

Original Article
Rehabilitation



Hyperbaric gaseous cryotherapy for postoperative rehabilitation enhances functional recovery of canine stifle joint: a report on a short-term study

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Conflict of Interest

The authors declare no conflicts of interest.

ABSTRACT

Background: Hyperbaric gaseous cryotherapy (HGC) is a type of cryotherapy used in human medicine for rehabilitation after orthopedic surgeries. Because HGC is known to reduce acute or chronic pain, research is needed to prove its effectiveness in veterinary medicine.

Objectives: To compare the effects of HGC between the HGC treatment group and the nontreatment (NT) group on postoperative swelling, range of motion, lameness score, postoperative pain, and kinetic measurements after stifle joint surgery in dogs.

Methods: Dogs were randomized in an HGC group or NT groups. In the HGC group, HGC was applied once a day for a total of 2 days after surgery. All parameters were measured postoperatively and at 1, 2, 10, and 28 days after surgery.

Results: Twenty dogs were enrolled: 10 in the HGC group and 10 in the NT group. Soft tissue swelling was not significantly different between groups at any time point. In the HGC group, pain scores decreased significantly 24 h after surgery and 48 h after surgery. Dogs in the HGC group showed a significantly decreased lameness and improvement for all kinetic measurements beginning 48 h after surgery. In addition, the HGC group indicated a significant increase in range of motion as compared with the NT group at 28 days after surgery.

Conclusions: HGC plays a powerful role in decreasing initial postoperative pain. Furthermore, the improvement in pain affects the use of the operated limb, and the continued use of the limb eventually assists in the quick recovery of normal function.

Keywords: Dogs; rehabilitation; cryotherapy; gait analysis

INTRODUCTION

Canine rehabilitation is one of the rapidly developing fields of veterinary medicine and is an important process to support recovery after canine orthopedic surgery [1]. Several prior studies have demonstrated the methods and effectiveness of rehabilitation. Thus, the questions, “How does it apply?” and “Which rehabilitation method will work better?” are now of interest in both human and veterinary medicine [2]. Cryotherapy is a rehabilitation method used to reduce edema and blood flow, decrease inflammation, and provide pain relief from decreased nerve conduction in the early postinjury or postoperative period [2-4].

Author Contributions

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Data curation: Han JY; Formal analysis: Han
JY; Funding acquisition: Kang BJ; Investigation:
Han JY; Methodology: Han JY; Supervision:
Kim WH, Kang BJ; Writing - original draft:
Han JY; Writing - review & editing: Kim WH,
Kang BJ.

Various available cooling mechanisms or devices include wet ice, submersion in a cold-water bath, cold packs, cold compression therapy, and hyperbaric gaseous cryotherapy (HGC) [5]. In veterinary clinics, cold packs or cold compression therapy are more frequently used, but these have the disadvantages of needing to be applied to one site for at least 15 min to achieve a proper effect [5,6].

HGC uses nitrogen-cold air or CO₂ microcrystals under high pressure (50 bar) at a very low temperature (-78°C) that are sprayed onto the skin, causing the skin temperature to fall very quickly [7-10]. This technique has the advantages of a traditional cooling mechanism and induces a physiological response called “thermal shock,” which results in additional vasoconstriction followed by vasodilation and stimulation of the peripheral cold receptors to increase blood flow at the muscle level, accelerating recovery [11,12]. In human medicine, Chatap et al. [13] showed that the use of HGC decreased pain scores in elderly patients with acute or chronic pain, and Guillot et al found that HGC might reduce cytokine levels in the synovial fluid in several inflammatory joint diseases [12]. According to Demoulin et al. [11], the application of HGC for 90 sec, when repeated 3 times a day, is effective in pain management and increases the range of motion (ROM) in postoperative care after orthopedic surgery.

The convenience and advantages of HGC have rendered it a method of rehabilitation in human medicine, but to the best of our knowledge, there are currently no veterinary medicine studies demonstrating the effectiveness of rehabilitation treatment using HGC. Therefore, the objective of this study was to compare the effects of HGC on swelling, ROM, lameness score, postoperative pain, and limb function between an HGC treatment group and nontreatment (NT) group after stifle joint surgery in dogs. We hypothesized that HGC applied after stifle joint surgery reduces inflammation and pain, which assists in the initial recovery and improves limb function. This study also presents HGC protocols using the postoperative treatment for each patient's weight.

MATERIALS AND METHODS

Case selection criteria

Dogs with unilateral stifle joint disease that underwent stifle joint surgery at the Veterinary Medicine Teaching Hospital at Seoul National University between February 2020 and February 2021 were included consecutively after dog's owners signed the informed consent. The protocol was declared and all procedures were approved by the Seoul National University Institutional Animal Care and Use Committees (SNU-200423-1). Dogs were excluded if they had bilateral stifle joint disease, severe orthopedic disease in another joint, or had previously undergone surgery of the operated joint. Dogs were also excluded if they had unilateral stifle joint disease attributable to an immunologic, neurologic, infectious, or neoplastic condition.

Anesthesia, analgesia, operation, and perioperative care of animals

All dogs received a standardized anesthesia/pain management protocol. Dogs were premedicated with intravenous injection of either acepromazine (5 µg/kg, Sedaject; Samu Median Co., Korea) and medetomidine (1–2 µg/kg, Domitor; Zoetis, Korea) according to the dog's American Society of Anesthesiologists grade. Induction of anesthesia was achieved with alfaxalone (2 mg/kg intravenously; Alfaxan; Jurox), and anesthesia was maintained with isoflurane (Ifran; Hana Pharm. Co., Korea) in oxygen using a vaporizer set at 1.2%–1.8%.

The same surgeon and assistant performed all operations. A tibial plateau leveling osteotomy (TPLO) or medial patellar luxation repair (MPLR) or tibial tuberosity fixation was performed on the affected stifle, as previously reported [14-16]. The method of surgery was determined by the surgeon's preference and experience. After stifle surgery, all dogs had a Robert Jones bandage (RJB) applied to the limb. The bandage was replaced once at 24 h after surgery and removed after 48 h. All dogs received firocoxib (5 mg/kg, orally every 24 h; Previcox, Boehringer-Ingelheim) and misoprostol (0.5 mg/kg, orally every 24 h; Alsoben, Unimed) for 28 days beginning the morning after the treatment.

HGC

Using a computer program, the HGC group and the NT group were randomly selected. In the HGC group, a CRYO300pet device (ISMED, Korea) was used. This device is made up of the main body, immersed tube, and a handpiece (**Fig. 1**). The main body is designed to contain a cylinder containing medical-grade liquid CO₂, and the handpiece part, shaped like a spray gun, consists of 5 different parts. The temperature of the skin can be measured through the part where the thermometer is mounted, and a laser-guided infrared measurement system can be used to pinpoint the location of the injection site. Gaseous CO₂ microcrystal is sprayed through a nozzle, with the light part displayed in blue during normal injection and turns red to avoid a risk of frostbite when the skin temperature drops to about 4°C. Lastly, there is a part in which a blue light-emitting diode is produced that can sterilize the epidermis.

In the HGC group, HGC was first applied 24 h after surgery when the bandage was replaced. This procedure was repeated 48 h after surgery when the bandage was removed. Based on the manufacturer's recommendation, the nozzle tip was placed 20 cm away from the skin, and the muscles of the operated limbs and stifle joint were sprayed overall in lateral recumbency. HGC was applied for 1 min and 30 sec initially. After that, the temperature of the skin was monitored, and if it did not reach 17°C–18°C, additional 30 seconds of HGC were applied. Finally, when the skin temperature reached 17°C–18°C, HGC was turned off, and the total HGC application time was recorded [17]. The NT group did not undergo any other forms of cryotherapy after the RJB was removed.

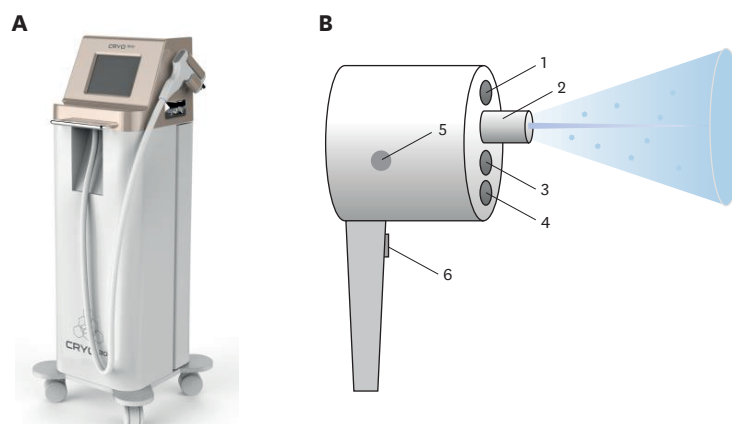


Fig. 1. (A) Cryo300pet device. (B) Schematic illustrations of Cryo300pet's handpiece part. (1) A blue light-emitting diode is produced that can sterilize the epidermis. (2) Nozzle, gaseous CO₂ microcrystal is sprayed. (3) Thermometer. (4) Laser-guided infrared measurement system. (5) Light part is displayed in blue during normal injection and turns red to avoid a risk of frostbite. (6) Gaseous injection switch

Measured parameters

The following parameters were measured before the operation and at 1, 2, 10, and 28 days after surgery. All measurements were measured by one person, and at 1 and 2 days after surgery, all parameters were measured after cryotherapy in the HGC group and after the bandages were removed in the NT group.

Assessment of edema, bruising, and soft tissue swelling

We measured the muscle mass circumference using conventional tapeline at 3 different anatomic landmarks as previously reported [18]. The femur was divided into quarter sections using a proximal landmark into the greater trochanter and distal landmark into the distal patella, and the circumference of the proximal quarter of the femur was used to measure femoral swelling. Stifle swelling was measured between the distal patella and the tibial crest, setting it to the anatomic landmark, and hock swelling was measured by encircling around the hock. All measurements were performed at a normal standing angle in lateral recumbency.

Assessment of postoperative pain

The visual analog scale (VAS) is a subjective measure of patient pain by the blinded examiner measured on a 10-cm line, with 0 cm indicating no subjective pain and 10 cm indicating the worst pain imaginable [19, 20]. The distance from the left end of the line (no pain) to mark in centimeters was the VAS pain score (range 0–10).

The criteria used by the examiner to evaluate the modified Glasgow Pain Scale (GPS) were vocalization, attention to wound area, mobility, response to touch, demeanor, and posture [21]. Each evaluation criterion was scored with a combined total score of 0 to 24, with 0 being pain free and 24 being severe pain. All dogs were measured using the VAS and modified GPS after all treatment was completed at 1 and 2 days after surgery and at the time of the patient's visit to the hospital postoperatively and at 10 and 28 days after surgery.

Assessment of limb function

To evaluate the lameness score, the dog walked 10 m at a nonslip place. The following subjective scoring system was performed for gait evaluation: 0 = normal, 1 = unclear/slight lameness, 2 = obvious weight-bearing lameness, 3 = obvious weight-bearing lameness with occasional non-weight-bearing lameness, 4 = non-weight-bearing lameness.

ROM was measured passive stifle joint flexion and extension angle in lateral recumbency using a conventional goniometer as previously described [22], with the center of the goniometer in the lateral epicondyle of the femur and the anatomical landmark set to the greater trochanter on the proximal side and lateral malleolus on the distal side. After measuring the stifle extension and flexion angle, we calculated the difference as the ROM [23].

We conducted objective gait analysis in a private room using a pressure-sensitive walkway (Strideway, Tekscan, USA). Recorded data were processed by specific software (Strideway Research, Tekscan), and we took the average of 5 measurements, including the dogs' heads looking forward in the walking direction and moving down the center of the mat [24]. All dogs have walked in hand at a uniform velocity ranged from 0.9 to 1.2 m/s. The following parameters were obtained from the system's gait analysis software: stance time, swing time, stride time, stride velocity, peak vertical force (PVF), vertical impulse (VI). The percentage of body weight distribution (%BWD) and symmetry index (SI) were calculated manually

using the following equation from both PVF and VI [25,26]. The SI was performed by normal hindlimb measurement (X1) and operated hindlimb measurement (X2).

$$SI = (X1 - X2)/(X1 + X2) \times 200$$

$$\%BWD = [(PVF \text{ from an operated limb}) / (\text{Total PVF from all limbs})] \times 100$$

Statistical analysis

We performed all statistical analyses using statistical software (GraphPad Prism 8.4.2, GraphPad Software Inc., USA). The variables muscle mass circumference, VAS score, modified GPS score, lameness score, ROM, PVF, VI, %BWD, and SI were analyzed using a 1-way analysis of variance with group as the independent factor and time point factor with repeated measurements. A resulting *p* value of ≤ 0.05 was considered statistically significant.

RESULTS

Clinical data

Twenty dogs were included, and **Table 1** lists the breeds, age, body weight, and operation method of patients in the experiment. The 10 dogs in the HGC group consisted of 6 females (4 neutered, 2 intact) and 4 castrated males. In the NT group, 5 dogs were female (4 neutered, 1 intact) and 5 were castrated male. The mean age at the time of initial examination was 4.1 ± 3.1 yr for dogs in the HGC group and 6.3 ± 2.9 yr for dogs in the NT group. Moreover, there were no significant differences between body weights (HGC group: 12.5 ± 12.6 kg; NT group: 14.79 ± 15.86 kg). The application time of HGC for dogs weighing 5 kg or less was 1 min 30 sec, 2 min 30 sec for those weighing between 5 kg and 10 kg, 3 min for dogs weighing between 10 kg and 20 kg, and 4 min for those greater than 20 kg (**Supplementary Table 1**).

All dogs that received TPLO surgery revealed positive cranial drawer and positive tibia compression tests. In the surgery of TPLO, the stifle joint was explored via arthrotomy and partial meniscectomy was performed to remove any damaged meniscus. Of the 9 dogs diagnosed with MPL, 7 were grade 3 and 2 were grade 2. In the case of grade 2 MPL, the lateral fascial imbrication was performed, and the block recession trochleoplasty was performed if the depth of the trochlear groove was not deep. In the case of grade 3 MPL, tibial tuberosity transposition was performed when the tuberosity has deviated, and medial retinacular release was performed if necessary. No major intraoperative or postoperative complications were observed.

Table 1. Breed, age, weight, and operation of all dogs taking part in this study

| | HGC group | | | | NT group | | | |
|----|-------------------|----------|-------------|----------------------------|--------------------|----------|-------------|-------------|
| | Breed | Age (yr) | Weight (kg) | Operation | Breed | Age (yr) | Weight (kg) | Operation |
| 1 | Yorkshire terrier | 7 | 3.75 | MPLR | Havanese | 12 | 7.4 | MPLR |
| 2 | Pomeranian | 1 | 3.6 | MPLR | Yorkshire terrier | 8 | 3.7 | MPLR |
| 3 | Pomeranian | 1 | 3.8 | MPLR | Chihuahua | 5 | 2.5 | MPLR |
| 4 | Pomeranian | 2 | 5.1 | MPLR | Poodle | 3 | 4.3 | MPLR |
| 5 | Cocker spaniel | 6 | 11.7 | TPLO | Poodle | 7 | 5.2 | TPLO |
| 6 | Golden retriever | 3 | 29.3 | TPLO | Maltese | 6 | 5.2 | TPLO |
| 7 | Shiba Inu | 5 | 7.3 | TPLO | Labrador retriever | 4 | 42.4 | TPLO |
| 8 | Golden retriever | 7 | 41.2 | TPLO | Maltese | 3 | 5.2 | TPLO |
| 9 | Poodle | 5 | 7.8 | TPLO | Labrador retrieve | 10 | 41 | TPLO |
| 10 | Shiba Inu | 1 | 11.5 | Tibial Tuberosity fixation | Jindo | 5 | 27.5 | MPLR + TPLO |

HGC, hyperbaric gaseous cryotherapy; NT, nontreatment; MPLR, medial patellar luxation repair; TPLO, tibial plateau leveling osteotomy.

Evaluated parameters

Assessment of edema, bruising, and soft tissue swelling

All dogs in the HGC group and NT group demonstrated increased edema, bruising, and soft tissue swelling until 48 h after surgery as compared with preoperatively. However, with regard to the parameter percentage change in swelling at each anatomic location, there were no statistically significant differences between HGC group and the NT group at any given time point (**Table 2**).

Assessment of postoperative pain

There were no significant differences in pain scores between the HGC group or the NT group before surgery, even if the patient's disease was different. Twenty-four hours after surgery, we recorded a statistically significant lower VAS ($p = 0.0356$) and lower modified GPS score ($p = 0.0320$) in the HGC group as compared with the NT group. At 48 h after surgery, both the VAS score ($p = 0.0474$) and modified GPS ($p = 0.0434$) in the HGC group significantly decreased as compared with the NT group. However, there were no statistically significant differences between the HGC group and the NT group in the VAS or modified GPS scores from 10 days after surgery onward (**Table 3; Supplementary Table 2**).

Assessment of limb function

There were no statistically significant differences in lameness score or ROM between the HGC group and the NT group before and 24 h after surgery. At 48 h after surgery, dogs in the HGC group showed a significantly lower lameness score ($p = 0.0184$). At 10 days after surgery, the HGC group had a statistically significant lower lameness score ($p = 0.0385$) as compared with the NT group. Twenty-eight days after surgery, the HGC group showed a significant

Table 2. Mean (\pm SD) of the percentage change in swelling at each anatomic location

| Group | Femur % change (mean \pm SD) | Stifle % change (mean \pm SD) | Hock % change (mean \pm SD) |
|-----------------------|--------------------------------|---------------------------------|-------------------------------|
| 24 h after surgery | | | |
| HGC group | 2.39 \pm 3.01 | 5.63 \pm 4.04 | 2.90 \pm 4.57 |
| NT group | 1.88 \pm 2.53 | 4.31 \pm 2.85 | 3.68 \pm 4.13 |
| 48 h after surgery | | | |
| HGC group | 3.07 \pm 3.88 | 5.88 \pm 4.98 | 2.91 \pm 6.56 |
| NT group | 1.12 \pm 2.17 | 3.05 \pm 2.14 | 2.42 \pm 4.08 |
| 10 days after surgery | | | |
| HGC group | 0.55 \pm 3.20 | 1.68 \pm 2.79 | 1.03 \pm 4.45 |
| NT group | 0.71 \pm 3.93 | 2.27 \pm 2.48 | 0.68 \pm 3.81 |
| 28 days after surgery | | | |
| HGC group | 2.90 \pm 4.57 | 1.89 \pm 2.90 | 1.96 \pm 3.70 |
| NT group | 0.99 \pm 3.48 | 0.78 \pm 3.11 | 0.77 \pm 4.07 |

HGC, hyperbaric gaseous cryotherapy; NT, nontreatment.

Table 3. p values of measured pain scores compared between study groups at all evaluated time points

| Comparison of groups | Visual Analogue Scale | Modified Glasgow Pain Scale |
|------------------------|-----------------------|-----------------------------|
| Before surgery | | |
| HGC group vs. NT group | 0.1458 | 0.0702 |
| 24 hours after surgery | | |
| HGC group vs. NT group | 0.0356* | 0.0320* |
| 48 hours after surgery | | |
| HGC group vs. NT group | 0.0474* | 0.0434* |
| 10 days after surgery | | |
| HGC group vs. NT group | 0.2876 | 0.4664 |
| 28 days after surgery | | |
| HGC group vs. NT group | 0.0611 | 0.0683 |

HGC, hyperbaric gaseous cryotherapy; NT, nontreatment.

*Statistically significant values.

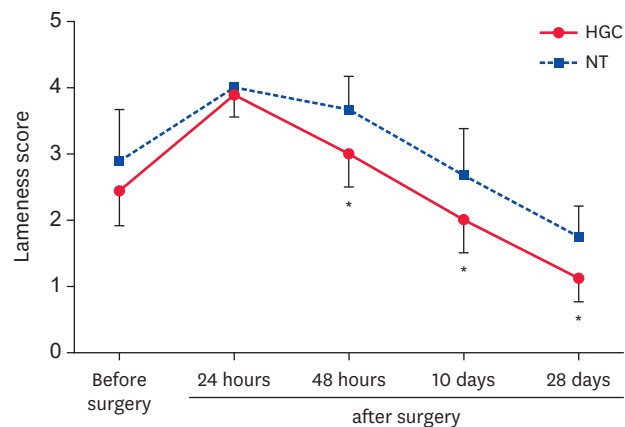


Fig. 2. Evaluation of lameness score (mean \pm SD) compared with the HGC group with the NT group at all measured time points (*indicates a significant difference, $p \leq 0.05$, between the HGC group and the NT group at a given time point).

HGC, hyperbaric gaseous cryotherapy; NT, nontreatment.

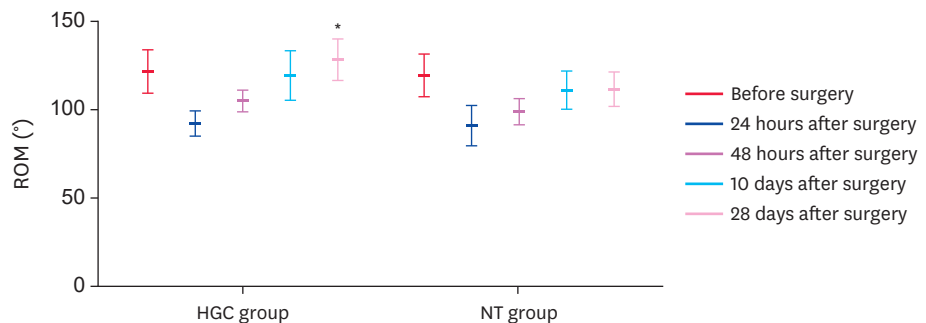


Fig. 3. ROM (mean \pm SD) of the stifle joint compared between the HGC and the NT groups at all measured time points (*indicates statistically significant difference between the HGC group and NT group at a given time point, $p \leq 0.05$). ROM, range of motion; HGC, hyperbaric gaseous cryotherapy; NT, nontreatment.

improvement in lameness score ($p = 0.0203$) and higher ROM ($p = 0.0107$) as compared with the NT group (Figs. 2 and 3; Supplementary Fig. 1).

None of the kinetic parameters showed a significant difference before surgery and 24 h after surgery. At 48 h after surgery, dogs in the HGC group showed a significantly lower SI of PVF ($p = 0.0227$), lower SI of VI ($p = 0.0041$), higher percentage weight distribution ($p = 0.0358$) compared with the NT group 10 days after surgery reported a statistically significant lower SI of PVF ($p = 0.0364$), lower SI of VI ($p = 0.0175$), and higher percentage weight distribution ($p = 0.0210$) as compared with the NT group. At 28 days after surgery, there were statistically significant differences for SI of PVF ($p < 0.001$), SI of VI ($p = 0.0260$), and percentage weight distribution ($p = 0.0433$) between the HGC group and the NT group (Figs. 4 and 5; Supplementary Figs. 2 and 3).

DISCUSSION

Cryotherapy (applied locally or to the whole body) has been widely and empirically used as an adjunct therapy in inflammatory diseases [12]. In human medicine, locally applied HGC was shown to decrease VAS pain scores and to accelerate recovery after gonarthrosis,

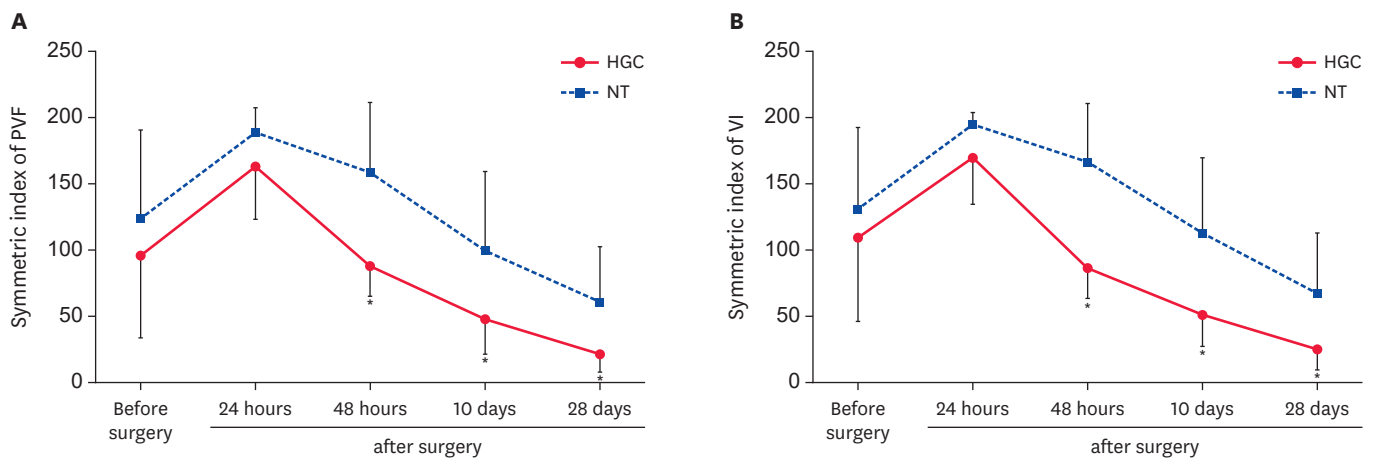


Fig. 4. (A) Comparison of the development of the symmetric index of PVF of the HGC group with the NT group. (B) Comparison of the development of the SI of VI of the HGC group with the NT group. The SI was calculated for the following analyses and accordingly converted (*indicates a statistically significant difference between the HGC group and the NT group at a given time point, $p \leq 0.05$).

PVF, peak vertical force; HGC, hyperbaric gaseous cryotherapy; NT, nontreatment; SI, symmetric index; VI, vertical impulse.

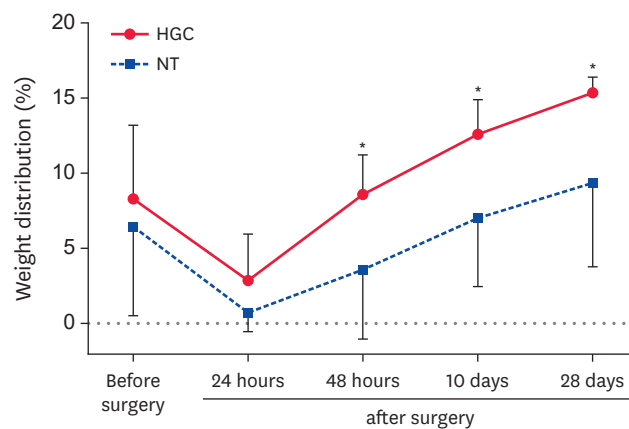


Fig. 5. Evaluation of the percentage of weight distribution (mean \pm SD) between the HGC group and the NT group at all measured time points. The weight distribution calculated for the following analyses was accordingly converted (*indicates a significant difference, $p \leq 0.05$, between the HGC and NT groups at a given time point). HGC, hyperbaric gaseous cryotherapy; NT, nontreatment.

coxarthrosis, and total knee arthroplasty [11,27,28]. The local application of HGC induces a thermal shock response based on the hunting reaction or cold-induced vasodilation [12,29]. Cold-induced vasodilation is a process of alternating vasoconstriction and vasodilation in the injection site exposed to cold. Although the mechanism underlying cold-induced vasodilation remains unclear, this technique has generally been presumed to provide a protective function by maintaining local tissue integrity and activating the autonomic nervous system via the release of cytokines and cortisol secretion [7,12]. Our study investigated the effectiveness of HGC and presented a protocol in which HGC is applied with these properties, which has never been demonstrated in veterinary medicine in actual patients.

Despite various research over the past 30 years, the HGC protocols (temperature, cycle, duration) have not been standardized. In human medicine, Demoulin et al. [11] applied HGC to patients for 90 seconds 3 times a day, and Guillot et al performed HGC twice a day for 2 min [12]. These protocols were based on experiments that measured the baseline human skin temperature after a cold pack, which has already been proven to be effective over

application time [7,30-32]. Akgun et al. [17] found that 17°C–18°C was the lowest baseline skin temperature on the femoral region after the application of a cold gel pack in an adult dog, and complications such as peroneal nerve damage occurred when the baseline skin temperature dropped below 17°C–18°C. Hence, we applied HGC until the baseline skin temperature reached 17°C–18°C around the surgical site. Our application of the HGC protocol showed that no matter how large a dog is, the HGC has a maximum cooling effect in less than 5 min.

In this study, postoperative swelling, as shown by the percentage change in circumference, did not significantly decrease between the HGC group and the NT group at any time point. It is well known that stifle surgery results in soft tissue catabolism in the first 48 h after operation, and soft tissue swelling is one of the most common postoperative complications [33,34]. Thus, we applied a traditional RJB for 48 h postoperatively to decrease swelling, provide immobilization, and protect the surgical incision. The compression function of the RJB may have affected the soft tissue swelling. However, Rexing et al. [18] revealed that applying RJB and cold compression therapy at the same time resulting in a significantly greater reduction in edema as compared with RJB alone. This can be seen as a function of the cryotherapy; cold compression therapy plays a major role in decreasing swelling, whereas RJB plays a minimal role. Unlike cold compression therapy, HGC takes the form of CO₂ microcrystal dispensing, so it has no function of pressure the soft tissue. Therefore, our results suggest that HGC does not play a major role in decreasing soft tissue swelling.

We also evaluated the assessment of pain through subjective pain scores. The VAS is widely used in animal studies because it evaluates pain in a simple way, and the modified GPS is a behavior-based composite scale that assesses pain via interactions with the animal's action and clinical observations [20,21]. Our results showed that HGC significantly decreases early postoperative pain. Guillot et al found that HGC might reduce cytokine levels in the synovial fluid through the nuclear factor- κ B and prostaglandin E2 pathway, and Algafly and George [35] reported that cryotherapy decreases the nociceptive conduction velocity [12]. Based on these studies, we thought that HGC exerted early pain management by reducing inflammation-acting anti-cyclo-oxygenase-2-like effects and increasing the threshold of pain. On the contrary, there was no significant difference in pain scores between the HGC group and the NT group 10 days after stifle surgery. The possible reason for our result is the long-term use of nonsteroidal anti-inflammatory drugs in all dogs, and the effect of nonsteroidal anti-inflammatory drugs with anti-cyclo-oxygenase-2 might have resulted in the pain reduction. Furthermore, because we applied HGC only until 48 h after surgery, the failure of the application of HGC before the 10- and 28-day measurements might have affected the evaluation of pain.

Recovery of limb function is the most important goal of early rehabilitation after the operation. After stifle surgery, changes occur in joint biomechanics and proprioception; thus, functional recovery is also directly related to good surgical outcome [36]. Our study found a significant decrease in lameness score from 48 h after surgery in the HGC group as compared with the NT group. This suggests that HGC significantly reduced pain from 24 h after surgery, which resulted in increased limb use, preventing muscular dystrophy, and enhancing weight bearing from 48 h onward. Interestingly, the HGC group's ROM increased significantly only 28 days after surgery. Monk et al. [37] found that early intensive postoperative physiotherapy did not show improvement in ROM within the first 2 weeks. Based on this study, we thought that it takes a certain amount of time to restore normal limb function. Moreover, because the ROM of the postoperative HGC group was 121.66 ± 12.24

degrees and the ROM of 28 days after surgery was 128.33 ± 11.72 , it can be seen that the HGC increases the ROM and allows an early return to normal ROM (**Supplementary Table 3**).

Next, a pressure-sensitive walkway system was used to objectively assess the function of the limbs. Thousands of pressure sensors analyzed the dog's walking and measured the PVF and VI, and based on these values, the SI and %BWD were calculated. In this study, the HGC group demonstrated significant differences in SI as compared with the NT group after 48 h of surgery. This indicates an enhancement in the initial operated limb use, with increased force acting perpendicular to the feet and increased contact area, resulting in a decrease in normal opposite-limb asymmetry. We also observed significant improvement in the %BWD after 48 h of surgery in the HGC group. This suggests that HGC for postoperative rehabilitation initially reduces pain and inflammation, helping to restore limb function and accelerating the return to normal weight bearing. In general, the normal %BWD of the hindlimb is 40% (20% right; 20% left), so a long-term experiment is needed to measure the period to return to normal.

Limitations of this study may be attributed to its small case number and discrepancy in diagnosis and surgical method. In addition, clinical factors such as chronicity of cranial cruciate ligament damage, presence or absence and type of meniscal tears, severity of articular and periarticular fibrosis, and severity of stifle joint osteoarthritis were not considered in the current study. Further studies including measurements of chronicity and osteoarthritis, standardized diagnosis and surgical methods, and long-term outcome measures would provide more comprehensive information on the benefits of HGC for patients undergoing stifle joint surgery. Furthermore, a comparative study of gaseous cryotherapy with more traditional forms of cryotherapy following stifle joint surgery is needed.

This paper is the first to investigate the potential use of HGC as early rehabilitation therapy after stifle surgery in dogs. We also present a powerful protocol for applying HGC in dogs of varying sizes and weights. Based on the results, we conclude that applying HGC once a day, for a total of 2 days, helps to restore limb function. The cooling effect is maximized by hyperbaric CO₂ microcrystal, which rapidly drops the baseline skin temperature in the surgical site, and the HGC can derive significant benefit in reducing pain. The use of HGC can enhance limb function, reduce initial pain after surgery, and allow for rapid recovery from hindlimb symmetry. These findings can potentially be significant when selecting cryotherapy for early rehabilitation after stifle surgery.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

The application time of hyperbaric gaseous cryotherapy

[Click here to view](#)

Supplementary Table 2

Pain scores (mean \pm SD) of the TPLO group and MPLR group at 24 h after surgery as well as 48 h after surgery of the HGC group and the NT group in each pain parameter

[Click here to view](#)

Supplementary Table 3

Mean (\pm SD) of the flexion angle and extension angle at before surgery and 28 days after surgery in the HGC group and the NT group

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Supplementary Fig. 1

Evaluation of lameness score (mean \pm SD) compared the HGC group with the NT group at all measured time points. The HGC group and the NT group were subdivided into the TPLO group and the MPLR group to show tendency in different colors.

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Supplementary Fig. 2

Comparison of the development of the symmetric index of peak vertical force of the HGC group with the NT group. The HGC group and the NT group were subdivided into the TPLO group and the MPLR group to show tendency in different colors.

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Supplementary Fig. 3

Evaluation of percentage of weight distribution (mean \pm SD) compared the HGC group with the NT group at all measured time points. The HGC group and the NT group were subdivided into the TPLO group and the MPLR group to show tendency in different colors.

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