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Working memory capacity and surgical performance whilst exposed to mild hypoxic hypoxaemia (3000m)

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Abstract – 250

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Abstract

INTRODUCTION: Medical Emergency Response Team (MERT) helicopters fly at altitudes of 3,000m in Afghanistan (9,843ft). Civilian hospitals and disaster-relief surgical teams may have to operate at such altitudes or even higher. Mild hypoxia has been seen to affect the performance of novel tasks at flight levels as low as 5,000ft. Aeromedical teams frequently work in unpressurised environments; it is important to understand the implications of this mild hypoxia and investigate whether supplementary oxygen systems are required for some or all of the team members.

METHODS: Ten UK orthopaedic surgeons were recruited and in a double blind randomised experimental protocol, were acutely exposed for 45 minutes to normobaric hypoxia (fraction of inspired oxygen (FiO_2) ~14.1% - equivalent to 3000m/10,000ft) or normobaric normoxia (sea-level). Basic physiological parameters were recorded. Subjects completed validated tests of verbal working memory capacity (VWMC) and also applied an orthopaedic external fixator (Hoffmann® 3, Stryker UK) to a plastic tibia under test conditions.

RESULTS: Significant hypoxia was induced with the reduction of FiO_2 to ~14.1% (SpO_2 87% vs. 98%). No effect of hypoxia on VWMC was observed. The pin-divergence score (a measure of frame asymmetry) was significantly greater in hypoxic conditions (4.6mm) compared to sea level (3.0mm), there was no significant difference in the penetrance depth (16.9 vs. 17.2mm). One frame would have failed early.

DISCUSSION: We believe that surgery at an altitude of 3000m when unacclimated individuals are acutely exposed to atmospheric hypoxia for 45 minutes, can likely take place without supplemental oxygen use but further work is required.

KEYWORDS: In-Flight Surgery, Resuscitation, Altitude, External Fixation

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Introduction

Medical Emergency Response Team (MERT) helicopters fly at altitudes of 3,000m (9,843ft) in Afghanistan and Iraq. Civilian hospitals and disaster-relief surgical teams may have to operate at such altitudes or even higher. An example is Leh Ladakh Hospital in India, situated at 11,400ft, which was the scene of a major humanitarian disaster after flash-flooding in 2010. Furthermore, Military Surgical Resuscitation Teams (SRT) may operate in-flight at 10,000ft or higher in non-pressurised aircraft such as the CV-22 Osprey Tilt-Rotor Aircraft. Should decompression occur in flight, or pressurization be unavailable; it is currently unclear as to whether the surgical team would benefit from supplemental oxygen by mask or other supportive measures.

Hypoxic impairment has been demonstrated at relatively low altitudes; increases in reaction time have been seen at 7,000ft¹⁴, in spatial awareness testing at 8,000ft⁷, arithmetic and decision-making errors at 12,000ft¹⁸ and working memory at 14,000ft⁹. The normal cabin pressure at a flight level (FL) of 28,000ft in most military aircraft is 5,000ft. Although preservation of simpler task completion seems to be maintained at FL altitudes, higher level decision-making (e.g., adapting to unforeseen circumstances under extreme time pressure), 3D and colour vision may be affected at this lower altitude⁴, particularly if hypoxia impairs aspects of cognitive functioning such as working memory capacity.

Working memory (WM) can be defined as a system of cognitive mechanisms that facilitate the completion of various tasks through their capacity for storing, retaining, and processing information^{1,2}. More specifically, working memory capacity (WMC) relates to the attentional processing component (i.e., central executive) of Baddeley and Hitch's² model of working memory. According to controlled attention

theory¹⁰, WMC represents a person's ability to regulate and direct attention, which is particularly important when completing tasks that are novel in some way, or need to be performed in contexts where interference or distractions are prevalent. Recent evidence from high-performance sport contexts^{11,17} suggests that WMC is a good predictor of how an athlete will perform under pressure (e.g., unfamiliar situations, distracting thoughts), with high WMC associated with greater success when attempting to block out task-irrelevant information. Although WMC is now a widely-studied topic within psychological science¹, it has received little attention within domains such as medicine and surgical skill.

Given that mild hypoxia has been seen to affect the performance of novel tasks (improved after practice) at levels as low as 5,000ft⁷, the purpose of this study was to examine whether there was deterioration in physiological parameters, surgical skill, and WMC when exposed to short, 45-minute periods of normobaric hypoxic hypoxaemia (3,000m / ~10,000ft). There are a small number of studies, which suggest that normobaric hypoxia is of less a physiological stress compared with hypobaric hypoxia⁶), even though the partial pressure of oxygen is the principal physiological stimulus to high altitude adaptation¹⁶. However, these data are potentially confounded, particularly by small samples sizes⁶, making it difficult to form a consensus. Therefore, simulating high altitude through the use of normobaric hypoxic in a controlled environment, was deemed appropriate for this pilot study, before taking the research into the field. This pilot study aimed to develop a model to test the effect of hypoxia on cognition and motor skills relevant to a surgical team. This would then inform whether supplemental oxygen or other novel solutions were required for unacclimated teams performing emergency surgery at altitude, either in an aircraft or if deployed rapidly to moderate altitudes.

Methods

Subjects

Ten UK orthopaedic surgeons were recruited to take part in this study. They were all consultants or trainees working in the North-east of England. Seven were male and three were female. The experimental procedures were fully explained to each participant before the study, and all subjects provided written informed consent. The protocol used during the investigation had received full institutional ethical approval from the Ethics Committee of Leeds Beckett University.

Each subject completed an initial health-screening questionnaire and underwent a basic medical examination. Exclusion criteria included pregnancy, active asthma, chronic obstructive airways disease, hypertension, past history of stroke, myocardial infarction, angina, diabetes and peripheral vascular disease. All female subjects were offered a pregnancy test, as hypoxic exposure may potentially be harmful to a foetus¹². Further, all subjects were non-smokers. Due to the nature of the adopted cognitive functioning test, subjects with dyslexia/dyspraxia were also excluded from the study. Only normal healthy volunteers were then allowed to enter the normobaric hypoxic chamber. All subjects were required to abstain from caffeine and alcohol for the 24 hours prior to the trial.

Experimental Design

In a double blind, repeated measures randomized control trial, subjects were acutely exposed for 45 minutes to normobaric hypoxia (fraction of inspired oxygen (FiO_2) of ~14.1% (considering water vapour pressure³, equivalent to 3,000m (~10,000ft, PiO_2 100 mmHg) or near sea level (absolute altitude ~113m (Leeds Beckett University,

Centre for Sports Performance, Leeds, UK)) under normobaric normoxia (FiO_2 of 20.9%) in an environmental chamber (TISS, Alton, UK and Sporting Edge, Sherfield on Loddon, UK).

However, only the lead researcher on each given testing day knew the environmental conditions, but their only interaction was the assessment of the physiological parameters. They kept the oxygen saturation recordings confidential from the other experimenters in the chamber who were assessing the verbal working memory capacity (VWMC) and surgical skills, to ensure the best possible blinding of the protocol. With regards the ambient breathing for the sea level condition, this was achieved by the system providing fresh air to the chamber at a high flow rate, drawn in by a compressor from the external environment. In contrast, during the altitude condition the hypoxic system filtered out oxygen from external ambient air, then supplied air with a reduced fraction of oxygen to the chamber. This was then mixed with ambient air once the set point was met, to maintain the hypoxic air within 0.15% of the required FiO_2 .

Both tests were performed on the same day, one in the morning and one in the afternoon with a minimum of a two-hour wash-out time between them. Subjects were unaware of the FiO_2 being inhaled, in that the control unit for the chamber was concealed from the subjects and the mechanical operating noise of the chamber was not dependent on its working FiO_2 .

Before entering the chamber, ten subjects completed baseline tests of VWMC. Subsequently baseline physiological parameters; heart rate, blood pressure, oxygen saturation (SpO_2) were measured. Subjects then entered the chamber and rested for 15 minutes simulating rescue flight time for casualty retrieval. This also allowed for internal physiological equilibration. After this “simulation period”, subjects

completed the test of VWMC for a second time, with further repeat measurement of baseline data. These physiological measurements were completed at 15 minutes from the start of the VWMC test. In the event of any subject feeling unwell, having an oxygen saturation of less than 75%, or heart rate of greater than 170 bpm, they would have been removed from the chamber. This was never necessary.

Ten subjects were then asked to apply an orthopaedic external fixator (Hoffmann[®] 3, Stryker UK) to a dry plastic tibia - hand-drilling four threaded pins into the bone and connecting them together with carbon-fibre bars. This application was assessed by a blinded observer in regard to; time taken and accuracy, alignment, efficacy and success of construct. Subjects were allowed a maximum of 15 minutes for this task. Ten subjects then repeated the VWMC test and were subjected to physiological measures for a third and final time 15 minutes after leaving the chamber. The chamber condition was then reset before the experiment was repeated at the alternate chamber parameter (e.g. sea-level to hypoxic).

Procedures

Subjects completed validated tests of VWMC, using an Operational Span protocol adapted from Conway, Kane, Bunting, Hambrick, Wilhelm, and Engle⁵. Tasks including mental calculations, reasoning, planning, and complex decision-making rely on WMC^{8,17}. For surgical teams to perform optimally, VWMC is important to allow for a) effective verbal communication, and b) accurate response to verbal information, especially under time pressure and in the face of multiple potential distractions. For example, in an emergency medical procedure, an MERT member would often be required to attend to and process multiple sources of verbal information such as instructions from other colleagues within the team.

Simultaneously, the MERT member would also need to hold other information in mind to inform the completion of tasks such as calculating, preparing, and administering appropriate doses of anaesthetics. With this example in mind, a test of VWMC was deemed an appropriate measure of cognitive function within this study, as it would closely replicate a number of cognitive challenges commonly faced by MERT surgeons at altitude.

The test (which takes between 4 and 10 minutes) consisted of eight separate trials, with each trial including a series of between two and five standardised mathematical problems. Trials were presented to subjects via a Laptop (Toshiba Tecra A50-A-151, Neuss, Germany) using *Microsoft PowerPoint®*. Within each trial, subjects were required to read aloud each mathematical problem, and state whether the answer provided was correct or incorrect, before reading an unrelated word aloud. Therefore, an example of a single problem and correct response would appear as follows:

Example problem presented to participant: Is $(6 \times 2) - 5 = 7$? Class.

Example correct response: "*Is six times two minus five equal to seven...yes...class.*"

The mathematical problem was the “interference” task necessary to obtain a measure of VWMC. In combination, the mathematical problem and recall of unrelated words represents a task sufficiently complex to test VWMC as well as inhibition (referred to as “selective attention”), another executive function closely associated with, yet distinct from WM⁸. The principal measure of VWMC was the number of unrelated words that subjects were able to accurately recall in sequence

order at the end of each trial. However, the number of mathematical problems accurately completed by subjects was also recorded as a manipulation check. Provided subjects responded correctly to 80% of the posed mathematical problems, their VWMC data could be included within the analysis. All subjects met this criterion, which indicated that subjects were expending sufficient levels of cognitive effort on both tasks simultaneously, ensuring that the total number of words recalled in correct sequence was sufficient as a reliable measure of VWMC⁵. VWMC test performance was assessed using the Partial-Credit Unit (PCU) scoring method, as advocated by Conway et al.⁵. PCU expresses the proportion of elements that are recalled correctly *in the order they were originally presented*. Applying the PCU method, the following would apply for the accurate recall of three unrelated words:

Order of words presented within a single trial: Table, Look, Melt.

Full Recall (all words recalled in order = score of 3/3): “*Table, Look, Melt*”.

Partial Recall (one word recalled in correct order = score of 1/3): “*Look, Table, Melt*”.

The PCU method was favoured over All-or-Nothing scoring (i.e., where no credit is awarded for partially accurate recall within a single trial) because it follows established procedures from the development and application of psychometrics⁵.

Given the novelty of this test, subjects were provided with instructions and a practice trial before every test of VWMC that was conducted.

Heart rate was obtained from a heart rate monitor (Polar RS400, Polar, Electro Oy, Finland). Blood pressure was measured using an automated blood pressure cuff M6 (Omron Healthcare, Milton Keynes, UK) with the participant sat upright at rest. Resting recordings of oxygen saturation (SpO₂) were performed using a Nellcor N-

20P pulse oximeter (Nellcor Puritan Bennett, Coventry, UK). According to the manufacturer's specifications, this device can operate at altitudes up to 6200m and SpO₂ within the range of 70-100% is accurate to $\pm 2\%$, when compared to arterial samples.

Extremity trauma and ballistic limb injuries are the wounds most often seen in military trauma. Such wounds require debridement and skeletal stabilization. Debridement adequacy; arterial, intestinal or vein repair were all considered as test skills but all authors agreed that these would be almost impossible to reproducibly test or score. Therefore, the application of an external fixator was chosen even though no standardised tests or scoring systems exist for measuring its accuracy or efficacy. That having been stated, the external fixator frame should however, be applied as symmetrically as possible: If the pins converge too much (or even touch) on the far side of the bone, they will exhibit reduced purchase and stress-risers can even cause a fracture. If the frame is overly asymmetric, it will be biomechanically disadvantaged. It is possible, however, to assess and compare pin penetration: in clinical practice, the pins should normally just millimetrically penetrate the far bone cortex to avoid deep tissue, nerve or vascular damage. For pin penetration, this was measured and summated for all four pins per fixator. Higher scores being perceived as worse, a 5,6,3,5mm penetration thus produces an overall penetration score of 19mm. Pin penetration data were unfortunately not recorded on the first test run. The time taken to fully apply the fixator was also recorded.

Pin alignment can also be measured (Figure 1): For biomechanical reasons, all pins should be parallel to each other. Although it would have been technically possible to directly measure the angular difference between pins, this could have led to parallax-based intra-observer error. The authors felt that a more accurate way to

measure angular change was to measure the successive horizontal differences in spacing between the pins (or pin-hole centres) as they passed through the near and far cortices of the studied bone. Between four pins, this then produces three near and three far millimetric linear values. These values can then be compared: For example, a score of 55,70,55mm (near) and 55,70,55mm (far) produces a 'difference or asymmetry score' of zero (0).

To explain this further, a 'near' figure of 55mm means that the distance between the first and second inserted pins is 55mm on the bone surface closest to the skin. The figure of 55mm on the 'far' surface demonstrates that this separation is absolutely maintained on the bone surface farthest away from the skin. These pins are parallel – which is best biomechanically. Four parallel pins with successively equally spaced entrance and exit holes, therefore produce an overall difference score of zero. Conversely, if the distance between two successive pins was 55mm near and 60mm far, this would indicate that these pins were skewed by 5mm. Therefore an asymmetry score of 85,70,80mm (near) and 88,70,88mm (far) produces a score of 11 - indicating skewed pins (diverging or converging) with over a centimeter difference in spacing between the entrance and exit holes. The higher the score, the worse the asymmetry. Skewed pins make frame construction more difficult and negatively alter the strength and biomechanics of the fixator construct

Statistical Analysis

All data were approximately normally distributed (Shapiro, Wilk) and are presented as mean \pm SD. Statistical analysis was conducted using IBM SPSS version 22 for the physiological and psychological data and Graphpad2 for the external fixator

data. A 0.95 level of confidence was predetermined to denote statistical significance ($p < 0.05$).

VWMC was analyzed using a Two-Way (Altitude (2 conditions; hypoxia and normoxia) x Time (3 time points) Repeated Measures Analysis of Variance (ANOVA). It is important to note that prior to final data analysis, one of the subjects disclosed that they found the test of VWMC difficult, possibly due to being dyslexic. Based on this information, it was decided not to include this participant's data within the final analysis.

One-way ANOVA was used to compare differences in the physiological variables. Where significance was detected, *post hoc* analysis was performed using a paired t-test with Bonferroni adjustment (alpha level of 0.05 per test ($0.05 / 10$)). There were five measurement points (baseline normoxia (T1), 15 min into the normoxic exposure (T2), 15 minutes post normoxic exposure (T3), 15 minutes into the hypoxic exposure (T4), and 15 minutes post hypoxic exposure (T5)) for the physiological variables, making ten paired comparisons (T1 vs. T2, T1 vs. T3, T1 vs T4, T1 vs. T5, T2 vs. T3, T2 vs T4, T2 vs T5, T3 vs T4, T3 vs T5 and T4 vs. T5), so the alpha level was adjusted accordingly to detect significance. A 2-Tailed t-test was used to analyse the external fixator data.

Results

Surgical skill: External Fixator Application

Time taken to apply the fixator was 272.22 seconds in the hypoxic environment and 293.87 seconds at sea-level (paired t-test, $p = 0.26$, NS). Table I shows that the divergence score was significantly greater in hypoxia (paired t-test, $p = 0.04$) compared to sea level. Table II shows that there was no significant difference in

the penetration depth between conditions (paired t-test, $p = 0.88$). One pin was not placed within the clamp.

[Insert Tables I & II]

Test of VWMC

Repeated Measures ANOVA did not reveal significant main effects of altitude condition ($df = 1$, $F = 0.046$, $p = 0.836$) or time point ($df = 2$, $F = 1.624$, $p = 0.229$). See Table III for Mean scores and SDs.

[Insert Table III]

Physiological parameters

There were no significant differences in systolic ($df = 4$, $F = 0.363$, $p = 0.883$) and diastolic ($df = 4$, $F = 0.827$, $p = 0.518$) blood pressure between time points, as was the case for heart rate ($df = 4$, $F = 0.835$, $p = 0.513$), Table IV. Further, there were no significant differences in SpO₂ over time when measurements were taken during normoxia (paired t-test, $p = 0.081$). However, SpO₂ was significantly lower (paired t-test, $p < 0.001$) following 15 minutes of hypoxic exposure compared to any other measurement time point in normoxia.

[Insert Table IV]

Discussion

Our study was designed to answer two questions: a) In an unpressurised environment, would surgical teams benefit from supplemental oxygen or other supportive measures? and b) If delivered to altitude to perform humanitarian assistance, should surgical teams need to wait (i.e., acclimatise) before performing complex tasks? The answer to both questions, based on the results of our pilot study, would appear to be not significantly.

Our subjects performed cognitive and surgical tasks under normobaric hypoxia ($\text{FiO}_2 \sim 14.1\%$) over a 45-minute period. Analysis of external fixation performance showed that although all frames would have worked (i.e., acutely stabilised a fracture), an aggregate measure of asymmetry in the pin's placement was greater in the hypoxic condition. Parallel pins are biomechanically better. Pin penetration was not significantly different. However, in one hypoxic application, one fixator pin was not placed in its fixator clamp. This therefore connected only one pin to the frame on that side of the fracture and its fixation (bone-hold) would likely have failed early when stressed. However, on expected handover to the next link in the casualty evacuation chain, this would normally be immediately remedied. External fixator application, like many orthopaedic techniques, requires 3D visualization of a number of reference points in space, central processing of these points and then by using fine and gross motor skills, rapidly and accurately placing a pin through both cortices of the relevant bone.

Performance would not have been influenced by acute mountain sickness, as signs and symptoms typically take more than four hours to develop and our subjects were only exposed to moderate altitude for up to 45 minutes. The altitude being simulated 3,000m ($\sim 10,000$ ft) is not an excessive altitude and although not encountered routinely within the UK, is common at the top peaks of European and

North American Ski Resorts (e.g., Steamboat Springs, Colorado, 11,000ft; Val Thorens Village, France 2,300m, highest lift 3,200m). In all our subjects, despite significant decreases in SpO₂, no significant effects of hypoxia on heart rate, systolic and diastolic blood pressure were observed.

Our data showed no effect of altitude on VWMC, indicating that impairment of this particular measure of cognitive function is unlikely to occur for surgeons performing at altitudes of 3,000m (~10,000ft) during a 45-minute period of acute hypoxic exposure. Although such findings are encouraging for initiatives related to the deployment of mobile surgical teams, it is important to consider the following implications regarding the experimental design and methods adopted in follow-up work. First, it is important that future studies include more extensive baseline/familiarisation periods in relation to tests of cognitive function (e.g., VWMC) and surgical skill. Second, it would be useful for subsequent work to employ and develop assessments of cognitive function (e.g., Stroop Tests for measuring Inhibition/Interference Control, Spatial Span Tests, Reasoning Tasks) that also reflect the context-specific demands placed on surgical teams.

Clearly, teams that have time to acclimate at altitude (e.g., those that live and work continuously at altitude in mountainous areas such as Kashmir or Nepal) should not theoretically have the same problems as unacclimated teams. However, acclimatisation is complex, involving various bodily systems, all of which adapt to altitude across different time periods ranging from days to weeks. Acclimatisation is not always possible, particularly as military air bases are usually situated (if possible) closer to sea level as the thicker air aids heavier take-off loads. There then often follows a rapid ascent to altitude, which is what we sought to simulate in our testing. Yet, even though SpO₂ was lower with acute hypoxic exposure, hypoxemia did not

influence VWMC or fixator task performance during a short 45-minute exposure period. Whether surgical skills and/or cognitive functioning are affected during longer exposures to altitude is yet to be established, and should be a focus for future research.

It is clear from aviation research that immediate exposure to altitudes higher than 10,000ft can produce profound and disabling, life-threatening hypoxaemia. Such non-explosive decompression might occur in compressor failure or the requirement to operate with 'doors-open' such as in a CV-22 Osprey or a CH-47 Chinook airframe. Exposure to this profound hypoxaemia was not our aim and we could not envisage any real-world scenario where our teams would be required to operate under these conditions. If an aircraft decompressed at this altitude, it would immediately descend to 5,000ft and proceed to the nearest airfield.

We accept the following potential confounders in our research: Individual performance within the study may have been influenced by prior medical experience and familiarity with the Hoffman 3 fixator. However, in using the subjects as their own controls in normoxia, we believe the potential impact of any learning effect was minimised. The number of subjects was small, and we did not test the full range of skills that may be impeded at altitude, meaning that definitive conclusions cannot be made at this stage. There are many other aspects of providing medical care in flight which may be affected under hypoxic conditions as well as situations which may potentiate any hypoxia such as exercise and stress, posture (e.g., crouched anaesthetists), environmental variables (e.g., heat, noise) on individual and team performance at altitude. Furthermore, this study used normobaric hypoxia, which may provide a different physiological stress compared to hypobaric hypoxia. These considerations should direct future research. Specifically, researchers are urged to

include extended periods of atmospheric-variable exposure within subsequent studies of this nature. In addition, it is important that researchers make greater efforts to incorporate a range of cognitive tests that are informed by and accurately reflect established models and theories of cognitive functioning ^{1,2}, attentional control ¹⁰ and decision-making ¹³. In conclusion: Surgery at an altitude of 3,000m (~10,000ft), when unacclimated individuals are acutely exposed to atmospheric hypoxia for 45 minutes, can likely safely take place without supplemental oxygen use. We observed changes in fine motor surgical skills under simulated conditions but further larger studies are required. Our future research will assess if more safeguards are required at higher altitudes or stressful environmental conditions to prevent performance deterioration in surgical teams.

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Tables:*Table I. Divergence scores (mm) under hypoxic and sea-level conditions*

Divergence (mm)		
	<i>Hypoxic (n = 10)</i>	<i>Sea-Level (n = 10)</i>
	4	0
	11	6
	2	4
	3	1
	5	3
	3	2
	4	4
	6	3
	3	3
	5	4
M	4.60*	3.00*
SD	2.55	1.70
SEM	0.81	0.54

*significantly different ($p < 0.05$)

Table II. Pin Penetration scores (mm) under hypoxic and sea-level conditions

Pin Penetration (mm)		
	<i>Hypoxic (n = 9)</i>	<i>Sea-Level (n = 9)</i>
	13	19
	20	23
	7	10
	18	21
	13	13
	21	16
	17	20
	20	20
	23	13
M	16.90	17.20
SD	5.04	4.41
SEM	1.68	1.47

Table III. VWMC performance of subjects ($n = 9$) in normoxia and hypoxia

	Baseline Normoxia	15 min Normoxia	15 min Post Normoxia	15 min Hypoxia	15 min Post Hypoxia*
Number of words recalled	16 \pm 3	19 \pm 8	17 \pm 6	19 \pm 5	18 \pm 4

*this score was recorded in normoxia.

Note: the maximum VWMC score was 28 in all conditions.

Table IV. Physiological parameters

	Baseline Normoxia	15 min Normoxia	15 min Post Normoxia	15 min Hypoxia	15 min Post Hypoxia*
Heart Rate (bpm)	68 \pm 13	71 \pm 10	69 \pm 15	74 \pm 14	70 \pm 14
SpO ₂ (%)	98 \pm 1	98 \pm 1	98 \pm 1	87 \pm 4**	97 \pm 3
Blood Pressure (mmHg)					
Systolic	124 \pm 11	123 \pm 12	122 \pm 11	125 \pm 11	124 \pm 10
Diastolic	77 \pm 9	78 \pm 10	76 \pm 9	74 \pm 6	78 \pm 8

*this measurement was made in normoxia. **significantly lower than all other time points ($p < 0.001$).

Figures:

Figure 1. The second pin from right is not contained within the clamp. Only one pin is holding this side of the fracture. The far right pin is also asymmetrically placed.