Randomized study comparing the effect of CO_2 insufflation on veins using two types of endoscopic and open vein harvesting.

Bhuvaneswari Krishnamoorthy[1,4] PhD, William R. Critchley[2] MRes, Janesh Nair[2] Bsc, Ignacio Malagon[1] PhD, John Carey[1] MD, James B Barnard[1] MD, Paul D Waterworth[1] MD, Rajamiyer V Venkateswaran[1] MD, James E. Fildes[2] PhD, Ann L Caress[3] PhD, Nizar Yonan[1] MD.

1. Department of Cardiothoracic Surgery, University Hospital of South Manchester NHS Foundation Trust, Manchester, UK, M23 9LT.
2. Manchester Collaborative Centre for Inflammation Research, Faculty of Medical and Human Sciences, University of Manchester, UK, M13 9NT.
3. School of Nursing and Midwifery, The University of Manchester, Manchester, UK, M13 9PL.
4. Faculty of Health and Social Care, Edge Hill University, Ormskirk, Lancashire, UK, L39 4QP.

Address for correspondence:

Dr. Bhuvaneswari Krishnamoorthy, Bsc(Hons), NMP, DNDM, Dip RCS, MPhil, PhD.
Lead Surgical Care Practitioner, Cardiothoracic surgery & Honorary Lecturer at Edgehill University.
University Hospital of South Manchester NHS Foundation Trust
Manchester, UK, M23 9LT.
bhuvaneswari.bibleraaj@uhsm.nhs.uk
Telephone: 0044 161 291 2078 and fax number: 0044 161 291 5024.

ISRCTN91485426 (VICO trial)

Randomized study comparing the effect of CO_2 insufflation on veins using two types of endoscopic and open vein harvesting.

Short title: Pressurised versus non-pressurised CO_2 tunnel.

Abstract:

Objective:

To assess whether the use of carbon dioxide (CO_2) insufflation has any impact on integrity of long saphenous vein comparing two types of endoscopic vein harvesting (EVH) and traditional open vein harvesting.

Methods:

A total of 301 patients were prospectively randomized into three groups. Group 1 control arm of open vein harvesting (OVH)(n=101), Group 2 closed tunnel (CO_2) EVH (CT-EVH) (n=100) and Group 3 open tunnel (CO_2) EVH (OT-EVH) (n=100). Each group was assessed to determine the systemic level of Partial arterial CO_2 ($PaCO_2$), end tidal CO_2 ($EtCO_2$) and pH. Three blood samples were obtained at baseline, 10 minutes after start of EVH and 10 minutes after the vein was retrieved. Vein samples were taken immediately after vein harvesting without further surgical handling to measure the histological level of endothelial damage. A modified validated endothelial scoring system was used to compare the extent of endothelial stretching and detachment.

Results:

The level of end tidal CO_2 was maintained in the OT-EVH and OVH groups but increased significantly in the CT-EVH group ($p=0.451$, $p=0.385$ and $p<0.001$). Interestingly, partial arterial CO_2 also did not differ over time in the OT-EVH group ($p=0.241$) whereas $PaCO_2$ reduced significantly over time in the OVH group ($p=0.001$). A profound increase in $PaCO_2$ was observed in the CT-EVH group ($p<0.001$). Consistent with these patterns, only the CT-EVH group demonstrated a sudden drop in pH over time ($p<0.001$) whereas pH remained stable for both OT-EVH and OVH groups ($p=0.105$ and $p=0.869$ respectively). Endothelial integrity was better preserved in the OVH group compared to OT-EVH or CT-EVH groups ($p=0.012$) and was not affected by changes in CO_2 or low pH. Significantly greater stretching of the endothelium was observed in the open tunnel endoscopic OT-EVH group compared to the other groups ($p=0.003$).

Conclusions:

This study demonstrated that the different vein harvesting techniques impact on endothelial integrity; however this does not appear to be related to the increase in systemic absorption of carbon dioxide or to the pressurised endoscopic tunnel. The open tunnel endoscopic harvesting technique vein had more endothelial stretching compared to the closed tunnel endoscopic technique; this may be due to manual dissection of the vein. Further research is required to evaluate the long term clinical outcome of these vein grafts.

Keywords: Coronary artery bypass surgery, Open vein harvesting, Closed tunnel endoscopic vein harvesting, Open tunnel endoscopic vein harvesting, endothelial integrity.

Introduction:

Open vein harvesting is the traditional long saphenous vein retrieval method for coronary artery bypass surgery (CABG) and is associated with significant morbidities[1, 2] post-surgery. Endoscopic vein harvesting (EVH) has been adopted for CABG as a result of reduced postoperative pain, reduced incidence of wound complications and improved patient satisfaction[2]. Whilst EVH is currently used routinely in our hospital, many centres in the United Kingdom have not adopted this technique or have ceased its use due to issues related to the quality of the vein[3], carbon dioxide insufflation[4, 5] and long term patency[6].

Carbon dioxide insufflation is a method used in EVH to create a subcutaneous tunnel in the leg, thereby opening up the tissue space for dissection and clear visualisation[7]. Previous studies have highlighted that the systemic absorption of CO_2[5] and rarely gas embolism[8] can lead to life threatening events, which has led to questions about the safety of EVH[8]. Careful attention is paid to EVH cases with safeguards in place, such as trans-oesophageal echocardiography (TOE) and end-tidal CO_2 monitoring, although but there is a paucity of information regarding vein tissue level absorption of the gas.

No previous studies have directly investigated the effect of CO_2 in relation to histological level vein tissue trauma. The aim of this study was to explore the effects of CO_2 insufflation and histological evidence of vein tissue damage on three different types of vein harvesting.

Methods:

The study was approved by the NRES committee North West-Greater Manchester East and written informed consent was obtained from all study participants in accordance with institutional research ethics review board guidelines. Between 2011 to 2015, 301 patients who underwent CABG were recruited (see CONSORT diagram, figure 1). Stratified block randomization for age and sex was performed by an independent statistician and patients were allocated into one of 3 groups:

Group 1 - (Control arm): 101 patients receiving traditional open vein harvesting (OVH).

Group 2 - (Intervention 1): 100 patients receiving closed tunnel CO_2 EVH (CT-EVH).

Group 3 - (Intervention 2): 100 patients receiving open tunnel CO_2 EVH (OT-EVH).

The allocation was made using sequentially numbered opaque, sealed envelopes. A designated and independent research assistant had responsibility for the list. The practitioner opened the envelope once the patient had been anaesthetised in the operating room to avoid any cancellation of surgery.

Vein sample tissue storage and handling was covered by the Human Tissue Act licence held at the Institution Research and Development office. All veins were harvested by an experienced surgical practitioner who had performed at least 250 cases in each EVH technique and more than 2000 open vein harvests.

Patients were excluded from the study if they required emergency CABG surgery, if they did not want to participate, if they had a previous history of varicose veins or had thin superficial veins[9, 10].

Sample storage and processing:

An un-distended 1cm vein sample was obtained from the proximal region of the vessel from each patient and processed immediately to assess the direct effects of CO_2 absorption without any potential confounding effects from surgical handling and distension. Samples were cut and placed into a solution of 4% formalin in distilled water (pH 7.4). The samples were immersed before inserting the vessel cannula into the vein for checking leakages and as such underwent no distension. There were a total of 301 vein samples obtained from these patients, which were then numerically coded to allow blinding of the laboratory histologist.

Histology and staining:

All embedded vein samples were sectioned at 5µm by a Leica 2255 fully automatic microtome. Hematoxylin and Eosin staining was performed by a Shandon Varistain™ 24-4 automatic slide stainer to evaluate endothelial preservation. Endothelial integrity was classified based upon endothelial preservation and severity of abnormality as used previously[11]: Grade 0 (normal endothelium), 1.1 (mild stretching), 1.2 (moderate stretching), 1.3 (severe stretching), 2.1 (mild detachment), 2.2 (moderate detachment), 2.3 (severe detachment).

Samples were blindly scored by five independent experienced assessors and a consultant histopathologist.

Surgical techniques:

In cases where 1-2 lengths of vein were required, these were harvested from mid-calf to thigh. When 3 lengths of vein were required, these were harvested from ankle to thigh.

Open vein harvesting - Control group:

For OVH, a skin incision was made between ankle and groin, with the site depending upon the length of vein required. In this study, we harvested two lengths of vein from approximately 9cm below the knee. If the patient required three lengths of vein then these were harvested from 4cm above the medial malleolus bone. All vein side branches were tied with 4-0 vicryl sutures and titanium clips[2].

Closed tunnel CO_2 EVH: Intervention group

The Vasoview Hemopro2® (Maquet, Wayne, NJ, USA) vein harvesting system was used in this study. This technique involves the introduction of a pressurised CO_2 tunnel for vein dissection. A 2-3cm incision was made below the knee (approximately 9cm) when 1 or 2 lengths of vein were required. All 3 lengths, when required, were taken from between the ankle and groin. The long saphenous vein was exposed and dissected using a West retractor and a Langenbeck retractor (Anetic Aid, Leeds, West Yorkshire, UK). The CO_2 insufflator was set to 3 litres/ min with 10mmHg pressure. Patients received full heparinisation upon completion of harvesting, which was followed by cardio-pulmonary bypass. All patients in the CT-EVH received 5000 units of heparin as standard before EVH to avoid intraluminal clot formation[12]. A 30mm, 0° endoscope with a sharp, clear dissecting cone on the tip was inserted through the skin incision. After 3cm of anterior dissection, the balloon was inflated with a minimal amount (10ml) of trocar cuff air to seal the incision port. The vein was dissected from the surrounding tissues anteriorly and posteriorly until reaching the femoral junction in the groin. The vein side branches were ligated with 4-0 vicryl ties and titanium clips[2] (Ethicon International, Johnson & Johnson Medical Ltd., New Brunswick, New Jersey, USA).

Most studies that have reported CO_2 embolism have used the company recommendation of 12–15mmHg pressurised tunnel in the leg[13, 14]. In our centre, we use 10mmHg pressure for the CT- EVH group in an attempt to avoid any local or systemic complications[15].

Open tunnel CO_2 EVH: Intervention group

The Clear Glide® (Sorin, Arvada, Colorado, USA) vein harvesting system was used for the OT-EVH group. This involves the formation of a non-pressurised CO_2 tunnel for vein dissection. A 2-3cm incision was made below the knee (approximately 9cm) when 1 or 2 lengths of vein were required. When 3 lengths were required, these were taken from between the ankle and groin. Initially, the long saphenous vein was exposed and dissected using a West retractor and a Langenbeck retractor (Anetic Aid, UK). A 30mm, 0° telescope with a ClearGlide dissecting retractor was introduced through the skin incision. The CO_2 insufflator was set up at a continuous flow rate of 3 litres per minute and 0mmHg pressure. The vein was dissected from the surrounding tissue anteriorly and posteriorly until reaching the femoral junction in the groin. The vein side branches were ligated with 4-0 vicryl ties and titanium clips (Ethicon, J&J Medical, Ltd. USA). The small leg wound was closed in layers and a dressing and pressure bandage was applied.

Standardisation:

A significant increase in $PaCO_2$ is associated with a decrease in arterial pH. In order to account for this, the anaesthetist normally adjusts the ventilator by increasing the patient’s minute ventilation[4]. In this study, artificial ventilation settings were not changed during the study period. All the patients were fully heparinised and went on bypass once the vein harvesting was completed. However, CT-EVH group patients received 5000 units of heparin before starting the retrieval in order to avoid intraluminal clot formation inside the vessel[12]. Patients who were on anticoagulant until the day prior to surgery were administered only 2500 units instead of 5000 units to avoid major bleeding inside the tunnel.

Systemic CO_2 measurements:

In addition to basic demographics, all patients had trans-oesophageal echo (TOE) performed for close monitoring of CO_2 bubbles. We collected three consecutive blood samples for CO_2 analysis at baseline after induction, 10 minutes after vein harvesting started and 10 minutes after vein harvesting was completed. The levels of Partial CO_2 (Pa CO_2), end-tidal CO_2 (Et CO_2), pH, respiratory rate, FiO_2 and tidal volume were also recorded to determine any pattern of acidity and hypercarbia.

Veins were considered to be exposed to CO_2 during the entire harvesting process from start of insufflation to removal of the vein from the leg.

Power calculation and Statistical analysis:

The primary outcome measure was to determine the extent of histological tissue damage by three vein harvesting techniques in relation to systemic CO_2 levels. The sample size required to address the primary end point was calculated on the basis of our previous pilot histological work due to unavailability of any previous studies in this area. With 91 patients in each of the three groups (CT-EVH, OT-EVH, OVH), i.e. 273 in total, the study would have 80% power to detect difference in the percentage with zero vein integrity of 20% or more (for example 20% vs 40%). This calculation is based on a comparison of just two groups using a simple chi-square test, with continuity correction at the 5% significance level. In total, 301 patients were recruited in order to allow for a 10% drop out rate.

All categorical data was assessed using the χ^2 test and expressed as number (percentage). The distribution of all continuous data was formally assessed via the Shapiro-Wilk test. Continuous data is expressed as mean±standard deviation or median [interquartile range] for parametric and non-parametric data respectively. Comparisons between the three groups were performed by ANOVA or Independent samples Kruskal-Wallis test for parametric and non-parametric data respectively.

Results:

Demographics:

A full description of pre-operative demographics is detailed in table 1. No conversions to traditional open harvesting from endoscopic groups were performed. A slightly higher BMI, more left main stem disease and more current smokers were observed in the CT-EVH group. No difference in the incidence of respiratory problems was observed between the groups.

Intraoperative details:

All surgical timings were recorded to establish the duration for which the vein conduit was exposed to CO_2 during retrieval and to determine the overall surgical duration required to obtain the veins. Our study demonstrates a greater vein harvesting time, and thus exposure to CO_2, in the CT-EVH group compared to the other groups ($p=0.028$), with the fastest retrieval achieved in the OT-EVH group. The rate of vein harvesting did not differ between groups, when accounting for length of conduit retrieved ($p=0.134$). This translated into an extended overall surgical time for the leg in the CT-EVH group ($p<0.001$) compared to the other groups, although the fastest time to completion of the leg surgery was in the OVH group. A full overview of vein graft harvesting timings is provided in table 2. The number of vein grafts required for the surgery did not differ significantly between the groups ($p=0.138$).

No differences in any recorded ventilator variables were observed. An overview of intraoperative data is provided in table 2.

Systemic CO_2 and pH measurements:

Baseline $EtCO_2$ levels were consistent across the 3 groups (median [interquartile range]: 4.40 [0.60] vs. 4.30 [0.65] vs. 4.50 [0.70] for OT-EVH vs. OVH vs. CT-EVH respectively, $p=0.137$). However, baseline $PaCO_2$ levels were significantly lower in the CT-EVH group compared to the other 2 groups (5.25 [0.9] vs. 5.40 [0.80] vs. 4.90 [0.90] for OT-EVH vs.

OVH vs. CT-EVH respectively, $p<0.001$). Baseline pH was also similar between groups (mean±standard deviation: 7.40±0.05 vs. 7.40±0.04 vs. 7.40±0.05 for OT-EVH vs. OVH vs. CT-EVH respectively, $p=0.666$).

$EtCO_2$ did not alter over time during harvesting in either OT-EVH or OVH group ($p=0.451$ and $p=0.385$ respectively); however, $EtCO_2$ increased significantly over time in the CT-EVH group ($p<0.001$, figure 2). Interestingly, $PaCO_2$ also did not differ over time in the OT-EVH group ($p=0.241$) whereas $PaCO_2$ reduced significantly over time in the OVH group ($p=0.001$). A profound increase in $PaCO_2$ was observed in the CT-EVH group ($p<0.001$, figure 2). Consistent with these patterns, only the CT-EVH group demonstrated a decrease in pH over time ($p<0.001$) whereas pH remained stable for both OT-EVH and OVH groups ($p=0.105$ and $p=0.869$ respectively, figure 3).

Endothelial integrity:

Conduit endothelial integrity was assessed in terms of intimal stretching and detachment and compared between groups on proximal undistended vein samples. The number of samples with normal preserved endothelium (defined as absence of stretching or detachment) varied between groups, with greatest preservation in the OVH group (54.0%), compared to either endoscopic group (39.0% and 34.0% for CT-EVH and OT-EVH respectively, $p=0.012$). Samples with intimal stretching were further evaluated and severity varied significantly between groups, with more stretching graded as severe in the OT-EVH group (13 (13.0%)), compared to OVH (0 (0.0%)) and CT-EVH (5 (5%)) groups ($p=0.003$, table 3). Samples with intimal detachment were further graded on severity, although no significant differences were observed ($p=0.245$, table 3).

Discussion:

It has been well documented that the use of CO_2 insufflation causes hypercarbia and tissue acidosis, yet despite this, EVH procedures still utilise this gas because of its non-flammable properties, low toxicity and low cost[4]. Other noble gases such as helium and argon have been suggested in laparoscopic surgeries but still there is no clear evidence for their benefit. Importantly, in this study we did not experience any CO_2 embolism or significant hypercarbia using the low pressure setting of 10mmHg**,** a relatively common complication experienced when using the recommended pressure of 12-15mmHg. We suggest that the use of a reduced pressure tunnel in the CT-EVH system may minimise any systemic complications. Our study also demonstrates significantly increased $PaCO_2$ level and significant decreases in arterial pH levels in the CT-EVH group. Despite this, tissue integrity remains similar or slightly better maintained compared to that observed in the OT-EVH group.

Our study data demonstrates that the method of vein harvesting utilised does impact on endothelial integrity. The OVH control group illustrated the greatest endothelial preservation compared to endoscopic techniques. However, veins obtained using the CT-EVH method demonstrated greater preservation of normal, continuous endothelium than veins retrieved by the OT-EVH technique. This enabled us to further our understanding about the effect of prolonged vein exposure to an acidic environment and pressurised CO_2 tunnel. The current literature suggests that the optimal pH for endothelial cell viability ranges between pH 7.3-7.4, below which the acidic environment can damage vessel viability[10, 16]. Our findings demonstrate that despite the drop in pH in the CT-EVH, conduit integrity is not adversely affected.

In this study, we observed longer harvesting time for CT-EVH compared to the other groups, which can increase the length of vein exposure to CO_2. Yet, the CT-EVH group more often required the retrieval of 3 lengths of vein compared to the other groups, although this did not demonstrate statistical significance. Longer harvesting time was not associated with reduced endothelial integrity.

Usually, the tunnel created by CO_2 insufflation (figure 5a) allows for easy dissection and visibility[17, 18]. This promotes absorption of CO_2 by the adjacent tissues, including, to some extent, the vein walls. Greater vessel compression is expected with CT-EVH because of the pressure in the tunnel produced by insufflation. Again, in our study, this did not impact upon the incidence of endothelial layer detachment or endothelial stretching.

The severe endothelial stretching observed in the OT-EVH group may be due to the increased manual handling of the vein due to lack of tunnel and the design of the EVH equipment. Open tunnel EVH (figure 5b) requires manual dissection and thus traction stresses on the vein. Additionally, it is required that the practitioner works very close to the vein, which is not the case in the closed tunnel technique due to the greater access created by the CO_2 tunnel. This helps the practitioner to work away from the vein and obtain the vein with surrounding tissues. The current evidence stresses that the veins harvested with surrounding tissues as a pedicle has a higher patency rate compared to skeletonised veins[19].

Limitations:

Patients who underwent CT-EVH for vein harvesting received heparin, which was not provided to those in the other two groups according to local standard endoscopic guidelines. We do not fully understand the role of heparin on the vascular structures and vessel wall, which may have complicated our findings. We did not perform any optical coherence tomography (OCT) to assess whether there was any intraluminal clot formation in the OT-EVH system. Our study focused only on the structural integrity of the vein but functional viability such as nitric oxide production, a potent endothelium-dependent vasorelaxant synthesised from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS)[20] are also very important. The endothelial denudation affects the functional capacity of the vein and leads to graft failure which weren’t explored in this study.

Our study also confirms that the veins obtained by the traditional open harvesting approach demonstrate a better preservation of the endothelium compared to the endoscopic groups. However, we cannot discount the fact that the practitioner had greater experience in OVH (>2000 cases at the start of the study) than endoscopic harvesting (>250 cases of each technique). This may have contributed to the differences observed.

Conclusion:

Our study demonstrates that the effect of CO_2 insufflation does not adversely affect the untouched proximal region of the conduit. No additional areas of the vein could be assessed due to the surgical handling and distension that they received. Furthermore, we highlight that the use of a less pressurised tunnel (10mmHg rather than 12-15mmHg) can maintain pH at levels suitable for endothelial integrity for the duration required for vein harvesting.

Clinical impact:

The use of a low pressure CO_2 tunnel does not impact upon the quality of the harvested vein on a histological level. So, EVH can be safely undertaken without concern about CO_2 exposure, acidic environment or risk of embolism.

Acknowledgement:

We would like to acknowledge the histopathology scientist Mr. Peter Walker BSc from the University of Manchester, Dr. Julie Morris PhD, Statistician and Reader from University of Manchester and all cardiothoracic surgeons and anaesthetists for their support on this study.

Conflict of Interest:

There was no conflict of interest.

Funding and support:

Mrs. Bhuvaneswari Krishnamoorthy is funded by a National Institute of Health Research (NIHR), Clinical Doctoral Research fellowship (CDRF), England.

This paper presents independent research funded by the National Institute of Health Research (NIHR). The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

References:

1. Andreasen JJ, Nekrasas V, Dethlefsen C. Endoscopic vs open saphenous vein harvest for coronary artery bypass grafting: a prospective randomized trial. *Eur J Cardiothorac Surg* 2008; 34(2):384-9.
2. Krishnamoorthy B, Critchley WR, Glover AT, et al. A randomized study comparing three groups of vein harvesting methods for coronary artery bypass grafting: endoscopic harvest versus standard bridging and open techniques. *Interact Cardiovasc Thorac Surg* 2012; 15(2):224-8.
3. Krishnamoorthy B, Critchley WR, Bhinda P, et al. Does the introduction of a comprehensive structured training programme for endoscopic vein harvesting improve conduit quality? A multicentre pilot study. *Interact Cardiovasc Thorac Surg* 2015; 20(2):186-93.
4. Neuberger TJ, Andrus CH, Wittgen CM, et al. Prospective comparison of helium versus carbon dioxide pneumoperitoneum. *Gastrointest Endosc* 1996; 43(1):38-41.
5. Vitali RM, Reddy RC, Molinaro PJ, et al. Hemodynamic effects of carbon dioxide insufflation during endoscopic vein harvesting. *Ann Thorac Surg* 2000; 70(3):1098-9.
6. Lopes RD, Hafley GE, Allen KB, et al. Endoscopic versus open vein-graft harvesting in coronary-artery bypass surgery. *N Engl J Med* 2009; 361(3):235-44.
7. Maslow AM, Schwartz CS, Bert A, et al. Endovascular vein harvest: systemic carbon dioxide absorption. *J Cardiothorac Vasc Anesth* 2006; 20(3):347-52.
8. Lin TY, Chiu KM, Wang MJ, et al. Carbon dioxide embolism during endoscopic saphenous vein harvesting in coronary artery bypass surgery. *J Thorac Cardiovasc Surg* 2003; 126(6):2011-5.
9. Davis Z, Jacobs HK, Zhang M, et al. Endoscopic vein harvest for coronary artery bypass grafting: technique and outcomes. *J Thorac Cardiovasc Surg* 1998; 116(2):228-35.
10. Krishnamoorthy B, Critchley WR, Venkateswaran RV, et al. A comprehensive review on learning curve associated problems in endoscopic vein harvesting and the requirement for a standardised training programme. *J Cardiothorac Surg* 2016; 11(1):45.
11. Hashmi SF, Krishnamoorthy B, Critchley WR, et al. Histological and immunohistochemical evaluation of human saphenous vein harvested by endoscopic and open conventional methods. *Interact Cardiovasc Thorac Surg* 2015; 20(2):178-85.
12. Brown EN, Kon ZN, Tran R, et al. Strategies to reduce intraluminal clot formation in endoscopically harvested saphenous veins. *J Thorac Cardiovasc Surg* 2007; 134(5):1259-65.
13. Chavanon O, Tremblay I, Delay D, et al. Carbon dioxide embolism during endoscopic saphenectomy for coronary artery bypass surgery. *J Thorac Cardiovasc Surg* 1999; 118(3):557-8.
14. Chen XC, Tang XY, Jiang YF, et al. Sudden cardiovascular collapse caused by carbon dioxide embolism during endoscopic saphenectomy for coronary artery bypass grafting. *Chin Med J (Engl)* 2006; 119(4):345-8.
15. Tamim M, Omrani M, Tash A, et al. Carbon dioxide embolism during endoscopic vein harvesting. *Interact Cardiovasc Thorac Surg* 2008; 7(4):659-60.
16. Rousou LJ, Taylor KB, Lu XG, et al. Saphenous vein conduits harvested by endoscopic technique exhibit structural and functional damage. *Ann Thorac Surg* 2009; 87(1):62-70.
17. Potapov EV, Buz S, Hetzer R. CO(2) embolism during minimally invasive vein harvesting. *Eur J Cardiothorac Surg* 2007; 31(5):944-5.
18. Banks TA, Manetta F, Glick M, et al. Carbon dioxide embolism during minimally invasive vein harvesting. *Ann Thorac Surg* 2002; 73(1):296-7.
19. Samano N, Geijer H, Liden M, et al. The no-touch saphenous vein for coronary artery bypass grafting maintains a patency, after 16 years, comparable to the left internal thoracic artery: A randomized trial. *J Thorac Cardiovasc Surg* 2015; 150(4):880-8.

20. Krishnamoorthy B, Critchley WR, Venkateswaran RV, et al. A comprehensive review on learning curve associated problems in endoscopic vein harvesting and the requirement for a standardised training programme. *J Cardiothorac Surg* 2016; 11:45.

Figure Legends:

Figure 1: CONSORT diagram

Figure 2: Graphs indicating the change in $PaCO_2$ and $EtCO_2$ levels over time in each harvesting group. $PaCO_2$ and $EtCO_2$ were consistent across all time points in the OT-EVH group (a). $PaCO_2$ was significantly reduced over time in the OVH group (b), although $EtCO_2$ remained constant. Significant increases in both $EtCO_2$ and $PaCO_2$ were observed in the CT-EVH group (c).

Figure 3: Graphs indicating the change in pH over time during vein harvesting. A significant drop in pH was observed in the CT-EVH group (c), whereas both OT-EVH (a) and OVH (b) groups maintained consistent pH throughout.

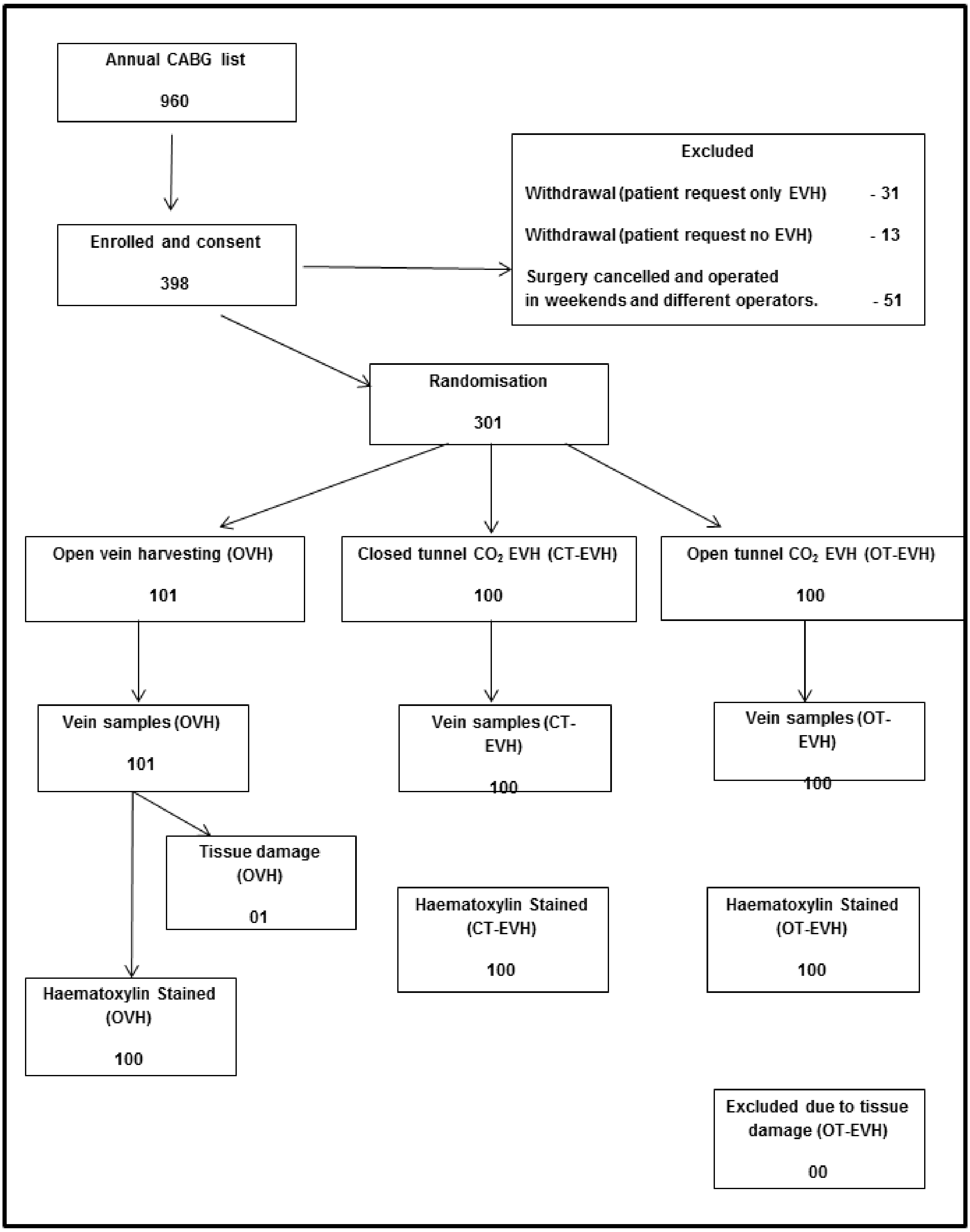
Figure 4a: Hematoxylin-eosin staining showing normal endothelium (a and b) and mild endothelial stretching (c and d).

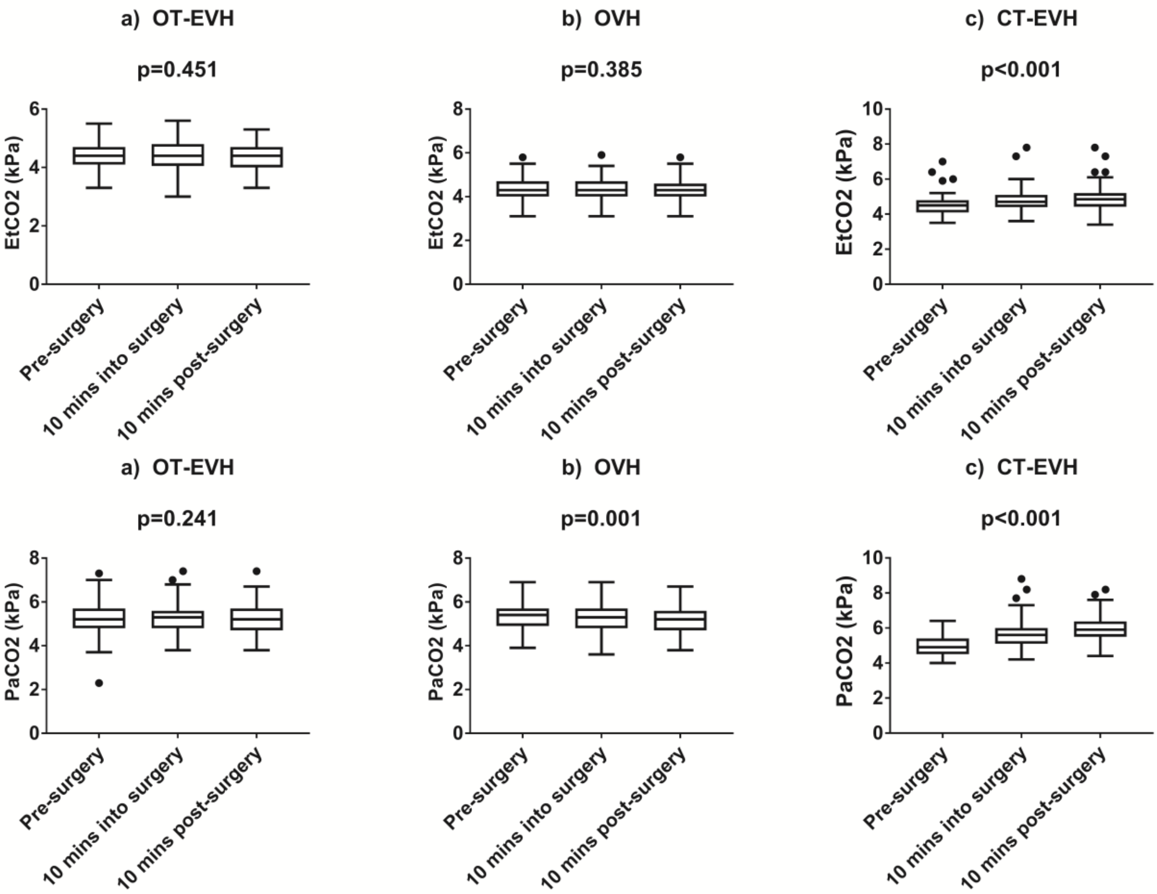
Figure 4b: Hematoxylin-eosin staining illustrating moderate & severe endothelial stretching (a and b) and mild and moderate endothelial detachment (c and d).

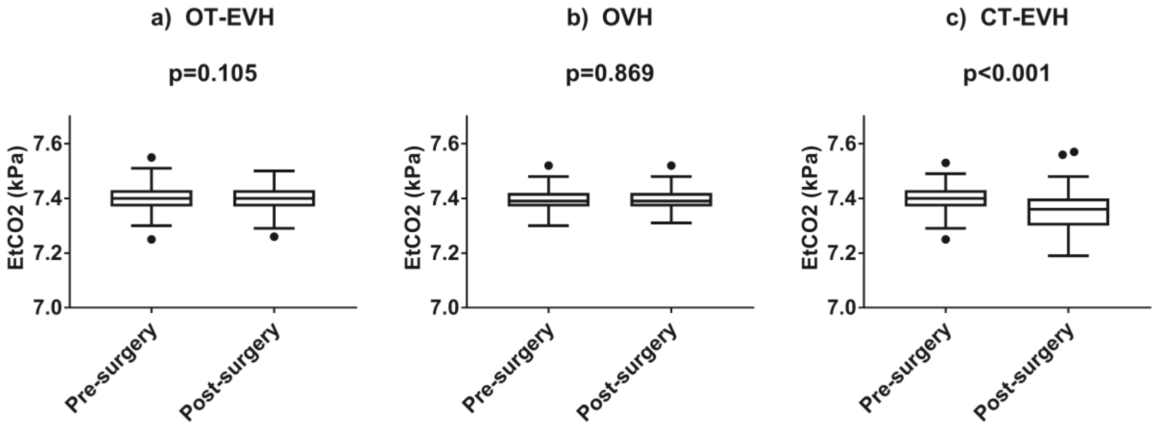
Figure 5a: Closed tunnel CO_2 EVH system. This picture demonstrates the location of the vein and the conical tip within the tunnel on the leg. The vasa vasorum and surrounding tissues are pushed away by the carbon dioxide insufflation.

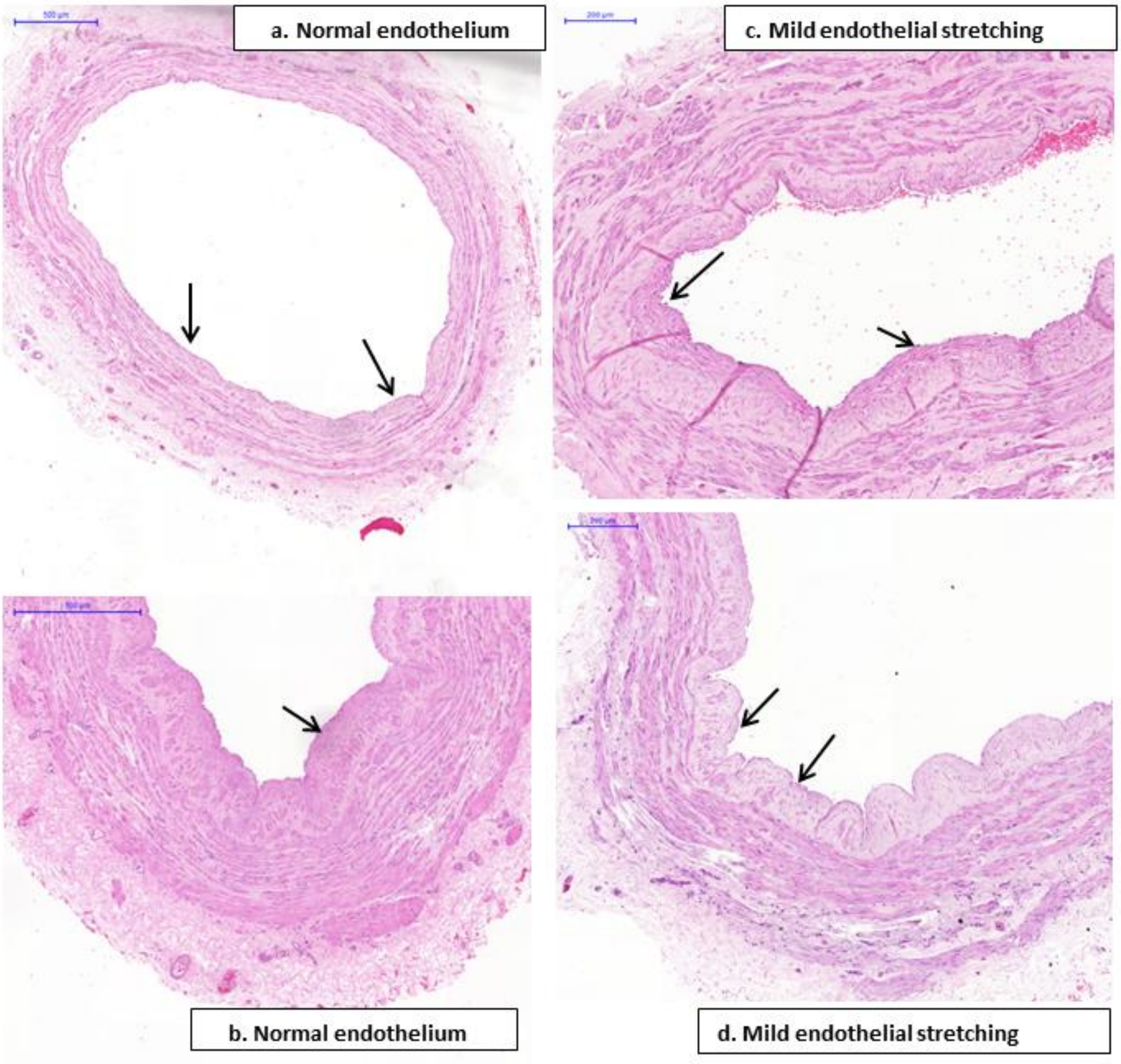
Figure 5b: Open tunnel CO_2 EVH system. This picture demonstrates the vein is harvested by the open tunnel technique. The bipolar diathermy is in use and also illustrates the proximity of the instruments close to the vein.

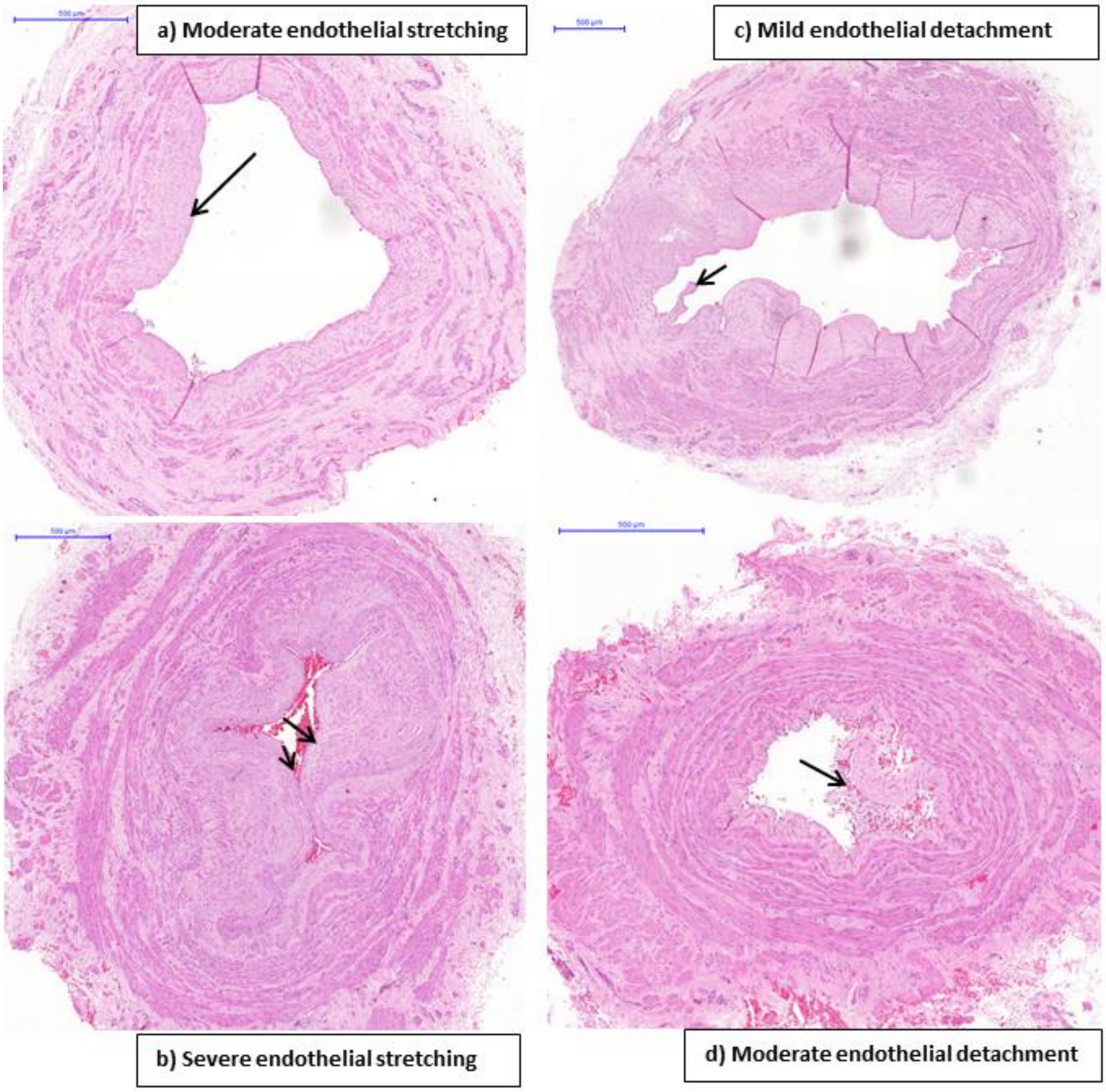
Figure 1: CONSORT diagram

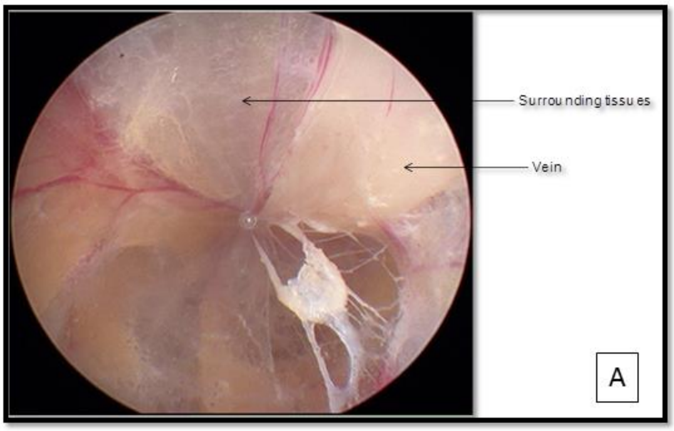


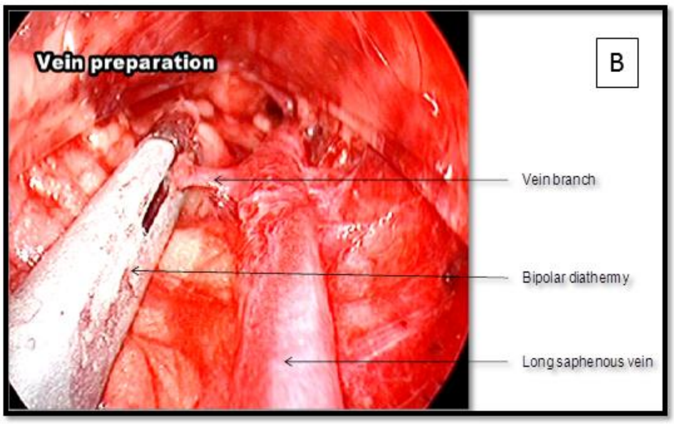

a) OT-EVH
p=0.451
EtCO2 (kPa)
b) OVH
p=0.385
EtCO2 (kPa)
c) CT-EVH
p<0.001
EtCO2 (kPa)
a) OT-EVH
p=0.241
PaCO2 (kPa)
b) OVH
p=0.001
PaCO2 (kPa)
c) CT-EVH
p<0.001
PaCO2 (kPa)
Pre-surgery
10 mins into surgery
10 mins post-surgery


a) OT-EVH
p=0.105
b) OVH
p=0.869
c) CT-EVH
p<0.001
EtCO2 (kPa)
7.6
7.4
7.2
7.0
Pre-surgery
Post-surgery


a. Normal endothelium
c. Mild endothelial stretching
b. Normal endothelium
d. Mild endothelial stretching


a) Moderate endothelial stretching
c) Mild endothelial detachment
b) Severe endothelial stretching
d) Moderate endothelial detachment


Surrounding tissues
Vein
A


Vein preparation
B
Vein branch
Bipolar diathermy
Long saphenous vein

Tables:

Table 1: Demographic data including pre-operative co-morbidities, risk factors and cardiac history. Categorical variables are expressed as number (percentage). Continuous variables are expressed as either mean±standard deviation (parametric data) or median [interquartile range] (non-parametric data). *PTCA=Percutaneous transluminal coronary angioplasty, OT-EVH = Open tunnel endoscopic vein harvesting, OVH = Open vein harvesting, CT-EVH = Closed tunnel endoscopic vein harvesting.

Demographic variables	**Group**		
	OT-EVH* (n=100)	**OVH* (n=100)**	**CT-EVH* (n=100)**
Age (years)	66.92±10.08	65.96±9.34	64.06±10.20
Sex (Male / Female)	82/18 (82.0%/18.0%)	79/21 (79.0%/21.0%)	79/21 (79.0%/21.0%)
Body Mass Index (BMI)	27.77 [6.41]	27.93 [5.45]	28.78 [6.54]
Urgency *Elective*	46 (46.0%)	49 (49.0%)	41 (41.0%)
Urgent	54 (54.0%)	51 (51.0%)	59 (59.0%)
Diabetes *Diet controlled*	8 (8.0%)	6 (6.0%)	4 (4.0%)
Tablet controlled	21 (21.0%)	27 (27.0%)	22 (22.0%)
Insulin controlled	8 (8.0%)	11 (11.0%)	4 (4.0%)
Canadian Cardiovascular Society *I*	17 (17.0%)	17 (17.0%)	12 (12.0%)
II	25 (25.0%)	29 (29.0%)	33 (33.0%)
III	45 (45.0%)	45 (45.0%)	46 (46.0%)
IV	13 (13.0%)	9 (9.0%)	9 (9.0%)
New York Heart Association *I*	27 (27.0%)	32 (32.0%)	40 (40.0%)
II	45 (45.0%)	35 (35.0%)	26 (26.0%)
III	26 (26.0%)	25 (25.0%)	29 (29.0%)
IV	2 (2.0%)	8 (8.0%)	5 (5.0%)
ST Elevated Myocardial Infarction	18 (18.0%)	19 (19.0%)	29 (29.0%)
Non ST Elevated Myocardial Infarction	42 (42.0%)	48 (48.0%)	44 (44.0%)
Previous PTCA*	16 (16.0%)	12 (12.0%)	20 (20.0%)
Previous Myocardial Infarction	52 (52.0%)	43 (43.0%)	54 (54.0%)
Multivessel disease	82 (82.0%)	81 (81.0%)	86 (86.0%)
Left main stem	25 (25.0%)	25 (25.0%)	40 (40.0%)
Hypertension	87 (87.0%)	83 (83.0%)	88 (88.0%)
Smoking *Never smoked*	32 (32.0%)	33 (33.0%)	23 (23.0%)
Previous smoker	52 (52.0%)	54 (54.0%)	47 (47.0%)
Current smoker	16 (16.0%)	13 (13.0%)	30 (30.0%)
Hypercholesterolemia	96 (96.0%)	90 (90.0%)	92 (92.0%)
Peripheral vascular disease	19 (19.0%)	20 (20.0%)	21 (21.0%)
Left ventricular ejection fraction *>50%*	74 (74.0%)	74 (74.0%)	72 (72.0%)
30-50%	21 (21.0%)	18 (18.0%)	22 (22.0%)
<30%	5 (5.0%)	8 (8.0%)	6 (6.0%)

Variable	Group			p-value
	OT-EVH*	OVH*	CT-EVH*	
Harvesting time (mins)	19.86 [11.64]	22.26 [17.65]	23.40 [12.48]	**0.031**
Harvesting rate (cm/min)	1.74 [0.96]	1.50 [0.86]	1.63 [0.93]	0.134
Full leg surgery time (mins)	42.93 [20.46]	42.73 [25.43]	53.50 [22.50]	**<0.001**
Total surgery time (mins)	226.77 [56.99]	222.65 [58.34]	228.46 [67.72]	0.806
Bypass time (mins)	93.00 [49.00]	90.00 [43.00]	92.00 [35.75]	0.698
Cross-clamp time (mins)	54.00 [37.00]	58.00 [34.75]	57.00 [23.00]	0.841
Number of vein grafts				
1	26 (26.0%)	26 (26.0%)	13 (13.0%)	0.130
2	54 (54.0%)	51 (51.0%)	57 (57.0%)	
3	20 (20.0%)	22 (22.0%)	30 (30.0%)	
4	0 (0.0%)	1 (1.0%)	0 (0.0%)	
Length of vein obtained (cm – mean±SD)	34.86±12.90	35.60±13.71	39.23±12.09	**0.039**
FiO2 (Fraction of Inspired Oxygen)	0.50 [0.17]	0.50 [0.10]	0.50 [0.11]	0.270
Respiratory Rate (bpm)	12.00 [2.00]	12.00 [2.00]	12.00 [2.00]	0.601
Tidal Volume (ml)	500.00 [67.50]	500.00 [100.00]	500.00 [50.00]	0.287

Table 2: Surgical data showing the full breakdown of surgical timings and the number of vein grafts harvested. Continuous data is expressed as either mean±standard deviation (parametric data) or median [interquartile range] (nonparametric data) and analysed by the Independent samples Kruskal-Wallis test. Categorical variables are expressed as number (percentage) and assessed by the χ^2 test. *OT-EVH = Open tunnel endoscopic vein harvesting, OVH = Open vein harvesting, CT-EVH = Closed tunnel endoscopic vein harvesting.

Group	Intimal Stretching				p-value
	Normal	Mild	Moderate	Severe	
OT-EVH*	34 (34.0%)	38 (38.0%)	13 (13.0%)	13 (13.0%)	0.003
OVH*	54 (54.0%)	35 (35.0%)	8 (8.0%)	0 (0.0%)	
CT-EVH*	39 (39.0%)	34 (34.0%)	20 (20.0%)	5 (5.0%)	
Group	**Intimal Detachment**				**p-value**
	No detachment	**Mild**	**Moderate**	**Severe**	
OT-EVH*	98 (98.0%)	1 (1.0%)	1 (1.0%)	0 (0.0%)	0.245
OVH*	97 (97.0%)	0 (0.0%)	2 (2.0%)	1 (1.0%)	
CT-EVH*	98 (98.0%)	2 (2.0%)	0 (0.0%)	0 (0.0%)	

Table 3: Histological data demonstrating the level of intimal stretching and intimal detachment in each group. Data is expressed as number (percentage) and was analysed using the χ^2 test. *OT-EVH = Open tunnel endoscopic vein harvesting, OVH = Open vein harvesting, CT-EVH = Closed tunnel endoscopic vein harvesting.