

# Effects of carbon dioxide insufflation on anastomosis remodeling at a carotid artery site in rabbits



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## Abstract

**Introduction:** Use of carbon dioxide (CO<sub>2</sub>) insufflation (CDI) on the surgical field during heart surgery has become widespread, and in some units routine.

**Aim:** To assess the effects of CDI on endothelial dysfunction in a carotid artery model in rabbits.

**Material and methods:** Twelve randomly selected rabbits were divided into two groups. Right carotid arteries of the animals were transected and sutured with running suture technique. Then, 1 l/min CO<sub>2</sub> insufflation was initiated with a 45° angle. In the control group, the anastomotic field was irrigated with 0.1 ml/s flow of 0.9% saline. At day 28, the carotid artery segments were removed and prepared for histological specimens.

**Results:** In the cross-sections of the control group vessel samples, thickening of the tunica intima was observed. Scoring the quantity of endothelial nitric oxide synthase (e-NOS) and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) positive staining revealed a nonsignificant difference between the experimental groups ( $p = 0.07$ ). In the CO<sub>2</sub> group, the intimal hyperplasia ( $p = 0.2$ ) and the thickness of the tunica media ( $p = 0.2$ ) were indistinguishable when compared to the control group. The mean luminal diameters and luminal areas of the experimental groups were all evaluated by histomorphometry and a significant difference was found between luminal areas ( $p = 0.016$ ). On the other hand, no significant difference was found between mean luminal diameters ( $p = 0.055$ ).

**Conclusions:** Our study indicated that CDI can affect endothelial cell damage and the mean luminal diameters.

**Key words:** carbon dioxide insufflation, carotid artery, intimal hyperplasia, endothelial damage.

## Streszczenie

**Wstęp:** Zastosowanie insuflacji dwutlenkiem węgla (CDI) w chirurgii serca jest szeroko rozpowszechnione, a na niektórych oddziałach stanowi rutynowy zabieg.

**Cel:** Ocena wpływu CDI na zaburzenia czynności śródbłonki naczyniowej w modelu tętnicy szyjnej u królików.

**Materiał i metody:** Dwanaście losowo wybranych królików podzielono na dwie grupy. Prawe tętnice szyjne zwierząt przecięto w poprzek i zszyto szwem ciągłym. Następnie rozpoczęto insuflację z przepływem 1 l/min CO<sub>2</sub> pod kątem 45°. W grupie kontrolnej pole anastomotyczne zostało przepłukane 0,9% solą fizjologiczną z przepływem 0,1 ml/s. W 28. dniu usunięto odcinki tętnicy szyjnej i przygotowano próbki do badań histologicznych.

**Wyniki:** W przekroju poprzecznym próbek naczyń grupy kontrolnej zaobserwowano pogrubienie warstwy wewnętrznej ściany naczynia. Pomiar ilości endotelialnej syntazy tlenu azotu (eNOS) i dodatni wynik barwienia  $\alpha$ -aktyny mięśni gładkich ( $\alpha$ -SMA) uwidocznily brak istotnej różnicy między grupami ( $p = 0,07$ ). W grupie CO<sub>2</sub> hiperplazja błony wewnętrznej ( $p = 0,2$ ) i grubość warstwy środkowej ściany naczynia ( $p = 0,02$ ) nie różniły się w porównaniu z grupą kontrolną. Średnie średnice światła naczyń i pola powierzchni światła naczyń w obu grupach oceniono w badaniach histomorfometrycznych; istotne różnice wystąpiły między polami powierzchni światła ( $p = 0,016$ ). Nie stwierdzono jednak istotnych różnic między średnimi średnicami światła naczyń ( $p = 0,055$ ).

**Wnioski:** W badaniu wykazano, że CDI może mieć wpływ na uszkodzenie komórek śródbłonki i średnią średnicę światła naczyń.

**Słowa kluczowe:** insuflacja dwutlenkiem węgla, tętnica szyjna, hiperplazja błony wewnętrznej, uszkodzenie śródbłonki.

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## Introduction

Insufflation of carbon dioxide (CO<sub>2</sub>) to the operative field has been used to prevent air embolisms and keep the surgical field bloodless during coronary artery anastomosis in cardiac surgery. Carbon dioxide blowing technique is simple to use without any major adverse effect or additional cost. However, a potentially harmful effect of carbon dioxide insufflation on postoperative endothelial dysfunction remains unproven in clinical trials. In addition, the damage mechanism responsible for this effect has not been elucidated. In the literature, data on outcomes of the effect of CDI on the surgical field are limited.

Arterial anastomosis models demonstrated that the main reasons for lumen stenosis are smooth muscle cell proliferation and intimal damage [1–3]. The pathological cause of restenosis and endothelial cell proliferation is migration of proliferated smooth muscle cells from media to intima [3]. When intimal damage develops, platelets coat the damaged area and release mitogenic growth factors [3]. Therefore, the proliferated smooth muscle cells migrate and cause intimal hyperplasia [4]. Studies on endothelial cell proliferation have demonstrated that the re-endothelialization was complete by day 14 [5]. The choice of surgical procedure is critical in the presence of vascular restenosis or in complex pathological settings such as arterial thrombosis where the atherosclerotic plaque plays a fundamental role.

## Aim

The aim of this study was to evaluate the effects of CO<sub>2</sub> insufflation and endothelial damage during a rabbit carotid artery model. In addition, it can demonstrate how to avoid endothelial dysfunction in coronary artery bypass surgery (CABG) induced by CO<sub>2</sub> insufflation to the coronary vessel, owing to its potentially harmful effect. Establishment of an animal model with CO<sub>2</sub> blowing over the surgical field in a carotid artery site would be useful for better understanding of the immunohistochemistry in human patients.

## Material and methods

This randomized, controlled, interventional experimental study was conducted at the Animal Laboratory and Research Center of Dokuz Eylul University Faculty of Medicine with the approval of the Ethics Committee for Animal Research on 3 March 2015 with the protocol number 06/2015. All procedures were performed according to the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Resources, National Academy of Sciences, Washington, DC, United States). The rabbits were given a 12-h dark/light cycle at temperature 24 ± 2°C and humidity 55–60%, and were maintained on *ad libitum* diets throughout the experiments.

### Surgical procedure

The experiment was performed on 12 male rabbits (weighing: 2–3 kg; mean age: 11 weeks). The rabbits were

divided into two groups randomly. Before the surgical procedure, intramuscular anesthesia was administered using ketamine hydrochloride (50 mg/kg, Pfizer, Istanbul, Turkey) and xylazine (5 mg/kg, Bayer Turk Kimya Sanayi, Istanbul, Turkey). Moreover, cefazolin sodium (50 mg/kg, Bilim Ilac Sanayi, Istanbul, Turkey) intramuscularly was used to prevent infections. Later on, right-sided carotid arteries were transected and preanastomotic anticoagulation initiated with intravenous injection of 150 U/kg heparin. The suture line was made with 7/0 polypropylene via running sutures. In the treatment group, 1 l/min CO<sub>2</sub> was blown at a 45° angle from 10 cm height with a standard flowmeter and gas insufflation cannula (Novatech aspirator microvac 2 × 70 mm) for approximately 5 min during the anastomosis process. In the control group, the carotid arteries were dissected, transected and restored in a similar fashion and the anastomotic field was irrigated with 0.1 ml/s flow of 0.9% saline solution from 10 cm height and a 45° angle for 5 min. There were no complications perioperatively. Following surgery, all rabbits were euthanized at the 28<sup>th</sup> day and the carotid artery segments were removed and prepared for histological dyeing.

### Histopathological evaluation

Carotid artery segments were removed and fixed in 10% neutral buffered formalin, dehydrated in a graded series of isopropyl alcohol (60% to 100%) and followed by xylol before being embedded in paraffin. Later on, 5 µm sections were taken with a rotary microtome (RM 2255, Leica Instruments, Nussloch, Germany) on poly-L lysine coated slides. The specimens were stained with hematoxylin and eosin (H + E, Surgipath, 01562E, Bretton, Cambridgeshire). Histomorphologic and morphometric measurements were evaluated with the images which were obtained from the selected areas and analyzed via a computer-assisted image analyzer system consisting of a microscope (Olympus BX-51, Tokyo, Japan) equipped with a high resolution video camera (Olympus, DP70, Japan). Aiming at further immunohistochemical examination, the specimens were also stained with endothelial nitric oxide synthase (eNOS) and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA). Following these dyeing processes, all images were digitally photographed and examined via a computerized video camera based image analysis system (UTHSCA Image Tool software version 3.0, University of Texas Health Science Center, San Antonio, TX, USA). The luminal diameters, luminal areas, and intima and media thickness were all measured and the groups were compared in terms of these parameters.

### Statistical analysis

Direction and significance of the association between variables were evaluated by the Mann-Whitney *U*-test. A *p*-value less than 0.05 was accepted as the significance level. In all calculations and statistical analyses, SPSS 15.0 (SPSS Inc, Chicago, IL, USA) and Excel (Microsoft, USA) software was used. Semiquantitative immune scoring variables were expressed as the mean ± standard deviation.

## Results

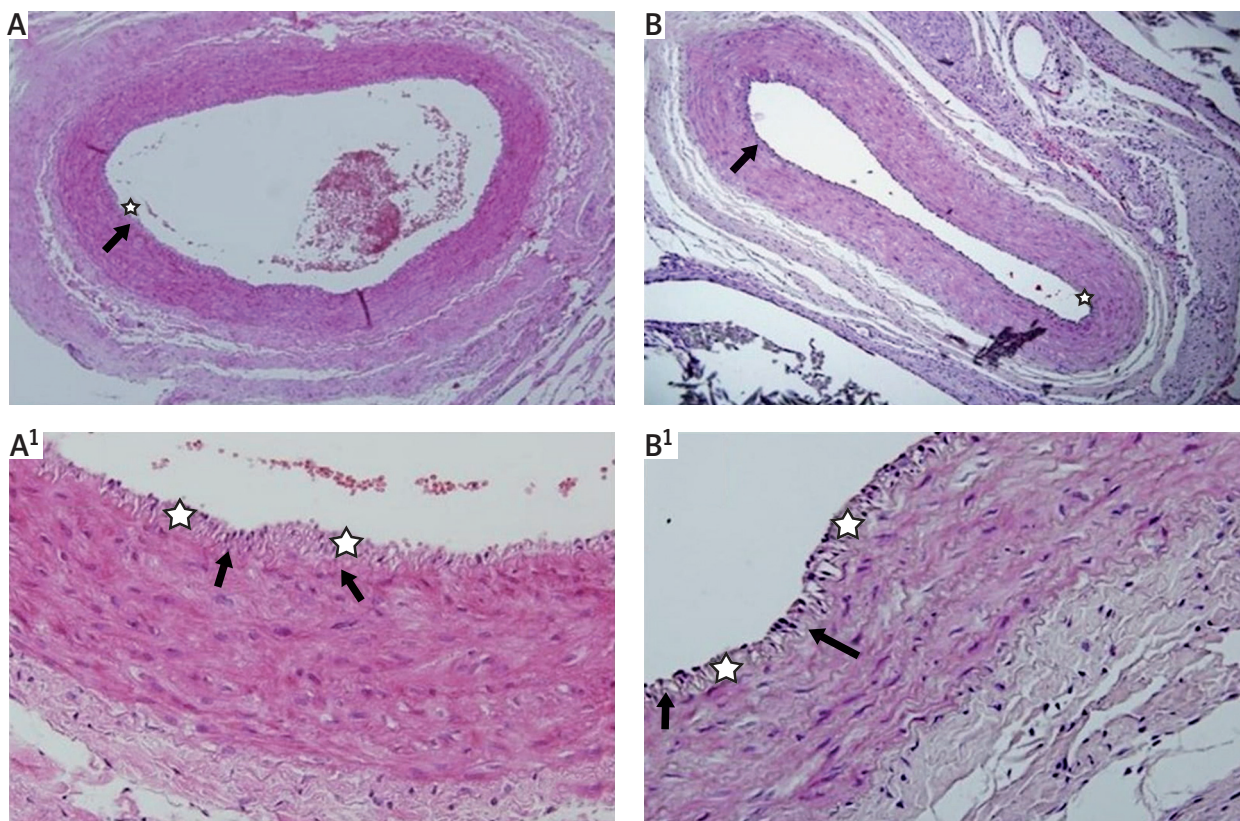
All histochemical and histomorphological analyses described above were performed by two investigators blind to rabbits' treatment. All carotid artery lumens of the rabbits were patent. When carotid artery segments were investigated, a difference in the thickness of the tunica media and the adventitia was seen between groups (Fig. 1). Scoring the quantity of e-NOS and  $\alpha$ -SMA positive staining revealed a nonsignificant difference between the experimental groups (in the Mann-Whitney *U*-test,  $p = 0.07$ ) (Fig. 2). The carotid arteries' mean intima thickness ( $\mu\text{m}$ ) was 25.8 (6.07–26.70  $\mu\text{m}$ ) in the  $\text{CO}_2$  group. It was 16.66 (13.75–44.46  $\mu\text{m}$ ) in the control group. The carotid arteries mean media thickness ( $\mu\text{m}$ ) was 150.2 (117.38–171.26  $\mu\text{m}$ ) in the  $\text{CO}_2$  group. It was 133.23 (127.65–179.02  $\mu\text{m}$ ) in the control group. In the  $\text{CO}_2$  group, the intimal hyperplasia ( $p = 0.2$ ) and the thickness of the tunica media ( $p = 0.2$ ) were indistinguishable when compared to the control group. The carotid arteries' mean lumen diameter ( $\mu\text{m}$ ) was 838.62 (535.63–885.55  $\mu\text{m}$ ) in the  $\text{CO}_2$  group. It was 711.02 (586.37–950.69  $\mu\text{m}$ ) in the control group. The carotid arteries' mean lumen area ( $\mu\text{m}^2$ ) was 518252.99 (142604.2–517199.2  $\mu\text{m}^2$ ) in the  $\text{CO}_2$  group. It was 260593.06 (254913.4–656958.0  $\mu\text{m}^2$ ) in the control group. The mean luminal diameters and luminal areas of the experimental groups were all evaluated by histomorphometry and a significant difference was found between luminal areas

( $p = 0.016$ ). On the other hand, no significant difference was found between mean luminal diameters ( $p = 0.055$ ) (Table I).

## Discussion

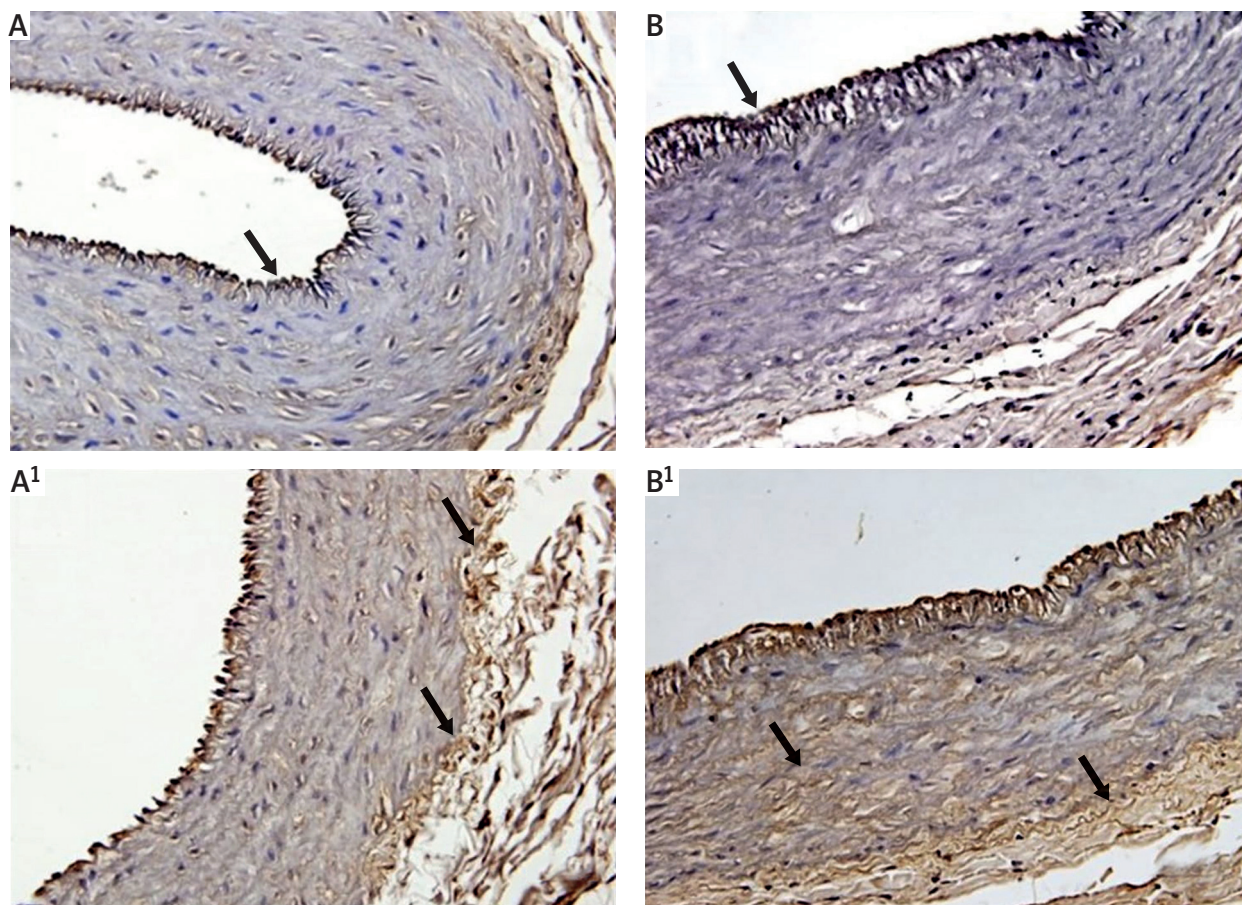
The present study demonstrated the effects of washing with saline solution and carbon dioxide gas blowing techniques on the cellular and ultrastructural changes and intimal hyperplasia in a rabbit carotid artery model. The experimental study model was performed by surgical intervention of the carotid artery followed by transection and restoration with side-by-side suture technique.

Beating heart coronary bypass surgery is often bloody because of blood from native arteries. Anastomosis quality must be maintained during the CABG operation. Different methods to ensure sufficient visibility in the surgical field are used for the anastomosis quality. These methods are intra-coronary shunt placement, aspiration and drying, washing with saline solution or  $\text{CO}_2$  blowing techniques. Saline irrigation may not be enough to clean the area of the anastomosis. On the other hand, application of intra-coronary shunts and occluders is difficult. Also, that can cause stress in the coronary artery wall and have a risk of coronary endothelial dysfunction. Gas blowing technique is the most effective method to maintain a bloodless surgical field, but this technique has some risks following physical stimuli [6, 7]. The importance of intimal hyperplasia, restenosis and endothelial proliferation processes is obvious in



**Fig. 1.** Histological sections of groups (hematoxylin + eosin stain, 10 $\times$  and 40 $\times$ ): **A** – treatment group, 10 $\times$ ; **A<sup>1</sup>** – treatment group, 40 $\times$ , **B** – control group, 10 $\times$ , **B<sup>1</sup>** – control group, 40 $\times$ . Internal elastic lamina marked with arrow and intimal hyperplasia marked with star





**Fig. 2.** Histological sections of groups (e-NOS and  $\alpha$ -SMA staining, 40 $\times$ ): **A** – treatment group, 10 $\times$ , **A<sup>1</sup>** – treatment group, 40 $\times$ , e-NOS staining; **B** – control group, 10 $\times$ , **B<sup>1</sup>** – control group, 40 $\times$ ,  $\alpha$ -SMA staining. Intimal hyperplasia marked with arrow

**Table I.** Comparison of mean lumen diameters and lumen area, mean intima/media thickness area values

Parameter		Treatment group (CO <sub>2</sub> )	Control group (Saline)	P-value
Lumen diameter [ $\mu$ m]	Mean	838.62	711.02	0.055
	Median	881.40	730.90	
	Std. deviation	132.11	103.13	
Luminal areas [ $\mu$ m <sup>2</sup> ]	Mean	518252.99	260593.06	0.016
	Median	546820.70	193291.21	
	Std. deviation	150864.68	150755.67	
Intima thickness [ $\mu$ m]	Mean	25.80	16.66	0.2
	Median	23.99	17.59	
	Std. deviation	11.77	7.98	
Media thickness [ $\mu$ m]	Mean	150.20	133.23	0.2
	Median	144.93	137.06	
	Std. deviation	20.75	13.53	

cardiovascular surgery. Impairment of vascular endothelial integrity occurs on the basis of arterial thrombosis. Accordingly, it continues to be promoted by thrombosis.

Carbon dioxide blowing can be performed continuously or intermittently. A standard method is undefined. This approach is used to clean the surgical field during coronary artery anastomosis. According to the experience of the sur-

gical team it is important to use different methods. Gas flow rate, catheter type and thickness, the distance of the anastomosis, and the differences in execution time can cause endothelial dysfunction in atherosclerotic vessels. Endothelial vascular tonus regulation can effect intimal hyperplasia [3–5]. Platelet adhesion to damaged endothelium causes aggregation and degranulation. Platelets are

released from platelet-derived growth factor dependent migration to the intima of smooth muscle cells and proliferation occurs. In addition, P-selectin, ICAM-1, and GP-IIb/IIIa cell adhesive factors can be secreted by damaged endothelium [6–8]. Thus, by contact of the blood components can cause aggregation and thrombosis.

Clinically, carbon dioxide insufflation on the surgical field was used as early as 1958 by Nichols *et al.* They demonstrated that carbon dioxide emboli absorbed faster than air emboli. Insufflation of CO<sub>2</sub> to de-air the heart and great vessels' wound markedly decreases the incidence of microemboli. Nowadays, the interest in minimally invasive heart surgery where de-airing techniques have to be more effective has led to resurgence in the interest in and use of CO<sub>2</sub> field flooding. Also, the interest in cardiac surgery is to prevent bleeding on the surgical field during coronary artery anastomosis. Altogether 205 articles were found using the reported search about CO<sub>2</sub> insufflation, and few of them were used to answer the clinical question of CDI on endothelial damage.

Burfeind *et al.* reported that high-flow carbon dioxide gas insufflation has a harmful effect on coronary endothelium and may cause both early and late-term graft failure [9]. Moreover, Alves *et al.* found a strong correlation between flow rate and endothelial injury of the internal mammary artery in their experimental model in goats [10].

As shown in another study, Martens *et al.* demonstrated that they could deliver CO<sub>2</sub> at up to 10 l/min without causing an increase in arterial PCO<sub>2</sub> or acidosis to determine the optimal technique for the delivery and amounts of CO<sub>2</sub> [7]. They measured arterial blood gasses. Martens *et al.*' prospective study of CO<sub>2</sub> insufflation compared to conventional de-airing techniques showed no statistically significant differences between the two groups in terms of mortality or neurocognitive function [6]. Otherwise, neurocognitive test outcomes did not reveal any advantages of CO<sub>2</sub> field flooding in two studies. Ganguly *et al.* recommend that CO<sub>2</sub> insufflation be performed in all open heart surgery cases to bring down the incidence of neurocognitive dysfunction. Also, they reported no requirement of major paraphernalia or additional costs with this technique [6–18].

In this content, the aim of our study was to evaluate the effects of CO<sub>2</sub> insufflation on the cellular and ultrastructural changes occurring in the carotid artery model in animals. e-NOS is known to be primarily responsible for the generation of NO in the vascular endothelium [16].  $\alpha$ -SMA is known to play a crucial role in fibrogenesis and correlates with the activation of myofibroblasts [17]. Also, SMA has an important role in fibrogenesis and myofibroblast generation [17]. The endothelium plays a major role in regulating usual blood vessel physiology. Intimal hyperplasia is an independent risk factor for the cardiovascular field. As a result of it, arterial elasticity and the muscle smooth muscle cell, collagen, elastic fibrils, and proteoglycans in the matrix are changed, and vessel wall injury occurs [17]. Medial thickening with effects of CO<sub>2</sub> insufflation can be attributable primarily to improved smooth muscle cell mass,

although enhanced deposition of collagen and elastin has a contributory role. Furthermore, many surgeons avoid operating on patients with I/R injury related intimal damage because of disastrous complications. In the animal model, we preferred to evaluate the effects of CO<sub>2</sub> insufflation in order to find activation of complements and ischemia/reperfusion (I/R) injury related damage. In the cross-sections of the control group vessel samples, thickening of the tunica intima was noted. When the other parts of the aorta were investigated, there was an increase in the thickness of the tunica media and prominent thickening in the adventitia in this group. Scoring the quantity of e-NOS and  $\alpha$ -SMA positive staining revealed a nonsignificant difference between the experimental groups (in the Mann-Whitney *U* test,  $p = 0.07$ ). In the CO<sub>2</sub> group, the intimal hyperplasia ( $p = 0.2$ ) and the thickness of the tunica media were indistinguishable when compared to the control group (Fig. 1 A, B). Also, the mean luminal diameters and luminal areas of the experimental groups were all evaluated by histomorphometry and a significant difference was found between lumen areas ( $p = 0.016$ ). On the other hand, no significant difference was found between mean luminal diameters ( $p = 0.055$ ). With the help of these findings, we found that CO<sub>2</sub> gas insufflation has a prominent destructive effect on luminal areas of the experimental groups. Because of this evidence, we think that CDI causes endothelial cell damage. Vascular endothelium is extremely sensitive to physical stimulation because of CDI.

### Study limitations

The primary limitation of our study was the lack of molecular data. We plan to perform our study on a higher budget and include immunohistochemistry data and oxidative stress parameters. In our opinion, further studies should be conducted to compare the effects of carbon dioxide insufflation on the surgical field during heart surgery.

### Conclusions

Carbon dioxide gas insufflation on the surgical field in an animal model seems to have harmful adverse effects on the mean luminal areas. In our opinion there is still a need for comparative studies to assess the endothelial effects of CO<sub>2</sub> insufflation in patients undergoing peripheral artery disease and coronary artery disease.

### Disclosure

The authors report no conflict of interest.

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