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*Original Citation:*

Increased renal formation of thromboxane A 2 and prostaglandin F 2 $\alpha$  in heart failure / S. Castellani; B. Paladini; R. Paniccia; C. Di Serio; B. Vallotti; A. Ungar; S. Fumagalli; C. Cantini; L. Poggesi; G.G. Neri Serneri. - In: AMERICAN HEART JOURNAL. - ISSN 0002-8703. - STAMPA. - 133:(1997), pp. 94-100. [10.1016/S0002-8703(97)70253-9]

*Availability:*

The webpage <https://hdl.handle.net/2158/771342> of the repository was last updated on

*Published version:*

DOI: 10.1016/S0002-8703(97)70253-9

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## Congestive Heart Failure

# Increased renal formation of thromboxane A<sub>2</sub> and prostaglandin F<sub>2α</sub> in heart failure

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**Renal formation of the vasoconstrictor prostaglandins thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) was investigated in 25 patients with cardiac failure, divided into New York Heart Association functional classes I to IV, and in eight healthy control subjects. Plasma renin activity (PRA) and hemodynamic parameters were also investigated. Renal vasoconstrictor eicosanoid formation, measured in urinary daily excretion, was not different between patients in class I and control subjects. Class II to IV patients showed progressively increasing production of PGF<sub>2α</sub> (F = 49.8, *p* < 0.001, analysis of variance) and TXA<sub>2</sub> (F = 37.8, *p* < 0.002). PGF<sub>2α</sub> excretion peaked in class IV (+1266% vs class I, *p* < 0.001). Compared with class I, urinary excretion of thromboxane B<sub>2</sub> was +816% in class III and +1561% in class IV (both *p* < 0.001). PRA was significantly increased only in class IV (+1558%, *p* < 0.001). The current results indicate a progressive increase in renal production of vasoconstrictor eicosanoids directly related to New York Heart Association class and suggest that these prostanoids may have a role in deterioration of renal function. (*Am Heart J* 1997;133:94-100.)**

The long-term outcome of patients with congestive heart failure (CHF) depends on progressive deterioration of ventricular performance and on neurohormonal reactions set in motion by the disease.<sup>1</sup> Sympathetic activation and enhanced activity of different vasoconstrictor systems, including the renin-angiotensin system and vasopressin, play a major role in maintaining perfusion pressure and blood volume.<sup>2</sup> The increased formation of vasoconstrictive mediators is associated with enhanced formation of vasodilating factors, such as atrial natriuretic peptide and renal prostaglandins E<sub>2</sub> and I<sub>2</sub>.<sup>3,4</sup> Their increase pre-

vents the deleterious effect of a nonmodulated vasoconstrictor system and maintains renal filtration.<sup>4</sup>

Whereas the synthesis of vasodilating eicosanoids is known to increase in heart failure,<sup>4</sup> the renal synthesis of vasoconstrictive prostanoids (i.e., prostaglandin F<sub>2α</sub> [PGF<sub>2α</sub>] and thromboxane A<sub>2</sub> [TXA<sub>2</sub>]) has not yet been investigated in cardiac failure despite their relevant vasoconstrictor activity. In animal studies, PGF<sub>2α</sub> has been shown to facilitate the response of sympathetic nerve stimulation and to increase responsiveness to norepinephrine.<sup>5</sup> Moreover, when systemically injected, it provokes an increase in blood pressure in animals and in human beings.<sup>6</sup> TXA<sub>2</sub> exhibits very potent constrictive effects in different vascular beds, including the renal bed, and within the kidney it causes a decrease in renal blood flow and glomerular filtration rate.<sup>7</sup> Thus, although in healthy subjects neither TXA<sub>2</sub> nor PGF<sub>2α</sub> seems to be involved in the maintenance of renal function,<sup>6,8</sup> their enhanced renal formation, particularly that of TXA<sub>2</sub>, may play a significant role in reducing renal perfusion and glomerular filtration in pathologic circumstances.<sup>9,10</sup>

The TXA<sub>2</sub> and PGF<sub>2α</sub> synthesized by the kidney are excreted in the urine as thromboxane B<sub>2</sub> (TXB<sub>2</sub>) and PGF<sub>2α</sub>. Therefore renal production of these prostaglandins can be evaluated by measuring their urinary excretion.<sup>11,12</sup> The aim of this study was to investigate the role of renal TXA<sub>2</sub> and PGF<sub>2α</sub> in patients affected by CHF with different degrees of disease severity according to New York Heart Association (NYHA) classification.<sup>13</sup>

## METHODS

**Study subjects and patient selection.** Twenty-five women affected by CHF (aged 28 to 79 years; mean age 46 ± 33 years) and 8 healthy, age-matched female control subjects (aged 26 to 82 years; mean age 51 ± 25 years) were studied. Only female subjects were studied because

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Received for publication March 19, 1996; accepted July 2, 1996.

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0002-8703/97/\$5.00 + 0 4/177198

renal prostaglandins were to be measured in urine, which may have an extrarenal origin in males.<sup>14</sup> None of the subjects was taking oral contraceptives. Patients were considered eligible for the study if CHF had been diagnosed by clinical examination or by echocardiographic or other instrumental evaluation (chest radiography or cardiac radioisotopic scanning). Exclusion criteria included diabetes, hypertension, liver disease, or malignant neoplasm as well as any primary or secondary cause of renal disease according to the results of clinical examination or of renal ultrasound examination with serum creatinine levels at  $\geq 2.0$  mg/dl and positive results on urinalysis. The types of heart disease underlying cardiac failure and their percentages in the different classes are described in Table I. Patients were subdivided into four functional classes.<sup>13</sup> All subjects refrained from taking nonsteroid antiinflammatory drugs for 15 days before being examined. All subjects gave their informed consent before participating in the study.

### Study protocol

**Experimental procedure.** In all patients, diuretic agents and angiotensin-converting enzyme inhibitors were withdrawn 48 hours before urine collection. Patients of class IV received close clinical surveillance. Among class IV patients, only those whose condition had remained stable after the 12-hour drug withdrawal period were admitted into the study and completed the 48-hour washout period according to the study protocol.

For the total period of hospitalization, the subjects received a diet that contained 108 mmol sodium chloride and 60 to 80 mmol potassium per day. Daily water intake was measured and controlled. At admission, after a 48-hour period of pharmacologic washout, 24-hour urine samples were collected for eicosanoid (urinary  $\text{PGF}_{2\alpha}$  and urinary  $\text{TXB}_2$ ), creatinine, and electrolyte (sodium and potassium) determinations. Venous blood samples were collected for serum electrolyte, serum creatinine, and plasma renin activity (PRA) determinations at 8:00 AM after overnight bed rest. Glass tubes devoid of anticoagulants (Vacutainer Systems, Becton Dickinson, Heylan Cedex, France) were used to collect the blood for serum electrolyte and creatinine determinations; blood samples for PRA were collected in cold glass tubes that contained 0.037 mol/L ethylenediamine tetracetic acid. Urinary collection and blood sampling were repeated whenever changes in clinical findings indicated that the patient's functional NYHA class had changed.

**Systemic hemodynamic parameters.** Arm blood pressure was measured with a sphygmomanometer. The first Korotkoff sound was taken as an index of systolic blood pressure (SBP) and the fifth as an index of diastolic blood pressure (DBP). Mean blood pressure was obtained by adding one third of the pulse pressure to the value of the diastolic pressure. An average of six blood pressure and heart rate recordings (measured at 6:00, 8:00, and 12:00 AM and at 4:00, 8:00, and 12:00 PM) was taken as an index of the 24-hour study period. If tricuspid regurgitation was present, mean pulmonary artery pressure ( $\text{PAP}_m$ ) was determined by Doppler echocardiography according to the method described by Currie et al.,<sup>15</sup> given that values thus obtained correlate well with those from invasive techniques. Only

when tricuspid regurgitation was not present,  $\text{PAP}_m$  was calculated by estimating pulmonary acceleration time, which has been shown to be directly and strongly correlated with  $\text{PAP}_m$ .<sup>16</sup> Venous pressure (VP) was determined by a direct method.<sup>17</sup> Pressure values were expressed as centimeters of water and were then converted into millimeters of mercury by dividing by a factor of 1.36. The values obtained by this method exhibit a high degree of correlation with the measurements in the right atrium as obtained by heart catheterization.<sup>17</sup>

**Sodium balance and volume status.** Variations in water and sodium metabolism were assessed during the hospitalization period by daily evaluation of serum and urinary electrolytes (sodium and potassium), patient weight, and water balance. Urinary and serum sodium and potassium were measured with a flame photometer (System 243 Instrumentation Laboratory, Lexington, Mass.).

**Plasma renin activity.** PRA was determined by radioimmunoassay with a commercially available kit (Angiotensina RIA CT, RADIM, Rome, Italy) according to Haber et al.<sup>18</sup> The intraassay coefficient of variation was 7.5% at 2.3 pg/ml, 5.4% at 8.8 pg/ml, and 9.9% at 13.5 pg/ml. The interassay coefficient of variation was 7.7% at 2.6 pg/ml, 8.1% at 8.6 pg/ml, and 11.5% at 13 pg/ml. The detection limit of the method is 0.15 ng/ml/hr, and the standard curve ranges between 0.15 and 50 ng/ml/hr.

**Urinary prostaglandin assay.** Immediately after collection, the urine was frozen and stored at  $-20^\circ\text{C}$  until extraction and purification procedures could be performed. Renal  $\text{PGF}_{2\alpha}$  and  $\text{TXB}_2$  were measured by radioimmunoassay after extraction from urinary samples by using an organic solvent and chromatographic purification in a silicic acid column. Details of the method have been reported elsewhere.<sup>19</sup> Carlo Patrono (Istituto di Farmacologia, Università di Chieti, Italy) provided the antibodies to  $\text{PGF}_{2\alpha}$ , obtained from the guinea pig. The antibody to  $\text{TXB}_2$  was provided by Luciano Caprino (Istituto di Igiene, Università Cattolica del Sacro Cuore, Rome, Italy). The average calculated recovery was  $58\% \pm 15\%$  for  $\text{PGF}_{2\alpha}$  and  $60\% \pm 10\%$  for  $\text{TXB}_2$ . The intraassay and interassay coefficients of variation were 9% and 10%, respectively. The data are expressed as picograms per gram urinary creatinine (UCr) per 24-hour period. Normalization was aimed at correcting any change in renal function in the various study groups.

**Statistical analysis.** Results are expressed as mean values  $\pm$  SD. Student *t* test was used to compare healthy subjects with patients of NYHA class I. Comparison among patient groups was performed by analysis of variance (ANOVA). Simple linear correlation (Pearson) was used to evaluate the relation among variables. If a variable was found to be significantly related to multiple dependent variables on linear correlation analysis, multiple regression analysis was carried out to rule out spurious correlations and to assess the best relation between different predictor variables and a dependent variable. Statistical significance was set at  $p < 0.05$ .

## RESULTS

No significant differences in hemodynamics, urinary volume, or urinary electrolytes were found be-

**Table I.** Etiology of heart disease and patients characteristics

Etiology	NYHA class			
	I (n = 6)	II (n = 6)	III (n = 7)	IV (n = 6)
Ischemic heart disease	n = 15 (60%)			
Dilated cardiomyopathy	n = 6 (24%)			
Valvular heart disease	n = 4 (16%)			
Patient characteristics				
Blood urea nitrogen (mg/dl)	0.5 ± 0.09	0.3 ± 0.06	0.4 ± 0.04	0.9 ± 0.05
Serum creatinine (mg/dl)	1.20 ± 0.28	0.96 ± 0.19	1.15 ± 0.13	1.46 ± 0.27
Serum sodium (mmol/dl)	140.7 ± 2.4	139.6 ± 2.3	138.8 ± 4.8	137.7 ± 3.2
Medication at time of 48 hr washout				
Digoxin (0.125-0.25 mg uid)	50%	66.7%	85.7%	83.3%
Furosemide (25 mg orally from uid to bid)	16.7%	16.7%	85.7%	100%
Potassium canrenoate (100 mg orally bid)	16.7%	33.3%	57.1%	50%
Captopril (25 mg uid)	0%	16.7%	28.6%	66.7%
Ramipril (2.5-5 mg uid)	16.7%	0%	0%	0%

bid, Two times per day.

uid, Once a day.

\*According to clinical status.

**Table II.** Hemodynamic variables among NYHA classes

	NYHA class				ANOVA for all patient groups	
	I (n = 6)	II (n = 6)	III (n = 7)	IV (n = 6)	F	p Value
SBP (mm Hg)	127.5 ± 3.5	126.9 ± 26.6	126.9 ± 10.3	115 ± 12.9	0.45	NS
DBP (mm Hg)	75 ± 7	72.5 ± 10.7	73.7 ± 10.3	78.7 ± 13.1	0.30	NS
PAP <sub>m</sub> (mm Hg)	28.8 ± 8.2	30.5 ± 5.4	41.7 ± 10.4*	58.8 ± 9.9†	14.7	<0.001
		‡				
		§				
VP (mm Hg)	5.2 ± 1.8	7.1 ± 1.6	9.2 ± 2.9*	11.9 ± 5.0†	5.2	<0.03
		‡				

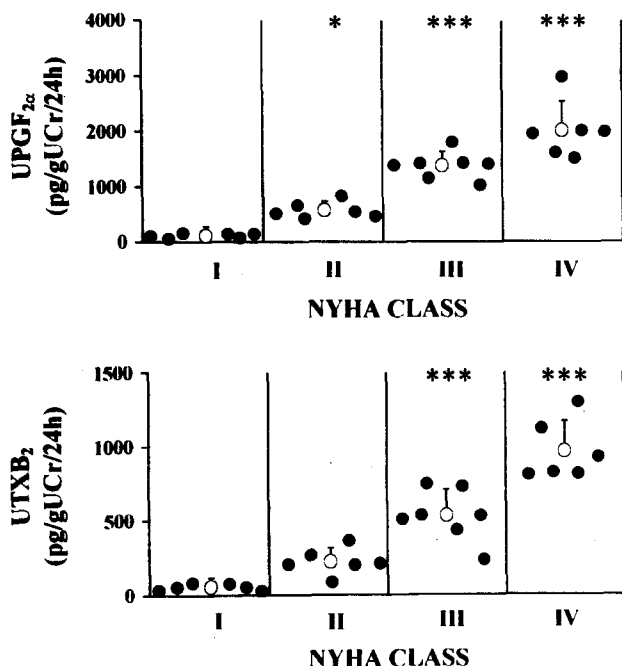
NS, Not significant. \* $p < 0.03$ , † $p < 0.001$  vs class I by Duncan ANOVA.‡ $p < 0.03$  and § $p < 0.001$  between classes by Duncan ANOVA.

tween healthy subjects and patients of NYHA class I (data not shown). SBP and DBP were similar in all patient groups (Table II). In healthy control subjects, average PGF<sub>2α</sub> urinary excretion was 374.8 ± 176.7 pg/gm UCr/24 hr and was not significantly different from that observed in class I patients (148.2 ± 52.6 pg/gm UCr/24 hr). Urinary excretion of PGF<sub>2α</sub> was significantly increased in class II patients compared with healthy control subjects and class I patients (+290%,  $p < 0.03$ ) (Table III and Fig. 1). Urinary excretion of PGF<sub>2α</sub> was higher in class III and IV patients than in class I patients (+835%,  $p < 0.001$  and +1266%,  $p < 0.001$ , for classes III and IV, respectively) and higher than in class II patients (+139.6%,  $p < 0.001$  and +250%,  $p < 0.001$ , for classes III and IV, respectively). Thus greater disease severity by NYHA functional class was associated with a progressive and significant increase in PGF<sub>2α</sub> excretion ( $F = 49.8$ ,  $p < 0.001$ , by ANOVA) (Table III and Fig. 1).

The average TXB<sub>2</sub> urinary excretion in control subjects was 98.1 ± 54.1 pg/gm UCr/24 hr. This value was not significantly different from that either in class I patients (58.2 ± 43.3 pg/gm UCr/24 hr) or in class II patients (214.3 ± 168 pg/gm UCr/24 hr) (Table III and Fig. 1). Urinary excretion of TXB<sub>2</sub> was markedly higher in class III patients (+816%,  $p < 0.001$  vs class I patients; +149%,  $p < 0.002$  vs class II patients) and even more so in class IV patients (+1561%,  $p < 0.001$  vs class I patients; +351%,  $p < 0.001$  vs class II patients). Thus, with greater disease severity according to NYHA class, TXB<sub>2</sub> excretion was progressively higher ( $F = 37.8$ ,  $p < 0.001$ ) (Table III).

In NYHA class III and IV patients, urinary TXB<sub>2</sub> was linearly related to PAP<sub>m</sub> ( $r = 0.62$ ,  $p < 0.02$ ) and PRA ( $r = 0.67$ ,  $p < 0.01$ ) (Fig. 2).

A progressive increase in PAP<sub>m</sub> and VP was also found in relation to functional class. The increase



**Fig. 1.** Urinary vasoconstrictive prostaglandins vs NYHA class.  $UPGF_{2\alpha}$ , urinary prostaglandin  $F_{2\alpha}$ ;  $UTXB_2$ , urinary thromboxane  $B_2$ . \* $p < 0.03$  and \*\*\* $p < 0.001$  vs patients of NYHA class I (ANOVA). Individual values are shown as solid circles; mean values are open circles.

was significant in NYHA class III and even more significant in class IV patients ( $PAP_m$  and VP,  $p < 0.001$  vs class I patients) (Table II and Fig. 3). Urinary volume decreased in class III and IV patients in comparison with control subjects and class I patients ( $p < 0.03$  for classes III and IV vs class I patients) (Table III). Urinary sodium was lower only in class III and IV patients ( $p < 0.03$  and  $p < 0.002$  respectively vs class I patients).

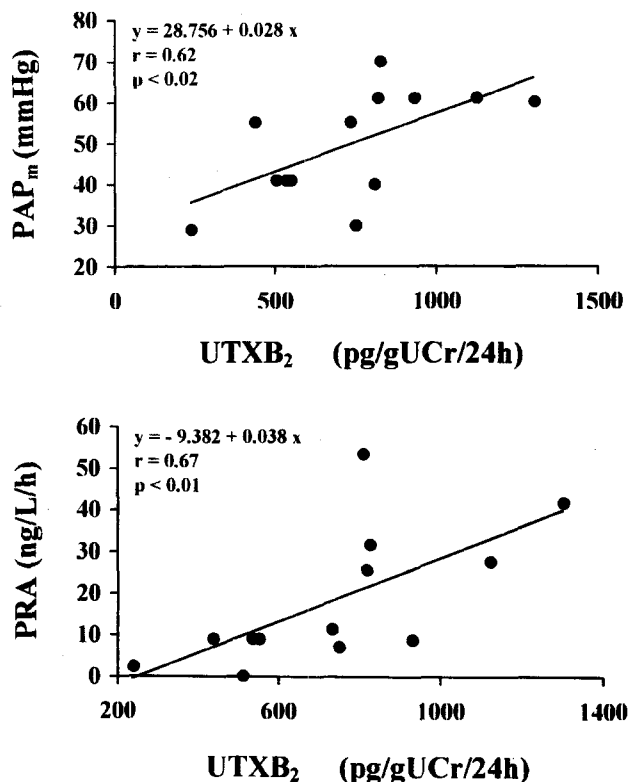
There was a consistent trend toward higher values of PRA in relation to greater severity by functional class, but the increase was significant only in class IV patients ( $p < 0.001$  vs class I patients) (Table III).

Multiple regression analysis indicated that urinary  $PGF_{2\alpha}$  was positively related to  $PAP_m$  ( $\beta = 0.65$ ,  $p < 0.001$ ), but not to VP (Fig. 4). Renal excretion of  $TXB_2$  correlated with  $PAP_m$  ( $\beta = 0.61$ ,  $p < 0.001$ ) and more weakly with VP ( $\beta = 0.33$ ,  $p < 0.04$ ) (Fig. 4). Neither urinary  $PGF_{2\alpha}$  nor  $TXB_2$  was related to SBP or DBP.

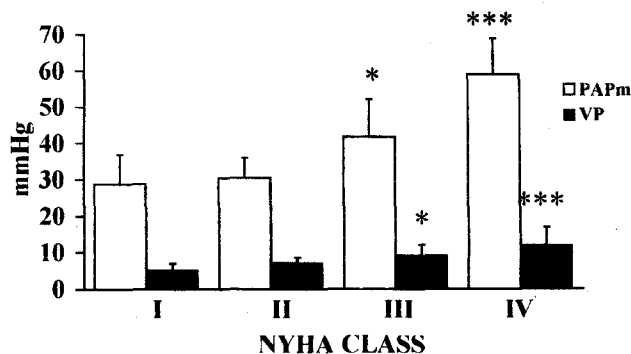
## DISCUSSION

Results of this study indicate for the first time that renal excretion of vasoconstrictor eicosanoids  $PGF_{2\alpha}$  and  $TXB_2$  is enhanced in patients with cardiac failure and parallels the severity of heart disease as evaluated by clinical data and  $PAP_m$  measurement.

The increase in  $PGF_{2\alpha}$  excretion was already evi-



**Fig. 2.** Regression analysis of relation between  $PAP_m$  and urinary thromboxane  $B_2$  ( $UTXB_2$ ) and relation between PRA and  $UTXB_2$  in patients of NYHA classes III and IV.



**Fig. 3.**  $PAP_m$  and VP vs NYHA class. \* $p < 0.03$  and \*\*\* $p < 0.001$  vs patients of NYHA class I (ANOVA).

dent in class II patients, whereas  $TXB_2$  excretion showed noteworthy increases only in class III and IV patients. However, both eicosanoids showed a strong correlation with  $PAP_m$  ( $\beta = 0.85$ ,  $p < 0.001$  for  $PGF_{2\alpha}$ ;  $\beta = 0.80$ ,  $p < 0.001$  for  $TXB_2$ ), suggesting that the progressive impairment of cardiocirculatory function may be important in causing increases in vasoconstrictor renal eicosanoid formation.

Because urinary excretion of  $PGF_{2\alpha}$  and  $TXB_2$  reflect their renal synthesis<sup>11, 12</sup> and increased formation of the vasodilating prostaglandins, prostaglan-

**Table III.** Hormonal variables, diuresis, and sodium among NYHA classes

	NYHA class				ANOVA for all patient groups	
	I (n = 6)	II (n = 6)	III (n = 7)	IV (n = 6)	F	p Value
Urinary PGF <sub>2α</sub> (pg/gm UC/r/24 hr)	148.2 ± 52.6	578.3 ± 103.8*	1385.6 ± 245.5‡	2023.7 ± 522.4‡	49.8	<0.001
		§				
		§				
			§			
Urinary TXB <sub>2</sub> (pg/gm UC/r/24 hr)	58.2 ± 43.3	214.3 ± 168.0	533.1 ± 174.6‡	966.7 ± 202.3‡	37.8	<0.001
		§				
			§			
PRA (ng/L/hr)	1.9 ± 1.3	2.3 ± 3.4	7.0 ± 4.1	31.5 ± 15.1‡	19.2	<0.001
		¶				
			¶			
Urinary volume (ml/24 hr)	1008.3 ± 156.3	850.0 ± 327.1	614.3 ± 254.5*	608.3 ± 241.7*	3.69	<0.03
Urinary sodium (mmol/24 hr)	100.0 ± 27.9	86.2 ± 34.2	55.3 ± 19.4*	50.3 ± 10.6†	5.93	<0.004
		§				
		§				

\**p* < 0.03, †*p* < 0.002, ‡*p* < 0.001 vs class I by Duncan ANOVA.§*p* < 0.03, ||*p* < 0.002, ¶*p* < 0.001 between classes by Duncan ANOVA.

din I<sub>2</sub> and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), have already been reported in severe congestive heart failure,<sup>4</sup> the current results indicate that renal prostaglandin system is globally involved in heart failure.

Increased renal formation of PGF<sub>2α</sub> and TXA<sub>2</sub> may be pathophysiologically relevant in cardiac failure. Biologic activity of renal prostaglandins is finely compartmentalized in the kidney and depends on the capacity of cyclooxygenase activity at the site of eicosanoid formation and on the potential of prostaglandin inactivation by prostaglandin dehydrogenases in renal compartments.<sup>20</sup> This functional organization may explain the very precise localization of their renal activity.

PGF<sub>2α</sub> formation is widely and homogeneously distributed among all renal structures, except the renal arteries,<sup>21</sup> and its inactivation takes place preferentially at the cortical level.<sup>20</sup> As a consequence, PGF<sub>2α</sub> may lead to a redistribution of renal blood flow from the more superficial nephrons toward the juxtamedullary nephrons that exhibit the greatest sodium reabsorption.<sup>22</sup> PGF<sub>2α</sub> acts as a vasoconstrictor; there is little evidence of any diuretic and natriuretic activity.<sup>23</sup> Therefore increased renal formation of PGF<sub>2α</sub> may result in enhanced sodium retention as a consequence of its vasoconstrictor activity on more superficial cortical nephrons. The increased PGF<sub>2α</sub> formation in patients of NYHA class II with its consequent slightly antinatriuretic effects probably contributes to the maintenance of effective plasma volume even during the early phases of heart disease and then during the more advanced stages with progressively increasing PGF<sub>2α</sub> formation.

Under physiologic conditions, renal production of TXA<sub>2</sub> is less than PGF<sub>2α</sub> formation, and it occurs mainly in the glomeruli.<sup>24</sup> Molecular biologic studies have indicated that thromboxane receptors are localized primarily within the mesangial cells<sup>25</sup> and in the smooth-muscle cells of the afferent and efferent arterioles<sup>26</sup>; in this way TXA<sub>2</sub> affects glomerular filtration rate both hemodynamically and through a reduction in the ultrafiltration coefficient. Renal TXA<sub>2</sub> acts preferentially at the preglomerular level by selective constriction of the afferent artery, thus leading to a decrease both in effective renal plasma flow and in glomerular filtration rate.<sup>27</sup> Increased TXA<sub>2</sub> renal formation, as found in class III and IV patients, results in a marked antidiuretic and antinatriuretic effect that potentially can help to maintain blood volume by reducing renal blood flow and the glomerular filtration rate.<sup>27</sup> However, detrimental effects on renal function can easily ensue because of the marked decrease in glomerular filtration. Thus, from the pathophysiologic point of view, increased renal formation of PGF<sub>2α</sub> and TXA<sub>2</sub> may result in augmented renal vasoconstriction and sodium retention, which may be transiently useful with regard to effective blood volume but ultimately may be dangerous in relation to renal function.

No conclusions can be drawn regarding the mechanisms responsible for the enhanced renal formation of PGF<sub>2α</sub> and TXA<sub>2</sub>. The increased formation of these prostanoids seems to reflect the progressive activation of the various renal vasoconstrictor systems. The earlier increase in PGF<sub>2α</sub> formation (present in class II patients) than in TXA<sub>2</sub> suggests that two

	$\beta \pm se$	$R^2$	p
PAP <sub>m</sub>	0.65 ± 0.18	0.44	< 0.001
VP	0.29 ± 0.18	0.41	ns
SBP	0.04 ± 0.18	0.46	ns
DBP	-0.14 ± 0.19	0.47	ns
Dependent variable PGF <sub>2α</sub>			

	$\beta \pm se$	$R^2$	p
PAP <sub>m</sub>	0.61 ± 0.15	0.44	< 0.001
VP	0.33 ± 0.15	0.41	< 0.04
SBP	-0.04 ± 0.15	0.46	ns
DBP	0.02 ± 0.16	0.47	ns
Dependent variable UTXB <sub>2</sub>			

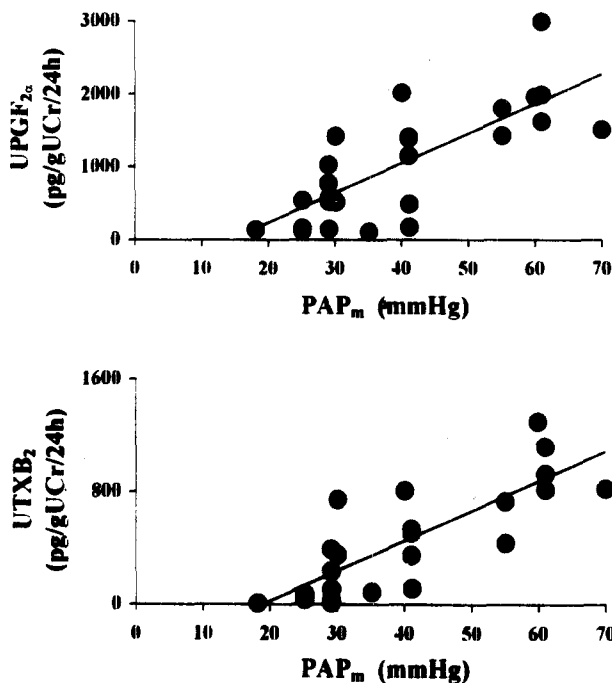


Fig. 4. Stepwise multiple regression analysis of relations between hormonal and hemodynamic factors in all patients. UPGF<sub>2α</sub>, Urinary prostaglandin F<sub>2α</sub>; UTXB<sub>2</sub>, urinary thromboxane B<sub>2</sub>.  $\beta$ , Multiple regression coefficient.

distinct mechanisms may be operating. The progressive lowering of cardiac output is considered to be responsible for the boosting of a broad spectrum of vasoconstrictor mechanisms, including the sympathetic nervous system, renin-angiotensin system, vasopressin, and endothelin systems,<sup>4,28</sup> each of which is able to activate arachidonic acid metabolism directly.<sup>29,30</sup> The sympathetic activation in the course of cardiac failure<sup>4</sup> is probably responsible for the early increase in PGF<sub>2α</sub> formation. Catecholamines are able to enhance the activity of phospholipase A and as a consequence to activate the arachidonic acid metabolism causing the global increase in renal formation of PGF<sub>2α</sub>, PGE<sub>2</sub>, and prostaglandin I<sub>2</sub> (PGI<sub>2</sub>).<sup>29</sup> At this early phase of heart failure, the activation of the renal prostaglandin systems still seems to be well balanced between vasodilating PGI<sub>2</sub> and PGE<sub>2</sub> and the vasoconstrictor PGF<sub>2α</sub> because TXA<sub>2</sub> formation was found to occur in the later stages (class III and especially class IV) (Table III and Fig. 1).

After activation of arachidonic acid, PGF<sub>2α</sub> can be formed directly from prostaglandin H<sub>2</sub> either by a reductase<sup>31</sup> or through the transformation of PGE<sub>2</sub> to PGF<sub>2α</sub> by PGE<sub>2</sub>-9-ketoreductase.<sup>32</sup> In addition, transformation of prostaglandin D<sub>2</sub> by 11-ketoreductase gives rise to an epimer of PGF<sub>2α</sub>, 9 $\alpha$ ,11 $\beta$ -PGF<sub>2α</sub>, that has been found in human beings.<sup>33</sup> This prostaglandin cross reacts with antisera to PGF<sub>2α</sub> and may contribute to the increase in PGF<sub>2α</sub> excretion. The

increased renal TXA<sub>2</sub> formation may have been due only in part to the activation of arachidonic acid metabolism by catecholamines because TXA<sub>2</sub> production in class II patients was not different from class I.

The enhanced formation of angiotensin II and probably endothelin may have been responsible for the increased production of renal TXA<sub>2</sub>. Both of these vasoconstrictor hormones have been reported to cause TXA<sub>2</sub> formation by selectively acting on specific receptors linked to phospholipase C.<sup>34</sup> Our patients with higher TXB<sub>2</sub> excretion were found to have higher PRA and PAP<sub>m</sub> values. Likewise, plasma endothelin concentration in patients with CHF has been shown to be closely related to pulmonary artery pressure values.<sup>28</sup>

In our study, PRA was significantly increased only in class IV patients. Our observations confirm and extend the findings of others who have shown that angiotensin activity is increased only in the more advanced phases of heart disease.<sup>35</sup> The dependence of the increase in renal thromboxane formation on angiotensin activation is underlined by its positive correlation with PRA, as observed in our study.

Even if the values of PGF<sub>2α</sub> and TXB<sub>2</sub> excretion were homogeneously distributed within the groups and the differences among the classes were highly significant, this study was not designed as prospective one. As a consequence, the urinary excretion of vasoconstrictive eicosanoids cannot be taken as a

predictive index of cardiac decompensation. Nonetheless, because the increase in urinary TXB<sub>2</sub> and urinary PGF<sub>2α</sub> was constantly associated with progressive severity in the course of heart disease, our results strongly suggest that the increased renal formation of the vasoconstrictor eicosanoids and their renal effects should be included among the mechanisms involved in the aggravation of heart disease. Therefore the inhibition of vasoconstrictive prostanooids and particularly that of the more powerful TXA<sub>2</sub> may be considered in the future treatment of more severe cases of CHF because this inhibition may reduce renal vasoconstriction.<sup>36</sup>

We thank Carlo Patrono (Istituto di Farmacologia, Università di Chieti, Italy) for his generous gift of PGF<sub>2α</sub> antiserum and Luciano Caprino (Istituto di Igiene, Università Cattolica del Sacro Cuore, Rome, Italy) for the TXB<sub>2</sub> antiserum.

#### REFERENCES

- Massie BM. Is neurohormonal activation deleterious to the long-term outcome of patients with congestive heart failure? *J Am Coll Cardiol* 1988;12:547-58.
- Schrier RW. Pathogenesis of sodium and water retention in high-output and low-output cardiac failure, nephrotic syndrome, cirrhosis and pregnancy. *N Engl J Med* 1988;319:1065-72.
- Dzau VJ, Packer M, Lilly LS, Swartz SL, Hollenberg NK, Williams GH. Prostaglandins in severe congestive heart failure: relation to activation of the renin-angiotensin system and hyponatremia. *N Engl J Med* 1984;310:347-52.
- Holmer SR, Riegger AJ, Notheis WF, Kromer EP, Kochsiek K. Hemodynamic changes and renal plasma flow in early heart failure: implications for renin, aldosterone, norepinephrine, atrial natriuretic peptide and prostacyclin. *Basic Res Cardiol* 1987;82:101-8.
- Brody MJ, Kadowitz PJ. Prostaglandins as modulators of the autonomic nervous system. *Fed Proc* 1974;33:48-60.
- Brus R, Herman ZS, Zabawska J. Comparison of central and peripheral actions of F<sub>2α</sub> prostaglandin on the circulation of rat and guinea pig. *Acta Med Pol* 1981;22:193-7.
- Zipser RD. Effects of selective inhibition of thromboxane synthesis on renal function in humans. *Am J Physiol* 1985;248:F753-6.
- Remuzzi G, FitzGerald GA, Patrono C. Thromboxane synthesis and action within the kidney. *Kidney Int* 1992;41:1483-93.
- Pierucci A, Simonetti BM, Pecci G, Mavrikakis G, Feriozzi S, Cinotti GA, et al. Improvement of renal function with selective thromboxane antagonism in lupus nephritis. *N Engl J Med* 1989;320:421-5.
- Badr KF, Kelley VE, Rennke HG, Brenner BM. Roles of thromboxane A<sub>2</sub> and leukotrienes in endotoxin-induced acute renal failure. *Kidney Int* 1986;30:474-80.
- Frohlich JC, Wilson TW, Sweetman BJ, Smigel M, Nils AS, Carr K, et al. Urinary prostaglandins: identification and origin. *J Clin Invest* 1975;55:763-70.
- Sinzinger H, O'Grady J, Demers LM, Granstrom E, Kumlin M, Nell A, et al. Thromboxane in cardiovascular disease. *Eicosanoids* 1990;3:59-64.
- Criteria Committee of the New York Heart Association. Diseases of the heart and blood vessels. Nomenclature and criteria for diagnosis. 6th ed. Boston: Little, Brown, 1964;114-24.
- Patrono C, Wennmalm A, Ciabattoni G, Nowak J, Pugliese F, Cinotti G. Evidence for an extrarenal origin of urinary prostaglandin E<sub>2</sub> in healthy men. *Prostaglandins* 1979;18:623-9.
- Currie PJ, Seward JB, Chang KL, Fyfe DA, Hagler DJ, Mair DD, et al. Continuous wave Doppler determination of right ventricular pressure: a simultaneous Doppler-catheterization study in 127 patients. *J Am Coll Cardiol* 1985;6:750-6.
- Kitabatake A, Inoue M, Asao M, Masuyama T, Tanouchi J, Morita T, et al. Noninvasive evaluation of pulmonary hypertension by a pulsed Doppler technique. *Circulation* 1983;68:302-9.
- Hussey HH, Jegers H. Practical considerations of venous pressure. *N Engl J Med* 1947;237:776-80.
- Haber E, Koerner T, Page LB, Kliman B, Purnode A. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J Clin Endocrinol Metab* 1969;29:1349-55.
- Castellani S, Del Rosso A, Chen JL, Camaiti A, Carnovali M, Masotti G. The renal excretory activity of atrial natriuretic factor is independent of renal prostaglandins in humans. *Prostaglandins Leukotrienes Essential Fatty Acids* 1991;42:23-9.
- Larsson C, Anggard E. Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. *Eur J Pharmacol* 1973;21:30-6.
- McGiff JC, Wong PYK. Compartmentalization of prostaglandin and prostacyclin within the kidney: implications for renal function. *Fed Proc* 1979;38:89-93.
- Barger AC. Renal hemodynamics in congestive heart failure. *Ann NY Acad Sci* 1966;139:276-81.
- Zook TE, Strandhoy JW. Mechanisms of the natriuretic and diuretic effects of prostaglandin F<sub>2α</sub>. *J Pharmacol Exp Ther* 1981;217:674-80.
- Dunn MJ, Zambraski EJ. Renal effects of drugs that inhibit prostaglandin synthesis. *Kidney Int* 1980;18:609-22.
- Hirata M, Hayashi Y, Ushikubi F, Yokota Y, Kageyama R, Nakanishi S, et al. Cloning and expression of cDNA for a human thromboxane A<sub>2</sub> receptor. *Nature* 1991;349:617-20.
- Abe T, Takeuchi K, Takahashi N, Tsutsumi E, Taniyama Y, Abe K. Rat kidney thromboxane receptor: molecular cloning, signal transduction and intrarenal expression localization. *J Clin Invest* 1995;96:657-64.
- Zhang B, Sassard J. Eicosanoid-dependence of responses of pre but not postglomerular vessels to noradrenaline in rat kidneys. *Br J Pharmacol* 1993;110:235-8.
- Wei CM, Lerman A, Rodeheffer RJ, Mc Gregor GA, Brandt RR, Wright S, et al. Endothelin in human congestive heart failure. *Circulation* 1994;89:1580-6.
- Loup R, Favre L, Vallotton MB. Effect of noradrenaline, vasopressin and angiotensin II on renal prostaglandins in man. *Clin Sci* 1986;70:371-7.
- Miura K, Yukimura T, Yamashita Y, Shimmen T, Okumura M, Imanishi M, et al. Endothelin stimulates the renal production of prostaglandin E<sub>2</sub> and I<sub>2</sub> in anesthetized dogs. *Eur J Pharmacol* 1989;170:91-3.
- Watanabe K, Yoshida R, Shimizu T, Hayaishi O. Enzymatic formation of prostaglandin F<sub>2α</sub> from prostaglandin H<sub>2</sub> and D<sub>2</sub>. *J Biol Chem* 1985;260:7035-41.
- Leslie CA, Levine L. Evidence for the presence of a prostaglandin E<sub>2</sub>-9-ketoreductase in rat organs. *Biochem Biophys Res Commun* 1973;52:717-24.
- Liston TE, Roberts LJ. Transformation of prostaglandin D<sub>2</sub> to 9α,11β-(15S)-trihydroxyprosta (5z,13E)-dien-1-oic acid (9α,11β-prostaglandin F<sub>2α</sub>): a unique biologically active prostaglandin produced enzymatically in vivo in humans. *Proc Nat Acad Sci USA* 1985;82:6030-4.
- Folkert VW, Yunis M, Schlondorff D. Prostaglandin synthesis linked to phosphatidylinositol turnover in isolated rat glomeruli. *Biochem Biophys Acta* 1984;794:206-17.
- Remes J, Tikkanen I, Fyhrquist F, Pyorala K. Neuroendocrine activity in untreated heart failure. *Br Heart J* 1991;65:249-55.
- Klausner JM, Paterson IS, Kobzic L, Rodzen C, Valeri CR, Shepro D, et al. Vasodilating prostaglandins attenuate ischemic renal injury only if thromboxane is inhibited. *Ann Surg* 1989;209:219-24.