Relation Between Iatrogenic Subcutaneous Emphysema And Fat Tissue Remodeling Induced By CO2 Gas

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Citation

J Sang, S Yoon, S Nam, Y Lee, J Oh, K Cho, C Choi. *Relation Between Iatrogenic Subcutaneous Emphysema And Fat Tissue Remodeling Induced By CO2 Gas*. The Internet Journal of Gynecology and Obstetrics. 2019 Volume 23 Number 2.

DOI: 10.5580/IJGO.54676

Abstract

Objective

Carbon dioxide (CO2) gas is commonly used in laparoscopy for abdominal inflation. Sometimes CO2 is injected into subcutaneous fat layer and it causes emphysema. However, there have few studies of what clinical changes in subcutaneous fat caused by unintended acute CO2 injection. In this study, we examined the histological and molecular changes in the acute CO2 injected adipose tissue.

Methods

To investigate the acute effects of CO2 injection, two different animal experiments were conducted. In experiment 1, mice were punctured in the left groin using a needle without gas, and the right groin was injected with 1cc of CO2. In experiment 2, mice were injected with 1cc of breathable air and 1cc of CO2 was injected into the left and right groin. At 6 hours post injection, adipose tissues were collected, and then examined by immunohistochemical analysis. Transcription level of genes associated with lipogenesis, angiogenesis and fibrosis were also measured using qPCR.

Results

There is no significant histological change in experiment 1 and 2. Adipose tissue injection with CO2 had marginally reduced mRNA levels of angiogenesis genes such as Vegf1 and Fgf1, but which did not attain the statistically significance. Overall, qPCR results with comparison to control group did not show any significant effects.

Conclusion

The acute CO2 injection did not lead to significant histological and transcriptional changes in subcutaneous adipose tissue. Even though the subcutaneous emphysema is induced by CO2, we found out that the subcutaneous emphysema is less significantly associated to the metabolism and remodeling of adipose tissue.

INTRODUCTION

In the early 1980s, the pioneers of laparoscopic surgery, Kurt Semm and Erich Muehe, developed laparoscopy from a diagnostic to a surgical procedure, which has since become a surgical treatment option in many fields [1]. Now, laparoscopic surgery is a gold standard procedure in reproductive (particularly gynecological) and digestive (such as cholecystectomy) surgeries and it has become more common in pediatric patients. In American pediatric tertiary care centers, the rate of laparoscopic appendectomy rapidly increased from under 10% in 1997 to over 95% in 2005 [2].

In laparoscopic surgery, insufflation by gas is required for securing an operative field of view. Carbon dioxide (CO2) is a widely used as an expansion gas in laparoscopic surgery since it has several advantages including colorless, easily breathable, nonflammable, and dissolving well in blood [3]. However, side effects such as subcutaneous emphysema, carbon dioxide retention, and air embolism have been also reported [4]. Particularly, subcutaneous emphysema incidence was reported to vary from 0.43% to 77% during surgical procedure [5-10].

Despite of high incidences, few studies have been

undertaken to examine the effects of CO2 on adipose tissue metabolisms. Balik et al. reported that chronic injection of CO2 affect the adipose tissue metabolism [11]. , which indicate that CO2 is a factor to regulate adipose tissue metabolism. However, these studies did not perform the effect of CO2 in acute phase which is condition of subcutaneous emphysema via laparoscopy surgical procedure. Currently, there is no research on how CO2 causes histological and molecular changes in subcutaneous fat which mainly account for the subcutaneous emphysema. In this study, we investigate the possibility of the alterations of adipose tissue metabolism in response to acute exposure of CO2 by using the animal model in respect with histological and molecular analysis.

METHODS

Animal studies

C57BL/6 male mice (Orient Bio, Korea) were purchased and fed with a high-fat diet (HFD) for 8 weeks. The experimental environment was controlled at 23 ± 2°C with a humidity of 55-60% in a 12-hour light/dark cycle. In experiment 1, HFD-fed mice (14 weeks old) were treated with sham operation in left groin and with CO2 injection in the right groin. For the sham operation, left subcutaneous fat was punctured using a needle without gas. For the CO2 treatment, 1cc of CO2 was injected to the right subcutaneous fat using a portable CO2 manufacturing machine (Fig. 1A). In experiment 2, 1cc of breathable air and 1cc of CO2 was administrated into the left and right groin of HFD-fed mice, respectively (Fig. 1B). At 6 hours post injection in which emphysema was ceased entirely, mice were sacrificed, then, groin white adipose tissues were collected, weighed, and stored at the -80°C freezer for the further analysis. Some tissues were fixed with 4% paraformaldehyde for histological analysis.

All of these procedures were carried out in accordance with ethics approval and the guidelines of the Institutional Animal Care and Use Committee of Soonchunhyang University (SCH16-0031).

Histochemical analysis

Paraffin-embedded tissues were sectioned at 5 m and mounted for histological staining. Fixative was removed using xylenes, tissues were rehydrated and then flushed for an additional 15 min in deionized water to remove any remaining fixative. Tissue sections were stained using Harris hematoxylin and eosin (H&E) staining. Blood vessel was stained by using isolectin and visualized as described in previous studies [12]. Sirius red staining was conducted as described previous studies to observe collagen fibers [13-14]. Photographs were taken under microscopic examination using light microscope (Leica DM1000).

Measurement of adipocyte size and vessels

Adipocyte size and vessel numbers in area were quantified by methods described previously [12, 15]. Briefly, 6 representative photographs of digital images from non-overlapping fields were taken from each slide for quantification using a Leica inverted microscope. Adipocyte diameter and number of vessels surrounding each adipocyte were calculated using Adiposoft® software.

Gene expression analysis

Total RNA was extracted from adipose tissue using TRIzol (Life Technologies, Seoul, Korea) according to the manufacturer's instructions. cDNA was generated from 1mg of RNA using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, CA, USA). PowerUp SYBR Green PCR Master Mix (Applied Biosystems) and the StepOnePlus System (Applied Biosystems) were used for real-time quantitative PCR (qPCR). Arbp expression was used as an internal control for data normalization. Samples were assayed in duplicate, and relative expression was determined using the 2– CT method. The PCR primers used are listed in Table 1.

Statistical analysis

All results were expressed as mean ± SD. Pairwise Student's t tests were used to analyze the difference between 'Sham' and CO2 injection groups, and between the breathable air and CO2 injection groups. All statistical analyses were performed using GraphPad Prism software (version 5.01; GraphPad Software, La Jolla, CA) and the criterion was $P < 0.05$.

RESULTS

In order to examine whether acute CO2 administration affect subcutaneous adipose tissue metabolism, we first evaluated histological differences between fat tissues treated CO2 and sham-operated tissue in same HFD-fed mice (Experiment 1). H&E staining showed that adipocyte size in CO2 injection groups is comparable to the them in Sham group (Sham vs CO2;2557 ± 653 m2 vs 3016 ± 1019 m2) (Fig. 2A) . Since vascularization is known to be a major regulator of adipose tissue remodeling [16-17], vessels of Sham and CO2

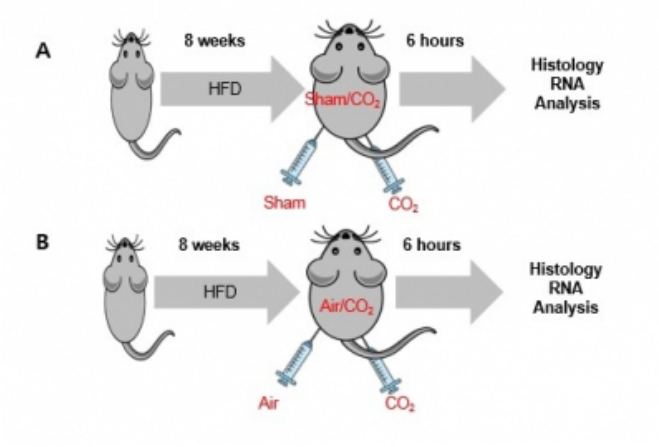
injected adipose tissue was stained by lectin. Vascularization analysis revealed that acute CO2 treatment did not affect the vessel numbers and volumes compared with Sham group (Fig. 2B). Fibrosis defined by excessive accumulation of fibrous connective tissue components such as collagen is the important remodeling process in response to injury [18-19]. Sirus Red staining to evaluate collagen accumulation showed that adipose tissue treated with CO2 had comparable amount of collagen to them of Sham group (Fig. 2C). Taken together, histological analysis by H&E, vasculature and fibrosis demonstrated that acute CO2 administration did not cause to significant histological changes in subcutaneous adipose tissue.

Since histological analysis have limitation to detect rapid changes of adipose tissue metabolism, we examined the effects of acute phase CO2 injection on transcription level of genes associated with lipogenesis, angiogenesis and fibrosis. mRNA abundances of peroxisome proliferator-activated receptor gamma 2 (Pparg2), fatty acid binding protein 4 (Fabp4) and fatty acid synthase (Fas) in groins from CO2 group were similar to them in Sham groups (Fig. 3A). Acute CO2 injection decreased the transcriptional level of vascular endothelial growth factor 1(Vegf1) fibroblast growth factor 1(Fgf1) by ~56% and ~25%, respectively, but it did not reach the statistical significances (Fig. 3B). Consistent to Sirus Red staining, mRNA levels of Col1a and Col6a in CO2 groups is comparable to them in Sham group while Acta2 mRNA was marginally reduced in CO2-treated adipose tissue.

In order to exclude the potential of the mechanical effects, we examined the difference of the CO2 and the room air administration in the subcutaneous adipose tissue metabolism from same HFD-fed mice (Experiment 2). Histological analysis of adipocyte size, vessel numbers and collagen accumulation showed that there was no significant difference in groups (Fig.4A-C). Expression levels of genes associated with lipogenesis such as Pparg2, Fas, and Fabp4 were similar between Air and CO2-treated adipose tissue (Fig. 5A). While angiogenesis related mRNA (Vegf1 and Fgf1) levels were marginally lower in CO2-injected adipose tissue, but it did not reach the statistically significance (P>0.05, Fig. 5B). Transcriptional analysis of genes involved with fibrosis did not show any significant effects of CO2 compared with Air in the acute treatment.

Figure 1

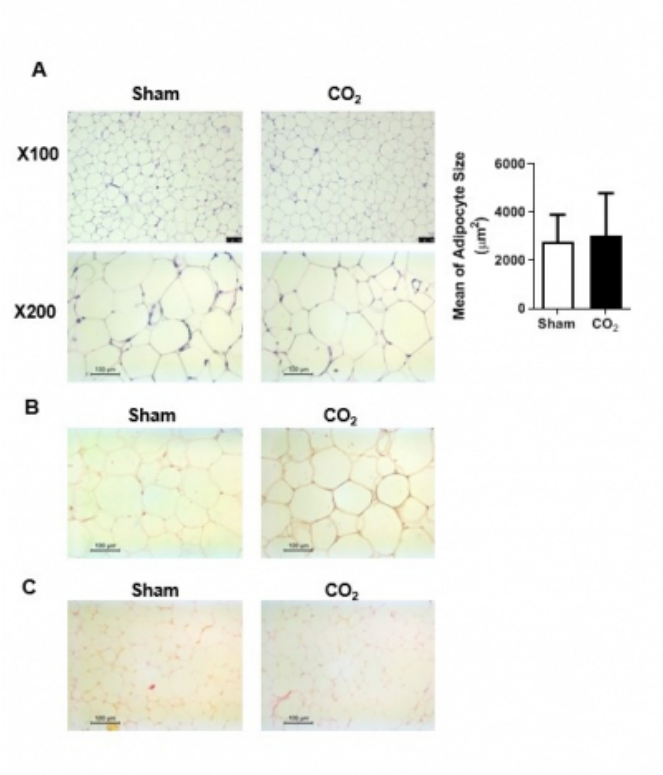
Schematic diagrams of experiment 1 (A) and 2 (B).



(A)–(C) HFD-fed male C57BL/6 mice were treated with a gasless needle puncture (Sham) to the left inguinal fat and CO2 injection (CO2) to the right inguinal fat. At 6 hours post injection, mice were sacrificed and inguinal adipose tissue were obtained. Histological analysis were performed using paraffin-embedded formalin fixed adipose tissues. (A) Representative Hematoxylin and Eosin (H&E) staining of the groin fat pads from Sham and CO2 group. Quantitation of average adipocyte size in subcutaneous fat treated with Sham or CO2. (B) Representative image of blood vessels in fat pads injected with CO2 and Sham. Blood vessels were stained with lectin. (C) Sirius red staining in in groin fat pads treated Sham and CO2.

Figure 2

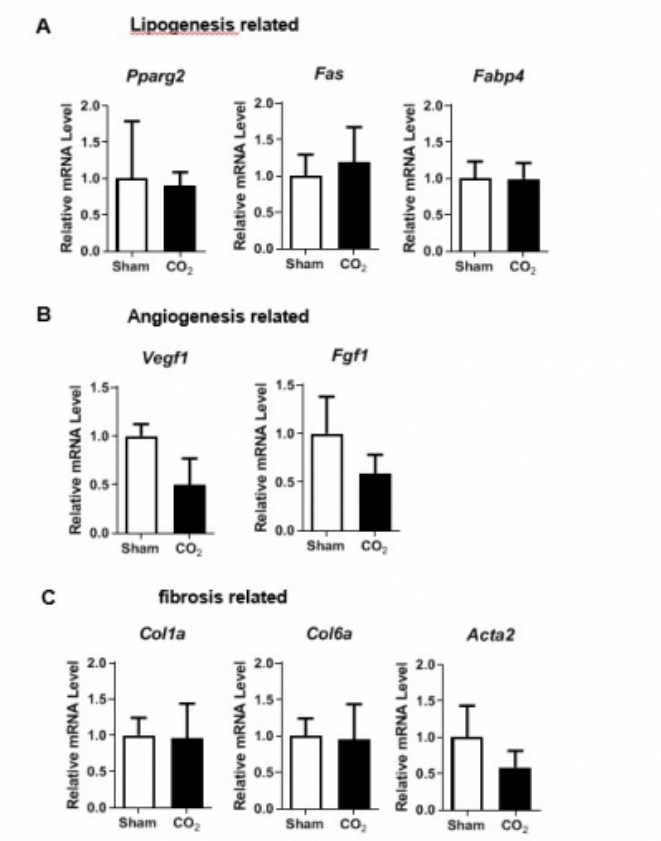
Histological effects of acute CO2 administration on the subcutaneous adipose tissue.



(A-C) HFD-fed male C57BL/6 mice were treated with a gasless needle puncture (Sham) to the left inguinal fat and CO2 injection (CO2) to the right inguinal fat. At 6 hours post injection, mice were sacrificed and inguinal adipose tissue were obtained. RNA was isolated from groin fat pads and synthesized cDNA was used to determine the gene expression by qPCR.(A) Expression of lipogenic genes, Pparg2, Fas, and Fabp4 in subcutaneous fat from Sham and CO2 group.(B) Effects of acute CO2 administration on angiogenesis-associated genes expression in local fat pads. RNA was isolated from groin fat pads and expression of Vegf1and Fgf1 was assessed by qPCR. Relative mRNA levels of the genes associated with angiogenesis were normalized to housekeeping gene Arbp expression. (C) Effects of acute CO2 administration on the expression of fibrosis genes in local adipose tissue. Expression of Col1a, Col6a, Acta2 was normalized to housekeeping gene Arbp mRNA and relative mRNA levels was expressed compared to Sham group. Data are given as the mean ± SEM.

Figure 3

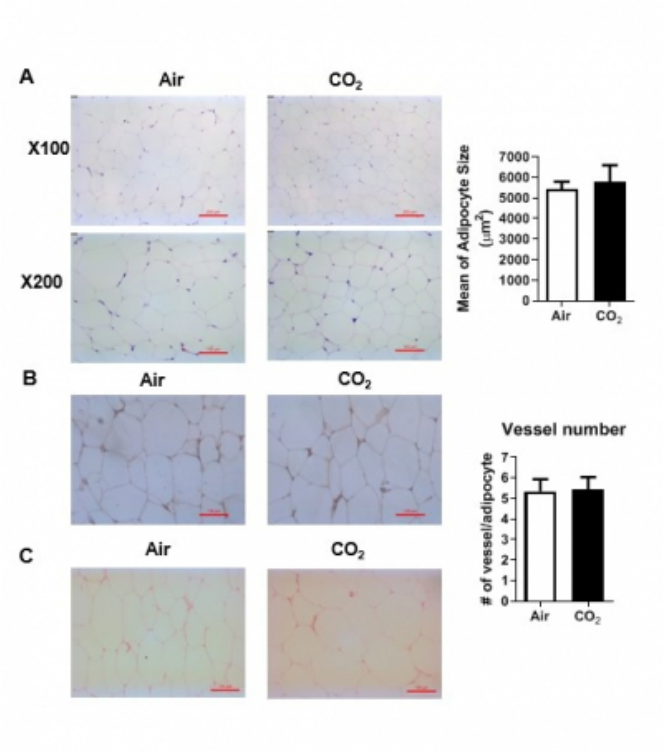
Effects of acute CO2 administration on the expression of genes associated with lipogenesis, angiogenesis and fibrosis in subcutaneous fat.



(A)–(C) Mice were treated with 1 cc breathable air (Air) to the left inguinal fat and CO2 injection (CO2) to the right inguinal fat. At 6 hours post injection, mice were sacrificed and inguinal adipose tissue were obtained. Histological analysis were performed using paraffin-embedded formalin fixed adipose tissues. (A) Representative H&E stain of groin fat pads injected with air or CO2. Quantitation of average adipocyte size in groin fat pads (right). (B) Representative lectin stain image of the subcutaneous fat layers after injection (left) and the quantitation of vessel numbers around adipocyte in groin fat pads (right). (C) Collagen stain by Sirius Red in groin fat pads injected with air and CO2.

Figure 4

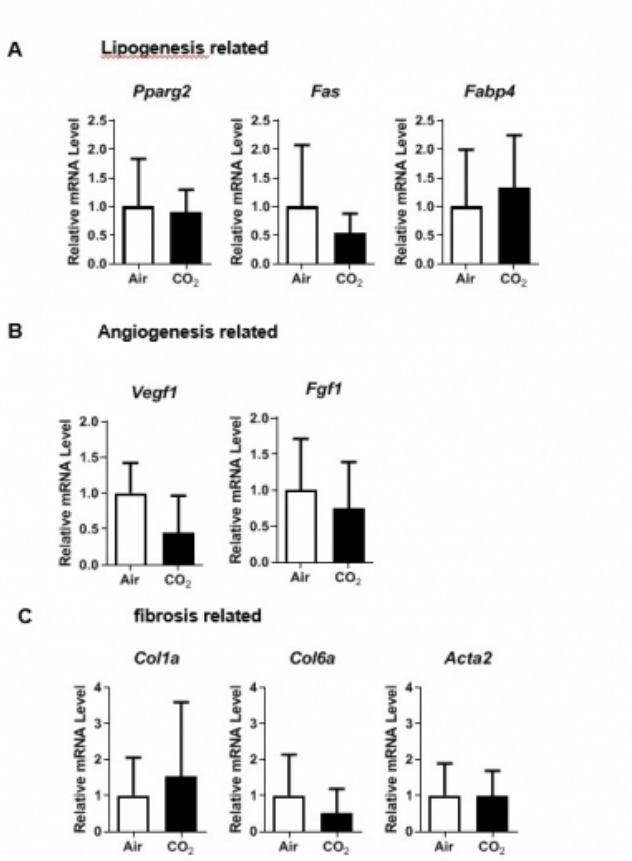
Comparison of histological analysis between Air and CO2-injected subcutaneous fat.



(A-C) HFD-fed male C57BL/6 mice were treated with a 1 cc Air (Air) to the left inguinal fat and CO2 injection (CO2) to the right inguinal fat. At 6 hours post injection, mice were sacrificed and inguinal adipose tissue were obtained. RNA was isolated and cDNA was synthesized. Gene expression was analyzed by qPCR and normalized to housekeeping gene Arbp expression. (A) Expression of lipogenic genes, Pparg2, Fas, and Fabp4 in subcutaneous fat treated with Air or CO2. (B) Expression of Vegf1 and Fgf1 in local fat pads treated with Air or CO2. (C) Expressions of genes, Col1a, Col6a and Acta2 in local fat pads. Data are expressed as the mean ± SEM.

Figure 5

Comparison of gene expressions between Air and CO2–injected subcutaneous fat.



DISCUSSIONS

The incidence of laparoscopic surgery has increased over the past 100 years due to the minimal invasion and rapid recovery and now it is serving as a cornerstone in most abdominal operations [20]. During the laparoscopic surgery, expansion of the abdominal wall by gases is needed. Among various gases, CO2 gas is widely used for insufflation because it is a colorless, odorless, nonflammable, highly water-soluble gas with acidic properties [21]. Complications caused by the use of carbon dioxide as expansion gas in laparoscopic surgery have been reported to include pneumoperitoneum including subcutaneous emphysema, mediastinal emphysema, pneumothorax, cardiac dysrhythmia, CO2 retention, postoperative pain caused by retention gas in the abdominal cavity, and air embolism due to vein damage [4]. Among them, subcutaneous emphysema is the most common complication. The reported incidence rate of known subcutaneous emphysema ranges from 0.43% to 2.34% [5-7]. It is reported that as much as 77% of laparoscopy patients have grossly undetectable subcutaneous emphysema but 20% have findings on postoperative chest

radiograph of pneumo-mediastinum [8-10].

McAlister et al. found that 56% of patients operated by laparoscopic cholecystectomy had undetectable subcutaneous emphysema using a computed tomography (CT) scan [22]. Subcutaneous emphysema is a common complication after laparoscopic surgery, and important because it may be unnoticed and overlooked. Although there are many observation studies on the effects of subcutaneous emphysema caused by laparoscopic surgery on subcutaneous fat tissues, there has been no study on the changes in acute subcutaneous fat tissues through animal experiments. The authors aimed to investigate the changes in subcutaneous fat tissues in an acute phase through animal experiments. In the experiment, we set the carbon dioxide capacity to 1cc in order to set to more than 50% of the body surface area and conducted the experiment.

This study suggests the possibility of adipose tissue alteration due to the subcutaneous emphysema during the laparoscopic surgical procedure. Here, our in vivo results demonstrate that acute CO2 injection had neither effect on histological changes nor effect on transcriptional regulations of genes associated with lipogenesis, vascularization, and fibrosis in subcutaneous adipose tissue.

CO2 has been identified as a regulator in adipose tissue metabolism. Kikuchi et al. observed that exposure of high CO2 regulates adipogenesis in vitro [23]. Clinical and animal studies have shown that chronic CO2 administration decrease adipose tissue mass through the reduction of adipocyte size [11, 24]. In contrast to previous studies, acute exposure of CO2 to subcutaneous fat did not show the significant changes in the adipocyte size and lipogenic mRNA levels compared with sham group (Fig. 2A & 3A). Furthermore, the difference was also not detected between CO2-treated groin and Air-injected groin (Fig. 4A & 5A). These results indicate that acute CO2 administration had no effect on the regulation of adipocyte size in the aspect of mechanical and chemical factors. This discrepancy between previous studies and current results is mainly due the duration of CO2 exposure (chronic vs. acute) and indicates that acute exposure of CO2 such as iatrogenic emphysema after laparoscopic surgery would not lead to modulate adipocyte size in fat.

Vascularization is known as an important regulator of adipose tissue remodeling [16-17]. Clinical studies have shown that chronic CO2 injection such as carboxytherapy increased vascular flow [24] Inconsistent to previous reports, one time CO2 injection did not lead to significant changes of adipose tissue vasculature (Fig. 2B and 4B) while mRNA levels of Vegf1 and Fgf1 were slightly reduced in CO2-treated adipose tissue (Fig. 3B & 5B). These results demonstrate that effects of CO2 administration acutely on vascularization has less pronounced than those of CO2 administration in chronic manner. However, we cannot rule out the possibility that 6 hours post injection might not be enough to distinguish the CO2-induced vasculature alteration since vascular changes such as angiogenesis take times.

Fibrosis has been observed when tissues are exposed to mechanical and chemical injury. Although subcutaneous emphysema after laparoscopic surgery is believed to have less complications, there has no direct evidence to provide the impacts of CO2 on adipose tissue fibrosis. As shown in Fig. 2C~5C, histological analysis and expression levels of Col1a, Col6a and Acta2 demonstrates that temporal exposure of CO2 does not cause the adipose tissue fibrosis. These results indicate that acute CO2 has no effects on adipose tissue fibrosis and support the property of CO2 gas to have less complication.

From the viewpoint of clinical applications, proper interpretation is necessary since this study was conducted using an animal model rather than humans. Especially, the dosage of CO2 (1cc for mice) and inflation time (less than 1 min) in this rodent model should be considerable. It would be more meaningful to investigate effects of CO2 at various doses and exposure time points on adipose tissue metabolism. Further experiments are also needed to investigate whether signal transductions associated with lipogenesis and vascularization may mediate CO2 administration-induced effects because alteration of protein amount and phosphorylation but not mRNA level could regulate these metabolism. Notwithstanding, this study was first reported that emphysema, which occurs iatrogenically in the subcutaneous fat after laparoscopic surgery, does not affect the subcutaneous fat lipogenesis, vascularization and fibrosis by histologically or molecular assessment and support the safety of CO2 usage in the laparoscopic surgery. So, acute subcutaneous emphysema during laparoscopic surgery does not require aggressive procedure, except in severe subcutaneous emphysema that cause chest and neck compression.

ACKNOWLEDGEMENTS

This work was supported by the Soonchunhyang University

Research Fund and the grant of Basic Science Research Program through the National Research Foundation (NRF) of Korea (2017R1C1B1004843)

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted. (Institutional Animal Care and Use Committee of Soonchunhyang University, SCH16-0031)

Author Contribution

CY Choi : Project development, Obtaining funding

KW Cho : Project development, Data management, Data analysis

JH Sang : Manuscript writing / editing

SK Yoon : Manuscript writing

SM Nam : Data analysis

YJ Lee : Data collection

J Oh : Data collection

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