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Non-thyroidal illness syndrome in chronic diseases: role of irisin as modulator of antioxidants

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Abstract. – OBJECTIVE: Non-thyroidal-illness syndrome (NTIS) refers to condition found in chronic diseases that is an adaptive mechanism. However, oxidative stress is related to NTIS in a vicious circle, due to deiodinases alteration and negative effects of low T3 on antioxidant levels or activity. Muscle is one of the main targets of thyroid hormones and it can secrete a myokine named irisin, which is able to induce the browning of white adipose tissue, energy expenditure and protect against insulin resistance. Inconclusive data have been reported about irisin role in chronic diseases. Moreover, no correlation with antioxidants has been investigated. Therefore, we performed a case-control study with the primary endpoint to evaluate irisin levels in two models of NTIS, such as chronic heart failure (CHF) and chronic kidney disease (CKD) during haemodialytic treatment. The secondary endpoint was the correlation with total antioxidant capacity (TAC) to establish a possible role of irisin in the modulation of antioxidant systems.

PATIENTS AND METHODS: Three groups of subjects were enrolled. Group A included CHF patients (n=18; aged 70.22 ± 2.78 ys; BMI ± 27.75 ± 1.28 kg/m^2); Group B included CKD patients (n=29; aged 67.03 ± 2.64; BMI 24.53 ± 1.01); finally, 11 normal subjects (Group C) have been enrolled as controls. Irisin has been evaluated by ELISA method and Total Antioxidant Capacity (TAC) by spectrophotometric method.

RESULTS: Irisin was significantly higher in Group B *vs.* A and C groups (Mean ± SEM: 20.18 ± 0.61 ng/ml *vs.* 2.77 ± 0.77 and 13.06 ± 0.56, respectively; $p<0.05$); a significant correlation between irisin and TAC was observed in group B.

CONCLUSIONS: These preliminary data suggest a possible role of irisin in the modulation of antioxidants in two chronic syndromes with low T3 (i.e., CHF and CKD) with differential pattern in these two models studied. Further insights are needed to confirm this pilot study, which could be the basis for a longitudinal investigation, to assess a prognostic role of irisin with possible therapeutic implications.

Key Words:
NTIS, Thyroid, Heart failure, CKD.

Introduction

Non-thyroidal-illness syndrome (NTIS) is a functional disorder present in acute and chronic sickness, in absence of thyroid disease, and considered an adaptive response rather than a real hypothyroidism[1]. The most common NTIS patient's condition is due to a low deiodination of fT4, leading to reduced circulating levels of fT3 ("low-fT3 syndrome"), that progressively involve also fT4 with low secretion[2]. In acute situations, this can be observed after starvation and critical illness, such as sepsis or major surgery, while low fT3 is commonly observed in chronic kidney and liver diseases, heart failure, and chronic inflammatory diseases[3]. A key role seems to be exerted by increased cytokines[4], but other mechanisms can be also involved, such as alterations in thyroid hormones transport, clearance and modifications of membrane transporters[2]. Also, selenium element is involved, since it converts thyroids hormones through a mechanism of deiodination by selenoproteins[5]. Although debated, thyroid

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hormone replacement therapy is not usually required[6,7]. Moreover, at cellular levels, a reduced T3 bioavailability can be unsafe and express a "maladaptive" rather than an "adaptive" response. As consequences, intracellular oxidative stress has been hypothesized[8,9] to exert an active role. In fact, oxidative stress (OS) could exacerbate NTIS condition in a vicious circle, due to deiodinases alteration and negative effects of low T_3 on antioxidant levels or activity[10]. Thus, chronic inflammation and OS exert reciprocal influences leading to a worse clinical progression of such conditions[11]. Accordingly, based on experimental and clinical studies[12], thyroid hormones (TH) levels in NTIS do not necessarily reflect its serum low concentrations and there is a tissue-specific thyroid hormone transport, receptor binding and hormone metabolism. Nevertheless, muscle is one of the main targets of TH and it is not surprising that many alterations are observed in NTIS condition. For instance, in mice, TH expression is decreased in sepsis and acute inflammation, but it seems not to be affected in chronic inflammation. The same scenario is observed in humans since the expression of monocarboxylate transporter 8 (MCT8) is lower after acute surgical stress than in prolonged illness[13], however, a compensatory increase in MCT8 has been described in chronic diseases in rabbits[13]. Different regulations of deiodinases 2 and 3 (DIO2 and DIO3) modulate T3 intracellular levels with a subtle tuning, to compensate the systemic TH unbalance[14].

Irisin is a 112 amino acid protein of 12 kDa molecular weight, identified for its ability to induce browning of white adipose tissue, protect from insulin resistance, and increase energy expenditure[15]. In human being, irisin is mainly expressed in skeletal and heart muscle[16]. Irisin is produced by a proteolytic cleavage of fibronectin type III domain containing protein 5 (FNDC5) a transmembrane protein whose expression is induced by exercise and/or by increasing peroxisome proliferator-activated receptor (PPAR)-γ co-activator 1α (PGC-1α). Interestingly, irisin has been confirmed to carry out a thermogenic role through a pathway involving PGC-1α. Moreover, it is proposed that PGC-1α activation and/or oxidative stress[17] controls the expression FNDC5, which, when released in the blood, produces irisin peptide[15].

Furthermore, irisin is highly expressed in myocardium, although its physiological effects in the heart remain not fully known and controversial[18]. While the precise physiological role of irisin in human being and its correlations with diseases remain unclear, some scholars[19] show that there is an association between irisin levels and comorbidities (e.g., type 2 diabetes mellitus and metabolic syndrome) .

Moreover, obesity, T2DM and the metabolic syndrome have been strictly associated with cardiovascular disease (CVD), including coronary artery disease (CAD) and acute coronary syndromes (ACSs). Some studies[19,20] have confirmed that circulating irisin levels are positively associated of the 10-year CVD risk, compared with the general Framingham risk profile[19] and diminished expression of FNDC5 could be related with reduced aerobic performance in patients with CVD[20].

Finally, a different model of NTIS is present in chronic kidney disease (CKD)[21]; consequently, low fT3 levels have been shown to be independent predictor of mortality in haemodialysis (HD) patients[22].

Therefore, this pilot study was designed to evaluate, in a case-control study, as primary endpoint the circulating irisin levels in two models of NTIS, such as chronic heart failure (CHF) and chronic kidney disease (CKD) during haemodialytic treatment. The secondary endpoint was the evaluation of total antioxidant capacity (TAC) and its correlation with irisin in order to establish a possible role of this myokine in the modulation of antioxidant systems.

Patients and Methods

Three groups of patients were selected to participate in this study and were enrolled after being explained the purposes and nature of the study, conducted in accordance with the declaration of Helsinki, as revised in 2013. The study protocol was approved by our Centre's Ethics Committee (School of Medicine, Catholic University, Rome, Italy) and written informed consent was obtained from all patients: patients affected by chronic heart failure (CHF, group A), chronic kidney disease treated by haemodialysis (HD, group B), and control group (group C).

All subjects involved in this study were admitted to the University Hospital "Policlinico Gemelli" Dept. of Internal Medicine. Two senior cardiologists separately confirmed the diagnosis of CHF (group A) based on clinical history, physical examination, laboratory and echocardiographic parameters, according to the European

Society of Cardiology Guidelines for the Management of Heart Failure[23]. Group A: 18 patients with Heart Failure with reduced ejection function (HFrEF) (15 males), aged 42-88 years (mean 69.2) were recruited. All of them were Caucasian; they were treated by conventional therapy according to ESC guidelines (beta-blockers 1, ACE-inhibitors 7, angiotensin receptor blockade 7, Diuretics 17). Comorbidities were present in HFrEF (33% T2DM, 39% hypertension, 44% atrial fibrillation, 6% peripheral atherosclerosis, 33% non-end stage chronic kidney disease, 16% COPD). All patients were classified according to NYHA classification (all belonged to class II or III) and levels of physical activity (which was confined to sedentary activity) were reported. Participants were excluded if they had uncontrolled hypertension (blood pressure > 140 mmHg/90 mmHg), alcoholism, drug abuse, abnormal hepatic function (transaminases > twice the upper limit of normal), end stage renal disease, malabsorption syndromes, gastro-esophageal reflux disease. Group B: 29 patients (16 males) affected by end-stage renal failure who received three times weekly hemodialysis for a period ranging from 8-336 months at the Hemodialysis Unit of the Catholic University, Rome, Italy, were screened for inclusion in the present study. Exclusion criteria were as follows: advanced heart failure (according to the criteria of the European Society of Cardiology), diagnosis of dementia based on DSM-IV criteria, history of alcohol or substance abuse, previous diagnosis of psychotic disorders, clinical instability requiring hospital admission. All patients received 4-h bicarbonate hemodialysis three times a week, according to the schedule employed in our Hospital[24]. The blood flow ranged from 250 to 300 mL/min, with a dialysis rate flow of 500 mL/min. All patients of Group B were treated with high-permeability membranes. Membranes were not reused. Comorbidity was quantified using the Charlson comorbidity score index[25]. Diagnoses were collected according to the International Classification of Diseases, ninth edition, and Clinical Modification codes (International Classification of Diseases (ICD). www.who.int/ classifications/icd/en/). All drugs assumed by participants were coded according to the Anatomical, Therapeutic, and Chemical codes. (ATC/DDD Index 2013. www.whocc.no/atc_ddd_index/). Interdialytic weight gain (IDWG) and pre-dialysis systolic blood pressure of 10 consecutive hemodialysis sessions (the same used to record IDH) were recorded and mean and median values were calculated[26]. Finally, a group of normal subjects (Group C), aged 50.4±3.42 ys, with BMI 25.07 ± 2.11 kg/m^2 was included as control group.

Between 08.00 and 09.00 a.m., at fasting, a venous withdraw was performed; the blood was collected in a test tube containing heparin as anticoagulant and immediately centrifuged (4°C at 2500× g for 10 min). Samples were subsequently aliquoted in 2 ml and stored at -80°C until assayed. In HD patients, the blood sample was collected during the long interval (72 h after the last dialysis, in order to minimize the effect of acute heparin administration) through the arteriovenous fistula or the central venous catheter immediately before their scheduled hemodialysis session at the beginning of the week.

Fasting glucose, total and HDL cholesterol, triglycerides, uric acid, transaminases and albumin levels were quantified using ADVIA 2400 automatic analyzer (Siemens, Italy). Plasma concentrations of NT-proBNP were measured by immunochemiluminometric assays on a Roche modular E170 analyzer (Roche diagnostic; Indianapolis, IN, USA). Plasma fT3, fT4, TSH were assayed by ECLIA. Lower T3 syndrome was defined as the presence of fT3 values under the median for each group (2.7 ng/ml in CHF and 2.3 ng/ml in CKD).

Total antioxidant capacity (TAC) was estimated with the method of Rice-Evans[27], as modified in our laboratory[28]. TAC determination is based on the interaction between the system H_2O_2-methmyoglobin with the chromogen ABTS, which radical form is spectrophotometrically detectable (at 740 nm). The latency time (LAG phase) expressed in second before the appearance of radical species is proportional to the antioxidant concentration in the sample.

An enzyme immunoassay kit (Cat. No. EK-067-029 from Phoenix Pharmaceuticals, Karlsruhe, Germany) was used for evaluation of circulating irisin concentrations according with the manufacturer's protocol. The intra- and inter-assay variations were less than 10% and 15%, respectively; detection limit ranges from 0.1 to 1000 ng/ml. The ELISA kit used for detection of irisin was previously validated by MS[29]. Optical density at 450 nm was measured, with a reading time of 1 sec., using a microtiter plate reader (Victor3; Perkin Elmer, Waltham, MA, USA) with precision at 450 nm < 0.5% and temperature control set at 25°C. Analyses were performed in duplicate.

Table I. Basal and anthropometric features in patients studied.

	Age (ys)	Sex (M/F)	BMI (Kg/m^2)	DM (%)	Hypertension (%)
CHF	70.22 ± 2.78	15/3	27.75 ± 1.28	46.2	46.7
HD	67.03 ± 2.64	16/13	24.53 ± 1.01	35	84.2

A complete echocardiographic evaluation was performed in CHF patients (Echocardiography Philips, Affiniti 70c), measuring the following parameters: left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), Septal thickness (IVS), Posterior wall thickness (PW), Peak E-wave velocity (E), Peak A-wave velocity (A), E/A ratio, Pulsed-wave TDI E' velocity (E'), E/E' ratio, Deceleration time (DT), left atrial volume (LAV), indexed left atrial volume (LAVI), systolic pulmonary artery pressure (SPAP), tricuspid annular plane systolic excursion (TAPSE) and tricuspid peak velocity (TPV).

Statistical Analysis

The Mann-Whitney U test was employed to evaluate differences between the two groups of subjects. A *p*-value of 0.05 was considered statistically significant. Chi-square test was employed to evaluate categorical variables (presence or not of Low fT3) and parts of whole. Linear regression analysis was employed to correlate irisin with the other studied parameters.

Results

Clinical characteristics and comorbidities of subjects affected by chronic heart failure (CHF, group A) and chronic kidney diseases treated by hemodialysis (HD, group B) are reported in Table I.

Mean (±SEM) values of hematochemical and thyroid hormones levels are reported in Table II and III, respectively. The prevalence of low T3 was similar in the two groups (Group A 43.75%; Group B 37.93%; n.s. using Chi-square test) as shown in Figure 1. Figure 2 shows the mean (±*SEM)* of plasmatic TAC values, which were not different between groups. Figure 3 shows the mean (±SEM) levels of irisin. Significantly higher levels of irisin were observed in HD patients (group B) *vs.* CHF (group A) and controls (group C); CHF also showed lower levels *vs.* controls.

When considering correlation studies, using irisin as independent variable, no significant correlation was showed between irisin and fT3/fT4/BMI/TAC; on the contrary, a significant positive correlation between irisin and LAG was present in patients with HD (shown in Figure 4).

Table II. Metabolic parameters in patients studied.

	CHF	HD
Glycemia (mg/dl)	80.2 ± 5.69	86.43 ± 3.24
Cholesterol (mg/dl)	153.62 ± 10.91	148.22 ± 6.06
HDL (mg/dl)	40.01 ± 3.68	41.45 ± 2.12
Triglycerides (mg/dl)	95.56 ± 9.14	160.23 ± 17.15
Uric acid (mg/dl)	5.81 ± 0.3	6.14 ± 0.2
Creatinine (mg/dl)	1.33 ± 0.21	9.48 ± 0.35
AST (U/l)	20.25 ± 1.61	11.78 ± 0.75
ALT (U/l)	17.54 ± 2.28	10.85 ± 1.03
Albumin (g/l)	39 ± 1.84	19.05 ± 4.77

Discussion

At the best of our knowledge, this is the first paper that reports a correlation between irisin and LAG in HD patients. The same correlation was not observed in CHF patients, in which a very low level of irisin were revealed.

The interest concerning irisin, in CHF condition, started from the experiments in zebrafish, in which it increases diastolic volume, heart frequency and cardiac output[30]. Interesting results were reported in murine models with diabetic cardiomyopathy: low dose of recombinant irisin treatment diminished cardiac fibrosis and ame-

Table III. Thyroid function parameters in patients studied.

	TSH mcUI/ml	fT3 pg/ml	fT4 pg/ml
CHF	2.5 ± 0.8	2.7 ± 0.15	11.82 ± 0.57
HD	2.01 ± 0.28	2.29 ± 0.08	9.26 ± 0.27

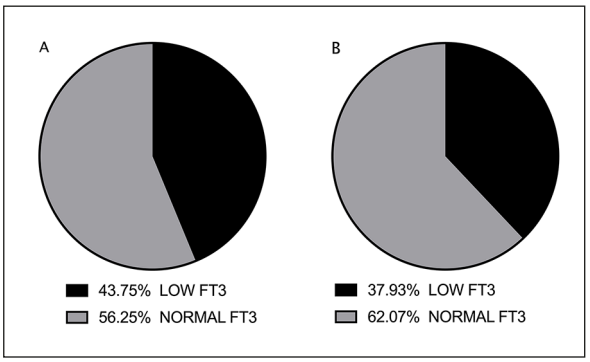

Figure 1. Prevalence of low T3 syndrome in the two groups of chronic diseases enrolled in the study (**A**= chronic heart failure with reduced ejection fraction; **B** = chronic kidney disease under haemodialytic treatment).

liorates left ventricular function; however, in higher doses the same effects were not observed; on the contrary, it increased collagen deposition, showing a dose-related effect. A possible explanation is an increased expression of matrix metalloproteinases *via* MAPK and proliferation of fibroblasts at myocardial level[31]. Accordingly, in murine model of chronic heart failure with reduced ejection fraction, the expression in skeletal muscle of FNDC5 and PGC-1a is depressed, with a consequent decrease of serum irisin levels[32].

Interestingly, the incubation of murine skeletal muscle cells with TNF-a and IL1B, or both, further decreased the expression of the same pathway suggesting an interference of chronic inflammation and endocrine function of muscle[32].

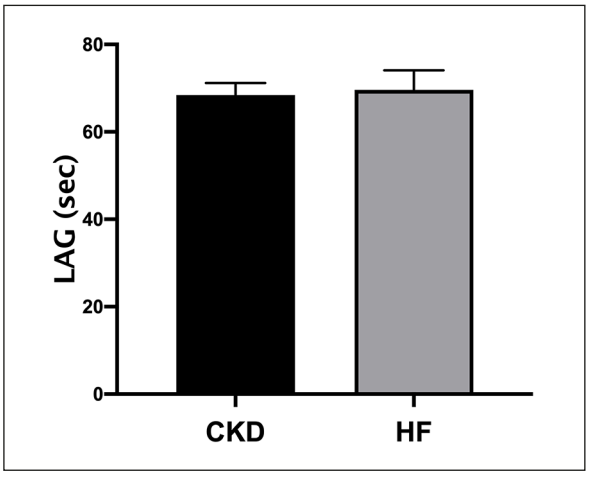

Figure 2. Mean ± SEM levels of LAG phase (sec) as measure of total antioxidant capacity in the two groups of chronic illness.

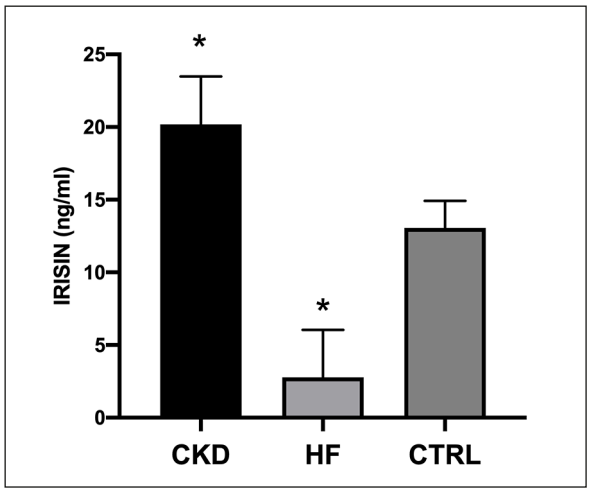

Figure 3. Mean ± SEM of circulating irisin levels in the two groups of chronic illness (CKD and HF) and control subjects (CTRL). *p<0.05.

Despite the correlation between inflammation and OS, no data were reported about the correlation between irisin and antioxidants.

Recently, we demonstrated however different pattern of irisin and antioxidants in two kinds of CHF, with preserved or reduced ejection faction. In the first, in fact, we observed higher levels than in the latter[33]; among the possible explanations, an increased oxidative stress, and a compensatory increase of irisin, was hypothesized[34].

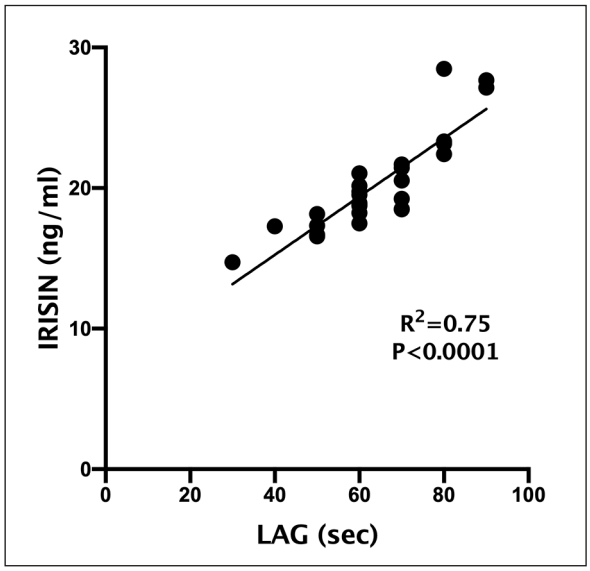

Figure 4. Correlation between circulating irisin levels and LAG in the CKD group of patients.

Accordingly, in the present work, we confirmed lower irisin levels in CHF with reduced ejection fraction (group B) compared with the other two groups.

In HD patients (group A), the situation is more complex. CKD is a condition with increased OS through different mechanisms[35], including lipotoxicity (both induced by reduced b-oxidation of free fatty acids, and lipid absorption by the CD36 receptor), increased mitochondrial ROS production and deregulation of mitophagy[36,37]. Furthermore, HD induces a chronic inflammation through the activation of polymorphonuclear cells and monocytes, and the consequent stimulation of myeloperoxidases and NADPH[38].

In the present study, we describe, in disagreement with data reported by other authors, higher levels of irisin in CKD than in controls and CHF patients. Surprisingly, results reported elsewhere in literature are still conflicting[39-43]. In patients with CKD irisin was associated with fat mass, BMI and glomerular filtration rate (GFR), with the lowest levels observed in the 5th stage of CKD[39]. Adult obese Chinese patients with higher irisin levels showed reduced prevalence of CKD[40]. A correlation with GFR was reported in some studies[41,42] but not with microalbuminuria[42]. Another article[43], confirming lower irisin levels in CKD, however, found that in peritoneal dialysis, irisin concentrations were higher than in HD. A recent meta-analysis[44], focused on irisin levels in CKD patients, confirmed that this peptide was decreased in CKD patients, mainly in dialysis patients.

To be clear, some limits could explain these contrasting results:

1) The normal range of circulating irisin in CKD is not known; conflicting data are reported about irisin levels in CKD[41]. Meta-analysis[44] showed that eight studies reported significantly different irisin levels in CKD *vs.* control, while other studies did not find this correlation. The CKD population was heterogeneous, including non-dialysis and patients treated with hemodialysis or peritoneal dialysis with differences that have been reported in these subgroups[44]. It has been also shown, in *in vivo* animal models, using irisin radiolabeled with ^{125}I and SPECT/TC imaging, that the metabolic clearance of irisin was related to liver and kidney, with an integrated action[45]. However, the specific contribution of these two systems could be modified in pathological states.
2) Several studies are observational and performed in a small sample, therefore a causal relationship between CKD and irisin is not still fully demonstrated. Moreover, the etiology of CKD could also be decisive. For instance, the study of Liu et al[46], which described the relationship between GFR and irisin, included patients with type 2 diabetes. It is known that diabetes itself can show irisin modifications which is important for the pathogenesis and clinical course of the disease[45].
3) Ethnic interferences can be important; a meta-analysis[47] showed higher levels of irisin in non-Asian than in Asian population. Accordingly, stratifying subgroups by geographic region, irisin concentrations was different, even if underlying mechanisms were not clarified.
4) The impact of the level of physical activity is not considered with irisin in most studies, although exercise is well known to affect irisin levels; different kind of effects are exerted by acute or chronic activity[15,48] and by resistance *vs.* endurance effort[49]. We have not measured the level of activity in our patients; however, it has been shown that neither a resistance training program[50] nor acute intradialytic strength exercise[51] were able to induce irisin variations in patients under hemodialytic treatment. In our cohort, the lack of a positive factor, such as exercise, was surely more determinant in chronic heart failure.
5) There is still considerable heterogeneity in reports on circulating levels of irisin in humans. Moreover, ELISA methods show significant variations depending on the manufacturer kit employed.
   In fact, irisin levels measured with different commercial ELISAs were reported in a range from picograms to micrograms per milliliter of plasma. Thus, measuring circulating irisin remains challenging.
6) Last but not least, the impact of malnutrition and sarcopenia on irisin levels is not always considered. Irisin, which originates from muscle, is evidently related to sarcopenia, even if this topic has been investigated especially in relation to cardiovascular risk factor[52]. Again, our cohort was consisting of normal weight subjects, therefore, the observed irisin values should not have been influenced by this factor.

Moreover, in our cohort, with normal weight patients, the higher irisin levels observed could be furthermore explained by its protective effect

on oxidative stress; this hypothesis is reinforced by the correlation with LAG and by the similarity with another condition characterized by increased OS as HFpEF[33].

The role of irisin and its antioxidant role is described in experimental animal models. Liu et al[53] demonstrated a protective effect of irisin on the damage produced in renal ischemia-reperfusion by suppressing p53. Other experiments supported this hypothesis: irisin treatment ameliorates OS induced by H_2O_2 in murine hepatocytes[54] and OS induced by palmitic acid in hepatocytes of mice and humans[55]. Other studies[56] confirmed similar result, demonstrating the antioxidant properties of irisin in a mice model of T2DM and in human cells of diabetic patients. Accordingly, irisin exerts a protective effect on endothelial function in the same models[57,58].

The lack of difference of LAG between HD and CHF patients is not contrasting with our hypothesis, since many factors can influence this parameter, such as the levels of uric acid, which is increased in HD, but very likely is the main component of LAG assay.

In spite of all precautions, our study may be still subject to certain biases, and some main potential restrictions should be considered, other than those above reported. The number of subjects in the groups is slightly small, so the statistical power of the study is limited; consequently, our findings will need to be confirmed in a larger population and still require further studies. Moreover, the moment of the disease history in which the patient is evaluated may affect irisin and antioxidants levels. Therefore, the study design and the power analysis cannot draw a cause-effect relationship.

Conclusions

These preliminary data suggest a possible role of irisin in modulation of antioxidants in two chronic syndromes with low T3 with differential pattern in these two models studied (i.e., CHF and HD). Further insights are needed to confirm this pilot study, which could be the basis for a longitudinal investigation, to assess a prognostic role of irisin evaluation with possible therapeutic implications.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Acknowledgements
None.

Informed Consent
Written informed consent was obtained from all patients.

Ethics Statement
The study protocol was approved by our Centre's Ethics Committee (School of Medicine, Catholic University, Rome, Italy). The study was conducted according to the Declaration of Helsinki.

Authors' Contribution
Conceptualization: AM, AS; Data collection: CB, EV, EC, AMN; Statistical Analysis: CB, EV; Writing: AM, EC, CB, EV; Supervision: AM, AS, EM, AM, AMRF, NP; Experimental procedures: AS, EM, AM.

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