -0.32, P<0.05) in the (51) patients as a whole group. TNF-α correlated negatively with body weight, BMI, EF, FS (r=-0.36, P<0.01 & r=-0.39, P<0.01 & r=-0.42, P<0.01 & r=-0.40, P<0.01 respectively) and correlated positively with fasting glucose (r=0.42, P<0.001) in the (51) patients (table 5) (figure2).

The correlation of leptin with BMI, fasting insulin and insulin sensitivity were preserved in the subgroups of ncCHF and cCHF patients (table 6).

Regression analysis of leptin showed significant positive correlation with fasting insulin and BMI (P<0.001 & P<0.01 respectively) (table 7). While regression analysis of TNF-α showed positive correlation of TNF-α with fasting plasma glucose (P<0.05) (table 8).The relative risk of incidence of cCHF with NYHA functional class (I &II) versus NYHA functional class (III & IV) was 0.427, P<0.05 (table 9).

Table (1): Comparison of some clinical and echocardiographic data of (23) healthy control subjects and total of (51) patients with chronic heart failure.

Variable	*Control N=23*	*Patients N=51*	*p*
Age (years)	55.92 ± 6.33	59.87 ± 6.91	NS
Wt (kg)	79.6 ± 3.49	68.98 ± 10.49	<0.01
Body mass index (kg/m²)	27.65 ± 1.59	24.17 ± 3.59	<0.01
LVES (cm)	3.34 ± 0.23	6.05 ± 0.7	<0.001
LVED (cm)	4.99 ± 0.28	6.94 ± 0.67	<0.001
EF	0.699 ± 0.033	0.331 ± 0.085	<0.001
FS	33.1 ± 2.69	12.8 ± 3.99	<0.001

Table (2): Comparison of some metabolic characteristics of (23) healthy control subjects and total of (51) patients with chronic heart failure.

Variable	*Control N=23*	*Patients N=51*	*p*
Serum creatinine (mg/dl)	0.95± 0.12	1.02 ± 0.15	NS
Serum Na (mEq/L)	141.1 ± 1.19	135 ± 2.92	<0.001
Fasting glucose (mg/dl)	87.78 ± 4.63	89.74 ± 5.26	NS
Fasting insulin (uU /ml)	7.41 ± 1.63	14.33 ± 4.4	<0.001
Insulin sensitivity ($min^{-1}.Uu.ml^{-1}.10^4$)	7.81 ± 1.8	5.35 ± 1.06	<0.001
Leptin (ng/ml)	4.66 ± 1.3	10.15 ± 3.72	<0.001
TNF-α (pg/ml)	25.34 ± 7.4	51.93 ± 14.57	<0.001

Table (3): Comparison of some clinical and echocardiographic data of healthy control subjects and chronic heart failure patients.

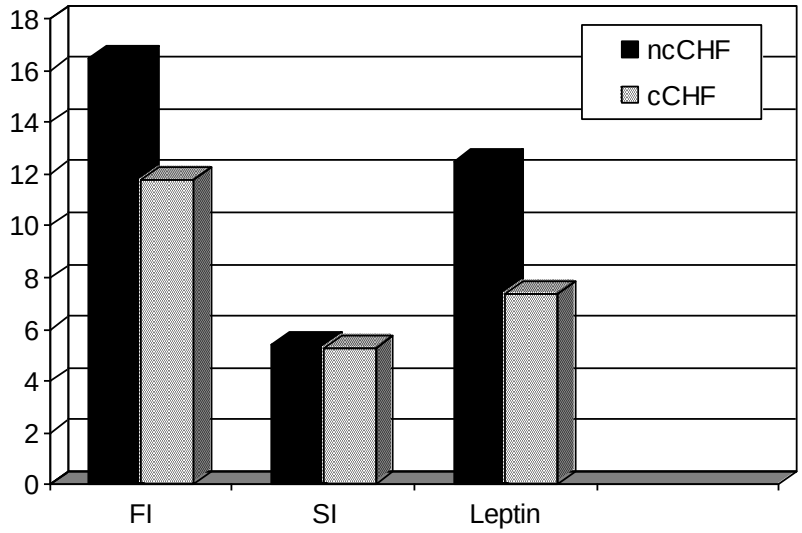
	Wt (kg)	***BMI*** (Kg.m²)	***LVES*** (cm)	***LVED*** (cm)	***EF***	***FS***
Control: Mean±SD	79.60±3.49	27.65±1.59	3.34±0.23	4.99±0.28	0.699±0.038	33.1±2.69
ncCHF: Mean±SD	77.5±4.96	27.18±1.60	5.93±0.68	6.99±0.67	0.382±0.07	15±3.47
cCHF : Mean±SD	58.64±3.69	20.51±0.62	6.2±0.71	6.89±0.68	0.27±0.056	9.9±2.32
P1	NS	NS	<0.001	<0.001	<0.001	<0.001
P2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P3	<0.001	<0.001	NS	NS	<0.001	<0.001

P1 value: control vs ncCHF P2 value: control vs cCHF P3 value: cCHF vs ncCHF

Table (4): Comparison of the metabolic characteristics of healthy control subjects and chronic heart failure patients.

	S.Na (mEq/L)	***Fasting Glucose*** (mg/dl)	***Fasting Insulin*** (uU/ml)	***Insulin Sensitivity*** $(min^{-1}.uU.ml^{-1}.10^4)$	***Leptin*** (ng/ml)	***TNF-α*** (pg/ml)
Control: Mean±SD	141.1±1.19	87.78±4.63	7.41±1.63	7.81±1.8	4.66±1.3	25.34±7.4
ncCHF: Mean±SD	137.93±1.09	86.64±4.6	16.45±2.92	5.42±1.09	12.47±2.95	42.45±6.78
cCHF : Mean±SD	133.5±2.56	93.5±3.16	11.76±4.6	5.27±1.06	7.34±2.39	63.44±13.16
P1	<0.001	NS	<0.001	<0.001	<0.001	<0.001
P2	<0.001	<0.01	<0.01	<0.001	<0.001	<0.001
P3	<0.001	<0.001	<0.01	NS	<0.001	<0.001

P1 value: control vs ncCHF P2 value: control vs cCHF P3 value: cCHF vs ncCHF



Figure(1): Comparison of Fasting insulin ,insulin Sensitivity and Leptin of ncCHF and cCHF

Table (5): Correlation of leptin and TNF-α with some clinical, echocardiographic and metabolic parameters in (23) control subjects and (51) CHF patients.

	Leptin				*TNF-α*			
	Control(23)		*Patients(51)*		*Control(23)*		*Patients(51)*	
	R	**P**	**R**	**P**	**R**	**P**	**R**	**P**
Wt	0.38	NS	0.402	<0.01	-0.02	NS	-0.36	<0.01
BMI	0.52	<0.05	0.406	<0.01	-0.20	NS	-0.39	<0.01
NYHA	-	-	-0.38	<0.01	-	-	0.21	NS
S. Creatinine	0.16	NS	0.14	NS	0.13	NS	0.11	NS
LVES	-0.04	NS	-0.30	<0.05	-0.01	NS	0.12	NS
LVED	-0.12	NS	-0.17	NS	-0.15	NS	0.002	NS
EF	0.17	NS	0.43	<0.01	0.01	NS	-0.42	<0.01
FS	0.09	NS	0.46	<0.001	0.09	NS	-0.40	<0.01
S.Sodium	-0.14	NS	0.28	<0.05	-0.05	NS	-0.36	<0.01
F. Glucose	-0.23	NS	-0.4	<0.05	0.28	NS	0.42	<0.001
F. Insulin	0.54	<0.05	0.59	<0.001	0.3	NS	0.13	NS
I. Sensitivity	-0.39	0.06	-0.32	<0.05	-0.42	<0.05	0.14	NS
Leptin	-	-	-	-	-0.04	NS	0.19	NS
TNF-α	-0.4	NS	0.15	NS	-	-	-	-

Table (6): Correlation of leptin and TNF-α with BMI, fasting insulin (FI) and insulin sensitivity in (27) ncCHF patient and (24)cCHF patients.

	Leptin				**TNF-α**			
	ncCHF(n=27)		**cCHF(n=24)**		**ncCHF(n=27)**		**cCHF (n=24)**	
	R	**P**	**R**	**P**	**R**	**P**	**R**	**P**
BMI	0.39	<0.05	0.701	<0.001	-0.21	NS	-0.43	<0.05
Fasting insulin	0.37	<0.05	0.77	<0.0001	-0.20	NS	0.21	NS
Insulin sensitivity	-0.46	<0.05	-0.503	<0.05	0.08	NS	0.25	NS

Table (7): Regression analysis of leptin in relation to other clinical, echocardiographic and metabolic variables in (51) chronic congestive heart failure patients.

Variables	*Partial R*	*SE of partial R*	*t*	*P*
Fasting insulin	0.48	0.08	5.83	<0.001
BMI	0.38	0.09	3.93	<0.01

Table (8): Regression analysis of TNF-α in relation to other clinical, echocardiographic and metabolic variables in (51) chronic congestive heart failure patients.

Variables	**Partial R**	**SE of partial R**	**t**	**P**
Fasting glucose	0.89	0.37	2.35	<0.05

Table (9): Relative risk (RR) of incidence of cCHF in NYHA functional class I&II versus NYHA functional class III&IV

	NYHA(I&II)		**NYHA(III&IV)**		**Sig**
	No	**%**	**No**	**%**	
cCHF	7	26.9	17	68.0	
ncCHF	19	73.1	8	32.0	
X²					4.014
P					0.045
RR of cCHF NYHA (I&II): NYHA(III&IV)					0.427

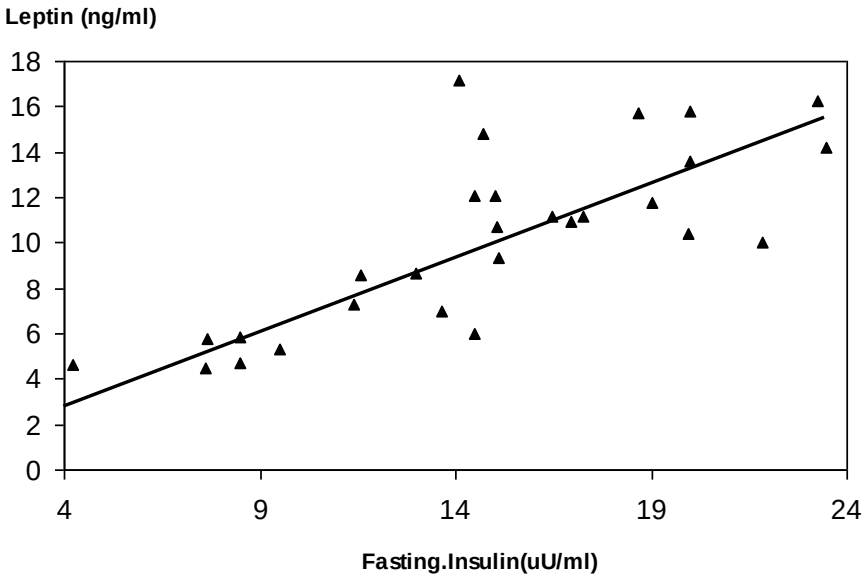


Figure (2): Correlation between fasting insulin and leptin in 51 patients with CHF

Discussion:

Chronic heart failure is a complex syndrome affecting many body systems. Some authors suggested that cardiac cachexia is a multifactorial neuroendocrine and metabolic disorder with a poor prognosis ***(Araujo et al., 2009)***. Simple starvation and anorexia can not be considered the main cause of cardiac cachexia as they lead to loss of fat tissue and reduced plasma albumin levels. Yet cachectic CHF patients suffer from fat, muscle and bone tissue loss. Serum albumin and liver enzymes levels were not decreased in previous studies ***(Akashi et al., 2005)*** and in our study. This would argue against a major contribution of starvation, gastrointestinal malabsorption or liver synthetic dysfunction in these patients. Also, it seems unlikely that physical inactivity is of great importance in the genesis of cardiac cachexia as atrophy in states of reduced activity is histologically significantly different from muscle atrophy in CHF ***(Miller et al., 2009).***

Leptin is postulated to represent the afferent hormonal signal to the brain including the hypothalamus in a feedback mechanism regulating fat mass. Leptin binds to leptin-receptors in hypothalamus, that plays a central role in the regulation of feeding behavior and energy balance in animal models, the result of this interaction is a decrease in food intake (***Ren 2004)***. There is now evidence that the effects of leptin on food intake are mediated by two limbs of weight control system: the appetite-stimulating peptide, neuropeptide Y (NPY) and the satiety melanocyte-stimulating hormone ***(Schulze et al., 2003)***. Furthermore, leptin may mediate energy expenditure by both increasing physical activity and activity independent thermogenesis, which in part appears to involve activation of brown adipose tissue. The recent finding that leptin receptors are also expressed in tissue other than the brain suggested that leptin might also be involved in the regulation of metabolism at a peripheral level ***(Doehner et al., 2001)***.

The increased levels of circulating leptin may contribute to anorexia and weight loss in some pathologic conditions. Importantly, it was reported that administration of endotoxin including TNF-α produced a prompt and dose dependent increase in serum leptin levels in both experimental animals and in humans ***(Ren and Relling 2005).*** However, it has been reported that T-cells have the signal-transducing leptin receptor and that leptin stimulates the proliferation of CD4+T cells and increase cytokine production ***(Fernández-Riejos et al., 2010)***. Leptin also influences FSH, LH, ACTH, cortisol and GH secretion ***(Wieselthaler et al., 2007)***.

This study revealed that plasma leptin was correlated with BMI and fasting serum insulin in chronic congestive heart failure patients. This finding is similar to that reported previously with healthy subjects ***(Doucet et al., 2000)***. Plasma leptin levels relate with body fat content, it is elevated in obesity and decreased in anorexia nervosa ***(Wabitsch et al., 2001)***.

The study also revealed that CHF patients had significantly higher leptin level although they had significantly lower body weight than controls. So this study proved that congestive heart failure is a hyperleptinaemic state. Significantly lower body weight and BMI in cCHF patients than ncCHF group can account for lower leptin levels in cachectic patients; however it is still significantly higher than that of the control. It is proved previously that leptin fluctuates considerably with body weight changes and

more particularly with adiposity ***(Filippatos et al., 2000; Araujo et al., 2009)***.

The study revealed that our CHF patients exhibited significantly higher fasting insulin levels than control subjects. The study supported the previous reports of increased fasting serum insulin levels and decreased insulin sensitivity in patients with CHF ***(Doehner et al., 2001)***. Insulin is considered the most powerful physiological anabolic hormone. The ability of insulin to stimulate glucose utilization differs enormously from person to person. One of the major causes of inter-individual variation is the presence of disease states that lead to the development of insulin resistance. Insulin increase is associated with an increase in sympathetic nervous system (SNS) activity ***(Hristova and Aloe 2006)***. Insulin has also been shown to inhibit food intake in animals by modulating the expression of neuropeptide Y ***(Xu et al., 2009)***. These experimental data fit well with population studies that demonstrated that individuals who display greater insulin levels are generally also those who experience the smallest weight gain over time ***(Folsom et al., 1998)***.

Another important finding is that our cardiac cachectic patients showed reduced insulin levels compared with non cachectic patients, but increased levels compared with normal control subjects.

We cannot explain from our study the cause for the up-regulation of leptin in CHF patients. The cause of hyperleptinaemia in congestive heart failure patients may be higher fasting insulin levels. Numerous previous studies confirmed that fasting leptin levels are influenced by insulin before and during weight loss in men and women ***(Doucet et al., 2000; Hintz et al., 2003)***. This fact may explain higher leptin levels in ncCHF group than cCHF group. Sustained hyperinsulinaemia stimulates leptin messenger RNA (mRNA) expression ***(Ren 2004)***. Serum leptin level emerged as the strongest predictor of growth hormone binding protein (GHBP) which corresponds to the extracellular domain of the GH receptor independent of age, BMI, total and regional fat mass, fasting insulin level and insulin sensitivity. So, leptin could be a regulating factor for the expression of tissue GH receptor/GHBP. Thus, lower leptin levels in cCHF may have a role through lower circulating GHBP levels that might be one of the molecular mechanisms of acquired GH resistance in cCHF ***(Doehner et al., 2001)***.

Moreover, leptin that is well known to be highly correlated with percentage of body fat and fall in response to weight loss ***(Weigle et al., 1997)***, correlated negatively with NYHA functional class ($r=-0.38$ & $P<0.01$). This finding suggests anabolic metabolism in NYHA class I and II and catabolic metabolism in advanced heart failure NYHA functional class III and IV, which might be of prognostic relevance. These findings coincide with previous researches ***(Richartz et al., 2001)***. This also explains the significantly higher relative risk of cCHF with NYHA functional class III and IV.

The relationship between insulin sensitivity and plasma leptin concentration in the study was similar to previous reports ***(Doehner et al., 2002)***. Fat accumulation may be suitable explanation for such relationship ***(Cnop et al., 2002)***.

The study also proved increased TNF-α in chronic congestive heart failure patients as a whole than controls. Subgrouping the patients on the basis of cardiac cachexia also reveals significantly increased TNF in cCHF patients. TNF-α in our study didn't correlate with NYHA functional class. This finding is similar to that confirmed by other studies ***(Richartz et al., 2001; Ren and Relling 2005; von Haehling et al., 2007; Greig et al., 2008)***.

TNF-α is one of the key cytokines important to the development of catabolism. These cytokines are produced primarily by monocyte/macrophages ***(*Konstantino *et al., 2007; Hirasawa et al., 2009)***, but also endothelial cells and the myocardium have been found to produce cytokines as TNF-α ***(Sigusch et al., 2000)***. At the myocardial level a chronic repetitive stress is thought to induce TNF-α production ***(Fantuzzi 2005)***. So, the failing human heart can directly produce TNF-α. Some authors postulate that bowel wall edema that occurs in heart failure leads to bacterial or endotoxin translocation with subsequent immune activation ***(Schulze et al., 2003)***. This endotoxin hypothesis may hold true and its relevance for the immune activation in cardiac cachexia remains to be seen.

Implantation of TNF producing tumor cells in skeletal muscles causes muscle wasting, whereas TNF producing cells in the brain causes anorexia ***(Tracey et al., 1990)***. Our finding of significant negative correlation of TNF-α with body weight and BMI supported that this cytokine activation may be a pathway of those closely related to the degree of wasting in cachectic CHF patients.

In our study TNF-α is correlated negatively with EF and FS. TNF-α can induce apoptosis, which might be important for such finding ***(Qiu et al., 2009)***. TNF-α also exerts effects on endothelial cells including rearrangement of the cytoskeleton, increased permeability to albumin and water, enhanced expression of activation antigens, induction

of surface procoagulant activity and IL-1 release ***(Aveleira et al., 2010)***. This action could all impair endothelial function. Previous studies documented that the strong inverse relationship between maximal blood flow and TNF-α level in CHF patients could support the idea of detrimental effects of long term increased TNF-α ***(Kan and Finkel 2003)***. Although the study did not demonstrate a significant correlation between TNF-α and insulin sensitivity, the possible role of TNF-α system in the development of insulin resistance can not be ignored and has been recently reported ***(Dzienis-Straczkowska et al., 2003; Wannamethee et al., 2004)***.

The study didn't reveal significant direct correlation between leptin and TNF-α as previously reported ***(Schulze et al., 2003; Ren and Relling 2005)***. The limited number of patients in our study may explain such conflicting results and further larger studies may be valuable.

Conclusion: CHF is associated with hormonal and immunologic disturbances. The state of cardiac cachexia is a systemic wasting process characterized by catabolic /anabolic imbalance in favor of catabolic metabolism, which may be a valid target for novel therapeutic interventions in CHF.

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