Increased inspired oxygen concentration does not adversely affect oxidative stress and the resolution of inflammation during reperfusion in patients undergoing knee replacement surgery

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ABSTRACT

The level of inspired oxygen during surgery may modify free radical release, and reperfusion injury. This controlled trial examined the effect of inspired oxygen on F_2-isoprostanes (F_2-IsoPs), isofurans (IsoFs) and specialised mediators of inflammation resolution (SPM) during knee replacement surgery. Patients received either 30% O_2 (Control n=21), 50% O_2 (n=20) or 80% O_2 (n=19) O_2, in a parallel design. Haemoglobin (Hb) was measured throughout the surgery and F_2-IsoPs, IsoFs and SPM were analysed by mass spectrometry. The effect of O_2 on F_2-IsoPs and IsoFs was examined during tourniquet inflation and after tourniquet release. SPM were measured at baseline and the end of surgery. There was a significant interaction between O_2 and Hb concentrations with plasma IsoFs during tourniquet inflation. An increase in plasma IsoFs over time was attenuated in the 80% O_2 group (P=0.012) compared with the 30% O_2 group after adjusting for Hb concentration. After tourniquet release, plasma F_2-IsoPs were significantly lower in the 50% and 80% O_2 groups (p=0.009 and P=0.001, respectively) compared with the 30% O_2 group after adjustment for Hb concentration. The SPM RvD2 and RvE2 were increased with 50% and 80% O_2 (RvD2, P=0.014 and P=0.002, respectively; RvE2, P=0.032 with 50% O_2) compared with the 30% O_2 group, in analyses that corrected for Hb concentration.We have shown for the first time that higher O_2 levels may be beneficial in reducing oxidative stress and increasing resolution of inflammation during surgery that involves reperfusion after application of a tourniquet.

Key words: inspired oxygen; lower limb surgery; oxidative stress; reperfusion injury; F_2-Isoprostanes, isofurans, resolvins, tourniquet inflation.

INTRODUCTION

Reperfusion injury is commonly encountered in surgical practice, including organ transplantation, coronary artery bypass surgery, free tissue transfers, and with the use of pneumatic tourniquets in orthopaedic practice[1, 2]. Short periods of ischaemia are well tolerated but when severe and prolonged, remote organ injury and whole-body inflammation may ensue[3]. The inflammatory response as a result of surgery has evolved to maximise healing potential and survival[4], but if excessive may be deleterious. [5] The patterns and magnitude of postoperative inflammatory responses appear to be predictive of adverse outcomes [5-8]. Hence, different strategies with a view to abrogating the inflammatory response and to limit tissue damage associated with reperfusion have been reported [9-11].

Oxidative stress due to the generation of toxic free radicals is one of the hallmarks of reperfusion injury[12]. F_2-isoprostanes (F_2-IsoPs) are currently the most reliable biomarkers of oxidative stress status and lipid peroxidation *in vivo* [13]-[14]. They formed from arachidonic acid, have some biological activity and may also act as mediators of oxidant injury [15, 16]. Isofurans (IsoFs) are also formed from arachidonic acid. They are chemically related to F_2-IsoPs, and their synthesis is favoured at higher oxygen tensions [17]. The resolution of inflammation is an active process that involves synthesis of specialized pro-resolving mediators (SPM) that promote the restoration of normal cellular function following inflammation and tissue injury. [18-20]. Dysregulation in SPM synthesis and signaling has been implicated in many disease states including immune suppression, chronic inflammation, organ fibrosis[19] and dementia[21]. The clinical relevance of F_2-IsoPs, IsoFs and SPM in reperfusion injury has not been investigated in detail and is at present undetermined.

Oxygen administration under anaesthesia is ubiquitous but it is unclear whether alteration of oxygen concentrations at the time of reperfusion of ischaemic tissues can influence the production of free radicals and subsequent immune system activation. Both

hyperoxic and hypoxaemic reperfusion have been suggested as strategies to attenuate the injury[9, 22-24], with the latter demonstrating some promise. In patients undergoing upper limb surgery with the use of a tourniquet, we have shown a positive relationship between plasma IsoFs concentrations and oxygen tension that was negatively influenced by haemoglobin, suggesting a buffering effect [25]. In patients undergoing lower limb surgery with the use of a tourniquet, we have demonstrated decreased concentrations of IsoPs and increased IsoFs in patients undergoing general anaesthesia compared to spinal anaesthesia [26], likely due to higher oxygen tensions during general anaesthesia.

In this controlled trial we aimed to determine whether IsoPs and IsoFs, as well as SPM concentrations would be influenced by altering inspired oxygen concentrations prior to reperfusion, in patients undergoing lower limb surgery that associated with a greater blood volume and potentially a larger reperfusion injury.

METHODS

Patient Selection

Patients were recruited from the preanaesthesia assessment clinic at Royal Perth Hospital, at least two weeks prior to surgery. Eligible patients were required be of American Society of Anaesthesiology class 1 or 2, undergoing elective total knee replacement under general anaesthesia where the indication was osteoarthritis of the knee, requiring the use of a pneumatic arterial tourniquet for a minimum of 60 minutes. Exclusion criteria included planned total intravenous anaesthesia, active systemic infection, surgery for a suspected prosthesis infection, use of immunosuppressive or disease-modifying medication, non-steroidal anti-inflammatory drugs (NSAIDs) within the previous 48h, body mass index (BMI) >40kg/m^2, chronic renal impairments with creatinine >125mmol/L, diagnosed or suspected obstructive sleep apnoea, the possible need for a fractional inspired oxygen concentration

greater than 0.3, or the likely requirement for multiple tourniquet episodes. Patients were advised to cease the consumption of NSAIDS or COX 2 inhibitors for 48 hours prior to surgery. Sixty patients were randomized using a computer-generated number sequence and allocated via an opaque sealed envelope to either 30%, 50% or 80% inspired oxygen concentration. Study personnel were not involved in providing anaesthesia to the study participants and gas administration equipment and monitoring was concealed from the investigators to maintain blinding where possible. Patients provided written informed consent prior to surgery.

The study was approved by the human research ethics committee of the South Metropolitan Area Health Service, Perth, Western Australia (EC 2008/211), and registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12617000306314).

Anaesthesia and Study Interventions

General anaesthesia induction was performed using fentanyl 1-2μg/kg (or alternative opioid), propofol 2-3mg/kg and midazolam co induction up to a maximum of 1mg. An appropriately sized endotracheal tube or laryngeal mask airway was inserted after adequate depth of anaesthesia and muscle relaxation was achieved (where necessary). Anesthesia maintenance comprised inhalation of sevoflurane in an oxygen/air mix to achieve end-tidal sevoflurane concentrations of 1.9–2.3%. Patients received intraoperative supplemental narcotic analgesia (fentanyl, hydromorphone or morphine) and paracetamol. NSAIDS and Parecoxib, where prescribed, were only given after the final blood sample was taken 35 minutes post-tourniquet release. Peripheral nerve blocks including femoral, adductor canal and sciatic nerve blocks were performed at the discretion of the anaesthetist. Routine antiemetics were allowed, except for dexamethasone which was contraindicated by the study protocol. After the induction of anesthesia, a 16-gauge cannula for blood sampling was inserted into the contralateral arm replacing the original cannula use for induction of anaesthesia. Tramadol,

ketamine, desflurane and nitrous oxide were avoided throughout the sampling period. In all patients the fractional inspired oxygen concentration was initially set at 30%. An arterial tourniquet was inflated to 300-350 mmHg (depending upon systolic blood pressure) for the purpose of haemostasis during the surgical procedure. Approximately 30 minutes prior to the deflation of the pneumatic tourniquet, the inspired oxygen concentration was either maintained at 30% or changed to 50% or 80% depending on the randomisation group of the patient. The target FiO2 was then maintained until emergence from general anaesthesia. Patients were transferred to the post-anaesthesia care unit (PACU) and received oxygen by facemask at 6 L/min until recovered from anaesthesia and deemed fit for discharge from the PACU.

Venous blood samples and blood gas concentrations were taken as shown in Figure 1: T1, prior to induction of anaesthesia on room air before administration of any sedative agents; T2**,** immediately prior to change in oxygen concentration, which was approximately 30 min before tourniquet release; T3**,** 10 min post oxygen concentration change; T4**,** 20 min post oxygen concentration change; T5**,** 30 min post oxygen concentration change and immediately prior to tourniquet release; T6**,** 5 min post tourniquet release; T7**,** 15 min post-tourniquet release; and T8**,** 35 min post-tourniquet release. Samples at T1 to T8 included analysis of venous blood gas concentrations and plasma IsoFs and F_2-IsoPs. Plasma SPMs were measured at T1 and T8.

Statistical Power

Based upon data from our previous study[26] and variability of plasma levels of F_2-IsoPs and IsoF's in our laboratory, we estimated that with 3 randomised groups observed over 4 time points (3 intervals), there is sufficient power using a repeated measures design to detect a small change in F_2-IsoP or IsoF concentration (100 units or 1.25% change) as statistically significant with power greater than 0.90 if 60 patients were block randomised into three

groups of 20. We block randomized 66 patients in three groups of 22 to allow for 10% dropout or protocol violation.

Processing of Blood Samples

Venous blood samples were drawn into 80IU of electrolyte-balanced heparin solution (PICO 50; Radiometer Medical ApS, Copenhagen, Denmark) and oxygen tension (venous oxygen partial pressure, PvO2), sodium, potassium, chloride, lactate, glucose and haemoglobin (Hb) were analyzed on a Radiometer ABL 800FLEX automated point-of-care device (Radiometer Medical ApS, Copenhagen, Denmark).

Measurement of Plasma F_2-Isoprostanes and Isofurans

Blood samples for F_2-IsoPs and IsoFs (T1 to T8) were collected into ice-cold tubes containing ethylenediaminetetracetic acid (EDTA), reduced glutathione and butylated hydroxytoluene and then centrifuged at 4 °C and the plasma was stored at −80 °C until assay. 15-F_{2t}-IsoP (8-iso-PGF2α) and 15-F_{2t}-IsoP-d4 (8-iso-$PGF_{2\alpha}$-d4) were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Pentafluorobenzylbromide and N,N-diisopropylethylamine were purchased from Sigma Chemicals (St. Louis, MO, USA). The silylating agent N,O-bis-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane was purchased from Pierce Chemicals (Rockford, IL, USA). Certify II cartridges were from Varian (Lake Forest, CA, USA). All solvents were of HPLC grade. IsoF standards were provided by Professor L.J. Roberts II (Department of Pharmacology, Vanderbilt University, Nashville, TN, USA) [24]. Total (free plus esterified) IsoFs and F_2-IsoPs were measured by gas chromatography–mass spectrometry (GCMS) using electron-capture negative ionization and a modification of our previously reported method [27]. Samples from each study participant were assayed in the one batch. Briefly, internal standard (5ng,15-F_{2t}-IsoP-d_4) was added to plasma (200µl) and samples were hydrolysed with potassium hydroxide in

methanol, acidified, and applied to pre-washed Certify II cartridges. After application of methanol:water (1:1), and hexane:ethyl acetate (75:25) the IsoFs and F_2-IsoPs were eluted with ethyl acetate:methanol (90:10), dried, and derivatised. IsoFs and F_2-IsoPs were quantitated by GCMS, using 15-F2t-IsoP-d4 (5 ng) as internal standard and monitoring ions at m/z 569, 573, and 585, for F_2-IsoP, 15-F_{2t}-IsoP-d4, and IsoF, respectively. The intra-assay and inter-assay coefficients of variation were 8% and 5.6% for plasma F_2-IsoPs, and 9%, and 10% for IsoFs.

Measurement of Plasma SPM

Blood samples were collected into EDTA on ice, centrifuged immediately and the plasma aliquoted and stored at -80^{o}C until assay. All samples from each participant were assayed in the one batch at the completion of the study. Plasma (1ml) was acidified to pH 3 and applied to a Bond Elut C18 solid-phase extraction cartridge (Agilent Technologies, Lake Forrest, CA, USA). The SPM were eluted with 2 mL ethyl acetate, dried under nitrogen and reconstituted for analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). SPM included 18-hydroxyeicosapentaenoic acid (18-HEPE), resolvin E2 (RvE2), resolvin E3 (RvE3), 18R-resolvin E3 (18R-RvE3), 17-hydroxydocosahexaenoic acid (17-HDHA), resolvin D1 (RvD1), 17R-resolvin D1 (17R-RvD1), and resolvin D2 (RvD2). SPM were analysed and quantified using LC-MS/MS on a Thermo Scientific TSQ Quantum Ultra triple quadrupole LC-MS system (Thermo Scientific) equipped with an electrospray ionization source operated in the negative-ion mode as previously described [28]. The LC-MS/MS chromatogram of each SPM standard and a representative plasma sample identified by retention time and multiple reaction monitoring (MRM) using two product ions, in addition to full mass spectral data that were used to identify characteristic MS-MS fragmentation patterns and diagnostic ions used for identification in human plasma, is available as Supplemental Data online

http://www.jlr.org/content/suppl/2014/09/03/jlr.M045583.DC1.html (see Supplemental Data in Reference [28]). Instrument control and data acquisition were performed with Xcalibur 2.0.7 software. The limit of detection and limit of quantitation for 18-HEPE, 17-HDHA, and 14-HDHA, were 8 and 15 pg/mL, respectively, and for all other SPM, 15 pg/mL and 20 pg/mL, respectively.

Statistical Analysis

All data were checked for normality and where necessary transformed using a natural logarithm for parametric analysis. Categorical data were examined using $\chi 2$ analysis. Haemolysed samples were excluded from the analysis and there was no significant association between the haemolysis and the intervention group (P=0.932). Between-group differences in F_2-IsoP and IsoF levels were examined over time using linear mixed models with restricted maximum likelihood estimation. This type of analysis adjusts for any differences at baseline. The analysis was conducted in 2 stages that examined the effect of different O_2 levels on plasma F_2-IsoPs and IsoFs during the period that the tourniquet was applied (T2-T5) compared with (T1) and then the effect during reperfusion (T6-T8) compared with (T5) immediately prior to tourniquet release. The intervention group was treated as a fixed effect and time was treated as a random effect. This approach to the analysis was chosen because of its flexibility with respect to variable distributions, power, and ability to cope with missing data (the haemolysed samples). The linearity of continuous variables was examined using reduced cubic splines. A corrected p value of less than 0.05 was taken to indicate a statistically significant result. Given our previous finding that haemoglobin modified the relationship between oxygen concentration and plasma IsoFs and F_2-IsoPs [25] we examined the relationship using modeling that accounted for the known buffering effects of haemoglobin. Plasma F_2-IsoP and IsoF were assessed with mixed-effects models that were

constructed for main effects with interaction terms for group and time, and group and haemoglobin during the period when the tourniquet was applied and then during the period after tourniquet release.

RESULTS

Table 1 shows the characteristics of the groups that were well matched for age, gender, BMI and smoking. The time taken for surgery and period during which the tourniquet was applied were not significantly different between groups. Table 2 shows the unadjusted data for plasma IsoFs and F_2-IsoPs from T1-T8. There was no significant effect of gender on plasma isoprostanes (p=0.161) or plasma isofurans (p=0.367) during the course of the study.

Plasma Isofurans and F_2-isoprostanes during application of the tourniquet

Table 3 shows that after adjusting for haemoglobin concentration, there was a significant association between oxygen concentration and plasma IsoFs, but not F_2-IsoPs, during the period when the tourniquet was inflated. Figure 2a (showing fitted values derived from the regression model) shows that compared with the 30% O_2 group there was an attenuation of the linear increase over time in the 80% O_2 (Group* Time interaction = -0.0014, P=0.130) and a further significant attenuation due to the effects haemoglobin in the 80% O_2 group (Group *haemoglobin interaction = -0.016, P=0.012) (Table 3). In contrast, compared with the 30% O_2 group there was no difference in plasma F_2-IsoPs over time in response to 50% or 80% O_2 (Group* Time interactions (P=0.102 and 0.491, respectively) and no significant interactions of 50% or 80% O_2 with haemoglobin (P=0.283 and 0.780, respectively) (Table 3)

Plasma Isofurans and F_2-isoprostanes after release of the tourniquet.

There was no significant difference in plasma IsoFs between the groups before or after

adjustment for haemoglobin (Table 4). In contrast, compared with the 30% O_2 group plasma F_2-IsoPs were significantly attenuated over time in the 80 % O_2 group (Group*Time interaction =-0.0025, P=0.030) and there was a significant attenuation in the 50% and 80% O_2 groups due to haemoglobin (Group*haemoglobin interaction, P=0.009 and P=0.001, respectively) when compared with the 30% O_2 group (Table 4). Figure 2b (showing fitted values derived from the model) shows that after adjusting for haemoglobin concentration the linear increase in plasma F_2-IsoPs over time was significantly diminished in the 50% and 80% O_2 groups.

Plasma SPM and the effects of inspired O_2

The unadjusted data for the SPM 17-HDHA, 18-HEPE, RvD1, 17-RvD1, RvD2, RvE3 and 18-RvE3 at T1 and T8 (baseline and post-surgery, respectively) are shown in Table 5. There were significant effects of increased inspired O_2 on plasma RvD2 and RvE2, such that 50% and 80% O_2 resulted in an increase in RvD2 (P=0.014 and P=0.002, respectively), and 50% O_2 increased RvE2 (P=0.032) compared with the 30% O_2 group, with these relationships modified by haemoglobin concentration (Table 6, Figure 2c and 2d).

Given the relationship between plasma F_2-IsoPs and inspired oxygen after release of the tourniquet was released we examined whether release of F_2-IsoPs modified the effect of inspired oxygen on RvD2 and RvE2. The relationship between RvD2 and inspired oxygen remained significant at 50% (P=0.010) and 80% (P=0.002) but higher plasma F_2-IsoPs was associated with a significantly lower RvD2 (Ln F_2-IsoPs co-efficient = -8.67 (-17.0, -0.35), P=0.041). The effect of plasma F_2-IsoPs on RvE2 was not significant (Ln F_2-IsoPs co-efficient = -6.20 (-14.87, 2.46), P=0.161).

DISCUSSION

We have shown for the first time that in the setting of knee replacement surgery there is a significant effect of higher oxygen concentrations on oxidative stress and mediators of inflammation resolution. Firstly, during the period of tourniquet application 80% O_2 suppressed the increase in plasma IsoFs over time when compared with 30% inspired O_2. Secondly, 50% and 80% O_2 associated with lower levels of F_2-IsoPs after tourniquet release compared with the 30% O_2 group. Lastly, the SPM RvD2 and RvE2 measured at baseline and after surgery showed that higher inspired O_2 during surgery significantly increased levels of these SPMs. Most importantly, all these relationships were significantly affected by haemoglobin levels during surgery.

There have been some concerns regarding the effects of oxygen therapy in the hospital setting. A meta-analysis of 25 randomised controlled trials that enrolled 16,037 patients showed liberal oxygen therapy increased in-hospital and 30 day mortality across a broad range of acute illnesses without improving other important outcomes, such as disability, risk of hospital-acquired pneumonia, risk of hospital-acquired infections, or length of hospital stay[29]. This scenario differs from that of patients undergoing surgery that may be exposed to high oxygen levels for shorter periods of time. A recent metanalysis showed that administration of 80% compared to 30% inspired oxygen did not impose safety related issues in adult patients undergoing surgical procedures [30]. The effect of increased inspired oxygen levels during surgery and the possible effects on reperfusion injury that may be due to induced oxidative stress has not been examined in the setting of lower limb surgery.

To date, there is only one report examining the effects of reperfusion on oxidative stress in response to lower leg ischaemia in humans. That study did not examine the effects of different oxygen concentrations but found that lower leg ischaemia for 20 minutes did not cause oxidative stress assessed by measuring muscle malondialdehyde [31]. Malondialdehyde

has been shown to be a poor maker of oxidative stress [32] whereas serial measurements of F_2-IsoPs and IsoFs such as those obtained in this study are considered to be reliable measures of oxidative stress [33]. Our study in patients undergoing knee surgery comparing the effects of spinal or general anaesthesia on F_2-IsoPs and IsoFs showed that F_2-IsoPs were reduced and IsoFs increased with general anaesthesia [26]. We suggested that the differences between spinal and general anaesthesia patients may have been due to increased inspired oxygen during general anaesthesia. The current randomized controlled trial showed that upon reperfusion F_2-IsoPs are reduced with with higher inspired oxygen (80%) but we did not observe a differential effect of of higher oxygen (80%) on IsoFs during reperfusion. Differences in the study design, levels of inspired oxygen between the studies, adjusting for the effects of haemoglobin and the timing and number of blood samples collected after tourniquet release could account for the different results between the studies.

This study confirmed our observation [25] that haemoglobin plays an important role in modifying the effect of different inspired oxygen levels on F_2-IsoPs and IsoFs during arm surgery. However, there are important differences between the two studies in terms of the timing when the blood samples were taken. The present study collected a larger number of blood samples, both when the tourniquet was applied and during reperfusion that allowed separate analysis of each period during surgery. Analysis during the period of tourniquet application showed that compared with the 30% O_2 group and after adjusting for haemoglobin concentration, the linear increase in plasma IsoFs over time was significantly attenuated in the 80% O_2 group. F_2-IsoPs were unaffected by different oxygen levels during the period of ischaemia. In contrast, during reperfusion 50% and 80% oxygen attenuated the time related increase in F_2-IsoPs seen with 30% oxygen, without affecting plasma IsoFs. These results suggest that high levels of inspired oxygen interact with haemoglobin and may differentially affect the pathways for oxidative stress during ischaemia and reperfusion. The

findings are consistent with the biology of the haemoglobin molecule in blood that allows the carriage of 30–100 times as much oxygen bound to the haemoglobin molecule than could be transported simply in the form of dissolved oxygen in blood without haemoglobin. They also emphasise the need for multiple blood sampling during the period when tourniquet is applied and then during reperfusion to ascertain these effects.

This is the first study to examine the effects of different inspired oxygen levels on SPM. The finding that RvE2 and RvD2 are increased with higher levels haemoglobin is novel and of particular interest. A recent report showing that 17R-RvD1 levels were reduced during hypoxia reperfusion in a mouse model of sickle cell anaemia, a genetic disorder of haemoglobin characterised by hemolytic anaemia [34], is in keeping with our findings for of a positive relationship between haemoglobin and RvD2, and RvE2. Indeed, the same report indicated that treatment with 17R-RvD1 diminished hypoxia reperfusion induced kidney damage and protected against progression of vasculopathy in sickle cell mice, suggesting that SPM may be important modulators of clinical outcomes resulting from reperfusion injury. The increase in RvD2 and RvE2 with higher inspired oxygen concentration may not only be relevant to the resolution of inflammation associated with surgery, but given their anti-nociceptive actions may directly affect post-operative pain control [35, 36]. The negative relationship between F_2-IsoPs and RvD2 under conditions of reperfusion injury could indicate a reduction in oxidative stress upon resolution of inflammation during this period that is independent of the effects of inspired oxygen on RvD2.

In conclusion, our study shows that higher inspired oxygen levels during lower limb surgery, reduced plasma F_2-isoprostanes during reperfusion and increased specialised mediators of inflammation resolution. It can be speculated that the reduction in oxidative stress with higher inspired oxygen is due to less inflammation that may to lead to a lower incidence of post-operative complications.

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Figure Legends:

Figure 1. Study design outlining the timing of blood sampling in relation to surgery and changes in oxygen levels. **Legend:** T1, prior to induction of anaesthesia on room air before administration of any sedative agents; T2, immediately prior to change in oxygen concentration, ~30 min before tourniquet release; T3, 10 min post oxygen concentration change; T4, 20 min post oxygen concentration change; T5, 30 min post oxygen concentration change and immediately prior to tourniquet release; T6, 5 min post tourniquet release; T7, 15 min post-tourniquet release; and T8, 35 min post-tourniquet release (just prior to removal of airway tube). Venous blood gas concentrations and venous blood for plasma IsoFs and F_2-IsoPs were taken at T1 to T8. Plasma SPMs were measured at T1 and T8.

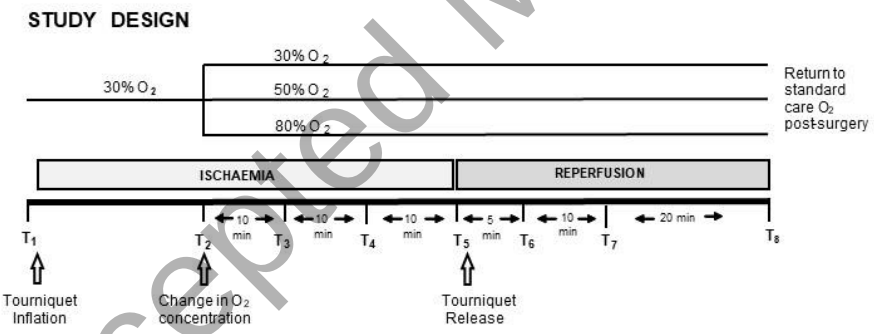

Figure 2. The effects of O_2 concentrations on (a) plasma IsoFs during tourniquet inflation adjusted for haemoglobin concentration using data from the fitted model. Group*haemoglobin interaction for 80% O_2 P=0.012; (b) plasma F_2-IsoPs after tourniquet release and adjusting for haemoglobin concentration using data from the fitted model. Group* Time interaction for 80%O_2 P=0.03, Group* Haemoglobin interaction for 80%O_2 P=0.001; (c) plasma RvD2 after adjusting for haemoglobin concentration (Group x time interactions for 50 % P=0.014 and 80%O_2 P=0.002) and (d) RvE2 after adjusting for haemoglobin concentration (Group x time interactions for 50 % P=0.032).

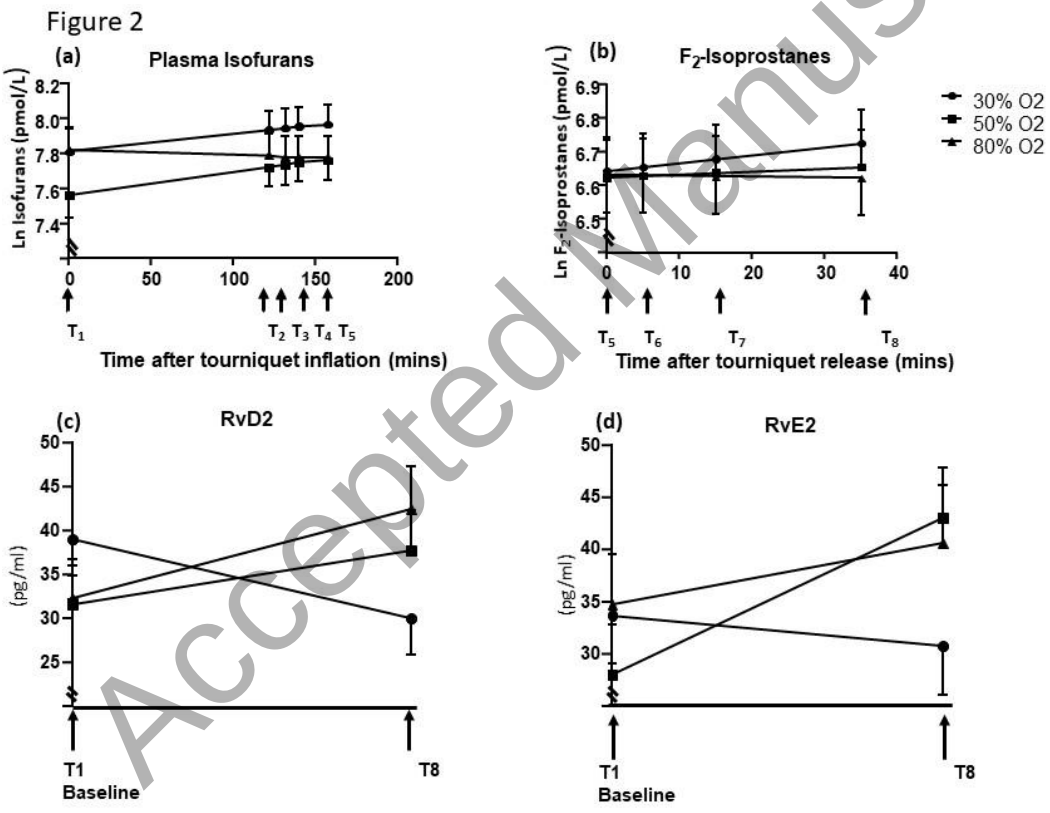

Table 1. Baseline Characteristics of the Study Groups

	Interventional Group		
	30% O2 (n=21)	50% O2 (n=20)	80% 02 (n=19)
Age (years)	68 ± 9	65 ± 13	66 ± 15
Gender (M/F)	15/6	11/9	8/11
BMI (kg/m^2)	29.1 ± 6.6	31.8 ± 4.6	31.8 ± 4.3
Tourniquet Time (min)	154 ± 35	158 ± 39	163 ± 60
Surgical Time (min)	192 ± 46	195 ± 40	194 ± 48
Smoker (n (%))	2 (9%)	0	2 (10%)
Aspirin Use (n (%))	6 (28%)	3 (15%)	4 (21%)
Comorbidities (n (%))	17 (81%)	15(75%)	16 (84%)
Cemented femoral prosthesis (n (%))	20 (95)	14 (70)	15 (79)
Cemented tibial prosthesis (n (%))	20 (95)	17 (85)	19 (100)

There were no significant differences between interventional groups for any of the variables shown. Values are mean ± SD

Table 2. Plasma IsoFs and F_2-IsoPs from T1-T8 according to whether the patients were allocated to 30%, 50% or 80% O_2

	Isofurans (pmol/L)			**F_2 -Isoprostanes (pmol/L)**		
	30% O_2	**50% O_2**	**80% O_2**	**30%**	**50%**	**80%**
T1 0 mins Prior to anaesthesia	2223 1737, 2847	1730 1388, 2156	2042 1525, 2738	852 686, 1057	743 582, 949	799 605, 1055
Tourniquet ON						
T2 Baseline before Δ in O2	3921 2497, 4334	2786 2193, 3566	2728 2104, 3538	826 675, 1011	755 600, 949	729 573, 926
T3 After 10 mins	2940 2172, 3979	2455 1962, 3070	2774 2148, 3582	781 611, 999	744 605, 914	711 558, 905

T4 After20 mins	2662 1935, 3662	2335 1926, 2829	2791 2129, 3657	781 616, 990	685 546, 859	732 581, 921
T5 After 30 mins	2415 1827, 3192	2156 1698, 2738	2679 2131, 3367	718 567, 909	733 596, 903	769 618, 958
Tourniquet OFF						
T6 5 mins	2588 2083, 3215	2149 1700, 2717	2390 2001, 2929	798 645, 986	739 581, 938	738 589, 925
T7, 15 mins	2631 2054, 3368	2475 2014, 3041	2401 1942, 2970	825 652, 1045	759 618, 932	748 601, 929
T8 35 mins	2631 2060, 3506	2475 1792, 2736	2401 2023, 3076	833 675, 1027	790 634, 984	752 604, 936

Values are geometric mean (95% confidence interval)

Table 3. The linear mixed model for the effects of oxygen concentrations on plasma IsoFs and F_2-IsoPs during the period of tourniquet inflation adjusting for haemoglobin concentration and the interactions between group and time, and group and haemoglobin concentration

	O_2		95% CI		
Plasma Isofurans (ln)	**Group**	**Coefficient**	**LCL**	**UCL**	***p***
Group	30%	0 (ref.)			
	50%	0.491	-1.44	2.34	0.604
	80%	2.093	0.333	3.85	0.020
Time (mins)		-0.001	-0.001	0.002	0.305
Group* Time interaction	30%	0 (ref.)			
	50%	0.0003	-0.001	0.002	0.747
	80%	-0.0014	-0.003	0.004	0.130
Haemoglobin concentration		0.001	-0.006	0.009	0.728
Group* Haemoglobin	30%	0 (ref.)			
interaction	50%	-0.006	-0.018	0.007	0.386
	80%	-0.016	-0.029	-0.004	**0.012**
Constant		7.64	6.54	8.73	<0.001
Plasma F_2-isoprostanes (ln)	**O_2**	**Coefficient**	**95% CI**		***p***

	Group		LCL	UCL	
Group	30%	0 (ref.)			
	50%	-0.710	-1.78	0.356	0.192
	80%	-0.189	-1.109	0.731	0.687
Time (mins)		-0.0004	-0.0009	0.0001	0.168
Group* Time interaction	30%	0 (ref.)			
	50%	0.0007	-0.0001	0.0016	0.102
	80%	0.0003	-0.0006	0.0012	0.491
Haemoglobin concentration		0.003	-0.0006	0.0007	0.109
Group* Haemoglobin	30%	0 (ref.)			
interaction	50%	0.004	-0.003	0.011	0.283
	80%	0.0009	-0.005	0.007	0.780
Constant		6.334	5.764	6.883	<0.001

Table 4. The linear mixed model for the effects of oxygen concentrations on plasma IsoFs and F_2-IsoPs over time during the period when the tourniquet is released adjusting for haemoglobin concentration and interactions between groups and time, and group and haemoglobin concentration.

	O_2		95% CI		
Plasma Isofurans (ln)	**Group**	**Coefficient**	**LCL**	**UCL**	***p***
Group	30%	0 (ref.)			
	50%	-0.783	-2.686	1.121	0.42
	80%	-0.343	-1.944	1.257	0.67
Time (mins)		0.00003	-0.003	0.003	0.982
Group* Time interaction	30%	0 (ref.)			
	50%	-0.0016	-0.006	0.003	0.448
	80%	-0.00006	-0.003	0.003	0.974
Haemoglobin concentration		-0.0026	-0.0121	0.007	0.604
Group* Haemoglobin	30%	0 (ref.)			
interaction	50%	0.007	-0.006	0.021	0.299
	80%	0.002	-0.009	0.013	0.705
Constant		8.21	6.79	9.63	<0.001

	O_2		95% CI		
Plasma F_2-isoprostanes (ln)	**Group**	**Coefficient**	**LCL**	**UCL**	***p***
Group	30%	0 (ref.)			
	50%	1.96	0.503	3.419	**0.008**
	80%	2.102	0.908	3.296	**0.001**
Time (mins)		0.0025	0.0003	0.004	**0.024**
Group* Time interaction	30%	0 (ref.)			
	50%	-0.0015	-0.0046	0.0016	0.353
	80%	-0.0025	-0.0048	-0.0002	**0.030**
Haemoglobin concentration		0.0144	0.007	0.022	**<0.001**
Group* Haemoglobin	30%	0 (ref.)			
interaction	50%	-0.0138	-0.0242	-0.0034	**0.009**
	80%	-0.0135	-0.0215	-0.0055	**0.001**
Constant		4.453	3.373	5.553	<0.001

Table 5. The effect of different inspired oxygen concentrations on SPM at baseline (T1) and after surgery (T8) (values are Mean ± SEM)

pg/ml	**Baseline (T1)**			**End of surgery (T8)**		
	30% O_2	**50% O_2**	**80% O_2**	**30%**	**50%**	**80%**
18-HEPE	220 ± 27	142 ± 27	211 ± 28	197 ± 27	140 ± 28	166 ± 29
17-HDHA	401 ± 55	230 ± 58	367 ± 57	449 ± 27	254 ± 58	279 ± 60
RvE2	33 ± 4.6	28 ± 4.8	34 ± 4.8	27 ± 4.6	41 ± 4.8	34 ± 5.0
RvE3	51 ± 12.4	48 ± 13.0	52 ± 13.5	51 ± 12.4	80 ± 13.0	55 ± 13.5
18R-RvE3	83 ± 14.9	56 ± 15.5	53 ± 15.6	58 ± 14.9	69 ± 16.0	50 ± 16.3
RvD1	47 ± 3.3	45 ± 3.6	44 ± 3.5	38 ± 3.3	42 ± 3.6	46 ± 3.5
17R-RvD1	63 ± 2.6	58 ± 2.8	62± 2.8	59 ± 2.6	59 ± 2.8	63 ± 2.9
RvD2	38 ± 3.8	32 ± 4.2	32 ± 4	27 ± 3.9	37 ± 4.3	37 ± 4.3

Table 6. The linear mixed model for the effects of oxygen concentrations on plasma RvD2 and RvE2 over time during the period when the tourniquet is released adjusting for haemoglobin concentration and interactions between groups and time, and group and haemoglobin concentration

	O_2		95% CI		
Plasma RvD2	**Group**	**Coefficient**	**LCL**	**UCL**	***p***
Group	30%	0 (ref.)			
	50%	-7.46	-19.5	4.56	0.224
	80%	-6.67	-18.58	5.24	0.272
Time (mins)		-0.36	-0.57	-0.15	**0.001**
Group*Time	30%	0 (ref.)			
interaction	50%	0.080	0.016	0.14	**0.014**
	80%	0.100	0.036	0.16	**0.002**
Haemoglobin *Time					
interaction		0.0023	0.0008	0.004	**0.004**
Constant		39.0	30.7	47.3	<0.001
	O_2		95% CI		
Plasma RvE2	**Group**	**Coefficient**	**LCL**	**UCL**	***p***
Group	30%	0 (ref.)			
	50%	-5.7	-18.8	7.38	0.393
	80%	0.74	-12.34	13.8	0.911
Time (mins)		-0.367	-0.618	-0.115	**0.004**
Group* Time	30%	0 (ref.)			
interaction	50%	0.094	0.008	0.180	**0.032**
	80%	0.047	-0.038	0.134	0.279
Haemoglobin *Time					
interaction		0.0026	0.0007	0.004	**0.007**
Constant		33.7	24.6	42.9	<0.001