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1 **Brain natriuretic peptide and NT-proBNP levels reflect pulmonary artery systolic pressure**
2 **in trekkers at high altitude.**

3 **Running title:** PASP and BNP/NT-proBNP

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1 **Summary**

2 Our objective was to evaluate the utility of the natriuretic peptides BNP (brain natriuretic
3 peptide) and NT-proBNP as markers of pulmonary artery systolic pressure (PASP) in trekkers
4 ascending to high altitude (HA). 20 participants had BNP and NT-proBNP assayed and
5 simultaneous echocardiographic assessment of PASP performed during a trek to 5150 m. PASP
6 increased significantly ($p=0.006$) with ascent from 24 ± 4 to 39 ± 11 mm Hg at 5150 m. At 5150 m
7 those with a PASP ≥ 40 mm Hg ($n=8$) (versus those with PASP < 40 mm Hg) had higher post-
8 exercise BNP (pg/ml): 54.5 ± 36 vs 13.4 ± 17 ($p=0.012$). Their resting BNP at 5150 m was also
9 higher: 57.3 ± 43.4 vs 12.6 ± 13 ($p=0.017$). In those with a pathological (≥ 400 pg/ml) rise in NT-
10 proBNP at 5150 m ($n=4$) PASP was significantly higher: 45.9 ± 7.5 vs 32.2 ± 6.2 mm Hg
11 ($p=0.015$). BNP and NT-proBNP may reflect elevated PASP, a central feature of high altitude
12 pulmonary oedema, at HA.

13 **Key words:** Hypoxia, altitude, BNP, NT-proBNP, Pulmonary artery systolic pressure

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1 **Introduction**

2 Large numbers of people trek to high altitude (HA) each year, of which a significant number
3 develop pulmonary hypertension and succumb to HA illnesses such as high altitude pulmonary
4 oedema (HAPE). Currently there is no useful bed-side blood test that can aid in the diagnosis of
5 pulmonary hypertension or HAPE.

6 The cardiac natriuretic peptides, BNP (active) and NT-proBNP (inactive), are derived from a
7 prohormone, proBNP, stored in cardiomyocytes (Hall 2005). Their release is stimulated
8 primarily by myocardial wall tension related to cardiomyocyte stretch and as such they are
9 classically associated with congestive cardiac failure (CCF) secondary to left ventricular
10 dysfunction (LVD) (Hall 2005). However, it is increasingly recognized that hypoxia may also
11 stimulate their release (Casals *et al.* 2009, Due-Andersen *et al.* 2008) and we have recently
12 reported a rise in BNP (Woods *et al.* 2011a) and NT-proBNP (Woods *et al.* 2012) at high altitude
13 (HA). Further, they may also be elevated in conditions associated with pulmonary artery
14 hypertension at sea-level (SL) (Blyth *et al.* 2007) and have been correlated with pulmonary
15 artery systolic pressure (PASP) pre- and post-exercise (Kerr *et al.* 2008).

16 The hypoxia of HA leads to pulmonary vasoconstriction, which in turn **contributes to** an increase
17 in PASP, which may be associated with elevated natriuretic peptides at SL. An exaggerated
18 pulmonary vascular response is of central importance in the development of high altitude
19 pulmonary oedema (HAPE) (Bartsch *et al.* 2005, Maggiorini *et al.* 2001) but symptoms of
20 HAPE can be difficult to distinguish in the early stages. Progressive shortness of breath with a
21 dry cough may occur but is difficult to distinguish from that which commonly occurs at HA
22 (Hall *et al.* 2011). Inspiratory lung crackles are a later feature of HAPE but again are not

1 pathognomic (Hall *et al.* 2011). Only as the disease progresses with orthopnoea through to the
2 typical pink frothy sputum of frank pulmonary oedema does the diagnosis become obvious.
3 Early detection of HAPE is therefore challenging and any simple test with the potential to
4 facilitate early diagnosis and treatment could be life-saving.

5 Conventionally, assessment of PASP either requires invasive assessment, not practical in the
6 field, or non-invasive assessment using trans-thoracic echocardiography that requires a skilled
7 operator and expensive equipment not usually available on conventional expeditions to HA. BNP
8 and NT-proBNP can be assayed on a small venous blood sample and BNP results can be readily
9 available in the field with little expertise using a point-of-care (POC) testing device.

10 Although we have previously reported an association between BNP and NT-proBNP and acute
11 mountain sickness (AMS) (Woods *et al.* 2011a, Woods *et al.* 2012) the evidence above prompted
12 us to establish if NT-proBNP/BNP could also be driven by, and act as a marker for, an elevated
13 PASP in previously healthy individuals exposed to HA.

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1 **Methods**

2 The study was approved by the Ministry of Defence Research Ethics Committee (MODREC),
3 UK and conformed to the standards set in the Declaration of Helsinki. All participants gave
4 written informed consent.

5 As previously described (Woods *et al.* 2012) twenty healthy participants from the UK military
6 participating in Exercise Khumbu Ramble, a trek in Nepal were studied. **Five of the subjects had**
7 **been trekking at high altitude previously, not in the preceding 6 months, and none were HAPE-**
8 **susceptible.** The group flew from Kathmandu (Kat) to Lukla (2840 m) by light aircraft and
9 commenced trekking on day 1. Three main altitudes were used to study participants: 3440 m
10 (day 2); 4270 m (day 6) and 5150 m after ascent of Kala Patthar (5643 m) on day 10. **Participants**
11 **were studied immediately on arrival after a day of trekking (“post-exercise”) and the following**
12 **morning at rest.**

13 Echocardiography and venous blood samples (from antecubital fossa) were performed post-
14 exercise and at rest at the main study altitudes. **Oxygen saturation (digitally on warm hands at**
15 **rest) was measured using a Nellcor NP-20 pulse oximeter (Covidian, MA, USA). BNP was**
16 **assayed using a point-of-care analyzer (Biosite Triage Meter with BNP tests strips, Alere Ltd,**
17 **Cheshire, UK) in the field and NT-proBNP was assayed on frozen samples on return to the UK**
18 **using the Roche NT-proBNP assay (Roche Diagnostics, Mannheim, Germany).** As NT-proBNP
19 is renally cleared renal function was assessed by serum creatinine. PASP was estimated from the
20 maximum velocity of the trans-tricuspid gradient using continuous wave Doppler imaging added
21 to a fixed value of 5 mmHg as previously validated (Yock and Popp 1984). Further off line

1 processing was carried out by a consultant cardiologist (CB), who was blinded to any of the
2 other data, to ascertain PASP.

3 For statistical calculations the software package SPSS 14.0 was used. Parametric or non-
4 parametric statistical tests were applied after performing the Shapiro-Wilks statistic.

5 Changes in dependent variables between altitudes were initially tested by Student's paired t test
6 or Wilcoxon signed ranks test. For independent variables an independent-samples t test or Mann
7 Whitney test was used. Either a within participants ANOVA or a Friedman test was performed to
8 investigate any serial changes in measures (eg PASP or BNP) with ascent. BNP and NT-proBNP
9 levels were also interrogated according to a PASP ≥ 40 mm Hg or < 40 mm since the maximal
10 PASP response in healthy participants to hypoxia is considered 40 mm Hg (Grunig *et al.* 2000).
11 Spearman's rank correlation between BNP, NT-proBNP and PASP was performed, as was a
12 ROC (receiver operating characteristic) analysis to investigate the utility of the natriuretic
13 peptides in identifying a PASP > 40 mmHg.

14 A p value < 0.05 was considered significant throughout. All data are reported as mean \pm SD (\pm
15 range where appropriate) except for figure 1 (mean \pm SEM).

16 Participants were excluded from analysis once they took drugs to aid acclimatization.

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1 Results

2 Patient demographics have been previously reported (Woods *et al.* 2012), in brief the 20 subjects
3 (9 female, 11 male) were 36.1 ± 1.9 years old (range 22-51 yrs); 174.5 ± 1.7 cm high and weighed
4 76.8 ± 3.1 kgs (all data mean \pm SEM). One subject had taken drugs known to affect acclimatization
5 by 4270 m, and 5 participants by 5150 m.

6 PASP rose significantly ($p=0.006$) with altitude from 24 ± 4 at baseline to 39 ± 11 mm Hg at 5150
7 m (table 1). Overall BNP significantly correlated with PASP ($\rho=0.45$, $p<0.001$) as did NT-
8 proBNP ($\rho=0.508$, $p<0.001$). **The correlation between BNP and PASP was strongest at 5150 m**
9 **post-exercise ($\rho=0.7$, $p=0.005$) (figure 1).**

10 At 5150 m post-exercise those with a PASP ≥ 40 mm Hg ($n=8$) (versus those with PASP <40 mm
11 Hg) had higher post-exercise BNP (pg/ml): 54.5 ± 36 vs 13.4 ± 17 ($p=0.012$). Their resting BNP at
12 5150 m was also higher: 57.3 ± 43.4 vs 12.6 ± 13 ($p=0.017$) (figure 2). In the same participants
13 there were non-significant differences in NT-proBNP (pg/ml) at 5150 m: 500 ± 516 vs 146 ± 251
14 (post-exercise, $p=0.083$) and 433 ± 432 vs 117 ± 162 (resting, $p=0.19$). BNP and NT-proBNP had
15 been significantly higher in these participants at the previous altitude of 4270 m (BNP: 40 ± 38.5
16 vs 16.3 ± 27 , $p=0.02$, NT-proBNP: 308 ± 356 vs 117 ± 207 , $p=0.028$). In those with a pathological
17 (≥ 400 pg/ml) rise in NT-proBNP at 5150 m ($n=4$) PASP was significantly higher than those
18 without: 45.9 ± 7.5 vs 32.2 ± 6.2 mm Hg ($p=0.015$) (figure 3).

19 ROC analysis (to investigate the utility of BNP and NT-proBNP measured at HA in identifying a
20 PASP >40 mmHg) revealed an AUC of 0.741 (95% CI 0.591-0.85, $p=0.001$) and 0.743 (95% CI
21 0.612-0.874, $p<0.001$) for BNP and NT-proBNP respectively. An NT-proBNP cut-off of 133
22 pg/ml gave a sensitivity of 70% and a specificity of 83% for identifying a high PASP.

1 Serum creatinine { $\mu\text{mol/l}$, mean \pm SD,(range)} at baseline was 78.11 \pm 8.8 (63-95) and at 5150 m
2 94.3 \pm 16.4 (76-142) (p<0.001).

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1 **Discussion**

2 The main, novel, finding of this study is that at HA those with a markedly elevated PASP appear
3 to have higher BNP and NT-proBNP than those with a more moderate PASP.

4 An abnormally high PASP is a key feature in the pathophysiology of HAPE (Bartsch *et al.* 2005,
5 Maggiorini *et al.* 2001) It has been suggested that the maximal PASP response to hypoxia (FiO₂
6 12%) in healthy participants is 40 mm Hg (Grunig *et al.* 2000) Indeed, HAPE susceptible
7 participants under hypoxic conditions have a much higher PASP than non-susceptible
8 participants (33±6 vs 50±9 mm Hg) (Berger *et al.* 2005) that can be further exacerbated by an
9 exercise stimulus (Dehnert *et al.* 2005)

10 Although markedly elevated levels of BNP and NT-proBNP are used as a marker of CCF they
11 are also moderately elevated in pulmonary hypertension at SL (Blyth *et al.* 2007) There is a
12 significant difference between their “normal” levels {median BNP in a healthy population age
13 <45 years is 7.7 pg/ml, (Alere Triage BNP test product insert); median NT-proBNP in a healthy
14 population age <50 years is 60.5 pg/ml (Bernstein *et al.* 2011)} and those conventionally used to
15 diagnose CCF (>100 and >400 pg/ml respectively). The levels we report associated with PASP
16 ≥40 mm Hg are above the normal range, occasionally above the diagnostic level for CCF, and
17 consistent with those seen in other states of pulmonary hypertension.

18 Only one previous study has examined the relationship between NT-proBNP and PASP at HA
19 (Toshner *et al.* 2008). This study reported no rise in NT-proBNP on arrival at 5200 m following
20 5 days acclimatization at 3600 m despite a rise in PASP from 16.1 mm Hg to 39.4 mm Hg. They
21 reported an NT-proBNP of 12.9 pg/ml on arrival compared to the 301 pg/ml we recorded at a
22 very similar altitude using the same assay. A critical difference between these studies may be the
23 exercise stimulus involved: our participants trekked to 5150 m, as opposed to arriving at 5200 m

1 by vehicle (Toshner *et al.* 2008). The fact that they reported very similar PASP to that which we
2 observed suggests that exercise, which also increases PASP, may be a key factor in the rise in
3 NT-proBNP at altitude. This is supported by our group's previous work that has shown no rise in
4 BNP during acute hypobaric hypoxia with minimal exertion (<5 minutes) (Boos *et al.* 2012,
5 Woods *et al.* 2011b) despite inducing a rise in PASP from 24.3 to 35 mm Hg (Boos *et al.* 2012).

6 Prolonged exertion at SL has been associated with a rise in natriuretic peptides (Leers *et al.*
7 2006, Neumayr *et al.* 2005, Scharhag *et al.* 2008) that has recently been suggested may relate to
8 ventriculo-arterial mismatch (Sahlén *et al.* 2012). Conversely, short duration exercise is
9 generally not associated with a rise in natriuretic peptides (Marumoto *et al.* 1995, Nishikimi *et*
10 *al.* 1997). Increases in BNP and NT-proBNP [under normoxic conditions](#) have been noted to
11 resolve within several to 24 hours (Leers *et al.* 2006, Neumayr *et al.* 2005, Scharhag *et al.* 2008)
12 and may explain why no rise was previously documented in trekkers at HA when samples were
13 only taken the following day at rest (Feddersen *et al.* 2009).

14 [In our study the rise in BNP and NT-proBNP](#) seen after exercise at HA not only persisted at rest
15 the following day but had often risen higher despite a fall in PASP. Exercise is likely to
16 exacerbate myocardial hypoxaemia at HA and be a greater stimulus to BNP release than hypoxia
17 alone. In support of this there are hypoxia-responsive elements in the promoter sequence of the
18 BNP gene (Luo *et al.* 2006) and hypoxia is known to stimulate natriuretic peptide release (Due-
19 Andersen *et al.* 2008, Möllmann *et al.* 2010). In isolated human heart muscle (where the
20 mechanical wall stress usually credited with stimulating BNP release has been removed) hypoxia
21 induces a rapid increase in myocardial BNP expression (Möllmann *et al.* 2010). Further, acute
22 myocardial hypoxaemia in pigs induces a marked rise in tissue BNP mRNA and a premature
23 mRNA BNP followed by rapid release (Goetze *et al.* 2004). We therefore speculate that the

1 initial rise in BNP and NT-proBNP following exercise at SL or HA may be due to release of
2 proBNP from the small stored pool in cardiomyocytes. If there has been sufficient stimulus then
3 in hypoxic conditions there may then be rapid up-regulation of BNP gene transcription that
4 accounts for the persisting and elevated levels at rest.

5 It also makes physiological sense that the stressed and hypoxic myocardium should release BNP
6 as it induces a natriuresis and is a vasodilator thereby acting to reduce myocardial wall stress.
7 Infused BNP is known to attenuate hypoxaemia-induced increases in PASP and pulmonary
8 vascular resistance (Cargill and Lipwoth 1995) and BNP levels are also employed to guide
9 vasodilator therapy in pulmonary artery hypertension (Yap 2004).

10 We are not proposing that a high PASP is the sole determinant of a high BNP level at HA. BNP
11 secretion may be stimulated by many factors. Although primarily stimulated by cardiomyocyte
12 stretch and end-diastolic wall stress (Hall 2005) BNP is also stimulated by ischaemia, cardiac
13 hypertrophy and oxidative stress (Nohria 2006). A high BNP may also occur in the context of
14 cardiac transplant rejection where it does not reflect haemodynamic changes but is thought to
15 reflect inflammatory changes within the myocardium (de Bold 2007). In the classical situation of
16 CCF there is still significant heterogeneity in BNP levels. Patients with a similar degree of left
17 ventricular systolic dysfunction demonstrate considerable inter-individual variation in BNP
18 levels (Nohria and Givertz 2006) but this has not prevented BNP from becoming a useful marker
19 of CCF. In a similar fashion we are not proposing that at HA PASP is an isolated stimulus to
20 BNP but that there is some relationship between high PASP and a high BNP or NT-proBNP at
21 HA that may ultimately prove useful in detecting not only high PASP but also cases of HAPE.

1 Limitations to our study include that although high PASP is a critical factor in the
2 pathophysiology of HAPE our study does not include overt, clinical cases of HAPE. As such this
3 report can only record an association between BNP and NT-proBNP and high PASP. Further
4 work is needed to establish any relationship with subsequent HAPE. In addition, creatinine rose
5 statistically significantly and theoretically a change in renal function could effect NT-proBNP
6 levels (since NT-proBNP is renally cleared). However, since the absolute rise was marginal and
7 only 1 subject had a creatinine $>125\mu\text{mol/l}$ ($142\ \mu\text{mol/l}$) we feel this is unlikely to have a
8 significant effect on the overall levels of NT-proBNP and the findings of this study.

9 The correlation between PASP and BNP and NT-proBNP were moderate but highly significant
10 and the ROC analysis suggests that both natriuretic peptides should be further evaluated for their
11 utility in identifying a high PASP at HA in a larger cohort. While an NT-proBNP cut-off of 133
12 pg/ml that gives a sensitivity of 70% and a specificity of 83% for identifying a high PASP may
13 not be perfect, in the context of the cohort studied it is encouraging.

14 In conclusion, HAPE is a serious condition with an incidence estimated at up to 4% at 4559m
15 (Maggiorini *et al.* 2001). The increasing number of visitors to HA represent a significant
16 challenge in the early recognition, treatment, and possible evacuation of those who succumb to
17 HAPE. Pulmonary hypertension, a key feature, is difficult to diagnose without either invasive or
18 non-invasive techniques that require expertise and equipment beyond the realm of most
19 conventional HA expeditions. Any association between high PASP and BNP or NT-proBNP at
20 HA could therefore prove clinically useful in differentiating HAPE from other medical
21 conditions, particularly in the early stages. Our data suggest that BNP and/or NT-proBNP may
22 have potential as a marker of pulmonary hypertension in previously healthy participants at HA

1 and as such, particularly with near-patient testing, may facilitate identification of a
2 pathophysiological state that without appropriate management may be life-threatening.

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1 **Table 1** Oxygen saturation, PASP, BNP and NT-proBNP with ascent to 5150 m in the Khumbu
 2 valley, Nepal (n=20 at 1300 and 3400 m, n=19 at 4270 m and n=15 at 5150 m). The BNP and
 3 NT-proBNP data have previously been reported (Woods *et al.* 2012) but not in the context of
 4 PASP.

Altitude	SpO2 (%)	PASP (mm Hg) (mean \pm -SD, range)	NT-proBNP (mean \pm - SD, range)	BNP (mean \pm - SD, range)
Kat (1300 m) (rest)	96 \pm 0.5	24.1 \pm 4.1 (18-32)	37 \pm 30 (9.4-111)	9.2 \pm 2 (5-35)
3400 m (post- exercise)		36.8 \pm 8.2 (23-56)	111.2 \pm 96 (35-379)	N/A
3400 m (rest)	87.5 \pm 1.2	31.6 \pm 8 (21-53)	334.8 \pm 541 (27-2081)	N/A
4270 m (post- exercise)		38.6 \pm 9.2 (25-62)	152 \pm 238 (18-994)	16.6 \pm 4 (5-56)
4270 m (rest)	85.1 \pm 1.2	34.8 \pm 7.7 (25-52)	207 \pm 291 (18-1045)	28.9 \pm 9 (5-101)
5150 m (post- exercise)		39.4 \pm 10.5 (25-57)	301 \pm 408 (15-1539)	32.3 \pm 8.8 (5-95.6)
5150 m (rest)	73.8 \pm 2	35.7 \pm 8.4 (25-53)	259 \pm 346 (18-1109)	33.3 \pm 9.7 (5-123)

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1 **Figure 1** At 5150 m post-exercise there was a significant correlation between BNP and PASP
2 ($\rho=0.7$, $p=0.005$).

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4 **Figure 2** BNP (mean \pm SEM shown) with ascent according to a PASP \geq 40 mm Hg (n=8) vs < 40
5 mm Hg at 5150 m post-exercise (n=20 at 1300 and 3400 m, n=19 at 4270 m and n=15 at 5150
6 m). Those with a PASP \geq 40 mm had post-exercise BNP (pg/ml) that was significantly ($p=0.012$)
7 higher (54.5 \pm 36 vs 13.4 \pm 17) as was resting BNP at 5150 m (BNP: 57.3 \pm 43.4 vs 12.6 \pm 13
8 $p=0.017$).

9
10 **Figure 3**

11 In those with a pathological (\geq 400 pg/ml) rise in NT-proBNP at 5150 m (n=4) PASP (mean \pm SD)
12 was significantly higher than those with a NT-proBNP <400 pg/ml (n=11): 45.9 \pm 7.5 vs 32.2 \pm 6.2
13 mm Hg ($p=0.015$).

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Figure 1

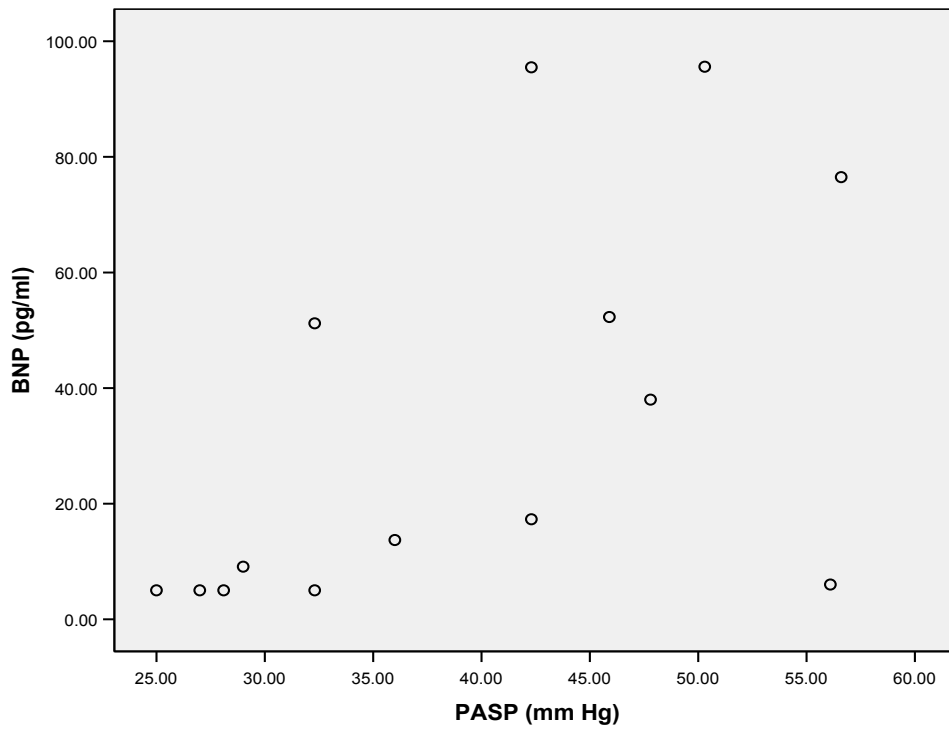


Figure 2

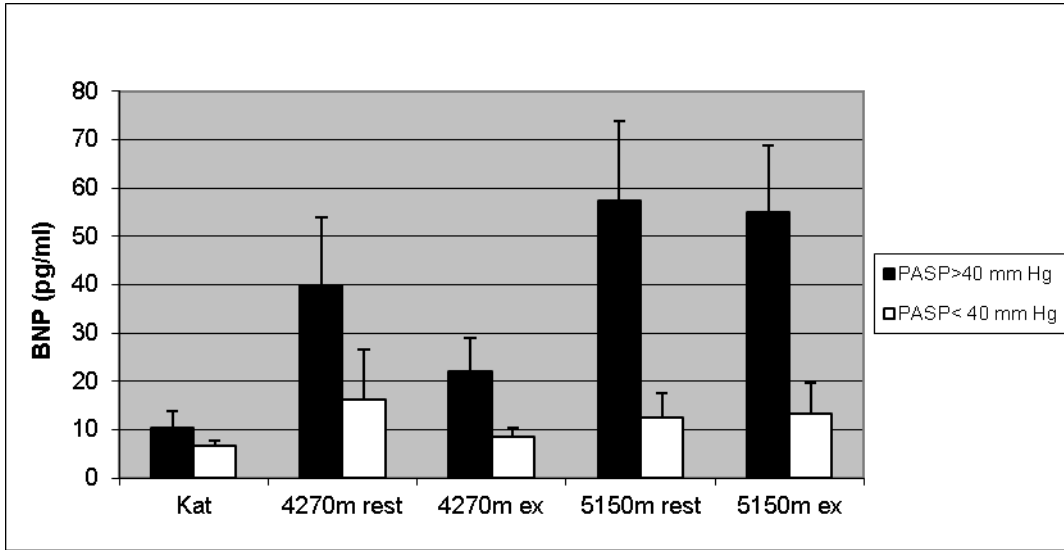


Figure 3

