

Oxidative stress as a possible mechanism underlying multi-hormonal deficiency in chronic heart failure

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Abstract. – OBJECTIVE: Chronic Heart Failure (CHF) is associated with multi-hormonal derangement depicting a prevalence of catabolic vs. anabolic axes. Moreover, thyroid adaption is characterized by the reduced conversion of thyroxine to the active hormone triiodothyronine. On the other hand, hormones modulate synthesis and utilization of antioxidant systems. Therefore, hormonal failure can cause unbalance between reactive radical species and the defenses, resulting in oxidative stress (OS). OS is well described in CHF, but the relationship with the hormonal picture is not entirely known.

In the present review, we firstly analyze the mechanisms of ROS production in the heart, discussing animal and human studies, and focusing on new discovered protective mechanisms such as sirtuins and fibroblast growth factor 21 (FGF21). The second section is dedicated to the role of main anabolic axes influencing antioxidant systems. Finally, we present some data supporting the hypothesis that OS could be the link between hormonal derangement and clinical outcome of CHF.

Key Words

Growth hormone, Anabolic hormones, Thyroid hormones, Antioxidants, Heart failure, Personalized therapy.

Introduction

Chronic heart failure (CHF) is a complex clinical syndrome defined as an unbalance between cardiac output and metabolic requirements of organism¹. This syndrome can result from any structural and functional disorder that reduces the ability of the ventricle to fill with or eject an adequate volume of blood.

The prevalence of CHF in European population is around 2-3% and, therefore, it has been singled out as an epidemic and staggering clinical and public health problem associated with significant mortality, morbidity, and healthcare costs, particularly among people aged >65 years². CHF often develops after the damaging or weakening of the heart by several causes, such as coronary heart disease, hypertension, diabetes mellitus, cardiomyopathies, heart valve diseases, arrhythmias, congenital heart defects, anemia, cocaine abuse, AIDS, thyroid disorders, radiation, and chemotherapy, etc.

Over the past several decades, clinical and experimental studies³⁻⁶ have provided substantial evidence that oxidative stress (OS), defined as an excessive production of reactive radical species compared to antioxidant defenses, is enhanced in heart failure (HF). The most important reactive oxygen species (ROS) are $O_2^{\cdot-}$, $\cdot OH$, H_2O_2 . In addition, when both $O_2^{\cdot-}$ and NO are synthesized in proximity, they will combine to form peroxynitrite ($\cdot ONOO$)⁷. ROS in the heart are involved in the lowering of contractile function, hypertrophy signaling, myocardial growth, matrix remodeling, fibroblast proliferation, and definitely in the breakthrough and progression of the disease⁸.

The dangerous effects of ROS are prevented by scavenging enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase, as well as non-enzymatic systems. Some of these are downregulated in HF. On the other hand, different endocrine systems are involved in modulation of anti-oxidants, as previously reviewed⁹. Multi-hormonal deficiencies are reported in CHF, suggesting a state of unbalance between anabolic

and catabolic systems. They could represent cause or consequence of the illness, but OS could underline both these phenomena¹⁰.

Therefore, in the present review, we firstly analyzed the mechanisms of ROS production in the heart, discussing animal and human studies, and focusing on new discovered protective mechanisms such as sirtuins and fibroblast growth factor 21 (FGF21). The second section is dedicated to the role of main anabolic axes influencing antioxidant systems. Finally, we present some data supporting the hypothesis that OS could be the link between hormonal derangement and clinical outcome of CHF.

Oxidative Stress and HF

Oxidative Stress and Heart Failure: Plasmatic Markers

The relationship between oxidative stress and heart failure can be evaluated thanks to several markers such as: uric acid (UA), malondialdehyde (MDA), C-reactive protein (CRP), Mn and CuZn superoxide-dismutase (SOD).

Positive correlations between uric acid concentrations and mean pulmonary artery pressure (mPAP) and pulmonary vascular resistance index (PVRI) have been shown, while correlations with left ventricular ejection fraction (LVEF) are negative. UA concentration is also a predictor of poor prognosis in heart failure patient¹¹. Probably it matches with an increase in ROS production and mitochondrial Mn-SOD activity¹².

CRP levels are in positive correlations with the activity of superoxide isoenzymes, so there is probably a link between ROS genesis and inflammation¹².

CuZnSOD, MnSOD and MDA activities positively correlate with mean pulmonary arterial pressure (mPAP) and pulmonary wedge pressure (PWP), whereas there are negative correlations between them and LVEF and no correlations between MDA and LVEF. MnSOD levels, which are in direct ratio to CuZnSOD and uric acid concentrations, are positively correlated with NT-proBNP¹². MnSOD increases as the patients pass from NYHA I, II to III, IV, while there's no change (or minimal changes) in CuZnSOD activity. This transition may be the key factor to explain MDA constant levels in the four classes of NYHA. MnSOD, uric acid, CRP and MDA are significantly higher in patients with dilated cardiomyopathy. They are also related to severity

of HF in patients with dilated cardiomyopathy except for MDA, in fact in two groups of patients with mild and severe limitation functional capacity (NYHA I, II, and NYHA III, IV) there are no significant differences in MDA level¹².

A significant negative correlation between MDA and left ventricular (LV) ejection fraction has been reported³.

ROS Production in the Heart

Table I synthesizes the main mechanisms leading to ROS production in the heart such as mitochondrial production, production by cytochrome P450 and different oxidases (NADPH, xanthine), uncoupling of nitric oxide (NO)-synthetase and auto-oxidation of catecholamines^{8,13-17}.

NADPH oxidases' (Nox) activity in the heart is first due to Nox2 and Nox4¹⁸⁻²⁰ which, despite their similar structure, differ in cellular localization and functionality.

Nox2 is located on the plasma membrane, and, in addition to binding p22phox, its activation involves binding of cytosolic subunits p47phox and p67phox (sometimes even p40phox), and Rac1-GTPase, which assure post-translational modulation of Nox2 activity through regulation of cytosolic subunit translocation²¹. Nox4 is located on perinuclear intracellular membranes, as mitochondrial ones¹⁸, and does not have cytosolic subunit binding, but it is regulated at a transcriptional level²¹. Nox activity is increased in end-stage failing human hearts²²⁻²⁴.

The expression of Xantine Dehydrogenases (XD) and Xantine Oxidases (XO) is elevated in failing human myocardium²⁵; it is not casual that long-term high-dose treatment with allopurinol, a XO inhibitor, reduces mortality in patients with HF²⁶.

Animal and Human Studies

While we present in table 2 what we actually know about studies in experimental animals²⁷⁻³⁸, we treat in more details studies in humans.

ROS and OS are involved in a number of pathological processes that contribute to HF, including vasoconstriction, ischaemia/reperfusion injury, cardiac hypertrophy, myocyte apoptosis, fibrosis, inflammation and myocardial stunning^{39,40}.

As above reported an alteration in oxidative balance can be the result of an overproduction of ROS or an inhibition of scavenging mechanisms.

Several studies have shown that O₂⁻ production and OS are significantly increased in the hearts of patients with dilated cardiomyopathy

Table I. Mechanisms of production of radical species in the heart.

Mitochondrial production	A source of ROS is the leakage of activated oxygen from mitochondria during oxidative phosphorylation The production of ROS fastly increases in conditions such as ischemia or hypoxia. Mitochondria from the failing heart produced more O_2^- ⁸
CYP450 production	Role in metabolism of different compounds including polyunsaturated fatty acids, in turn associated to cardiovascular disease ¹³
Production by NADPH oxidase	Vascular endothelial cells and activated leukocytes are the cells involved in ROS production via NAD(P)H oxidase. It is a family of membrane-bound enzymes that produces O_2^- by transferring an electron from NAD(P)H to O_2 NADPH oxidase activity can be increased by several stimuli that are relevant to the pathophysiology of HF (mechanical stretch, angiotensin II, endothelin-1 and tumor necrosis factor) ¹⁴ .
Xanthine oxidase	XO transfers electrons to O_2 , resulting in formation of urate and O_2^- Increased xanthine oxidase expression and activity has been reported in HF ⁸
Uncoupled NO synthetase	Uncoupled NOS can lead to ROS production through the oxidation of the essential NOS cofactor BH_4 . NOS3 becomes structurally unstable and generates ROS after the exposure to oxidative stress or the deprivation of BH_4 or L-arginine ¹⁵
Auto-oxidation of catecholamines	Aminolutine's production causing coronary spasms, arrhythmias and cardiac dysfunction ^{16,17}

ROS = reactive oxygen species; XO = xanthine oxidase; NOS = nitric oxide synthase

and HF. In fact, lipid peroxides and 8-iso-prostaglandin F2 (major biochemical markers of ROS generation) levels are elevated in the plasma and pericardial fluid of patients with HF and also

positively correlated with its severity⁶. Electron spin resonance (ESR) spectroscopy combined with the nitroxide radical 4-hydroxy-2,2,6,6-tetramethyl-piperidine-N-oxyl granted a direct evidence for

Table II. Studies in experimental animals concerning oxidative stress and heart.

A deficiency of NAD(P)H oxidase protects the heart from LV remodeling and dysfunction after MI in mice lacking p47phox (p47phox ^{-/-} mice) ²⁷ .
Angiotensin II can mediate mitochondrial dysfunction via the activation of NAD(P)H oxidases in vascular endothelial cell ²⁸ .
Left ventricle (LV) contractile function and myocardial efficiency is improved by the treatment of HF animals with allopurinol ²⁹ .
Chronic allopurinol treatment of animals with MI significantly reduces adverse LV remodeling ³⁰ .
In spontaneously hypertensive/HF rats with established dilated cardiomyopathy XO, Nox subunits, Nox2 and p67phox, increase, while only XO activity is elevated above normal ³⁰ .
Uncoupled NOS3 contributes to LV remodeling in response to chronic pressure overload in mice ³¹ .
Nox2 and its cytosolic cofactors increase during the progression of cardiac hypertrophy to HF in guinea pigs subjected to pressure overload and in hypertensive Dahl salt-sensitive rats ^{32,33} .
Mice subjected to transverse thoracic aortic constriction have reduced BH_4 levels and uncoupling of eNOS in association with LV dilatation and contractile dysfunction ³¹ .
eNOS/mice subjected to aortic constriction develop worse contractile function, greater hypertrophy, and more interstitial fibrosis ³⁴ .
Post-myocardial infarction (MI) LV remodeling is more extensive in eNOS/mice ³⁵ .
The activity of electron transport chain complex I, III, and IV decreases in mice subjected to MI ³⁶ .
COX III overexpression results in a decreased abundance of COX I and a decrease in COX activity, accompanied by increased apoptosis in HF following MI ³⁷ .
Significant improvement in survival after MI in MMP-2 knockout mice ³⁸ .

MI = myocardial infarction; XO = xanthine oxidase; Nox = NADPH oxidase; NOS = nitric oxide synthase; HF = heart failure; LV = left ventricle; COX = cyclo-oxygenase; MMP = matrix metallo-proteinase.

enhanced generation of ROS within the failing myocardium⁴¹. In the heart obtained from pacing-induced HF no decrease in the activities of the scavenging enzymes, including SOD and catalase, is observed; GSHPx activity is even increased⁴². OS in HF might be primarily due to the enhancement of ROS generation rather than to the decline in antioxidant defense within the heart.

ROS activate a wide variety of hypertrophy signaling kinases and transcription factors⁴³. ROS stimulate the tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, mitogen-activated protein kinases (MAPK), and Jun-nuclear kinase (JNK). Low levels of H₂O₂ are associated with MAPK activation and protein synthesis, whereas higher levels stimulate MAPK, JNK, p38, and protein kinase B (Akt) kinases to induce apoptosis⁴⁴.

ROS induce apoptosis by DNA and mitochondrial damage and activation of pro-apoptotic signaling kinases⁴⁵. ROS cause DNA strand breaks, activating the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1), which regulates the expression of plenty of inflammatory mediators.

ROS can also activate metalloproteinases (MMPs) post-translationally interacting with critical cysteines in the propeptide auto-inhibitory domain. ROS also stimulate transcription factors nuclear factor- κ B, Ets, and activator protein-1 to stimulate MMP expression. MMP activity increases in the failing hearts^{46,47}. Furthermore, an MMP inhibitor can limit LV dilatation after an experimental myocardial infarction (MI)⁴⁸. An $\cdot$ OH scavenger, dimethylthiourea, suppresses the activation of MMP-2 and the development of LV remodeling and failure after MI⁴⁹. Enhanced OS can be a stimulus for myocardial MMP activation, which plays an important role in the development and progression of HF.

ROS directly influence contractile function by modifying proteins involved in excitation-contraction coupling⁵⁰. This includes modification of critical thiol groups (-SH) on the ryanodine receptor to enhance its open-probability, the suppression of L-type calcium channel, and oxidative interaction with Ca²⁺ ATPase in the sarcoplasmic reticulum to inhibit Ca²⁺ uptake.

The relationship Between Angiotensin II and ROS

As reviewed by Zablocki and Sadoshima⁵¹ angiotensin II (ATII) is a source of OS in human body, in fact it has the faculty to enhance

ROS production. ATII-induced ROS upregulate growth factors and cytokines, activate kinases and modulate gene expression activating transcription factors. Moreover, ATII-induced OS can cause mitochondrial damage and dysfunction.

In HF patients, OS markers are raised and related to circulating ATII levels and are highest in patients with increased ATII type 1 receptor (AT1R) responsiveness to ATII due to homozygosity for the AT1R A1166C gene polymorphism⁵². The cleavage of angiotensinogen (AGT) by renin requires a conformational change caused by the formation of a disulfide bridge between two cysteines as a result of oxidation⁵³, suggesting the presence of a positive feedback mechanism by which ATII-induced ROS further increase ATII formation⁵¹. The link between ROS and ATII is stronger than we previously thought.

OS plays an important role in ATII-induced myocyte apoptosis, fibrosis and inflammation in cardiac hypertrophy and ultimately in cardiomyocyte contractile dysfunction⁵¹.

ATII-induced apoptosis is mainly due to Nox activation, with a production of ONOO- and consequent DNA damage and p53 activation, leading to an increased Bax/Bcl-2 ratio, caspase-3 cleavage⁵⁴. Since p53 also binds to and activates the promoters of AGT and AT1R⁵⁵ a mechanism of self-amplification of ATII signaling has been hypothesized. ROS generated by Nox are responsible for other cascades, as previously reviewed⁵¹: activation of Ca²⁺/calmodulin dependent kinases (CaMK)-II⁵⁶⁻⁵⁹; the same CaMK-II phosphorylates class II histone deacetylases (HDACs), especially HDAC4 which lead to the de-repression of transcription factors linked to cardiac hypertrophy⁶⁰⁻⁶¹; activation of NF κ B, TGF- β , MMPs, and growth factors, and consequently to increased fibrosis, extracellular matrix degradation, and tissue remodeling, and ultimately cardiac contractile dysfunction^{33, 62-69}.

TNF α , activated by ATII, can favor proteolytic cleavage of xanthine dehydrogenases (XD) and increased expression of xanthine oxidase (XO)⁷⁰; XO activity can also be regulated by substrate availability⁷¹. In addition, XO activity is directly increased by ATII⁷². Several evidences show that ROS can increase eNOS expression via activation of redox-sensitive transcription factors or mRNA transcript stabilization. However, TNF α , activated by ATII, may reduce eNOS expression⁷³.

Metabolomic profiling and electron microscopy have demonstrated that mitochondrial biogenesis and morphology are altered in the presence

of increased RAS component expression⁷³⁻⁷⁵. Mitochondrial autophagy is enhanced by ATII-induced ROS^{76,77}. Moreover, ATII can increase the expression of uncoupling proteins and decrease the expression of mitochondrial respiratory chain proteins^{77,78}. ROS from other sources may cause self-augmenting ROS release⁷⁹, damaging mitochondrial components and oxidation of the membrane permeability transition pore.

Different studies indicate that catalase expression and activity are alternately upregulated, downregulated, or unchanged in the hearts of patients with end-stage HF⁸⁰⁻⁸³. This discrepancy may be caused to differences in the patient populations. Thioredoxin (Trx) can be oxidized, so inhibited by ROS. It is upregulated by ATII in patients with chronic HF⁸⁴⁻⁸⁶. Trx protects cardiomyocytes against apoptosis via ubiquitination and degradation of apoptosis signal-regulating kinase^{187,88}, preventing oxidation-dependent nuclear export of HDAC4¹⁸, and inhibiting ATII-induced cardiac hypertrophy thanks to the upregulation of miR-98/let-7 microRNAs (miR) and downregulation of cyclin D2^{86,89}.

The role of miR in ATII-induced hypertrophy is one of the key point to understand the molecular basis of this phenomenon. In rats with myocardial hypertrophy, miR-181a is downregulated increasing the ATG5-induced cardiomyocytes autophagy, which, in turn, enhances the expression of hypertrophic genes⁹⁰.

What are the mechanisms by which ATII induces OS? A better knowledge of the problem has been obtained thanks to a great amount of experiments in animals. Some of them consist in ATII infusion and measurement of marker of OS, some others, on the other hand, assess the link between ATII and ROS, evidencing the decrease of OS after angiotensin receptor blockers (ARB) therapy. ATII infusion for 2 weeks, which causes cardiac hypertrophy, doubled OH production in mouse hearts acting on AT1R⁹¹. In rats, increased OS after MI overlaps with an increase in RAS components in infiltrating macrophages, suggesting the presence of local ATII production. This can be blocked by treatment with an ARB⁹². Similarly, ARB treatment has protective effects against myocarditis-induced HF and hypertensive diastolic HF in rats and pacing-induced HF in dogs are related to decrease in ROS production and OS^{78,93,94}.

As suggested, an important role in coronary heart disease and its consequences is played by AT1R, which can increase ROS and calcium release, promoting heart damage⁹⁵. Wang et al⁹⁶

demonstrated the beneficial effect of the ARB Valsartan in culture of endothelial progenitor cells, involved in repairing coronary damage in coronary heart disease; this effect is due to the inhibition of AT1R-induced apoptosis by regulating ROS and Ca²⁺. Moreover, Valsartan decreases AT1R-induced activation of ERK and modulates caspase-3, Bcl-2, p-eIF2 α , and CHOP.

The role of NADPH-oxidase in this relationship is one of the most elaborate and complex, involving especially Nox2 and Nox4. Radical production induced by Nox2 involves ion channels, contributing to myocardial contractile dysfunction⁹⁷⁻¹⁰², as confirmed in models of knockout mice^{62,69,103}. However, data on Nox4 are conflicting; in fact, in young mice neither the overexpression nor the suppression of Nox4 results in any baseline abnormality^{18,103,104}.

In vitro and *in vivo* studies in rats and mice have shown that ATII treatment stimulates an increase in ROS production in the heart that is significantly inhibited by diphenylene iodonium (DPI, an inhibitor of flavoproteins such as Nox), apocynin (a Nox inhibitor), or dominant negative Rac, but not by NG-nitro-L-arginine methyl ester (L-NAME, a NOS inhibitor), rotenone (a complex-I mitochondrial electron chain inhibitor), or XO inhibitors, suggesting that Nox is the major source of ATII-induced ROS in cardiomyocytes^{20,59,75,78,105,106-108}. This increase in Nox activity and oxidative stress is not detected in female mice⁸⁴. Similarly, rats known to have increased ATII levels, such as Ren2 and hypertensive Dahl salt-sensitive rats, show an AT1R-dependent increase in ROS generation, inhibited by DPI^{75,78,106}. ATII increases translocation of p47phox to the membrane^{53,65,76}, as well as membrane-associated Rac1 GTP-binding activity¹⁰⁹, both of which are involved in Nox2 activation. Furthermore, RAS inhibition by ACE inhibitors or ARBs favors the downregulation of Nox subunits p22phox, Nox2, p47phox, and p67phox in rats and mice with pathological cardiovascular conditions^{69,75,78,94,110-112}. Finally, it has been recently described that atorvastatin can ameliorate heart oxidative stress in heart-failed Wistar rats by blocking the system of Rac1/p47phox/p67phox and the subsequent ROS release¹¹³. The scientific community has produced poor evidence of XO role in the binomium ROS-ATII. One of the few studies¹¹⁴ shows how in mice with ATII-induced diastolic dysfunction allopurinol reduces oxidative stress and improves cardiac function in a blood pressure-independent manner.

Another important player is eNOS, whose expression is downregulated in the hearts of spontaneously hypertensive stroke-prone rats, but upregulated to normal levels in the presence of RAS inhibition¹¹⁵. NOS inhibition removes the protective effect of treatment with losartan, against left ventricular remodeling in hamsters with cardiomyopathy, and I/R injury in Dahl salt-sensitive rats^{116,117}, suggesting that NOS activity is protective against the effects of ATII. Likewise, adenoviral gene transfer of the human eNOS gene into rat hearts after MI resulted in increased cardiac NO production and reduced cardiac ROS generation and remodeling¹¹⁸. However, in rat aortas, ATII treatment enhances eNOS expression and decreases NO production, indicating the presence of eNOS uncoupling¹¹⁹; furthermore, treatment with captopril normalizes the growth in eNOS expression observed in the LVs of hamsters with chronic congestive HF¹¹⁹. Losartan treatment increases eNOS expression and NO production in the cardiomyocytes of obese rats, while in control animals it has the opposite effect¹²⁰, suggesting that the relationship between ATII signaling and eNOS function may be context-dependent.

A large piece of research on this topic has focused its attention on mitochondrial role. Mitochondrial DNA copy number is not modified by ATII stimulation of cultured neonatal rat cardiomyocytes¹⁰⁷, while ATII-treated mice express a significantly decreased mtDNA copy number and increased mtDNA deletions, as well as a poor mitochondrial respiratory capacity and increased mitochondrial damage⁷⁶ accompanied by hypertrophy, fibrosis, and diastolic dysfunction.

Another line of research concerns the evaluation of antioxidants. In rats subjected to MI, MnSOD expression in the infarct area and activity of both MnSOD and Cu/ZnSOD are reduced. Treatment with RAS inhibitors abolished these effects¹²¹. In rats with MI-induced congestive HF, the activities of SOD, catalase, and GPx are all diminished, but only the GPx activity is improved by losartan treatment¹²², suggesting that ATII is involved in the downregulation of GPx, but not of the other antioxidants, in this model. Treatment with an ACE inhibitor increases GPx activity in rats with chronic MI¹²¹. However, GPx expression and activity do not change in ATII-stimulated rat cardiac fibroblasts and HF patients^{80-82,123,124}, while GPx mRNA expression is increased during the transition to congestive HF in hypertensive Dahl salt-sensitive rats (re-

versible by treatment with an ARB)³³. Moreover, catalase is downregulated in the hearts of AGT transgenic mice and in cardiomyocytes treated with ATII¹²⁵. In spontaneously hypertensive stroke-prone rats, expression and activity of MnSOD are unchanged, but those of Cu/ZnSOD are decreased. This phenomenon is mitigated by treatment with an ACE inhibitor and abolished by ARB treatment¹¹⁵.

An important modulator of antioxidant tissue response is represented by sirtuins.

Sirtuins and Oxidative Stress in Heart

Sirtuins are a group of proteins included in class III histone deacetylases, which use one molecule of NAD⁺, as a co-substrate, during each deacylation cycle¹²⁶. Silent information regulator 2 (SIR2) from *Saccharomyces cerevisiae* is the first sirtuin protein identified, it has also been found in *Caenorhabditis elegans* and in *Drosophila melanogaster*^{126,127}. Sirtuins play a role in several mammalian processes, such as aging (with anti-aging functions), transcription apoptosis, oxidative stress and inflammation. There are seven mammalian sirtuins, SIRT1-7, which are localized in separate cellular compartments. SIRT3, which has the greatest deacetylating activity, SIRT4 and SIRT5 are located in mitochondria and, in this site, they control metabolic enzymes and moderate oxidative stress¹²⁸. SIRT1, SIRT6 and SIRT7 are nuclear sirtuins, they regulate genes expression epigenetically¹²⁸.

Initial studies which tried to know sirtuins "signature" on oxidative stress in animal hearts were focused on SIRT1. SIRT1 is decreased in the heart of 12 or 15-month mice. There is also a significant decline in left ventricular systolic function until 18 months of age in the C57BL/6J mice¹²⁹. The expression of SIRT1, AMP-dependent kinase (AMPK) and MnSOD decrease in the old mice (aged 12 months) in comparison with younger mice (3 months); however, nicotinamide phosphoribosyl transferase (Namt), an enzyme involved in many processes including a positive regulation of NOS, is increased. The expression of phospho-AMPK/total AMPK is significantly reduced in the old mice¹³⁰. A study¹³¹ with transgenic mice has revealed that increased expression of SIRT1 in heart prevented programmed cell death and aging-associated alterations. In a hamster model of chronic HF, the induction by resveratrol of nuclear SIRT1 increases MnSOD levels in cardiomyocytes and enhances resistance against oxidant load, reduces

oxidative stress and suppresses apoptosis¹³². It is clear that SIRT1 has an antioxidant role in all these situations.

New evidences show that SIRT3 has a relevant value in HF pathophysiology. SIRT3^{-/-} mice develop, spontaneously, cardiac hypertrophy, more pronounced compared with non-transgenic control, with increasing age¹³³⁻¹³⁶. SIRT3 has various targets in mitochondria, which are hyperacetylated in SIRT3^{-/-} mice. In this situation it has been shown that many pathways (fatty acid oxidation, glucose oxidation, Krebs cycle and oxidative phosphorylation) slow down with subsequent myocardial energy depletion^{135,137}. Another important mechanism, which can explain the previous data, may be an imbalance between ROS production and expression and activity of antioxidant enzymes: SIRT3^{-/-} mice show, in fact, increased levels of 4-hydroxynonenal (HNE) and thiobarbituric reactive substances (TBARS), which are indexes of lipid peroxidation^{134,138}. Increased fibrosis in aged SIRT3^{-/-} mice may be related to a disinhibition of TGF- β 1 signaling and hyperacetylation of glycogen synthase kinase 3 β (GSK3 β) by SIRT3 deficiency, resulting in increased expression of pro-fibrotic genes¹³⁹. Otherwise, overexpression or pharmacological activation of SIRT3 can improve and even block cardiac hypertrophy and interstitial fibrosis in response to pressure overload or hypertrophy induction with ATII infusion^{134,136,140}. This is probably due to the prevention of a drop in catalase and MnSOD expression after ATII infusion, likely related to an increase in transcription factor forkhead box (FOX) O3a signaling¹³⁴. Moreover, SIRT3 is able to deacetylate MnSOD, increasing its activity and attenuating oxidative stress¹³⁴. Protection from oxidative stress may also attenuate activation of the ROS-sensitive MAPK/ERK and PI3K/AKT signaling pathways, which are known to play a major role in the development of cardiac hypertrophy¹⁴¹. Indeed, SIRT3 expression is reduced and global mitochondrial protein lysine acetylation is increased in rodent models of heart failure, suggestive of impaired SIRT3 activity¹⁴². This may be the consequence of an alteration in peroxisome proliferator-activated receptor gamma co-activator (PGC)-1 α -SIRT3 signaling or NAD⁺ depletion or a decrease in the NAD⁺/NADH ratio. PARP-1, which overacts in heart failure, uses NAD⁺ as a co-substrate¹⁴³, narrowing SIRT3 possible activity. In the last decade, several studies have been published on this topic in human being. SIRT1 improves heart tolerance against ischemia

and oxidative stress enhancing eNOS¹⁴⁴. SIRT1/FoxO3a controls the transcription of Sod2¹⁴⁵. Lu et al¹³⁰ demonstrate that SIRT 1 expression in left atrium is downregulated in heart-failed patients, moreover MnSOD, Trx1 and Bcl-xL, some of the most important molecules in SIRT1-signaling, are all decreased in advanced heart failure. FoxO1, a transcriptional factor which can modulate the expression of MnSOD, is decreased. On the other hand, the expression of Bax, which is a protein involved in pro-apoptotic signal, is increased in the same patients; this is probably due to the increase of acetylated p53¹³⁰. AMPK and Nampt, both involved in the activation of SIRT1, are reduced in advanced heart failure¹³⁰. Besides heart situation, even peripheral blood one has been analyzed, showing that the transcript of SIRT1 in leukocytes significantly decreases in patients with compensated and decompensated HF¹⁴⁶, furthermore, as a result of redox balance disorder in HF, SIRT1 mRNA levels correlate negatively with oxidative stress index and total oxidant status, and positively with serum total antioxidant status and HDL levels in both patient groups with cHF and dHF¹⁴⁶. Finally, it is important to know that the antioxidant properties of SIRT1 in the heart are partially mediated by FGF21¹⁴⁷.

The Role of FGF21

Fibroblast growth factor 21 (FGF21) is a protein mainly produced in the liver, which regulates glucose homeostasis, ketogenesis and insulin sensitivity¹⁴⁸. These functions are provided by the interaction with FGF receptors especially FGFR1 and FGFR4^{149,150}.

Plenty of information has been given by mouse models. In FGF21-null mice hearts biological markers of oxidative stress are altered¹⁴⁷. FGF21 has a protective effect on myocardium after the I/R injury, which is based on oxidative damage¹⁵¹. In FGF21-null mice the enhancement in apoptotic rate is related with Ucp3 downregulation. Activation of Ucp3 protects the heart against ROS damage¹⁵². In fact, Ucp3-null mice develop exaggerated apoptotic cell death and enhanced signs of cardiac damage^{152,153}. It is possible that Ucp3 play an important role activating the protective action of FGF21 against cardiac oxidative stress under conditions of cardiac hypertrophy.

SIRT1 and pro-oxidative stimuli can induce FGF21 expression. One of the most representative example is lipopolysaccharide (LPS) one: Lps is an oxidative inducer¹⁵⁰, which can also induce Fgf21

in cardiomyocytes; secreted FGF21 promotes the expression of antioxidant genes (e.g., UCP2, UCP3, and Sod2) and prevents ROS formation. Therefore, FGF21 stimulates the same response by acting in an autocrine manner¹⁴³. ERK pathway has a primary role in the transcriptional control of Sod2¹⁵¹ and FGF21 activates the ERK pathway in the heart¹⁵². Therefore, the secreted FGF21 may act through the ERK pathway to activate Sod2 and prevent ROS formation in the context of SIRT1 overexpression. However, SIRT1 mRNA levels are not found to be induced in failing human hearts; it is possible that the up-regulation of FGF21 in failing hearts may still be mediated by post-transcriptional changes in SIRT1.

The Hormonal Control of Antioxidants

Thyroid Hormones

We previously reviewed how antioxidant systems are influenced by thyroid hormones, both in ovarian physiopathology¹⁵⁷ and in relationship with non-thyroidal illness¹⁵⁸. Both the extremes of thyroid function (hyper- and hypo-) can induce OS¹⁵⁹, but probably with different mechanisms: augmented ROS production in hyperthyroidism and decreased antioxidant system in hyperthyroidism. OS can be responsible of some complications of hyperthyroidism at tissues level¹⁶⁰. The presence of the phenolic group makes thyroid hormones per se oxidant agents¹⁶¹. The synthesis of thyroid hormones is coupled with oxidoreactive reactions, leading to the production of H₂O₂. This potentially dangerous mechanism is contrasted by unic anatomical conformation of thyroid follicles, which are separated from circulation. Therefore, the risk of oxidative damage is confined in these structures called thyroxisome. A special role is covered by selenoproteins and membrane oxidases, contributing to lowering radical species inside the cell¹⁶². The importance of such oxidases is confirmed by cases of hypothyroidism due to mutation of DUOX or DUOXA genes in the literature^{163,164}. Moreover complex molecular mechanisms, involving cytokine release and other oxidases, such as NOX4, have been hypothesized in Hashimoto's thyroiditis and also in thyroid neoplasia^{165,166}.

An important review¹⁶⁷ described other phenomenon contributing to OS: among these an increased NO production, due to augmented Nitric Oxide Synthase (NOS) gene expression.

NO, which is arterial vasodilator and endothelial protector, becomes dangerous in an oxidant milieu, due to the production of peroxynitrites and preferential expression of eNOS¹⁶⁷. Moreover the activation of hepatic NF- κ B with the consequent increase in cytokines levels induces ROS production.

Besides, some mechanisms try to counteract oxidative status induced by thyroid hormones through autoloop feedback. For example an antioxidant role can be exerted by Uncoupling Protein (UCP)-2 and -3^{168,169}. The regulation of UCP is quite complex and recognizes a major role of T₃ in comparison to T₄^{170,171}, while a repression of UCP, causing ROS increase, is exerted by estrogens¹⁷². Finally mitochondria damaged by oxidative stress are removed by mitoptosis process¹⁷³ also regulated by peroxisome proliferator-activated receptor gamma coactivator-1, which in turn is upregulated by T₃ administration¹⁷⁴.

As a consequence, thyroid hormones influence lipid composition of tissues and consequently the susceptibility to OS. However, the response is tissue-specific, and discrepant effects of T₃ and T₄ have been reported.

Index of lipid-peroxidation (TBARS and lipid hydroperoxydes) have been shown in liver of rats made hyperthyroid by T₃ administration^{167,174,175}. However a four-week T₄ treatment did not induce the same results¹⁷⁴. Similarly in other organs like testes, hyperthyroidism did not modify lipo-peroxidation, inducing, on the contrary, an increase of carbonyls¹⁷⁷. In addition T₃ and T₄ exerted differential effects on antioxidant enzymes in different tissues¹⁷⁸. Vitamin E reduced the damaging action of peroxyl radicals through the preservation of polyunsaturated fatty acids in biological membranes and the lowering of NADPH oxidase action¹⁷⁴. The thyroid itself can be damaged by OS; this phenomenon is evident in case of iodine excess, studied both in vitro and in animals models fed with a diet rich in iodide^{179,180}. On the same wavelength another study has shown how iodide stimulates hydrogen peroxide generation in thyroid slices and, at high concentrations, induces the apoptosis of the thyroid cell¹⁸¹.

Moving from tissues to systemic level, hyperthyroidism has been associated with low circulating levels of alpha-tocopherol^{182,183} and CoQ₁₀^{183,184} in humans. Thus CoQ₁₀ has been defined as a sensitive index of tissue effects due to thyroid hormones, in situations such as treatment with amiodarone¹⁸⁵ and low T₃ syndrome¹⁸⁶.

On the other hand, conflicting data have been collected on hypothyroidism and OS in humans. The lower metabolic rate in hypothyroidism should induce lower ROS generation, however other mechanisms are operative, for example the marked increase in lipoprotein plasma levels and the reduction *in vitro* due to blockage of beta-carotene conversion. In a group of patients with primary hypothyroidism it has been detected high plasma levels of NO and malondialdehyde (MDA), the last one a marker, formed by lipo-peroxidation, of OS, SOD levels not significantly different from controls ones and a diminished activity of paraoxonase (PON)-1, an antioxidant enzyme synthesized in the liver¹⁸⁷. An interesting hypothesis emphasizes the possibility that in hypothyroid patients the pro-oxidant environment could take part in the development of atherosclerosis. Elevated MDA levels were also detected in subclinical hypothyroidism¹⁸⁸. In this scenery the alteration of oxidative status is primarily due to the decrease in antioxidants levels, secondarily the altered lipid metabolism, demonstrated by a significant correlation among LDL-cholesterol, total cholesterol triglyceride levels and MDA. Total antioxidant status (TAS) was similar in overt hypothyroidism, subclinical hypothyroidism and controls.

Augmented TSH directly produces OS¹⁸⁹. Other works show lipid peroxidation, indicated by MDA levels, and protein oxidation, evidenced by protein carbonyls elevation, both in overt and subclinical hypothyroidism¹⁹⁰. Both the TSH increase and the MDA elevation contribute to protein damage. Finally, different investigations described NO elevation^{191,192}.

Data on other parameters are more conflicting. Both in hypo- and hyperthyroidism PON-1 activity has been found decreased¹⁹³, while in other studies no significant differences have been detected in comparison to controls¹⁹¹. Increased levels of antioxidant (SOD, CAT, Vitamin E), but also TBARS have been reported¹⁹⁴. All these parameters correlated with T_3 and the correlation between T_3 and CAT remained significant also when corrected for total cholesterol. The discussion is present even for TBARS levels, which are elevated in both overt and subclinical hypothyroidism for some investigations^{192,195}, but some others did not detect this finding^{191,196}.

Another involved topic is whether OS is related to hypothyroidism *per se* or to the alterations of lipid profile caused by thyroid dysfunction, as above stated. Santi et al¹⁹⁷ reported OS, in

particular an increase of TBARS and CAT and an arylesterase decrease, in subclinical hypothyroidism, but they attributed this pattern to hypercholesterolemia.

We evidenced low Total Antioxidant Capacity (TAC) levels in hypothyroid patients and increased CoQ₁₀ plasma levels in secondary hypothyroidism. This latter finding can be explained reminding the metabolic role of CoQ₁₀ in the mitochondrial respiratory chain and its consequent reduced cell use in patients with hypothyroidism. In secondary hypothyroidism, the scenery is more complex due to concomitant alterations of other pituitary-dependent axes, which can have different effects on CoQ₁₀ and its plasma levels. Low CoQ₁₀ plasma concentrations have been found in hypoadrenalism and acromegaly, however, when they are associated with hypothyroidism, this latter has a overwhelming effect^{9,198}.

Another work in patients affected by subclinical hypothyroidism secondary to Hashimoto's thyroiditis didn't show any significant difference in MDA levels between patients and controls; however, after using the pro-oxidant 2,2'-azobis-(2-amidinopropane) hydrochloride MDA levels were strongly augmented in hypothyroid patients. This response was not followed by any change in LDL fraction: in fact, the production of MDA induced by copper was reduced only in patients with overt hypothyroidism, while it was not significantly different from controls in subclinical hypothyroidism¹⁹⁹. Since both tissue and systemic inflammation are present in thyroiditis, these studies should be interpreted with caution.

The procedures by which hypothyroidism is induced affect the OS findings. Decreased OS in heart²⁰⁰ and kidney²⁰¹ was detected in hypothyroidism obtained by surgical thyroid resection in rats. Drug-induced hypothyroidism was linked with increased lipo-peroxidation in amygdala²⁰² and hippocampus in rats^{202,203}. It seemed that other cerebral areas, including the cerebellum, remained unaffected²⁰⁴, but this was not confirmed in other studies^{202,203}. Similarly, in animals, methimazole treatment is associated with cell damage in various organs (heart, kidney, lung, liver and spleen), while thyroidectomy does not²⁰⁴. However some authors think that the organ damage is not consequent to the hypothyroidism *per se*, but to the drug itself^{205,206}.

In the latest years, the focus has been given on the OS induced damage in organs such as liver, bone, skeletal muscle and particularly the heart¹⁷⁴.

The metabolism of cardiomyocytes depends on serum T₃, in fact in these cells the deiodinase activity is lacking²⁰⁷.

Increased, decreased or unmodified levels of total SOD, Mn-SOD, Cu, Zn-SOD, GPx, GSH, Vitamin E, CoQ₉, CoQ₁₀ and TAC have been reported in cardiomyocytes in response to hypothyroidism¹⁷. These results indicate that the evaluation of a single OS parameter is not a reliable index of cardiomyocytes oxidative status and the evaluation of TAC depends on the measurement method used.

Growth Hormone

Data concerning the role of anti-oxidants and GH/IGF-1 axis are still not completely understood.

GH modulates functions and life cycle of defense cell systems. In human polymorphonuclear neutrophils (PMNs), cultured in vitro, GH pretreatment inhibits apoptosis by down-regulation of Fas expression. However, it up-regulates intermediate reactive oxygen production, stimulated by phorbol myristate acetate system²⁰⁸. This potentially harmful aspect is counterbalanced by enhanced life span, therefore the authors concluded that GH may increase host defense. Reactive oxygen intermediate production is also augmented in cultured monocytes; no effect was reported on apoptosis in monocytes or lymphocytes. It has been shown that GH can have a deleterious effect on OS, both increasing ROS production²⁰⁹ and reducing antioxidants like glutathione²¹⁰. Therefore, the role is not clearly established.

As rat models is concerned, Wister rats were tested after caloric restriction during a 6 weeks period, resulting in decreased ROS production and oxidative DNA damage in heart mitochondria; this was reverted by insulin treatment and by GH/insulin administration²¹¹. However, in the liver, GH and insulin decreased mitochondrial ROS generation, while increased oxidative damage to mitochondrial DNA. GH and insulin decreased three different markers of oxidative liver protein modifications, but increased lipoperoxidation-dependent markers, probably by the increasing of phospholipid unsaturation. Therefore, GH seems to drive both pro-oxidant and protective effects, depending on parameters and tissue considered.

Ames dwarf mice (df/df), which are deficient in GH, prolactin and TSH, have a longer lifespan, where transgenic mice with GH overexpression show premature ageing and reduced life-time.

The evaluation of antioxidant systems showed lower liver levels of glutathione and ascorbate in dwarf animals; TAC activity in dwarf liver and kidney, instead, was higher than in the other groups, suggesting that GHD mice may contrast oxidative stress more efficiently than normal or GH-overexpressing mice²¹⁰.

This hypothesis was not in agreement with the observations of Hauck et al²¹² in long-living GH receptor/binding protein gene knockout (GHR-KO) mouse. The authors discovered lower SOD and higher GPX in kidney; GHR-KO mice had lower TAC and higher LP males and were also more susceptible to paraquat toxicity. In contrast, LP was higher only in female mice. In the liver, female GHR-KO mice had lower GPX. Even if the authors concluded that the longevity in this experimental model was not due to an improved free-radical scavenging, at least in liver and kidney, their experiments showed an important sex-related modulation of these systems and a differential response of various tissues.

Another interesting model is the Apo-e^{-/-} mice, characterized by marked hyperlipidemia which induces atherosclerosis. It was used to explore the role of IGF-1 on atherogenesis. The infusion of IGF-1 for 12 weeks ameliorated atherosclerotic plaques with a concomitant reduction of urinary 8-isoprostane, index of OS at systemic level²¹³. Very interestingly, these effects were not reproduced by the infusion with GH-releasing-peptide-2 (GHRP2) capable of stimulating both GH and IGF-1, suggesting that GH per se can contrast the positive action of IGF-1²¹⁴. The last observation suggests the effect of GH could be dose related.

Male Wister rats treated by aortic stenosis to induce heart failure, a model with high OS, showed beneficial effects of 1 mg/kg GH administration (lower lipoperoxidation, higher glutathione peroxidase), while the opposite was observed after 2 mg/kg GH²¹⁵. In apparent contrast, mice with IGF-1R inactivation had a mean elongation of lifespan in comparison to wild-type mice and a better resistance to OS²¹⁶. However, this model is similar to animals undergoing caloric restriction, therefore suggesting a link with caloric status of the former²¹⁷.

Studies on humans, concerning mechanisms of oxidative stress, have been conducted both in prepubertal and adult GH deficiency (GHD). Pre-pubertal GHD showed significantly reduced Lag phase and vitamin E, with a correlation between these parameters and IGF-1 or IGFBP-3;

on the contrary MDA was significantly increased and inversely correlated with IGF-1/IGFBP-3²¹⁸. The study was repeated after 1 year of rGH therapy, which induced an increase in Lag phase and a decrease in MDA, which reached normal levels.

Observations in GHD patients, by Scacchi et al²¹⁹, found higher peroxide levels and lower Lag phase, measured by a fluorescence kinetic method; no correlation was observed, at baseline, with IGF-1 levels. The patients were retested after a 4 months rGH treatment: peroxide levels decreased and Lag time increased, reaching values of controls; a correlation with IGF-1 – direct in the case of LAG time and indirect in the case of lipoperoxides – was restored. Therefore, the short-term GH administration enhanced anti-oxidative patterns, at a dose, which increased, but not fully normalized, IGF-1 levels. These results may appear in contrast with Smith et al²²⁰ data: neutrophil O_2^- generating capacity measured in a group of GHD adults was found to be lower than in normal controls, and it was raised by GH treatment. Moreover, adult GHD was associated with reduced lipid peroxidation, evaluated both as plasma lipid hydroperoxides, measured with ferrous oxidation with xylenol orange assay, and as LDL susceptibility to peroxidation, measured with copper-catalyzed Lag phase. Reduced lipid peroxidation and impaired PMN O_2^- generation were reverted with a 3-month rGH treatment. But these findings were coupled with a higher LDL-cholesterol and triglyceride and lower HDL-cholesterol²²⁰.

Similarly to the above reported data in experimental animals, it has been reported that human centenarians can be carrier of mutation in the IGF-1R gene²²¹. However, patients with GH receptor deficiency, which had severe IGF-1 deficiency, did not show any effect on life-time duration, even if presented lower diabetes and cancer incidence²²².

Previously we presented our data in a group of 7 adult GHD, aged 24-77 years, with hypopituitarism due to empty sella, removal of non-secreting tumors or craniopharyngioma. Our study did not show significant differences with normal subjects; however, this could indicate a “relative” deficiency of antioxidants, if referred to the altered lipid pattern and increased cardiovascular risk of these subjects⁹.

DHEAS

As regards DHEAS, some data are available on exogenous DHEA, that can exert a dual ef-

fect (antioxidant or pro-oxidant) depending on the dose and tissue specificity^{223,224}. It prevents oxidative injury in obstructive jaundice in rats. When administered to male Wistar rats it produces significant differences in some parameters of oxidative stress in rat hearts, suggesting a pro-oxidant answer in this model²²⁵.

DHEA treatment may reduce weight gain and lipogenesis in rats²²⁶, delay atherosclerosis in rabbits²²⁷, increase insulin sensitivity²²⁸ and insulin secretion in rats²²⁹ and reduce cardiac fibrosis in diabetic rats²³⁰.

Several studies have shown a potential antioxidant effect of DHEA. Yorek et al²³¹ stated that DHEA reduces plasma thiobarbituric acid-reactive substances (TBARS) and superoxide anion production in arterioles from diabetic rats, while Aragno et al²³² observed ROS reduction in hearts from diabetic rats treated with DHEA. It also narrows oxidative stress-induced skeletal muscle damage in diabetic rats²³³. *In vitro* studies have shown direct effects of DHEA on oxidative stress parameters: DHEA reduces NF- κ B activation in endothelial cells treated with TNF α ²³⁴, and stimulates endothelial cells proliferation²³⁵ and protects them against apoptosis²³⁶. The results of *in vivo* and *in vitro* studies have shown that DHEAS limits lipid peroxidation^{237,238}. Moreover, oxidative stress parameters in plasma and in peripheral blood mononuclear cells in diabetic subjects are meaningfully decreased by DHEA treatment²³⁹.

DHEA treatment also prevents oxidative stress in obstructive jaundice in rats by increasing SOD activity in liver²²³, enhances SOD activity in aorta from aged rats²⁴⁰, and in liver from diabetic rats²⁴¹. DHEA treatment in ovariectomized rats, an experimental model of menopause, has positive effects on oxidative balance, in fact it reduces ROS (assessed by superoxide anion content in aorta of rats), increasing the expression of Cu/Zn-SOD (downregulated in this model), and enhancing eNOS phosphorylation and NO production; according to this, it improves vascular function and reduces blood pressure²⁴².

Goy et al²⁴³ have demonstrated that serum DHEAS levels are positively correlated to BMI in postmenopausal women, furthermore BMI negatively correlates with the MDA/DHEAS ratio and can be a possible predictor of this ratio. MDA/DHEAS ratio can be used as a marker of oxidative stress in postmenopausal women.

It's also important to underline that the administration of supra-pharmacologic doses of DHEA, as demonstrated by Emer et al²⁴⁴ induces various histologic cardiac lesions in rats includ-

ing misshapen cell nuclei, leukocytic infiltrates and disorganized myocardial fibers. Furthermore, echocardiography shows increased left ventricular posterior wall thickness, ejection fraction (EF), and fractional shortening (FS). Left ventricular internal diameter in systole (LVIDS) was increased with no concomitant increase in left ventricular internal diameter in diastole (LVIDD). Moreover, such doses of DHEA increases TBARS, SOD and glutathione peroxidase (GSH-Px) levels; the last two modifications are probable rescue mechanisms to counteract oxidative stress induction.

Testosterone

Testosterone (T) is the predominant and most important androgen, playing a major role in the development of the male reproductive system^{245,246}, but it's involved in anabolic processes making it fundamental in relation with myocardial function and contractility. Previously we reviewed a dual action of T on radical production, depending on doses and kind of cellular model studied⁹.

The role of T in determining redox-status of biological systems is well documented in animals. Eleawa et al²⁴⁷ demonstrated that testosterone deficiency in orchidectomized (ORX) rats produced a reduction of CAT, SOD and increased levels of MDA in comparison with controls. Moreover, the administration of T improved levels of the same parameters in treated ORX rats if compared with non-treated ORX ones.

Interesting differences are noted when they considered myocardial contractility parameters on isolated myocardium. ORX rats showed significant decrease in left ventricular developed pressure (LVDP) and the peaks of the positive and negative pressure derivatives compared to controls; treatment with T in ORX rats seemed to improve myocardial contractility²⁴⁷.

Several studies have underlined the effect of T as a pro-oxidants factor. Chignalia et al²⁴⁸ reported that T induces ROS generation in cultured Vascular Smooth Muscle Cells (VSMC), with greater production of ROS in cells from hypertensive compared with normotensive animals, by upregulation of Nox4²⁴⁸. T metabolites, such as 6 β -hydroxytestosterone generated by cytochrome p450 activity, contribute to ATII-induced hypertension and its associated cardiac damage. All changes are accompanied by increasing of NAD(P)H oxidase activity and ROS generation²⁴⁹. Similarly, Xanthine oxidase, source of superoxide

production, is stimulated by T via activation of Androgen Receptor (AR) and the PI3 kinase-Akt signaling cascade²⁵⁰.

Finally, Skogastierna et al²⁵¹ demonstrated that supra-physiologic dose of T induced NO production and oxidative stress in healthy volunteers, by evaluation of NO metabolites in urine samples after 48h administration of 500 mg of testosterone enantate.

On the contrary, previous *in vivo* studies have shown a protective T effect: men with coronary artery disease (CAD) have significantly lower concentrations of bioavailable T than men with normal angiograms²⁵², and prevalence of hypogonadism in a group of men with CAD is about twice that observed in the general population²⁵³. Hypotestosteronemia is associated with an atherogenic lipid profile (elevated low density lipoproteins and triglycerides, decreased high density lipoprotein), high fibrinogen with a hypercoagulable state, an increase in insulin resistance and hyperinsulinaemia, and higher systolic and diastolic blood pressure²⁵⁴. When T is instilled into the left coronary artery, vasodilatation ensues and coronary flow increases²⁵⁵. More importantly, acute administration of intravenous T improves exercise tolerance and reduces angina threshold in men with CAD^{256,257}. Non-genomic effects of T on vascular smooth muscle cells were more extensively studied²⁵⁸.

Therefore, the impact of T on oxidative stress is strictly dependent on the experimental model, with an optimal hormonal level that could balance both the detrimental effects of deficiency and excess.

Heart Failure as a Multihormonal Disease

HF has been depicted as a multi-hormonal disease²⁵⁹. The imbalance between anabolic and catabolic system, in favor of catabolism, is a key feature of patients with severe chronic heart failure. This is related to the activation of inflammatory and neuroendocrine systems, to symptoms, exercise tolerance and the onset of cachexia²⁶⁰⁻²⁶². An example of these hormonal changes comes from the phenomenon of cardiac cachexia. There is no uniformly accepted definition of cardiac cachexia, but it can be considered as a weight reduction > 10% compared to normal^{262,263}. It has been observed that the alteration of the balance of anabolic and catabolic systems with preva-

lence of catabolic ones, which leads to cachexia, and neuro-hormonal activation (such as catecholamine increase) are linked each other²⁶³. It has also been demonstrated that cachexia in patients with chronic heart failure is also associated with acquired GH resistance. This resistance, which also justifies the poor biochemical response to substitution therapy in some cases, seems to be related to a reduction in tissue distribution of GH receptors²⁶⁴.

The anabolic hormone deficiency is characterized by a distortion in the GH/IGF-1 axis. However, this phenomenon is more complex, including abnormalities in adrenal and gonadal axes^{260,264}. In healthy men, these hormonal abnormalities are a crucial element of the normal aging process, even though they are related to adverse consequences such as sexual dysfunction, depression, abdominal obesity, metabolic syndrome and cardiovascular disease²⁶⁵. In male population, deficiencies in circulating levels of DHEAS and IGF-1 are associated with an increased risk of both cardiovascular and total mortality and reduced levels of IGF-1 promote the development of heart failure in older patients^{266,267}.

An important report of 208 males with chronic heart failure has demonstrated a high prevalence of reduced serum concentrations of anabolic hormones in this population. The 3 hormones analyzed reflect the main anabolic endocrine axes: the gonadal, adrenal and somatotrophic axes. It has been shown that reduced levels of total T, DHEAS and IGF-1 are strong markers of worse prognosis independently of conventional risk factors for cardiovascular disease²⁶⁰. A lack of T was observed in the group of younger men with heart failure (<45 years) in 39% of cases. In this group, the hormonal deficiency is particularly meaningful as it significantly affects the quality of life. In the group of men >66 years, total T deficiency was found in 1/3 of cases. This group also presents a higher prevalence of HF. DHEAS and IGF-1 deficiencies were present in most heart-failed patients in all the groups under 65 years.

The deficiencies of these hormones are not just a surrogate of the severity of the underlying disease. The only significant association was between a reduction in total T and DHEAS and the symptoms of heart failure according to NYHA classes. IGF-1 levels remained low despite the NYHA class. Moreover, nor T neither IGF-1 correlated with left ventricular function indexes such as LVEF or NT-proBNP. These last two parameters were only related to the circulating

levels of DHEAS. The presence of multi-hormonal deficiencies in men affected by chronic heart failure leads to a worse prognosis²⁶⁰.

DHEAS deficiency is an independent risk factor for ischaemic heart disease and a predictor of increased mortality for all causes²⁶⁸. There are no data on the relationship between total T levels and mortality. However, it has been noted that low levels of T are an independent determinant of endothelial dysfunction in men: in fact total and free T levels are related to %flow-mediated dilatation (FMD) independently of age, BMI, hypertension, hyperlipidemia, diabetes mellitus and smoke. Whereas, DHEAS levels were not significantly related to %FMD²⁶⁹. Increased total and cardiovascular mortality and increased incidence of heart failure can be seen in IGF-1 deficit. In addition, a progressive association between the number of altered anabolic axes and 3-year mortality for all causes has been reported. Patients with alterations of at least two endocrine axes had the worst survival, with a 3-year mortality rate of 50% and 75% in those with alterations corresponding respectively to two and three hormonal axes²⁶⁰. These observations demonstrate the clinical utility of considering all three anabolic hormonal axes in the overall assessment of long-term prognosis of patients with chronic heart failure in addition to parameters such as the NYHA class, ejection fraction, NT-proBNP, and renal function, classically used in these patients. However, these alterations have been stated in observational studies and need further examination to understand the mechanisms that may be responsible for.

The origin of age-related decline in anabolic hormones remains uncertain. One hypothesis is that the aging process leads to a constant exacerbation of inflammatory processes with high circulating levels of cytokines that can inhibit the secretion of sex steroids from gonads and breast. It is presumed that reduced DHEA secretion in patients with chronic heart failure is due to insulin resistance and hyperinsulinemia. Insulin resistance is frequently detected in heart failure. In fact in comparison with subjects with normal blood glucose levels, insulin values in patients with heart failure were higher. Furthermore, insulin resistance is also related to the severity of heart failure. Insulin is a physiological inhibitor of DHEA secretion in healthy subjects²⁷⁰. Therefore, it is clear that alterations in major anabolic hormone axes contribute to a worse prognosis in patients with chronic heart

failure. Studies on the elderly with DHEAS and T deficiency have been performed to see if hormone replacement therapy could successfully modify body composition, sexual function, and psychological state in the aging process. Restoring normal T levels with substitution therapy can improve muscle mass, prevent osteoporosis, improve mental status and improve libido especially in older patients²⁷¹. Malkin et al²⁷² have noticed that 12-month replacement therapy with men with heart failure results in an improvement in NYHA class and functional capacity measurable through walking test.

Due to the fundamental role of hormones in modulating antioxidant systems, as above described, in our opinion OS could be the link between hormone deficiencies and HF.

As a personal experience (preliminary unpublished data), in order to evaluate the relationships between anabolic hormones and indexes of OS and the impact on HF, we have studied a group of 21 patients (18 males 3 females, age 49-73 years) affected by HF (NYHA II-III; EF<40%), evaluating metabolic parameters (glycaemia, total and fractioned cholesterol, uric acid, triglycerides, proteins), hormonal parameters (IGF-1, DHEAS, T, freeT₃, freeT₄, TSH, NT-proBNP) and total plasma antioxidant capacity (TAC). TAC was evaluated by a spectrophotometric method, using H₂O₂-metmyoglobin system, which, interacting with the chromogen ABTS, induces the appearance of its radical forms with a latency phase

(LAG) proportional to antioxidant content of the sample^{273,274}. Hormones were measured by enhanced chemiluminescence assay.

The most prevalent hormonal deficiencies were those of IGF-1 (83%) and DHEAS (82%). The association of multiple hormonal deficiencies correlated with levels of NT-proBNP (no deficit, n=5, 882±483.1; one deficit, n=5, 787±307.4, two deficits, n=4, 4199.3±2167.7; three or four deficits, n=7, 7968.8±5123.9 pg/ml). LAG values were significantly elevated in patients with one or more deficit vs. patients with normal hormone pattern (106±11.3 vs. 66.7±6.7 s), but while patients with single hormonal deficiency showed the greatest levels (123.3±6.7), suggesting a compensatory increase in antioxidant systems, while no further increase was observed with the worsening of hormonal picture (two deficits, 106.7±31; three or four deficits 92.5±20.1) (Figure 1).

Therefore, we can hypothesize that OS in early stages can be counteracted by defense mechanisms, but multiple hormone deficiencies are unable to balance the worsening of OS and consequently the course of illness.

Conclusions

These preliminary data, while indicating that multiple hormonal deficiencies are associated with the severity of HF, suggest that an increased antioxidant defense can be observed in patients

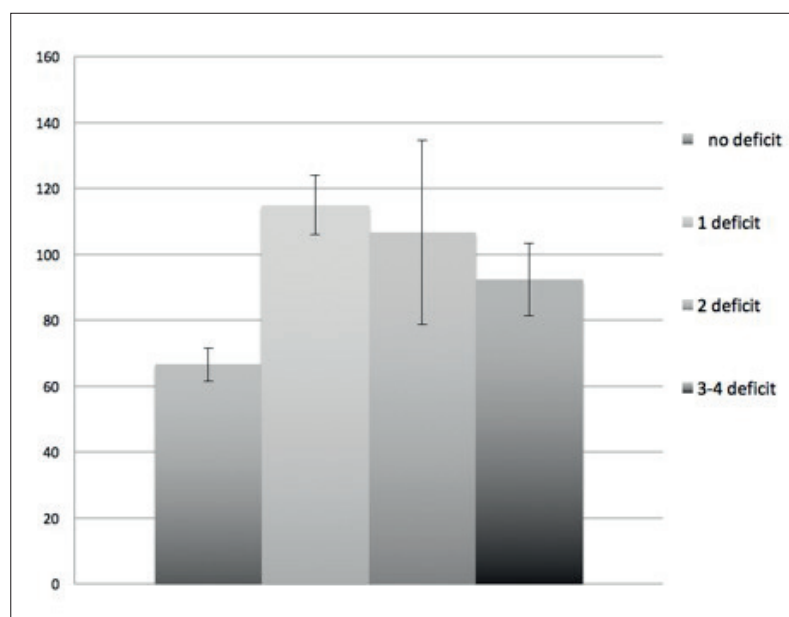


Figure 1. Mean ± SEM values of plasma LAG (measure of total antioxidant capacity, expressed in sec) in patients with chronic heart failure, divided according to the absence or presence of multiple hormone deficits.

with only one anabolic hormone deficiency, but this system could not be effective in contrasting the ingravescence of the hormonal picture, perhaps contributing, in a reciprocal way, to influence hormone levels themselves.

The relevance of the topic of OS and its modulation by hormones confirms the systemic involvement in progression of HF and opens the field of longitudinal researches combining antioxidants administration and hormonal replacement therapies.

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Conflict of interest

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