Ergometric exercise testing and sensitivity of cyclic guanosine 3',5'-monophosphate (cGMP) in diagnosing asymptomatic left ventricular dysfunction

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Abstract

Objective—Increased plasma concentrations of cyclic guanosine monophosphate (cGMP) have been reported in patients with manifest heart failure. At rest, however, cGMP concentrations in patients with asymptomatic left ventricular dysfunction or heart failure in New York Heart Association (NYHA) functional class I do not differ significantly from those of healthy subjects. The purpose of this study was to investigate whether graded exercise on an ergometer improves the sensitivity of cGMP in diagnosing asymptomatic left ventricular dysfunction.

Patients—Plasma cGMP concentrations were compared in 17 healthy controls and 98 patients with asymptomatic left ventricular dysfunction or congestive heart failure of different stages (asymptomatic left ventricular dysfunction or NYHA functional class I, 56 patients; NYHA class II, 31 patients; NYHA class III, 11 patients).

Results—Before exercise plasma cGMP concentrations in patients with clinical heart failure (NYHA functional classes II and III) were significantly higher than those in healthy controls. In patients with asymptomatic left ventricular dysfunction or heart failure of functional class I plasma cGMP concentrations were not significantly different from those in healthy subjects. Thirty minutes after exercise, however, cGMP concentrations in patients with asymptomatic left ventricular dysfunction or class I heart failure were significantly higher than those in healthy controls.

Conclusion—Measurement of plasma cGMP concentrations 30 minutes after ergometric exercise testing allows better discrimination between healthy subjects and patients with symptomless left ventricular dysfunction or mild heart failure (NYHA class I) than measurement of such concentrations before exercise.

(Br Heart J 1995;73:145-150)

Keywords: cyclic guanosine monophosphate (cGMP); heart failure; left ventricular dysfunction; exercise

Heart failure is not simply a haemodynamic disorder and its physiological abnormalities cannot be assessed simply by measuring pressure, volume, and flow. The interplay of neurohormonal and haemodynamic forces defines the syndrome of heart failure.¹ For example, atrial natriuretic peptide, which has potent diuretic and natriuretic activity, is released in response to volume loading in normal subjects and in some pathological conditions such as heart and renal failure.² Once released, atrial peptides exert potent direct vasodilator and natriuretic actions by virtue of their ability to increase release of the intracellular second messenger cyclic guanosine monophosphate (cGMP).³

Exogenously administered atrial natriuretic peptide raises plasma cGMP concentration in accordance with its physiological effects.^{4,5} Raised plasma concentrations of atrial natriuretic peptide are found in patients with congestive heart failure, and concentrations also increase with exercise in healthy subjects⁶⁻⁸ and patients with congestive heart failure.⁹ As cGMP is extruded from cells after interaction with atrial natriuretic peptide, it is a possible marker for atrial natriuretic peptide. Moreover, because it has a longer half life in plasma than atrial natriuretic peptide (15 minutes *v* 1-2 minutes) cGMP can be regarded as a more sensitive marker of atrial natriuretic peptide release than atrial natriuretic peptide itself in many situations.^{5,10,11} We showed that cGMP is a sensitive and specific marker for overt heart failure in the absence of severe renal functional impairment.¹² In patients with heart failure close correlations have been found between plasma concentrations of cGMP and atrial natriuretic peptide at rest. Both atrial natriuretic peptide and cGMP correlated closely with the severity of congestive heart failure.¹³

Knowledge about cGMP during physical exercise is, however, scant. Early identification of patients with symptomless left ventricular dysfunction and early pharmacological intervention may have an impact on the outlook for patients with heart failure. Screening for symptomless left ventricular dysfunction will require the use of simple, validated, and non-invasive objective measures of cardiac function.¹⁴ We therefore investigated whether exercise improves the diagnostic sensitivity of cGMP for symptomless heart failure.

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Accepted for publication
8 August 1994

Subjects and methods

HEALTHY SUBJECTS

We investigated 17 healthy volunteers (seven women, 10 men) aged between 17 and 47 years (mean 24 (7) years). Their mean achieved maximal workload was 219 (54) W, ranging from 150 W to 325 W, which corresponds to 119% (23.3%) (range 75–155%) of expected values corrected for age, sex, weight, and height.

PATIENTS

We investigated 98 patients (11 women, 87 men) aged between 32 and 79 years (mean 60 (9) years). In all patients left ventricular function at rest measured by radionuclide ventriculography was below normal values (< 0.55) or patients showed symptoms of heart failure. Patients were classified into three groups according to their cardiac symptoms (asymptomatic left ventricular dysfunction or New York Heart Association (NYHA) functional class I, 56 patients; NYHA class II, 31 patients; NYHA class III, 11 patients). Patients with heart failure of NYHA class IV were excluded.

The clinical characteristics of patients with heart failure are summarised in the table. Symptomatic or asymptomatic coronary artery disease was the underlying cause of heart failure in 83 patients. Sixty five patients had a history of myocardial infarction and 35 of coronary artery bypass grafting (18 patients had had both). Six patients suffered from valvar heart disease, and nine had cardiomyopathy. Maximal workload was 97 (38) W, ranging from 25 W to 200 W, which corresponds to 61.4% (20.1%) (range 25–100%) of expected values corrected for age, sex, weight, and height. Plasma creatinine concen-

trations were within the normal range in all patients (90.5 (17.2) $\mu\text{mol/l}$ (range 53.0 $\mu\text{mol/l}$ to 132.6 $\mu\text{mol/l}$).

BICYCLE ERGOMETRIC TESTING

Each subject underwent symptom limited ergometric exercise testing according to the guidelines of the Austrian Society of Cardiology. Exercise was started with a workload of 25 W and was increased by 25 W every two minutes until one of four end points was reached: excessive weakness or exhaustion, shortness of breath, severe angina, or frequent complex arrhythmias. The electrocardiogram was continuously monitored throughout the exercise test. Blood pressure was measured at the end of each interval. In the patient group left ventricular ejection fraction was determined by supine radionuclide ventriculography before exercise, at maximal workload, and 30 minutes after exercise.

RADIONUCLIDE VENTRICULOGRAPHY

Supine electrocardiogram gated equilibrium radionuclide ventriculography was performed after an in vitro-in vivo labelling of red blood cells with technetium-99m (740–925 MBq). We used an Elscint Apex SR camera interfaced to an Elscint Apex 1 computer with a semiautomatic operator-interactive ejection fraction program. Resting scintigrams were obtained in the anterior, 40° left anterior oblique, and left lateral positions for a preset time of four minutes. During peak exercise we attempted an acquisition time of four minutes (at least 300 heart cycles) so as to obtain high quality scintigrams. Scintigraphic evaluation of left ventricular ejection fraction in our laboratory correlates well with angiographically obtained left ventricular ejection fraction ($r = 0.9$). For determination of right ventricular ejection fraction, end diastolic and end systolic regions of interest were isolated in the 40° left anterior oblique view. After background was subtracted the right ventricular ejection fraction was calculated. Normal values in our laboratory are 0.48 (0.05) for the right ventricular ejection fraction, and 0.62 (0.07) for the left.^{15 16}

BLOOD SAMPLES

Blood was taken from the cubital vein after a resting period of 15 minutes just before the exercise test, during the last 30 seconds of the maximal workload, and at the end of a recovery period of 30 minutes. All specimens were collected in tubes containing EDTA (1.5 g/l of blood). After centrifugation for 10 minutes at room temperature, plasma was stored at -20°C until assayed.

cGMP DETERMINATION

We extracted cGMP from plasma by adding 1000 μl ethanol to 250 μl plasma, mixing and centrifuging for 15 minutes. We collected the supernatant. The precipitate was washed with 500 μl ethanol and centrifuged for 15 minutes again. The supernatants were combined and evaporated to dryness at 56°C under a stream of nitrogen. The residues were redissolved in

Clinical characteristics of patients with heart failure or asymptomatic left ventricular dysfunction

	Heart failure (NYHA class):		
	I (n = 56)*	II (n = 31)	III (n = 11)
Mean age (years)	59 (8)	63 (7)	62 (9)
No (%) of men	53 (95)	26 (83)	8 (75)
No (%) with:			
Coronary artery disease	53 (95)	27 (86)	9 (83)
Cardiomyopathy	3 (5)	8 (10)	2 (17)
Valvar heart disease		8 (10)	2 (17)
Mean blood pressure at rest (mm Hg)	130/83	137/85	125/82
Drug treatment (No (%) of patients)			
ACE inhibitors	14 (25)	10 (31)	6 (58)
Glyceryl trinitrate	20 (35)	7 (24)	5 (42)
Digitalis	5 (9)	9 (28)	6 (50)
Diuretics	12 (21)	11 (34)	5 (42)
β Blocker	24 (42)	4 (14)	
Calcium antagonists	22 (39)	12 (40)	1 (8)
Aspirin	22 (39)	11 (34)	4 (33)
Mean (SD) maximal workload (% of expected)	67 (18)	55 (19)	50 (24)
LVEF (%):			
At rest	51 (14)	37 (13)	28 (7)
At maximal workload	50 (16)	33 (13)	27 (7)
30 Minutes after exercise	51 (16)	37 (15)	29 (7)
RVEF (%):			
At rest	47 (5)	42 (8)	41 (8)
At maximal workload	47 (7)	40 (7)	39 (10)
30 Minutes after exercise	53 (6)	41 (10)	39 (12)
Creatinine ($\mu\text{mol/l}$)	93 (16)	87 (18)	87 (16)

*Includes patients with asymptomatic left ventricular dysfunction.

ACE, angiotensin converting enzyme.

LVEF, left ventricular ejection fraction.

RVEF, right ventricular ejection fraction.

Patients who had more than one disease or who had more than one drug were listed in each group separately.

1 ml assay buffer and 500 μ l were then used in the assay for acetylation. Immunoreactive cGMP was measured by radioimmunoassay using iodine-125 (cGMP assay RPA 525; Amersham International, Amersham, Buckinghamshire). The upper limit of the reference interval using this procedure is 6.6 nmol/l.¹²

CREATININE

Creatinine concentrations in plasma were determined enzymatically with reagents from Boehringer Mannheim (Mannheim, Germany).

DATA ANALYSIS

Results are expressed as means (SD). Linear correlation coefficients were calculated to describe the association between continuous variables. Between group comparison was performed using analysis of variance and Student's *t* test. Significance was defined as $P < 0.05$. Sensitivity, specificity, and receiver operating characteristic curves were calculated to describe the diagnostic performance of cGMP.

Results

HEALTHY VOLUNTEERS

Under resting conditions cGMP concentrations were 4.35 (1.45) nmol/l, ranging from 2.42 to 7.35 nmol/l. In response to exercise, at maximal workload cGMP increased significantly to 6.26 (2.62) nmol/l (range 3.46 nmol/l to 13.72 nmol/l; $P = 0.0042$ compared with pre-exercise values). Thirty minutes after exercise cGMP decreased significantly to 4.86 (2.05) nmol/l (range 2.35 nmol/l to 9.64 nmol/l, $P = 0.0003$). cGMP concentrations 30 minutes after exercise were not significantly different from values before testing ($P = 0.25$) (fig 1).

Both plasma cGMP concentrations ($P = 0.0002$) and haemodynamic parameters, such as heart rate, systolic blood pressure, and the product of rate and pressure ($P = 0.0001$)

increased significantly with increasing workloads. There was, however, no significant correlation between the increase in plasma cGMP concentrations and the respective increase in heart rate ($r = 0.30$, $P = 0.29$), systolic blood pressure ($r = 0.12$, $P = 0.66$), and the rate-pressure product ($r = 0.25$, $P = 0.39$) during exercise in healthy subjects. We found significant correlations between the maximal achieved workload and cGMP concentrations at maximal workload ($r = 0.60$, $P = 0.011$) and after exercise ($r = 0.61$, $P = 0.012$). We also found significant correlations between the maximal workload expressed as a percentage of the expected workload, which was predicted according to age, sex, weight, and height of the tested individual, and cGMP concentrations at maximal workload ($r = 0.69$, $P = 0.003$) and 30 minutes after exercise ($r = 0.68$, $P = 0.004$).

PATIENTS WITH CONGESTIVE HEART FAILURE

Under resting conditions cGMP concentrations in patients with heart failure (NYHA classes I-III) were 5.62 (3.09) nmol/l, ranging from 1.74 nmol/l to 17.73 nmol/l. In response to exercise cGMP increased significantly to 7.47 (3.84) nmol/l (range 1.27 to 20.68 nmol/l; $P = 0.0001$ compared with pre-exercise values).

Thirty minutes after exercise cGMP was 9.01 (3.52) nmol/l (range 3.31 nmol/l to 18.12 nmol/l). Plasma cGMP concentrations 30 minutes after the exercise test further increased and were significantly higher than values before exercise and than values measured at maximal workload ($P = 0.0001$). Both plasma cGMP concentrations and haemodynamic parameters, such as heart rate, systolic blood pressure, and the rate-pressure product, increased significantly with increasing workloads ($P = 0.0001$). There was no close correlation between the increase in plasma cGMP concentrations and the respective increase in heart rate ($r = 0.38$, $P = 0.0003$), systolic blood pressure ($r = 0.23$, $P = 0.0293$), and the rate-pressure product ($r = 0.37$, $P = 0.0005$). In patients with heart failure there was no significant correlation between maximal workload and cGMP concentrations. We did not find a close correlation between the plasma cGMP concentration and the right or left ventricular ejection fraction before exercise (right: $r = 0.29$, $P = 0.005$; left: $r = 0.28$, $P = 0.007$), at maximal workload (left: $r = 0.15$, $P = 0.18$; right: $r = 0.22$, $P = 0.052$), or 30 minutes after exercise (left: $r = 0.33$, $P = 0.0123$; right: $r = 0.14$, $P = 0.37$).

In patients with asymptomatic left ventricular dysfunction or mild heart failure (NYHA classes I and II) cGMP concentrations significantly increased during the whole observation period (from rest to maximal workload: $P = 0.0001$ and $P = 0.048$, respectively, and from maximal workload to 30 minutes after the exercise: $P = 0.0001$ and $P = 0.001$, respectively) (figure 1). In patients with severe heart failure (NYHA class III) there was no significant increase in cGMP concentrations from

Figure 1 Mean plasma cGMP concentrations during ergometric exercise in healthy subjects and patients with heart failure. Vertical lines indicate 1SD.

* $P < 0.05$ for difference between patients and healthy subjects at the same time point. ALVD, asymptomatic left ventricular dysfunction.

rest to maximal workload ($P = 0.22$) or from maximal workload to 30 minutes after exercise ($P = 0.053$). Thirty minutes after exercise, however, plasma cGMP concentrations were significantly ($P = 0.013$) higher than values before testing (fig 1).

When we compared cGMP concentrations in healthy controls and in patients with heart failure we found significantly raised cGMP concentrations in patients with left ventricular dysfunction or heart failure at rest ($P = 0.049$) and 30 minutes after exercise ($P = 0.0001$). At maximal workload there was no significant difference between the groups ($P = 0.11$). At rest cGMP concentrations both in healthy controls and in patients with asymptomatic left ventricular dysfunction or heart failure of NYHA class I differed significantly from cGMP concentrations in patients with heart failure of NYHA class II ($P = 0.011$, $P = 0.003$, respectively) and of class III ($P = 0.006$, $P = 0.002$, respectively) (fig 1). However, there was no significant difference between plasma cGMP concentrations in healthy subjects and in patients with heart failure of NYHA class I or asymptomatic left ventricular dysfunction ($P = 0.4536$). In contrast, 30 minutes after exercise patients with asymptomatic left ventricular dysfunction or heart failure of NYHA class I showed significantly increased cGMP concentrations ($P = 0.0003$) compared with controls. After exercise cGMP concentrations in patients with heart failure of NYHA classes II and III were also significantly higher ($P = 0.0001$, $P = 0.001$, respectively) (fig 1). At maximal workload there was no significant difference between groups.

In a subgroup of 23 patients with angina or who had undergone revascularisation who had normal left ventricular function at rest cGMP concentrations did not differ significantly from those in controls at rest or at maximal workload ($P > 0.84$). Thirty minutes after exercise, however, cGMP concentrations in these patients were significantly higher than those in healthy controls (7.60 (3.20) nmol/l *v* 4.86 (2.05); $P = 0.005$). In these patients we did not find a significant increase in left ventricular and right ventricular ejection fraction during exercise ($P > 0.13$).

With a cut off value of 6.6 nmol/l the sensitivity of cGMP for asymptomatic left ventricular dysfunction or heart failure of NYHA class I was 0.18 (specificity 0.94) before exercise; sensitivity increased to 0.64 , 30 minutes after exercise (specificity 0.88). Figure 2 shows receiver operating characteristic curves. The area under the curve increased from 0.52 at rest to 0.81 30 minutes after exercise.

Discussion

Early diagnosis and treatment of patients in the symptomless phase of left ventricular dysfunction is now of increasing clinical relevance.¹⁷ Consequently, a test of symptomless left ventricular dysfunction is needed that is easy to apply and suitable for screening large populations. Recently the natriuretic peptides N-terminal proatrial natriuretic peptide and brain natriuretic peptide have been proposed for this purpose.^{18, 19}

These peptides have drawbacks for routine use. They are easily degraded (susceptible to the actions of non-specific factors in vitro). For example, to keep atrial natriuretic peptide stable for a month it needs to be stored at -196°C in liquid nitrogen.²⁰ N-terminal proatrial natriuretic peptide has a longer biological half life than the other natriuretic peptides and cGMP.¹⁹ However, current information on the in vitro stability of N-terminal proatrial natriuretic peptide in blood samples is scant. In contrast, even at room temperature cGMP is stable for at least five days in plasma samples containing EDTA.¹² To measure natriuretic peptide concentrations plasma samples have to be extracted before determination by reverse phase chromatography, which is time consuming. In contrast, there is no need for such extraction of plasma samples before determining cGMP concentration,²¹ which makes cGMP suitable for routine laboratory use.

In accordance with other study groups,²² we previously showed an increase in plasma cGMP concentrations during ergometric exercise in healthy subjects.²³ Our current study shows that plasma cGMP concentration increases during exercise both in healthy controls and in patients with heart failure. Some investigators, however, have reported that plasma cGMP concentrations remained unchanged during exercise in healthy people.^{24, 25} But exercise is a complex haemodynamic event, which may explain these conflicting results.

Our patients with heart failure were older than our controls. This should not affect our results, however, because basal plasma cGMP concentrations do not change with age in healthy subjects.²⁶ We found a highly significant rise in plasma concentrations of cGMP during physical exercise, reaching maximal values at maximal workload in healthy people and 30 minutes after stopping exercise in patients with heart failure or asymptomatic left ventricular dysfunction. Compared with controls, patients with heart failure had

Figure 2 Receiver operating characteristic curves of cGMP for diagnosing symptomless left ventricular dysfunction or heart failure of NYHA class I before (A) and 30 minutes after (B) ergometric exercise. The larger the deviation from the line representing a worthless test the better is the discriminative power of a test under scrutiny. *Diagnostic performance of the cut off value of 6.6 nmol/l.

significantly raised plasma cGMP concentrations at rest and 30 minutes after exercise. At rest patients with heart failure of NYHA class I or asymptomatic left ventricular dysfunction could not be discriminated easily from healthy subjects, either by clinical symptoms or by measuring cGMP concentrations in plasma. In contrast, 30 minutes after an ergometric exercise, the patients showed significantly raised plasma cGMP concentrations compared with healthy controls. With exercise the sensitivity of cGMP for patients with pure left ventricular dysfunction or NYHA class I heart failure increased from 0.18 to 0.64 (cut off value 6.6 nmol/l). Thirty minutes after exercise patients with coronary artery disease whose resting left ventricular ejection fraction was within normal range but did not increase during exercise also had significantly higher cGMP concentrations than controls. These observations support a role for cGMP as a non-invasive marker of symptomless left ventricular dysfunction.

Whereas Haug *et al* reported close inverse correlations between atrial and brain natriuretic peptide plasma concentrations and ejection fraction,²⁷ we did not find a close correlation between plasma cGMP concentrations and right or left ventricular ejection fraction assessed by radionuclide ventriculography, either at rest or at maximal workload. The lack of correlation between ventricular ejection fraction and cGMP in chronic heart diseases may be explained by downregulation of natriuretic peptide receptors, which results in a depressed release of cGMP.^{4,28} Changing or chronically raised atrial or brain natriuretic peptide concentrations might cause down regulation of the number, expression, or sensitivity of natriuretic peptide receptors.¹⁴ Despite appreciable atrial or brain natriuretic peptide secretion, this may lead to inhibition of the signal transduction and decreased cGMP release into plasma.^{4,28}

Several limitations of our study have to be considered. Natriuretic peptides and as a consequence cGMP are released in response to diastolic abnormalities associated with heart failure—that is, increased filling pressures of the left and right ventricles and increased left and right atrial size. They need not in themselves correlate with measures of systolic function such as left and right ventricular ejection fraction. We did not correlate diastolic dysfunction and cGMP concentrations, which may also enhance our understanding of cGMP concentrations in heart failure. We included all patients with clinical signs of heart failure or a reduced left ventricular ejection fraction at rest. The range of drugs taken by patients with asymptomatic left ventricular dysfunction or heart failure of NYHA class I reflects the high prevalence of coronary artery disease. The high use of β -blockers and calcium antagonists reflects the standard treatment of patients after myocardial infarction who have only a mildly impaired left ventricular ejection fraction. Clinical symptoms of heart failure were classified according to NYHA criteria. Exercise limitation was mea-

sured by expressing the maximal achieved workload as a percentage of expected values corrected for age, sex, weight, and height. We did not measure maximal oxygen uptake during exercise, which would have allowed a more accurate classification of heart failure.

In summary, our study tried to measure exercise performance in heart failure with a new approach—that of measuring cGMP, which is the second messenger of the natriuretic peptide system. Our main finding was that ergometric exercise improves the diagnostic sensitivity of cGMP for patients with asymptomatic left ventricular dysfunction or heart failure with minimal symptoms. These observations support a role for cGMP as a non-invasive marker for symptomless left ventricular dysfunction. As we described previously,²¹ measurement of cGMP does not require extraction of plasma samples and is, therefore, suitable for routine laboratory use.

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