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## Atrial Natriuretic Peptide and Acute Changes in Central Blood Volume by Hyperthermia in Healthy Humans

Thomas Wiis Vogelsang<sup>1,2</sup>, Jens Marving<sup>1</sup>, Craig G. Crandall<sup>4,5</sup>, Chad Wilson<sup>4,6</sup>, Chie C. Yoshiga<sup>3</sup>, Niels H. Secher<sup>3</sup>, Birger Hesse<sup>1</sup>, and Andreas Kjær<sup>\*,1,2</sup>

<sup>1</sup>Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, Denmark

<sup>2</sup>Cluster for Molecular Imaging, University of Copenhagen, Denmark

<sup>3</sup>Department of Anesthesia, Copenhagen Muscle Research Center, Rigshospitalet, University of Copenhagen, Denmark

<sup>4</sup>Institute for Exercise and Environmental Medicine, Presbyterian Hospital of Dallas, Dallas, TX, USA

<sup>5</sup>Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

<sup>6</sup>Department of Medicine, Pennsylvania State College of Medicine, Hershey, Pennsylvania, USA

### Abstract

**Background**—Hyperthermia induces vasodilatation that reduces central blood volume (CBV), central venous pressure (CVP) and mean arterial pressure (MAP). Inhibition of atrial natriuretic peptide (ANP) could be a relevant homeostatic defense mechanism during hyperthermia with a decrease in CBV. The present study evaluated how changes in plasma ANP reflect the changes in CBV during hyperthermia.

**Methods**—Ten healthy subjects provided with a water perfused body suit increased body core temperature 1 °C. In situ labeled autologous red blood cells were used to measure the CBV with a gamma camera. Regions of interest were traced manually on the images of the whole body blood pool scans. Two measures of CBV were used: Heart/whole body ratio and thorax/whole body ratio. CVP and MAP were recorded. Arterial (ANP<sub>art</sub>) and venous plasma ANP were determined by radioimmunoassay.

**Results**—The ratio thorax/whole body and heart/whole body decreased 7 % and 11 %, respectively (p<0.001). MAP and CVP decreased during hyperthermia by 6.8 and 5.0 mmHg, respectively (p<0.05; p<0.001). Changes in both thorax/whole body (R=0.80; p<0.01) and heart/

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\*Address correspondence to this author at the Department of Clinical Physiology, Nuclear Medicine & PET, KF-4012, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark; Tel: +45 35 45 42 16; akjaer@sund.ku.dk.

### CONFLICT OF INTEREST

None declared.

whole body ratios ( $R=0.78$ ;  $p<0.01$ ) were correlated with changes in ANP<sub>art</sub>. However, there was no correlation between venous ANP and changes in CBV, nor between ANP<sub>art</sub> and MAP or CVP.

**Conclusion**—Arterial but not venous plasma concentration of ANP, is correlated to changes in CBV, but not to pressures. We suggest that plasma ANP<sub>art</sub> may be used as a surrogate marker of acute CBV changes.

### Keywords

ANP; natriuretic peptides; central blood volume; heating; blood pool imaging; nuclear medicine

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## INTRODUCTION

Hyperthermia increases core and skin temperature. This leads to peripheral vasodilatation with a decrease in central blood volume (CBV), central venous pressure (CVP), mean arterial pressure (MAP) and an increase in heart rate [1-4]. Atrial natriuretic peptide (ANP) provides a potent physiological defense mechanism against volume overload by causing natriuresis, vasodilatation and suppressing of the renin-angiotensin-aldosteron-system [5]. Therefore, during hyperthermia with decrease in CBV, inhibition of ANP would be expected and could be a homeostatic defense mechanism. Circulating ANP is released mainly from the right atrium and its plasma concentration is higher in the pulmonary artery than in the arterial blood which, in turn, carries a higher concentration than that of the venous blood [6]. ANP exerts its effect through the natriuretic peptide receptors [7].

The ANP/natriuretic receptor system is important not only in acute, but also chronic regulation of volume homeostasis, since deletion of ANP or natriuretic receptors leads to chronic arterial hypertension or hypervolemia [8]. The ANP response to volume changes is rapid [9], which makes ANP a suitable hormone in the investigation of the acute effect of volume displacement during hyperthermia. Therefore, the aim of the present investigation was to evaluate the relation between changes in CBV induced by hyperthermia and arterial plasma concentration of ANP (ANP<sub>art</sub>).

## METHODS

### Subjects

Ten healthy men volunteered to participate in the study. The subjects were  $29 \pm 5$  years and had a height of  $182 \pm 6$  cm and a body weight of  $80 \pm 11$  kg (mean  $\pm$  SD). Subjects did not take any medication. The study was approved by the local scientific ethical committee (K22-090/04) and in all cases informed, written consent was obtained prior to the experiments. All experiments were performed in accordance with the Declaration of Helsinki.

### Thermal Protocol

Each subject swallowed a telemetry pill for the measurements of intestinal temperature (HQ Inc, Palmetto, FL, USA). The subjects were also instrumented for the measurement of mean skin temperature from the weighted average of six thermocouples attached to the skin. Each subject was placed in the supine position wearing a tube-lined suit that covered the entire

body surface with the exception of the hands, feet, head, and the forearm from which skin blood flow was assessed.

During baseline (i.e. pre-heat stress) thermoneutral water (34 °C) was perfused through the tube-lined suit. For the experimental subjects, upon completion of baseline data collection, the temperature of the water perfusing the suit was elevated to 46-48 °C with a goal of increasing internal temperature 1.0 °C (heat stress).

### **Blood Pool Scintigraphy**

*In vitro* isotope labelling of autologous red blood cells was performed with 800 MBq Tc-99m by a kit (Ultra-Tag RBC, Mallinckrodt, St. Louis, MO, USA). During labelling, for about 45 min, the subject rested on the gamma camera table. Approximately 15 min after re-injection of the labelled red cells, scanning from the head to just below the knees was performed at 12 cm/min with a dual-headed gamma camera (Skylight, Philips Medical System) with the detectors positioned in anterior and posterior views. Typical acquisition time was 10-12 min. Data were acquired in a 512 by 1024 matrix and stored in a dedicated computer (Pegasys, ADAC, Milpitas, CA, USA) and subsequently transferred to, and analysed, in another work station (eNTEGRA, General Electric, Milwaukee, WI, USA). For calculation the anterior and posterior projections were combined by calculation of the geometric mean of opposite views. This calculation is necessary to compensate for different counting efficiency of the labelled red blood cells if they are located superficially or deep in the body, and if they are distributed in the anterior or posterior direction. Attenuation compensation was also applied to compensate for red cells redistribution between regions with different thickness and thereby differences in count efficiency.

A second whole body scanning was performed when the core temperature was raised 1°C (typically ~90 min. after injection of the labelled red cells) with the position of the subject maintained and with the same image acquisition protocol as used in the first scan.

For analysis, regions of interest (ROI) of the thorax, heart, and proximal femur were traced manually (Fig. 1). The ROIs were copied from the baseline to the hyperthermia images.

### **Estimation of Changes in CBV**

With the injected labelled red cells, the radioactive count rate from ROIs was determined. To estimate whether CBV changed two ratios were calculated: the ratio between the counts in the chest ROIs and that over whole body ROI and, second, the ratio between the number of count in the cardiac ROI and the whole body ROI. A decreased ratio from the baseline to hyperthermia, would indicate redistribution of blood from either the heart or thoracic region to other parts of the body. We also calculated the proximal femur ROI/whole body ROI to evaluate whether blood pooling occurred in this compartment during hyperthermia. Since ratios were used, no decay-correction was necessary.

### **Measurement of Central Venous Pressure and Mean Arterial Pressure**

Central venous pressure (CVP) was measured upon cannulation of a vein in the arm (typically the basilic vein), with the catheter advanced to the superior vena cava. Correct

placement was identified *via* the pressure waveform. Arterial pressure was obtained following cannulation of the brachial or radial artery of the non-dominant arm. Both catheters were connected to fluid filled pressure transducers that were zeroed to atmospheric pressure 5 cm below (i.e., dorsal) the subject's supra-sternal notch. Mean values of these pressures were obtained by integration of the respective waveforms *via* data analysis software (Acknowledge, Biopac, CA). Heart rate was quantified *via* R-wave detection of the subject's ECG.

### Blood Sample Analysis

Blood was sampled from the brachial artery or from the central venous catheter in tubes containing EDTA. The samples were centrifuged and plasma was kept at  $-80^{\circ}\text{C}$  until analyzed. ANP was measured by RIA of plasma extracted by means of C18 cartridges according to a previously described procedure [10]. The sensitivity of the assay was 3.1 pg/ml and the intra- and interassay coefficient of variation were 4% and 5%, respectively.

### Statistical Analysis

Data are shown as mean  $\pm$  SEM. Values obtained during heating were compared to baseline values by a paired *t*-test. Changes in CBV, central venous pressure or MAP were compared with those in arterial and venous plasma ANP concentrations by linear regression.  $P < 0.05$  was considered statistical significant.

## RESULTS

### Haemodynamic Changes

The subjects wore the water tube-lined suit for  $56 \pm 4$  min, which increased the body core temperature from  $36.9 \pm 0.0$  to  $37.9 \pm 0.1$   $^{\circ}\text{C}$  ( $p < 0.0001$ ). Heart rate increased from  $51.7 \pm 2.3$  bpm at baseline to  $85.7 \pm 5.8$  bpm during heat stress ( $p < 0.001$ ). MAP decreased 8% during hyperthermia (from  $87.6 \pm 2.3$  to  $80.8 \pm 2.4$  mmHg,  $p < 0.02$ ). CVP decreased 86% during hyperthermia (from  $5.8 \pm 0.6$  to  $0.8 \pm 0.7$  mmHg;  $p < 0.001$ ).

During hyperthermia the thorax/whole body and heart/whole body blood pool ratios decreased 7% ( $0.34 \pm 0.01$  vs  $0.31 \pm 0.01$ ;  $p < 0.001$ ) and 11 % ( $0.11 \pm 0.01$  vs  $0.10 \pm 0.01$ ;  $p < 0.0001$ ), respectively. Proximal femur/whole body ratio increased 12% during hyperthermia ( $p < 0.001$ ).

### ANP and Haemodynamic Parameters

Induction of 1  $^{\circ}\text{C}$  of hyperthermia lead to a decrease from 41% to an increase of 24% in ANP<sub>art</sub>. The average ANP<sub>art</sub> was similar before ( $76.2 \pm 11.6$  pg/ml; range: 34.6-150.7 pg/ml) and during hyperthermia ( $69.4 \pm 22.3$  pg/ml; range: 28.1-113.7 pg/ml). The average venous plasma ANP concentration was similar before ( $58.5 \pm 10.6$  pg/ml; range: 34.2-113.6 pg/ml) and after hyperthermia ( $61.1 \pm 8.2$  pg/ml; range: 34.8-103.4 pg/ml). On average, there were no significant A-V differences either at baseline ( $14.3 \pm 13.4$  pg/ml) or during heat ( $8.4 \pm 5.2$  pg/ml). Changes in ANP<sub>art</sub> were correlated to changes in thorax/whole body and heart/whole body ratios ( $R = 0.80$  and  $R = 0.78$ , respectively,  $p < 0.01$ ; Fig. 2). No

correlation was observed between venous ANP and CBV changes, nor did we observe any correlations between either MAP or CVP and ANP<sub>art</sub>.

## DISCUSSION

The major finding of the present study was a correlation between changes in CBV and those in ANP<sub>art</sub> during passive heating.

Our finding of a 7-11 % displacement of CBV during hyperthermia is in line with previous findings during hyperthermia [11]. Other methods for manipulating CBV, e.g. head-up tilt and mild lower body negative pressure (LBNP) [12-14] also showed a CBV displacement comparable to values obtained in the present study. We observed an increase in the proximal femur/whole body blood volume ratio of 12 % during hyperthermia, changes comparable with data obtained by gravitational stress in a head-up study supporting the idea of a venous blood pooling in the legs [15].

The two blood pool ratios, applied to express changes in volume distribution in response to heating, showed an almost identical displacement of CBV, and the correlation between the two ratios was strong ( $R=0.93$ ,  $p<0.0001$ ). Therefore, to find an estimate for the CBV using our methodology, we suggest that it is possible to use either the thoracic blood volume or the blood volume in the heart.

The plasma concentration of ANP correlates well with atrial distension which allows for an indirect evaluation of CBV [16-20]. Accordingly, we found a positive correlation between changes in CBV and those in ANP<sub>art</sub>. In accordance with this, it has previously been found that a post exercise decrease in central blood volume was paralleled with a decrease in ANP concentrations [21].

The present investigation did not show any correlation between CVP and ANP<sub>art</sub>. The commonly parallel changes of CVP with the CBV, pressures make it tempting to relate plasma ANP to pressures rather than CBV. In concert with our results, it has previously been shown that when there is a discrepancy between pressure and volume, plasma ANP follows the changes in CBV [22]. In accordance with this, it has previously been shown that the stimulus for release of ANP into the bloodstream is mainly caused by volume-induced distension of the atria rather than changes in pressure [23-25]. Furthermore, plasma ANP continues to decrease during sustained head-up tilt, even when central venous pressure remains stable [26].

Our finding indicate that the delta values of ANP may represent a sensitive marker for changes in CBV. Thus, ANP<sub>art</sub> might be used as a simple marker of CBV.

CBV is widely monitored in physiological and pathophysiological studies, but there is not any consensus of its definition. Two different blood pool ratios were used in the present study for determination of CBV redistribution, the thorax/whole body ratio and the heart/whole body ratio. It is not surprising that the correlation with ANP is almost identical in the two blood pools, since ANP is released from the atria, and the atria are included in both

blood pools and, probably more important, strongly correlated with the blood volume contents of the ventricles as well as the large central and pulmonary veins.

In conclusion, we found that changes in arterial ANP are directly related to changes in CBV but not to CVP or MAP. Thus, ANP<sub>art</sub> might be used as a marker of CBV in studies with acute intervention when CBV is not measured directly.

## ACKNOWLEDGEMENTS

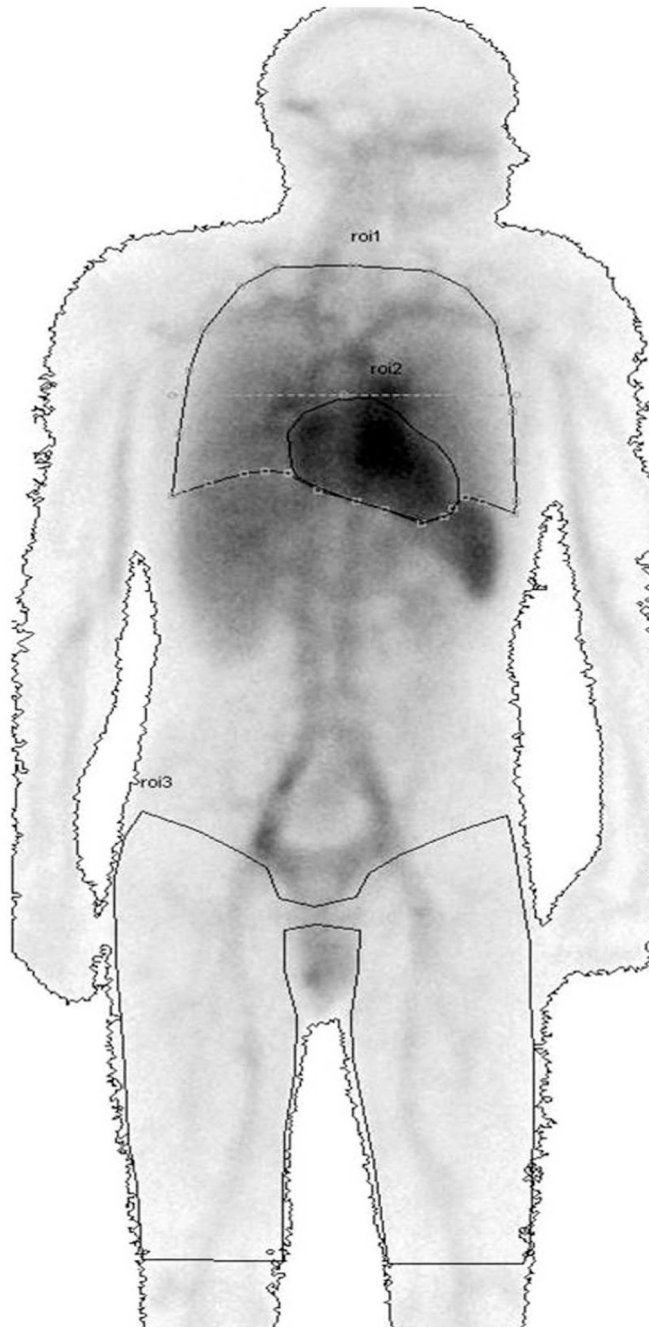
None declared.

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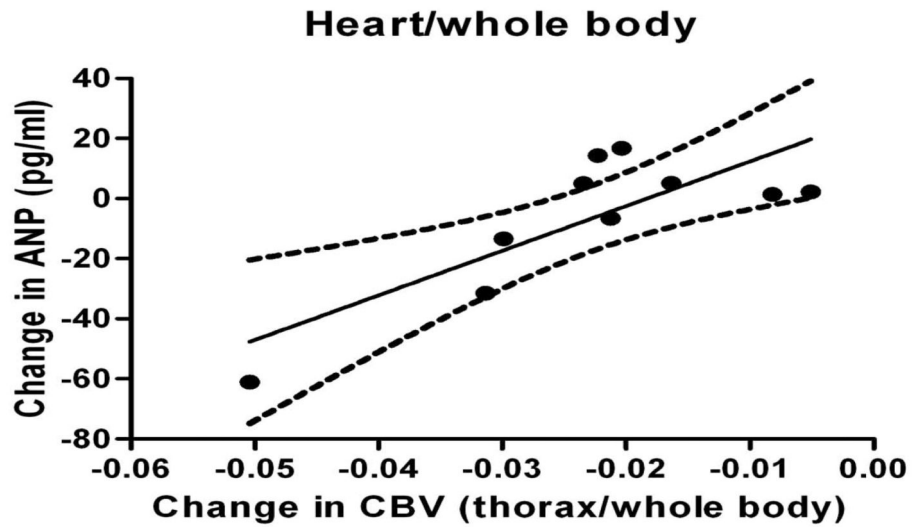
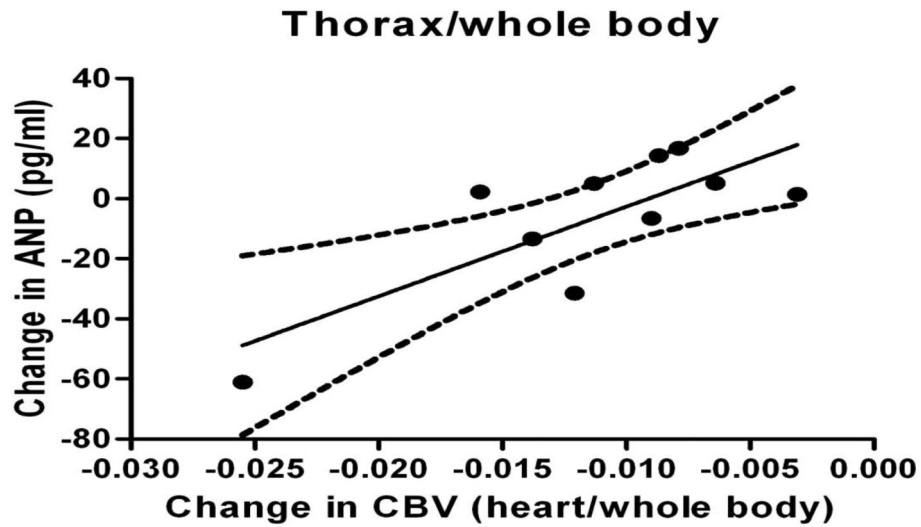
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**Fig. (1).** Regions of interest (ROI) of the thorax, heart, and proximal femur as traced manually on scintigrams obtained using *in vitro* Tc-99m labeled autologous red blood cells.





**Fig. (2).**

Linear regression between changes in CBV (expressed as change in the ratio of the blood pool in the thoracic region relative to the whole body; “thorax/whole body”) and absolute change in arterial plasma ANP concentration in pg/ml (upper panel:  $R=0.80$ ;  $p<0.01$ ) and between CBV (expressed as change in the ratio of the blood pool in the heart region relative to the whole body; “heart/whole body”) and absolute change in arterial plasma ANP concentration in pg/ml (lower panel:  $R=0.78$ ;  $p<0.01$ ).