

Increased Oxidative Stress in Patients With Congestive Heart Failure

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Objectives. We sought to study the markers of lipid peroxidation and defenses against oxidative stress in patients with varying degrees of heart failure.

Background. Despite advances in other areas of cardiovascular disease, the morbidity and mortality from congestive heart failure (CHF) are increasing. Data mainly from animal models suggest that free radical injury may promote myocardial decompensation. However, there are no studies in humans correlating the severity of heart failure with increased free radical injury and antioxidants.

Methods. Fifty-eight patients with CHF and 19 control subjects were studied. In addition to complete clinical and echocardiographic evaluations, the prognosis of these patients was established by measuring the levels of soluble tumor necrosis factor- α receptors 1 and 2 (sTNF-R1 and sTNF-R2). Oxidative stress was evaluated by measuring plasma lipid peroxides (LPO), malondialdehyde (MDA), glutathione peroxidase (GSHPx) and vitamin E and C levels.

Results. The patients' age range, cause of heart failure and drug intake were comparable across the different classes of heart failure. Heart failure resulted in a significant increase in LPO ($p < 0.005$), MDA ($p < 0.005$), sTNF-R1 ($p < 0.005$) and sTNF-R2 ($p < 0.005$). There was a significant positive correlation between the clinical class of heart failure and LPO, MDA, sTNF-R1 and sTNF-R2 levels. There was an inverse correlation between GSHPx and LPO. With increased lipid peroxidation in patients with CHF, the levels of vitamin C decreased, but vitamin E levels were maintained.

Conclusions. These data demonstrate a progressive increase in free radical injury and encroachment on antioxidant reserves with the evolution of heart failure; they also suggest that oxidative stress may be an important determinant of prognosis. The therapeutic benefit of administering antioxidant supplements to patients with CHF should be evaluated.

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Despite a remarkable decline in the death rate from cardiovascular disease over the past 30 years, both the morbidity and mortality from congestive heart failure (CHF) have been steadily increasing (1). There have been considerable advances in our understanding of the functional alterations associated with the early adaptive phases of myocardial hypertrophy and the terminal stages of heart failure; however, critical questions remain about the evolution of cardiac decompensation. Presently available therapeutic interventions have not been shown to substantially improve the long-term survival of patients with dilated cardiomyopathy and CHF. The underlying heart disease is relentlessly progressive in almost all patients who develop symptoms of overt failure, and mortality continues to be unacceptably high (2). Trying to enhance cardiac function during the later stages of heart failure cannot be done over the

long term. The solution lies in defining and preventing the causes of myocardial failure or arresting and reversing its evolution.

Recent investigations suggest that free radicals may be important contributors to the deterioration of the decompensating myocardium (3,4). This finding is not surprising because a number of factors associated with heart failure, such as increased plasma catecholamines (5), cardiac sympathetic tone (6), microvascular reperfusion injury (7,8), cytokine stimulation (9,10) and mitochondrial deoxyribonucleic acid mutations (11) (particularly complex I), are known stimuli for peroxidative damage (12-14). Markers of oxidative stress, such as elevated levels of breath pentane (15) or plasma and urinary malondialdehyde (MDA) (16,17), have been reported. However, there are no studies in human CHF evaluating markers of peroxidation, antioxidant levels, functional class and indexes of prognosis.

In this study, we examined markers of lipid peroxidation and oxidative defenses in patients with different degrees of heart failure. Soluble tumor necrosis factor- α receptors 1 and 2 (sTNF-R1 and sTNF-R2), which have been shown to be indexes of patient prognosis, were also measured to determine the clinical severity of CHF. Our findings strongly support the

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Abbreviations and Acronyms

CHF	= congestive heart failure
GSHPx	= glutathione peroxidase
LPO	= lipid peroxide
MDA	= malondialdehyde
sTNF-R1 and -R2	= soluble tumor necrosis factor receptors 1 and 2

adverse effects of free radicals in human CHF and suggest that antioxidant supplements may be of therapeutic benefit.

Methods

Study group. Fifty-eight patients (43 men, 15 women) with CHF and 19 control subjects (12 men, 7 women) were studied (Table 1). Patients with recent cardiac events such as myocardial infarction, those on dialysis, those with decompensated liver disease or malignancy and those taking antioxidant vitamin supplements were excluded. Control subjects were healthy hospital staff volunteers of the same age and gender mix and with no history of diabetes or lipid abnormalities. The Ethics Committee of the University of Toronto approved the study protocols; written informed consent was obtained from all study participants.

Clinical assessment. A detailed clinical assessment was done by one investigator (A.G.) using a standardized format. The diagnosis of heart failure was confirmed in all patients by clinical and noninvasive assessment of myocardial function. Echocardiograms were available for detailed review in 48 patients. Left ventricular ejection fraction, left ventricular end-systolic diameter and left ventricular end-diastolic diameter were measured by one investigator (A.O.) who had no knowledge of the clinical state or laboratory data. Previous reproducibility studies performed in the Toronto Hospital Echocardiography Laboratory have determined a retest variability (95% limits) of 3 mm in left ventricular end-diastolic diameter.

Blood sampling. Immediately after the initial interview, 20 ml of blood was obtained by venipuncture and was centrifuged at 3,000 rpm at 4°C for 7 min (Sorval refrigerated centrifuge, Sorval Instruments), and the plasma was separated and stored in a frozen state at -20°C for analysis. All measurements of lipid peroxide (LPO) were done within 1

month of drawing the blood. In separate experiments we found that in 10 normal subjects, LPO levels measured on the same day that the blood was drawn was $2.22 \pm 0.19 \mu\text{mol/liter}$, and after 1 month of storage in the conditions described previously, the levels were unchanged at $2.22 \pm 0.20 \mu\text{mol/liter}$.

Measurement of LPO. Lipid peroxides were measured using a commercially available kit (Kamiya Biomedical Co.) (18,19). Absorbance was measured spectrophotometrically at 675 nm using a Hitachi U2000 spectrophotometer.

Analysis of MDA. The procedure used was adapted from that of Draper et al. (20,21). Briefly, samples were injected into a high performance liquid chromatography column (Waters C18) with the UV/Vis variable detector (Shimadzu, SPD-6) set at 532 nm. An integrator (Shimadzu CR-3A) was connected to the detector and net peak areas were calculated. 1,1,3,3-Tetraethoxypropane (Sigma Co.) was used as the MDA standard.

Plasma tocopherols. Plasma vitamin E was analyzed by a modification of the method of Bieri et al. (22). A reverse-phase high pressure liquid chromatographic system (LC-6A, Shimadzu) with a C18 column was used for the separation of tocopherols. The concentration of the vitamin in the sample was calculated from the peak areas of standard and sample curves. Although plasma vitamin E represents only a small proportion of total body membrane-bound stores, nonfasting plasma vitamin E, uncorrected for plasma lipoproteins, appears to be an excellent reflection of an individual's actual vitamin E level (23,24).

Glutathione peroxidase activity. The activity of the selenium-dependent glutathione peroxidase (GSHPx) in plasma was measured by using the coupled assay procedure of Paglia and Valentine (25), as modified in our previous report (26).

Vitamin C (ascorbic acid). The method used determines the total biologically active vitamin C (L-ascorbic acid and dehydro-L-ascorbic acid) colorimetrically (27). The color density was read at 521.0 nm against a reagent blank and compared with a calibration curve of known standards.

Measurement of sTNF-R1 and sTNF-R2. Human sTNF-R1 (p55) and sTNF-R2 (p75) were measured in 61 patients, using commercially available enzyme-linked immunosorbent assay kits (Quantikine, R&D Systems). The standards were prepared from 7.8 to 500 pg/ml for sTNF receptors. All samples were run in duplicate.

Table 1. Characteristics of Patients With Heart Failure

NYHA Functional Class	No. of Patients	Gender (M/F)	Age (years)	Ejection Fraction (%)
I	9	6/3	63.7 ± 7.5	40.6 ± 5.8 (n = 8)
II	19	13/6	54.6 ± 2.44	33.5 ± 3.1 (n = 19)
III	18	15/3	60.0 ± 4.6	25.1 ± 2.4 (n = 14)
IV	12	9/3	61.5 ± 3.5	30.0 ± 4.5 (n = 8)

Data are presented as number of patients or mean value ± SEM. F = female; M = male; NYHA = New York Heart Association.

Table 2. Cardiac Medications in Patients in Each Functional Class

	NYHA Functional Class			
	I (n = 9)	II (n = 19)	III (n = 18)	IV (n = 12)
ACE inhibitor*	8 (0.89)	14 (0.74)	14 (0.78)	8 (0.67)
Diuretic agent†	4 (0.44)	16 (0.84)	16 (0.89)	10 (0.83)
Digoxin	6 (0.67)	11 (0.58)	11 (0.61)	6 (0.5)
Beta-blocker	1 (0.11)	5 (0.26)	5 (0.28)	3 (0.25)
Amiodarone	2 (0.23)	4 (0.21)	0 (0.00)	3 (0.23)
ASA	5 (0.55)	6 (0.32)	8 (0.44)	5 (0.42)

*Includes various angiotensin-converting enzyme (ACE) inhibitors. †Includes loop and thiazide diuretic agents. Data are presented as number (%) of patients. ASA = acetylsalicylic acid; NYHA = New York Heart Association.

Statistical analysis. Data are presented as the mean value \pm SEM. The significance of the differences between control subjects and patients in the different heart failure classes was determined by the nonparametric Kruskal-Wallis test. The relations of New York Heart Association functional class heart failure to various variables of oxidative stress were analyzed by the Spearman rank correlation. The differences in age, the pattern of the drug intake and the comorbid conditions among the different heart failure classes were tested using the chi-square test.

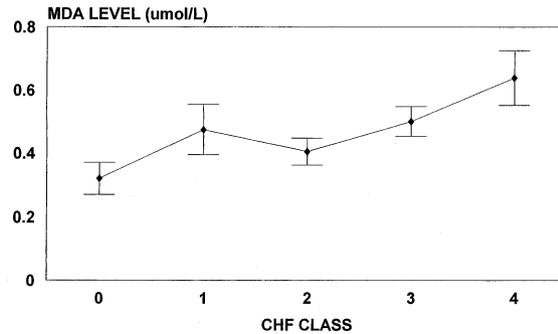
Results

Clinical features. *Age.* The age distributions across the heart failure classes were not significantly different (Table 1).

Causes of heart failure. The most common cause of heart failure in these patients was ischemic heart disease, with the second most common cause being idiopathic cardiomyopathy. The distribution of the disease across different classes of heart failure was not significant (chi-square 0.6662, *df* 12).

Comorbid conditions. The most frequent comorbid conditions were diabetes, hypertension and hyperlipidemia. The distribution of these comorbid conditions was not significantly different among heart failure groups (chi-square 0.1144, *df* 21). Only two patients smoked.

Medication intake. Review of the medications disclosed that the majority of the patients were taking angiotensin-

**Figure 2.** Relation of plasma MDA levels to heart failure functional class.

converting enzyme inhibitors and diuretic agents. A significant number of patients were also taking digoxin and aspirin (Table 2). The intake of angiotensin-converting enzyme inhibitor, acetylsalicylic acid, furosemide, beta-blockers, inotropic agents and amiodarone was not significantly different between the heart failure groups.

Markers of oxidative stress and their relation to heart failure. *LPO and heart failure.* The LPO levels were significantly different between control subjects and patients with CHF ($p < 0.005$). There was a significant relation between the patients' LPO levels and functional class ($r_s = 0.75$, $p < 0.01$) (Fig. 1).

MDA and heart failure. The MDA levels were significantly different between control subjects and patients with CHF ($p < 0.005$). There was a significant relation between the patients' MDA levels and functional class ($r_s = 0.37$, $p < 0.01$) (Fig. 2).

LPO in relation to cardiac function. In contrast to the relation of oxidative variables to functional class, there were no statistically significant correlations between LPO and ejection fraction or left ventricular function or dimensions as determined by echocardiography.

Antioxidant levels. *GSHPx.* The levels of this enzyme varied inversely with the levels of LPO ($r_s = -0.241$, $p < 0.051$) (Fig. 3). There was no significant relation between GSHPx levels and the severity of heart failure (Table 3).

Vitamins C and E levels. The level of vitamin E did not differ between patients with CHF and control subjects (Table

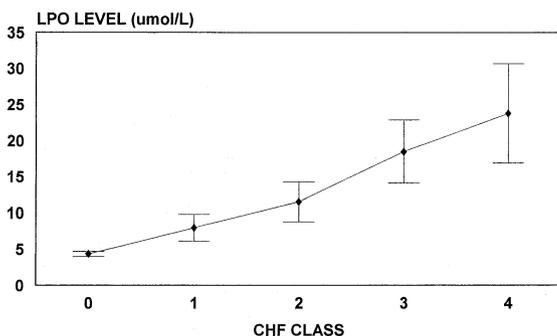
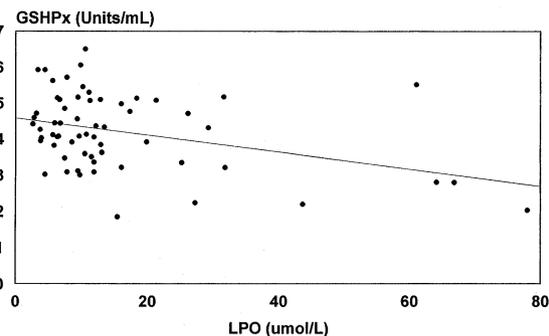
Figure 1. Relation of plasma LPO levels to heart failure functional class.**Figure 3.** Relation of plasma GSHPx activity to LPO levels.

Table 3. Antioxidant Levels and New York Heart Association Functional Class

	Vitamin C ($\mu\text{mol/liter}$)	Alpha-Tocopherol ($\mu\text{mol/liter}$)	GSHPx (U)*
Control subjects	71.4 \pm 3.8	11.3 \pm 1.0	5.4 \pm 0.27†‡
Patients in class I or II	63.3 \pm 8.4	14.2 \pm 1.0	4.5 \pm 0.2
Patients in class III or IV	61.1 \pm 6.8	14.3 \pm 0.7	4.2 \pm 0.2

*See Methods for definition of units. †Data previously published from our laboratory (38). ‡ $p < 0.01$ compared with patients with congestive heart failure. Data are presented as mean value \pm SEM. GSHPx = glutathione peroxidase.

3). However, vitamin C levels were significantly higher in control subjects than in patients with CHF (Table 3).

TNF- α , sTNF-R1 and sTNF-R2 levels. The sTNF-R1 ($p < 0.005$) and sTNF-R2 ($p < 0.005$) levels were significantly different between control subjects and patients with CHF. There was a rise in both sTNF-R1 and sTNF-R2 with the functional class (sTNF-R1: $r_s = 0.31$, $p < 0.02$; TNF-R2: $r_s = 0.4$, $p < 0.01$) (Fig. 4 and 5). There was a significant correlation between sTNF-R1 and MDA levels ($r_s = 0.36$, $p < 0.0001$), sTNF-R2 and MDA levels ($r_s = 0.514$, $p < 0.0002$), sTNF-R1 and LPO levels ($r_s = 0.313$, $p < 0.04$) and sTNF-R2 and LPO levels ($r_s = 0.227$, $p < 0.05$).

Discussion

Sources of oxidative stress in CHF. The formation of LPDs, through oxidative destruction of polyunsaturated fatty acids within cell membranes, is an important mechanism of free radical-mediated cellular injury (28). This process is initiated by the extraction of hydrogen atoms (H^+) from polyunsaturated fatty acids to form fatty acid radicals. These radicals, in turn, react with oxygen to form fatty acid dioxy radicals, which then react with other lipids and to a lesser extent with other cell constituents (proteins, nucleic acids), propagating the transfer of electrons (i.e., free radical formation) and perpetuating a chain reaction destructive to cell membranes and molecules. The process ultimately terminates in the formation of stable products such as MDA. It can also be terminated by an enzymatic reduction of the lipid hydroperoxides by antioxidant enzymes such as GSHPx or by free radical scavengers. The most important scavenger in cell membranes

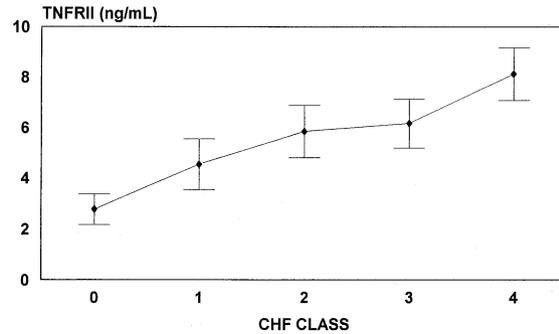


Figure 5. Relation of sTNF-R2 to heart failure functional class.

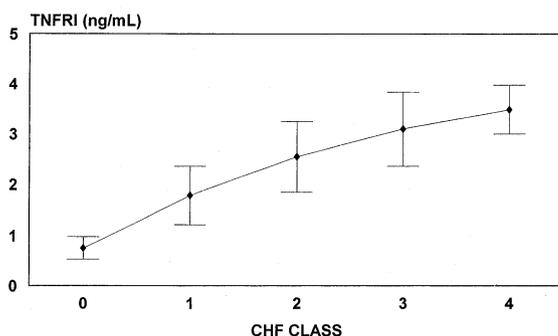
is vitamin E (alpha-tocopherol); this nutritional antioxidant is dependent on vitamin C for its regeneration (29). The generation of these products and their level in blood and tissues (LPO and MDA) are a measure of free radical injury (28).

In the present study, we have shown a significant increase in the plasma level of LPO and MDA in patients with CHF. The data were similar when examined separately in patients with ischemic cardiomyopathy ($n = 37$) or dilated cardiomyopathy ($n = 24$). The increase in LPO and MDA was related to the functional severity of heart failure, with the highest levels being observed in patients in functional class III and IV. Only two of our patients had renal insufficiency and three had hepatic insufficiency; therefore, impairment of MDA and LPO metabolism or clearance is unlikely to have contributed to our observations.

Our data do not show the tissue origins of the markers of increased lipid peroxidation; both poorly perfused peripheral muscles and the myocardium (3,30-33) could have contributed. Poor nutritional status and increased metabolic rate (34) may also play a role. Similar to the observations of Ferrari et al. (35), we also found a rise in both sTNF-R1 and sTNF-R2 in relation to the functional class. Increased sTNF receptor levels have been shown to have adverse functional consequences. Kapadia et al. (36) have shown that these receptors modulate the negative inotropic effect of TNF- α , and Ferrari et al. (33) have shown that "sTNF-R2 was a more powerful independent indicator of mortality than TNF- α , sTNF-R1, NYHA class, norepinephrine and atrial natriuretic peptide." In this study, we have shown a progressive rise in sTNF receptor levels and markers of lipid peroxidation, as well as decreased antioxidant reserves with increasing severity of CHF. Thus, there is strong circumstantial evidence that oxidative stress is a prognostic factor in CHF.

Our patients not only had evidence of increased lipid peroxidation, but also had changes that showed that increased lipid peroxidation in relation to functional class was of biologic significance, because it inversely correlated with GSHPx levels and a reduction in vitamin C. These observations in our patients indicated that vitamin C may have been consumed to regenerate vitamin E and that GSHPx was buffering increasing peroxidation. Similar findings were noted in subjects with increased peroxidation due to smoking (37); giving large doses

Figure 4. Relation of sTNF-R1 to heart failure functional class.



of vitamin E increased GSHPx levels. Therefore, these data support the possibility that in heart failure, increasing levels of peroxidation result in a relative deficiency of antioxidant factors.

Conclusions. Free radical injury is increased and antioxidant reserves decreased in patients with CHF. This increase correlates with functional class and with an objective marker of prognosis—namely, sTNF receptors. These observations provide strong evidence for an adverse role of oxidative stress in CHF and suggest that antioxidant supplementation may improve myocyte function or survival, or both, and thus prove to be an important addition to our contemporary treatment of patients with CHF.

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