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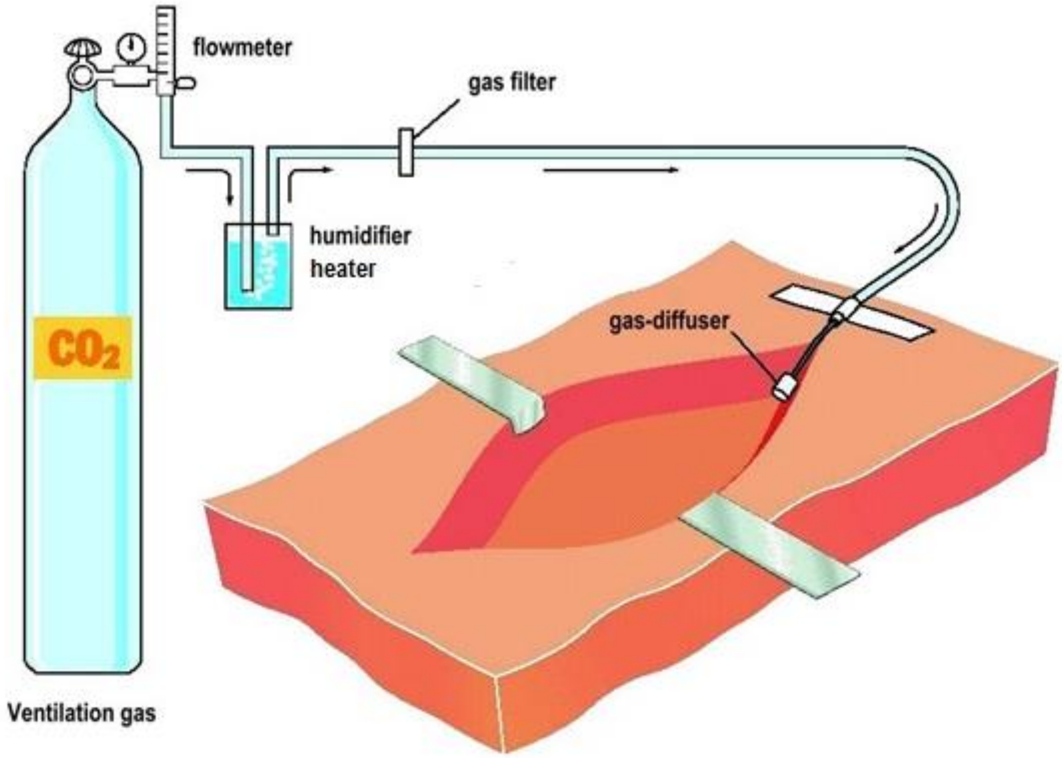
EVALUATION OF CARBON DIOXIDE INSUFFLATION INTO THE OPEN SURGICAL WOUND; INFLUENCE ON WOUND TEMPERATURE, CORE TEMPERATURE, AND POSTOPERATIVE OUTCOME

Joana Frey, MD

STOCKHOLM 2016



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EVALUATION OF CARBON DIOXIDE INSUFFLATION INTO THE OPEN SURGICAL WOUND; INFLUENCE ON WOUND TEMPERATURE, CORE TEMPERATURE, AND POSTOPERATIVE OUTCOME

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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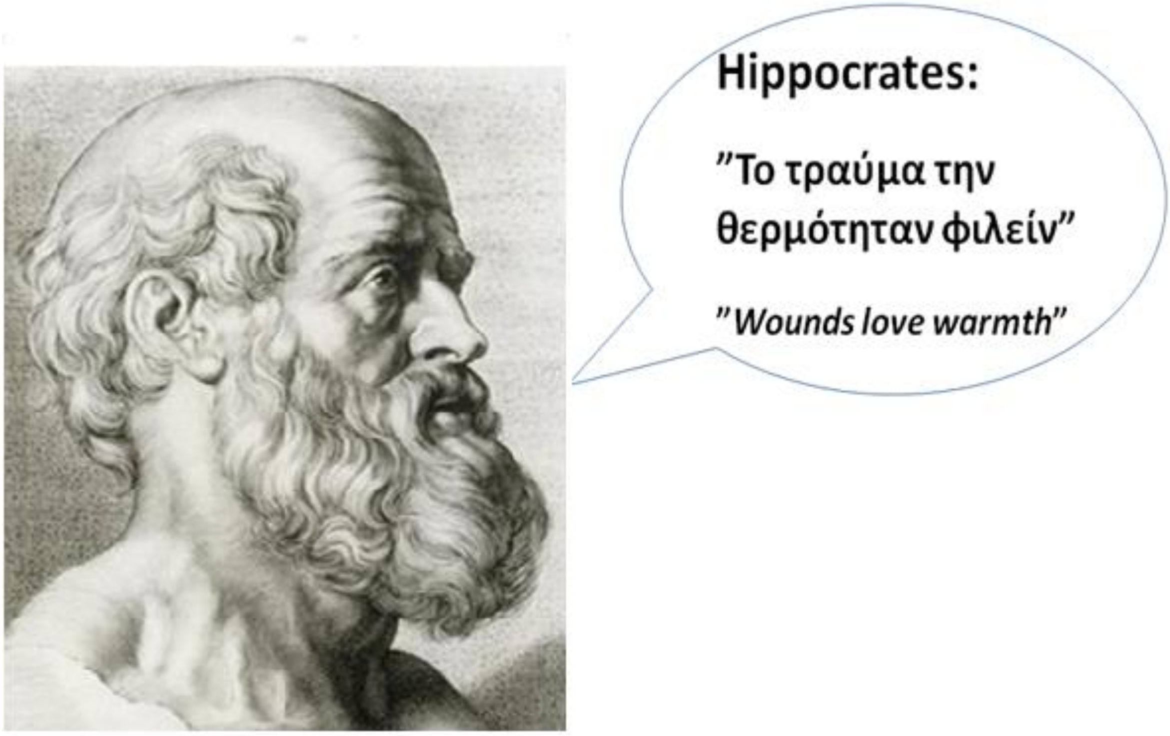


The concept of keeping the wounds moist, warm, clean and protected is not new. There is documentation indicating that the ancient Mesopotamians dressed their wounds with fine linen soaked in oil. The Greeks applied animal fat and wrapped the wounds with greasy sheepskin and the Roman applied ashes, oil and herbs. So the old expression from Hippocrates, one of the most outstanding figures in the history of medicine, still holds true today, 2500 years later.

The Healing Hand: Man and Wound in the Ancient World by Guido Majno - 1991

To my mother who still believes that I am going to get the Nobel Prize, one day!

ABSTRACT

Introduction: When the internal organ tissues are abruptly exposed to the relative cold and dry ambient air during open surgery, body heat is lost through radiation, evaporation, and convection. Also general and neuraxial anesthesia contributes to a decrease in core temperature, mainly due to a shift of the threshold for thermoregulatory defense mechanism toward lower temperatures. It is well known that perioperative hypothermia is disadvantageous for the patient, since it increases the risk of surgical wound infections, blood loss, morbid cardiac events and postoperative shivering. Guidelines to keep the patient warm during surgery are today common practice, but despite routine preventive measures, mild intraoperative hypothermia is still common and contributes to postoperative morbidity and mortality. The aim of this thesis was to investigate if local insufflation of CO_2 could increase both the open surgical wound temperature and core temperature, and affect postoperative outcome.

Methods: I. In 10 patients undergoing cardiac surgery, the sternotomy wound was insufflated with dry, room-tempered CO_2 via a gas diffuser for two minutes. A heat-sensitive camera measured the wound temperature before, during and after insufflation. **II**. 80 patients undergoing open colon surgery were randomized to either standard warming measures (n=39) or additional local wound insufflation (n=41) of warmed (30°C) humidified (93% rH) CO_2 via a gas diffuser. A heat-sensitive camera measured the wound temperature and an ear thermometer measured the core temperature. **III.** 83 patients undergoing open colon surgery were randomized to either standard warming measures (n=39) or additional local wound insufflation (n=40) of warmed (37°C) humidified (100% rH) CO_2 via a gas diffuser. A heat-sensitive camera measured the wound temperature and an ear thermometer measured the core temperature. **IV.** This is a post hoc retrospective study of study II and III, where patients were randomized to warmed humidified CO_2 (n=80) or not (n=78).

Results: I. Exposure to dry CO_2 increased the median temperature of the whole wound by 0.5°C (p=0.01). The temperature of the area distant to the diffuser increased by 1.2°C ($p<0.01$) whereas in the area close to the diffuser it decreased by 1.8 °C ($p<0.01$). **II.** The median wound area and wound edge temperatures were 1.2ºC ($p<0.001$) and 1.0ºC (p=0.002) higher in the CO_2 group, respectively, than in the control group. The mean core temperature after intubation was the same (35.9ºC) in both groups, but at end of surgery the two groups differed with a mean of 36.2 ± 0.5 ºC in the CO_2 group and 35.8±0.5ºC in the control group (p=0.003). **III**. The mean wound area temperature during surgery was 31.3ºC in the CO_2 group compared with 29.6ºC in the control group ($p<0.001$). Also, the mean wound edge

temperature during surgery was 30.3ºC compared with 28.5ºC in the control group ($p<0.001$). Mean core temperature before start of surgery was similar with 36.7 ± 0.5ºC in the CO_2 group versus 36.6 ± 0.5ºC in the control group. At end of surgery the two groups differed significantly with 36.9 ± 0.5ºC in the CO_2 group versus 36.3 ± 0.5ºC in the control group ($p<0.001$). **IV.** A multivariate analysis adjusted for age ($p=0.001$) and cancer ($p=0.165$) showed that the larger the temperature difference between final core temperature and wound edge temperature, the lower the overall survival rate ($p=0.050$). A lower end-of-operation wound edge temperature was negatively associated with mortality (OR=0.80, 95%CI=0.68-0.95, P=0.011), whereas age (10-year increase, OR=1.78, 95% CI=1.37-2.33, P<0.001) and cancer (OR=8.1, 95% CI=1.95-33.7, P=0.004) were positively associated with mortality.

Conclusions: The major finding of this thesis is that insufflation of dry room-tempered CO_2 with a gas diffuser increases the average surface temperature in an open wound cavity. Insufflation of warmed humidified CO_2 in an open surgical wound cavity results in significant increases of the surgical wound temperature as well as the core temperature. Insufflation of warmed fully humidified CO_2 in an open surgical wound cavity increases surgical wound and core temperatures, and helps to maintain normothermia. A small end-of-operation temperature difference between final core and final wound edge temperature was positively associated with patient survival in open colon surgery, and a lower end-of-operation wound edge temperature was negatively associated with mortality.

SAMMANFATTNING

Introduktion: När de inre organens vävnader plötsligt exponeras för den relativt kalla och torra omgivande luften under öppen kirurgi så förloras kroppsvärme genom strålning, avdunstning och konvektion. Även generell och regional anestesi bidrar till en sänkning av kroppstemperaturen, mestadels beroende på en nedsatt tröskel för termoregulation och därmed ett minskat försvar mot kyla. Det är välkänt att perioperativ hypotermi är till nackdel för patienten eftersom det leder till ökad risk för postoperativa sårinfektioner, ökad blödning, kardiovaskulär morbiditet och mortalitet samt postoperativ shivering. Guidelines för att hålla patienten varm är idag rutin på operationsavdelningar men trots detta är mild intraoperativ hypotermi vanligt och bidrar till postoperativ morbiditet och mortalitet. Syftet med denna avhandling var att undersöka om lokal tillförsel av koldioxid kunde öka både sårtemperaturen och kroppstemperaturen under öppen kirurgi, samt om det påverkade det postoperativa förloppet.

Metoder: I. Hos 10 patienter som genomgick öppen hjärtkirurgi lät vi tillföra torr, rumstempererad CO_2 till det kirurgiska såret via en gas-diffusor i 2 minuter. En värmekänslig kamera mätte sårtemperaturen före, under och efter tillförseln. **II**. 80 patienter som genomgick öppen kolonkirurgi blev randomiserade till antingen rutinmässiga värmningsmetoder (n=39) eller dessutom lokal tillförsel (n=41) av värmd (30°C) och befuktad (93 % rH) CO_2 via en gas-diffusor. En värmekänslig kamera mätte sårtemperaturen och en öron termometer mätte kroppstemperaturen. **III.** 83 patienter som genomgick öppen kolonkirurgi blev randomiserade till antingen rutinmässiga värmningsmetoder (n=39) eller dessutom lokal tillförsel (n=40) av värmd (37°C) och befuktad (100 % rH) CO_2 via en gas-diffusor. En värmekänslig kamera mätte sårtemperaturen och en öron termometer mätte kroppstemperaturen. **IV.** Detta är en post hoc, retrospektiv studie av studie II och III där patienter blev randomiserade till värmd och befuktad CO_2 (n=80) eller inte (n=78).

Resultat: I. Exponering för CO_2 ökade mediantemperaturen i hela såret med 0.5°C (p=0,01). Temperaturen i området distalt om diffusorn ökade med 1.2°C (p<0,01), medan i området nära diffusorn minskade temperaturen med 1.8°C (p<0,01). **II.** Mediantemperaturerna i sårytan och sårkanterna var 1.2ºC (p<0,001) respektive 1.0ºC (p=0,002) högre i CO_2-gruppen jämfört med kontrollgruppen. Medelkroppstemperaturen efter intubation var samma (35.9ºC) i båda grupper, men vid slutet av kirurgin skilde sig de båda grupperna åt med en medeltemperatur på 36.2 ± 0.5 ºC i CO_2-gruppen och 35.8 ± 0.5ºC i kontrollgruppen (p=0,003). **III**. Medeltemperaturen i sårytan under kirurgi var 31.3ºC i CO2-gruppen jämfört med 29.6ºC i kontrollgruppen (p<0,001). Dessutom var medeltemperaturen i sårkanterna under kirurgi 30.3ºC jämfört med 28.5ºC i kontrollgruppen (p<0,001). Medelkroppstemperaturen före start av kirurgi var nästan densamma med 36.7 ± 0.5ºC i CO_2-gruppen jämfört med 36. ± 0.5ºC i kontrollgruppen. Vid slutet av kirurgin skilde sig de två grupperna signifikant åt med 36.9 ± 0.5ºC i CO_2-gruppen jämfört med 36.3 ± 0.5ºC i kontrollgruppen (p<0,001). **IV.** En multivariat analys justerad för ålder (p=0,001) och cancer (p=0,165) visade att ju större temperaturdifferens mellan slutlig kroppstemperatur och slutlig sårkantstemperatur desto lägre total överlevnad (p=0,050). En lägre sårkantstemperatur vid

slutet av kirurgi var negativt associerad med mortalitet (OR=0.80, 95 % CI=0.68-0.95, P=0,011), medan ålder (10-års ökning, OR=1.78, 95 % CI=1.37–2.33, P<0,001) och cancer (OR=8,1, 95 % CI=1,95–33,7, P=0,004) var positivt associerade med mortalitet.

Slutsats: De huvudsakliga fynden i denna avhandling är att tillförsel av torr, rumstempererad CO_2 med en gas-diffusor ökar den genomsnittliga areatemperaturen i en öppen kirurgisk sårhåla. Tillförsel av värmd och befuktad CO_2 i en öppen kirurgisk sårhåla resulterar i en signifikant ökning av sårtemperaturen liksom av kroppstemperaturen. Tillförsel av värmd och 100 % befuktad CO_2 i en öppen kirurgisk sårhåla ökar sår- och kroppstemperaturen och bidrar till att bibehålla normal kroppstemperatur. En liten differens mellan slutlig kroppstemperatur och slutlig sårkantstemperatur var positivt associerat med bättre överlevnad efter öppen kolonkirurgi. En lägre sårkantstemperatur vid slutet av kirurgi var negativt associerad med mortalitet.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers that are referred to by their Roman numerals as follows:

I. **CO_2 insufflation influences the temperature of the open surgical wound**
Frey J, Svegby H, Svenarud P, van der Linden J
Wound Rep Reg 2010; 18: 378-382

II. **Intraoperative local insufflation of warmed humidified CO_2 increases open wound and core temperatures: A randomized clinical trial**
Frey J, Janson M, Svanfeldt M, Svenarud P, van der Linden J.
World J Surg 2012; 36:2567–2575

III. **Local insufflation of warm humidified CO_2 increases open wound and core temperatures during open colon surgery: A randomized clinical trial.**
Frey J, Janson M, Svanfeldt M, Svenarud P, van der Linden J
Anesth Analg 2012; 115:1204–1211

IV. **Relation of intra-operative temperature to postoperative mortality in open colon surgery - an analysis of two randomized controlled trials**
Frey J, Holm M, Janson M, Egenvall M, van der Linden J.
Int J Colorectal Dis 2015; published online Dec 13, 2015

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ºC	Degree Celsius
CO_2	Carbon dioxide
kPa	Kilopascal
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
PtO_2	Tissue oxygen tension
rH	Relative Humidity
SSI	Surgical site infections

INTRODUCTION

The human body temperature is strictly regulated to range from 36.5ºC to 37.5ºC. This temperature threshold is set by complicated and not fully understood postsynaptic potentials in the hypothalamic neurons. Very roughly the body can be divided in two compartments; a core thermal compartment that consists of the well-perfused tissues in the trunk and head, and a peripheral that consists of the arms and legs. Within the core compartment the temperature remains relatively uniform and changes very little between the different tissues and over time while in the periphery the temperature is non-homogeneous, has a much greater variability over time, and is 2-4ºC below core temperature under normal conditions. This creates a core-to-peripheral temperature gradient that is lowered by a warm environment or vasodilatation and increased by a cold environment or vasoconstriction. The only natural internal source of heat production is metabolism and it is mainly the brain and major organs in the trunk that are metabolically active. All produced metabolic heat must eventually by dispersed to the environment to keep thermal steady state. A waste majority, 95%, of this heat is lost through the skin surface while the rest, 5%, is lost through respiration[1].

The definition of normal core temperature in this context has to be clarified. The ‘expected normal core temperature range of adult patients…’ is ‘…between 36.5ºC and 37.5ºC’ according to the National Institute for Health and Clinical Excellence guideline [2]. Moreover, the comprehensive review in Anesthesiology by D. Sessler states that ‘Normal core temperatures in humans typically range from 36.5ºC and 37.5ºC; values less than 36ºC or greater than 38ºC usually indicate loss of control or a thermal environment so extreme that it overcomes thermoregulatory defenses’[3].

PERIOPERATIVE CORE HYPOTHERMIA

Under neuraxial and general anesthesia there is always a drop in temperature of 1-3ºC typically, depending on type and dose of anesthesia, type and duration of surgery, and ambient temperature. This development of perioperative hypothermia follows a characteristic three-phase pattern with the highest temperature drop of 1-1.5 ºC during the first hour, a slower, linear drop during the following 2-3 hours and finally a plateau phase during which core temperature remains constant. The first phase depends on a redistribution of heat from the core to the periphery caused by vasodilatation mainly due to a shift of the centrally mediated threshold for thermoregulatory defense mechanism toward lower temperatures, but also as a direct effect of the anesthetic drugs. The next linear phase is simply a result of heat loss exceeding heat production, since metabolic rate is reduced by 15-40% during general anesthesia[3]. Heat loss through the skin surface is mediated by radiation, convection, conduction, and evaporation. Radiation usually contributes the most to heat loss but does not depend at all on the temperature of the surrounding air. Convection is the second most important source of heat loss during surgery and anesthesia and is a direct transfer of heat from one surface to another adjacent surface, depending on the temperature gradient between them. Conduction and evaporation contributes normally very little to heat loss through intact skin but from a surgical incision the loss can be substantial, which presumably explains why major operations results in more pronounced core hypothermia than small procedures [4]. It is during this linear phase that efforts to reduce heat loss, like for example passive insulation or active warming, are most effective.

CONSEQUENCES OF OPEN SURGERY

Open surgery abruptly exposes the internal organ tissues to a new hostile environment of relatively dry and cold ambient air in the operating room. With the protective barrier of the skin broken, the impact of radiation, convection, and evaporation from the open surgical wound can be assumed to cause local desiccation, a decrease of open wound temperature, and possibly also lower core temperature.

GUIDELINES

Growing awareness of the negative consequences of perioperative hypothermia has led to the development of guidelines and to increased use of countermeasures to avoid hypothermia. Widely applied warming strategies include passive insulating measures or, even more effective, active transfer of heat to the body with resistive-heating or forced-air warming blankets, as well as fluid warming systems.

NICE guidelines (National Institute for Health and Care Excellence)

NICE clinical guidelines[2] are recommendations about the treatment of care of people with specific diseases and conditions in the NHS (National Health Service) in England and Wales, and have a strong impact all over Europe. This guidance represents the view of the Institute, which was arrived at after careful consideration of the evidence available. However, the guidance does not override the individual responsibility of health care professionals to make decisions appropriate to the circumstances of the individual patients. NICE defines hypothermia as a patient core temperature of below 36.0°C. The key priorities for implementation in this guideline provide strong direction for healthcare professionals in helping to prevent perioperative hypothermia in adults undergoing surgery. The guidelines state that during the intraoperative phase:

- The patient's temperature should be measured and documented before induction of anesthesia and then every 30 minutes until the end of surgery.
- Induction of anesthesia should not begin unless the patient's temperature is 36.0°C or above (unless there is a need to expedite surgery because of clinical urgency, for example bleeding or critical limb ischemia).
- Intravenous fluids (500 ml or more) and blood products should be warmed to 37°C using a fluid warming device.
- Patients who are at higher risk of inadvertent perioperative hypothermia and who are having anesthesia for less than 30 minutes should be warmed intraoperatively from induction of anesthesia using a forced air warming device.
- All patients who are having anesthesia for longer than 30 minutes should be warmed intraoperatively from induction of anesthesia using a forced air warming device.

NEED FOR IMPROVEMENT

Warming the open surgical wound

Despite all efforts made to keep the patient warm, about half of general surgery patients undergoing abdominal operations become hypothermic during surgery, and a significant proportion are still hypothermic on arrival in the recovery room [5-7]. Considering the well-known clinical complications of hypothermia it seems wise to keep not only the patient but also the surgical wound at normal temperatures during surgery. Nowadays, forced air-warming can be regarded as standard of care for skin-surface warming, but obviously this method cannot be effective when skin-surface covering is limited for surgical reasons. Even if the rest of the patient is well protected, there will still be a substantial evaporative heat loss from the unprotected open surgical wound [3].

Humidifying the open surgical wound

Desiccation is one of the causes of inadvertent loss of peritoneal mesothelium [8-10]. Traditionally, desiccation is reduced by use of irrigating lavage and by placing wet packs into the abdominal cavity. However, criticism is growing against the use of intra-peritoneal lavage, as it may increase the risk of post-operative complications by disrupting the peritoneal mesothelium. Furthermore, it is not effective in reducing the risk of surgical site infection [11, 12]. Also, rubbing the peritoneum with a wet pack can probably cause mesothelial damage [13, 14]. Insufflation with humidified CO_2 will differ fundamentally from this standard practice of care. Warmed fully humidified CO_2 will humidify not only the wound edges but the complete surface of the wound cavity, without any direct contact with foreign material on the tissue surface, thus preventing any possible contact injury.

QUALITIES OF LOCAL CO_2 GAS

Several both experimental and clinical studies have shown that an artificial CO_2-atmosphere within the open surgical wound cavity protects against heat loss. In order to create an atmosphere of almost 100% CO_2 the gas has to be delivered via a specially designed gas diffuser from within a wound cavity at a low velocity while at a flow rate high enough to create a local environment with a high concentration of CO_2[15]. First, CO_2 is heavier than air and settles in the wound cavity like a protective layer. This minimizes both water diffusion and the convective air currents caused by the operating room ventilation. Second, CO_2 is a greenhouse gas that reflects radiant heat from the wound[15]. Third, the addition of warm water vapor to the insufflated CO_2 has experimentally been shown to increase its heat transfer, prevent evaporation and desiccation, and result in an increased local wound temperature[16]. Fourth, CO_2 has a bacteriostatic feature compared with air, where the bacterial growth rate is exponential. This specific effect of CO_2 is dependent on a high concentration and explains why CO_2 gas is used as a preservative in the food industry[17]. Fifth, CO_2 insufflation of the wound via a gas diffuser results in a continuous laminar overflow of CO_2 from the wound opening that repels and transports bacteria-carrying particles away from the surgical area. Experimentally the method reduces direct airborne contamination by approximately 80%[18]. This may be of importance since the first few hours following bacterial contamination constitute a decisive period during which infection is established[19]. The effects of hypothermia induced hypoperfusion are thus especially important during the intraoperative period. Techniques aimed at improving resistance to surgical wound infections are therefore most likely to succeed if implemented intraoperatively[19].

AIMS OF THE THESIS

The specific aims of this thesis were to investigate if:

- Dry, room-tempered insufflation with CO_2 gas in an open surgical wound could affect the surgical wound temperature.

- Local insufflation of warmed (30°C) humidified CO_2 (93% rH) could increase both the open surgical wound and core temperature.

- Local insufflation of warmed (37°C) fully humidified CO_2 (100% rH) could increase both the open surgical wound and core temperature, and maintain normothermia.

- Increased wound and core temperatures during major open colorectal surgery induced by local insufflation of warmed humidified CO_2 could affect long term overall mortality.

PATIENTS AND METHODS

STUDY I

Study I was conducted in 10 adult patients undergoing cardiac valve procedures, because they routinely obtain CO_2 insufflation to prevent air-embolism, at the Department of Cardiothoracic Surgery & Anesthesiology at the Solna site of Karolinska University Hospital in Sweden during 2007. Using our earlier published data[15] we calculated that only 6 patients were needed in each group to find a statistical difference (alpha-error 5% level, statistical power 80% level). Moreover, by using each patient as his own control we avoided the influence of inter-individual differences. Still, since we used a less controlled environment in this study, we expanded the calculated needed number to 10.

Medical CO_2 gas was delivered at a flow rate of 5 L/minute from a pressurized gas cylinder, controlled with back-pressure–compensated flow meters (AGA gas AB, Stockholm, Sweden) via a single use gas diffuser device (CarbonAid™, Cardia Innovation AB, Stockholm, Sweden). It consists of a PVC tube with an inner diameter of 1/4 inch (6.35 mm), a gas filter, and a distal 2.5 mm tube with a diffuser made of polyurethane plastic foam at its end. The polyurethane foam together with the cylindrical shape diverts the gas jet into multiple directions via the many small paths inside the foam. The gas is thus uniformly distributed and the large diffuser surface greatly reduces the velocity of the outflow[20].

The delivered CO_2 was dry (0% rH) and had a constant temperature equal to that of the air in the operating room, approximately 20°C. After the pericardium had been opened and before cannulation, the diffuser was positioned at the caudal end of the wound cavity, close to the diaphragm approximately 5 cm below the wound edge. During the first two minutes, the CO_2 flow was not turned on, followed by two minutes with a CO_2 flow of 5 L/minute. Then, the CO_2 flow was kept turned off for two minutes. We anticipated that the effect lasts as long as the open wound is insufflated with CO_2 to create a local CO_2 atmosphere. Once insufflation is stopped, the effect will be reversed by the counteracting effect of diffusion, which will disperse CO_2 from the wound cavity and fill the wound with ambient air[21].

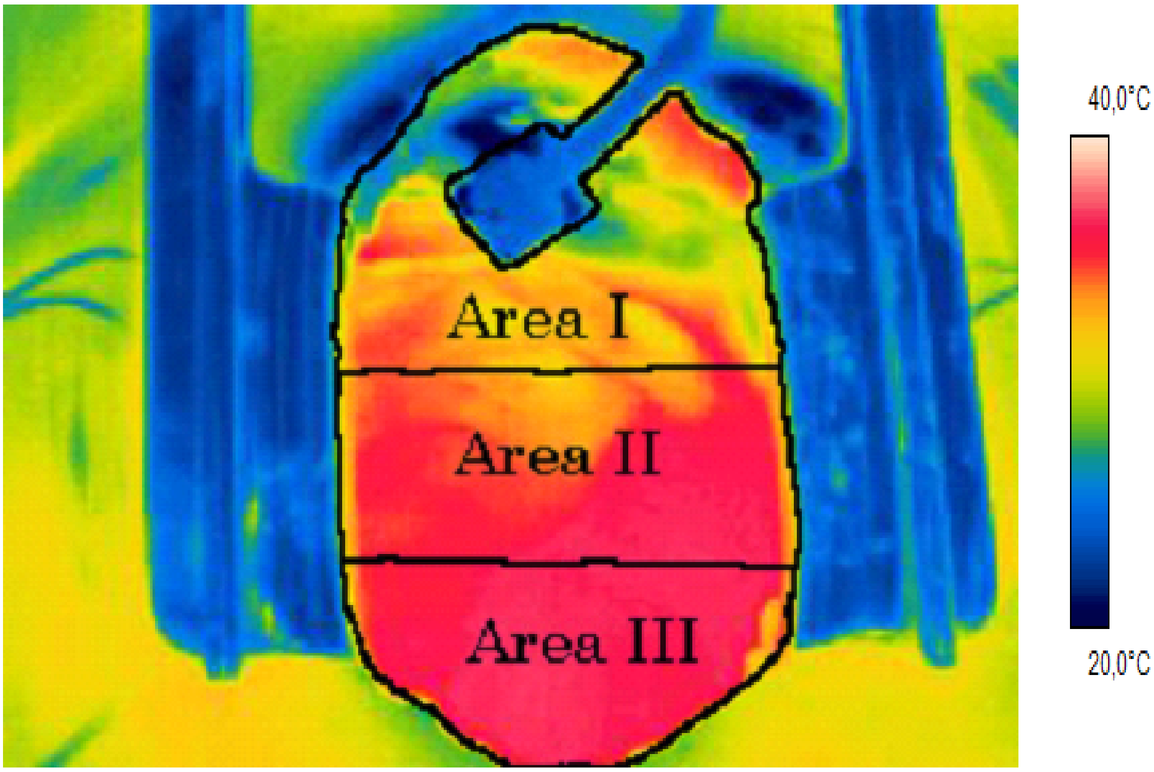


Figure 1. The thermal image of the open cardiothoracic wound area was divided into three equal thirds. Area I consists of the caudal area excluding the diffuser and its tube. Area II is the middle area, and Area III is the cranial third

The temperature in the wound cavity was measured with a heat-sensitive infrared camera (ThermaCAM™ B2, FLIR Systems AB, Danderyd, Sweden). Images were taken every two minute during 6 minutes. This resulted in three images per patient. First, the wound area was divided into three equal thirds. Then, the following five areas were created: Area I consisted of the caudal third but excluded the diffuser. Area II represented the middle third, and Area III the cranial third. Area IV was created by adding Areas II and III and Area V was created by adding Areas I, II, and III After delineating the respective areas, the program automatically calculated the average temperature for each area with its corresponding standard deviation (Figure 1).

STUDY II AND III

Study II and **III** were conducted at the Huddinge site of Karolinska University Hospital between March 2007 and May 2008 (**Study II**) and between November 2008 and February 2010 (**Study III**). Only adults above 18 years who were included to undergo elective colon surgery were eligible for inclusion in the study. Exclusion criteria were acute surgery and preoperative core temperature of ≥37.5ºC. In **Study II** 80 adult patients were randomized to a CO_2 group (n=39) or a control group (n=41) and in **Study III** 83 adult patients were randomized to a CO_2 group (n=42) or a control group (n=41). Randomization was achieved with sealed envelopes, each of which was opened as the patient was being transported to the operation room. Nurses from an independent hospital department had generated the random allocation sequence and they were kept uninformed of all other parts of the study. When arriving to the pre-anesthetic room, all patients were covered with heated blankets. The operating room temperature was kept at 20-22°C. Standard warming procedures included maximal heating with forced-air heating blankets over the upper part of the body and whenever possible over the lower part of the body, insulation of the limbs and the head, and preheated fluids that were additionally warmed to 39-40°C via a fluid warmer when given to the patient. A thoracic epidural catheter was placed in all patients, except two in the humidified CO_2 group (**Study II**), and three in the humidified CO_2 group and five in the control group (**Study III**), respectively. A 3 ml test dose of marcain 5 mg/ml + epinephrine 5 ug/ml (AstraZeneca AB, Södertälje, Sweden) was immediately given, after which an infusion of ‘‘Breiviks mix’’ (bupivacain 1 mg/ml + fentanyl 2 ug/ml + epinephrine 2 ug/ml, Apoteket AB, Stockholm, Sweden) was started at a rate of 12–15 ml/h. All patients were given standardized premedication, anesthesia, and analgesia. Anesthesia was induced with fentanyl (B. Braun Medical AB, Danderyd, Sweden) 1–2 ug/kg, propofol (B. Braun Medical AB, Danderyd, Sweden) 1–2 mg/kg, and atracurium (Glaxo-SmithKline AB, Solna, Sweden) 0.5 mg/kg or rocuronium (Schering-Plough AB, Stockholm, Sweden) 0.6 mg/kg, and continued with inhalation of Sevoflurane (Baxter Medical AB, Kista, Sweden). The lungs were mechanically ventilated using a circle circuit with absorption of CO_2 at 1 L/ min fresh gas flow. Mechanical ventilation was adjusted to keep end-tidal CO_2 at 4-5 kPa. Phenylephrine or norepinephrine was given i.v. at the discretion of the individual anesthetist to keep a mean blood pressure above 70 mmHg. Atracurium or rocuronium was given intermittently during the operation to keep a train of four value of 0, and reversed with neostigmin 50 ug/kg + glykopyrron 10 ug/kg (Meda AB, Solna, Sweden). Intraoperatively, all patients received fluids intravenous according to a standardized protocol: glucose 25 mg/ml (Glukos Buffrad, Baxter, Kista, Sweden) 1 ml/kg per h, hydroxyethyl starch 60 mg/ml (Voluven, Fresenius Kabi AB, Uppsala, Sweden), 2 ml/kg per h, and Ringer’s acetate 2 ml/kg per h (Baxter Medical AB, Kista, Sweden). Additional Voluven or Ringer’s acetate was given if the attending anesthetist diagnosed hypovolemia. Transfusions were given at the discretion of the attending anesthetist. Six respectively seven senior surgeons participated in **Study II** and **III**, and they all used a circular abdominal wall retractor (GO400/GO450 General Retractor System, Omni-Tract Surgical, St. Paul, MN). The competence of the bowel anastomosis was, whenever applicable, tested by filling the abdominal cavity with 200-500 ml of preheated (37ºC) saline (0.9 %), and followed by insufflation of air into the rectum. The fluid was thereafter removed with a suction device.

Patients randomized to the CO_2 group of **Study II** and **III**, respectively, received insufflation of warmed humidified CO_2 into the open wound cavity. The gas diffuser was positioned inside the open abdominal wound cavity (in the right cranial quadrant) at a depth of approximately four cm from the skin as soon as the surgeon had fixated the wound edges with the circular abdominal wall retractor. Two additional abdominal retracting pads, each consisting of a flat metal rod enclosed in polyurethane foam measuring 4.5 x 4.5 x 5cm (Disarp, Unomedical A/S, Birkerød, Denmark), were then positioned around the abdominal wound edge to increase the depth of the surgical wound cavity.

Figure 2. To the left the noncommercial humidification system. To the right the more effective commercial humidification system, and the gas diffuser.

In **Study II** dry medical CO_2 was delivered at a flow rate of 10 L/min at a pressure of 4.5 bar from a pressurized gas cylinder (AGA gas AB, Stockholm, Sweden) via a 1/4 in. (6.35 mm) PVC tube to a plastic bottle containing sterile water (Hudson Oxygen humidifier without jet adaptor, Meteko Instrument AB, Esbo, Finland), which humidified the passing CO_2. The bottle was submerged in a heated water bath (Water thermostat-bath WB-4MS, BioSan, Riga, Latvia), which was kept at 40ºC. The humidified and warmed CO_2 then passed through a 2.5 meter long PVC tube with polyurethane foam at its end, the gas diffuser (CarbonAid™ Cardia Innovation AB, Stockholm, Sweden) (Figure 2). The CO_2 delivered to the patient had a temperature of 30ºC and an rH of 93%. This corresponds to 28.2 mg of water per liter CO_2.

In **Study III** the same flow rate was used but a more effective heating system delivered humidified CO_2 at 37°C and 100% rH to the surgical wound (HumiGard™, Fisher & Paykel HealthCare Ltd, Auckland, New Zealand). This corresponds to 43.9 mg of water per liter CO_2, which is 55% more than delivered with the noncommercial device used in **Study II**. The system used in **Study III** consists of a bacterial filter, and a humidification chamber filled with 180 ml sterile water, positioned on a humidifier controller that includes an integrated temperature and flow sensor. The outlet of the humidification chamber is connected to the CarbonVita™ gas diffuser (Cardia Innovation AB, Stockholm, Sweden) that maintains temperature and humidity of the gas to its outlet (Figure 2). The arrangement is very simple and does not need any attention after start except when after approximately 8 hours an alarm will indicate that water has to be refilled. Insufflation of warmed humidified CO_2 was thereafter started and continued until the abdominal retractor and the rector pads were removed just before surgical closure of the wound.

The temperature in the wound cavity was measured with a heat sensitive infrared camera (Figure 3) that was mounted on a rigid pod one meter above the wound to ensure that all images from each patient showed the wound surface area from the same position (Figure 4). Images were taken every 10th minute with start just before the surgical incision until the wound was closed. Core temperature was measured at the tympanic membrane every 30th minute with a thermometer (CORE-CHECK Tympanic Thermometer System, Cardinal Health, Dublin, OH) from the time that the patient was anesthetized until end of surgery. The used tympanic thermometer has an accuracy ± 0.1°C. All temperatures were measured by a specially trained nurse to avoid and minimize inter- and intra-examiner variability, respectively.

In **Study II** the temperatures are presented for every 30: th min for the first 3 h only, as thereafter fewer than 50 % of the patients were available for comparison. The median group temperatures were calculated by using the median temperature for each patient during the operation i.e., the area under the curve. Thus, the problems of analyzing repetitive measurement as well as differences in operative time among the patients were avoided. In **Study III** the wound and core temperatures are presented for every 10th and 30th minute, respectively, during the first 4 hours, after that only around 40% of the patients remained. Also in this study, the mean group temperatures were calculated by using the mean temperature for each patient during the operation, i.e., the area under the curve.

STUDY IV

This study is a post hoc retrospective single-center study of **Study II** (n=72) and **III** (n=70) and 16 additional patients, who had all been randomized to a warmed humidified CO_2 group (n= 80) or a control group (n=78). All patients underwent elective major open colon surgery between March 2007 and November 2013 at Karolinska University Hospital. In addition to the exclusion criteria described for **Study II** and **III**, patients who underwent colostomy surgery were excluded, since the focus was on major colon surgery. Postoperative morbidity and mortality were obtained in May, 2015 from the hospital's medical records that are linked to the national Swedish database on mortality, the Total Population Registry. In **Study II** a noncommercial system that delivered humidified CO_2 at 93% rH and 30°C was used, whereas in **Study III** and in the 16 additional patients a commercial system delivered 100% rH and 37°C to the surgical wound (HumiGard™, Fisher & Paykel HealthCare Ltd, Auckland, New Zealand). All but 10 patients (6%) received a thoracic epidural blockade in addition to general anesthesia.

Heat sensitive infrared camera

A heat sensitive infrared camera was used in **Study I-III** (ThermaCAM™ B2, FLIR

Figure 3. ThermaCAM™ B2

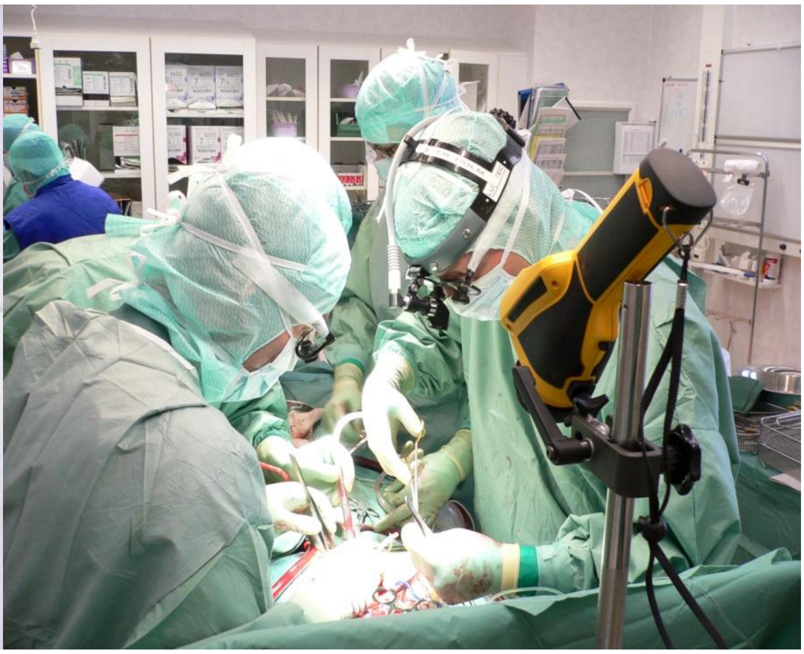


Figure 4. The ThermaCAM™ B2's position during the measurements. The camera is mounted on a rigid pod

Systems AB, Danderyd, Sweden) to take images from the open surgical wound whereby it measures the emitted infrared radiation from the open wound surface. The fact that radiation is a function of object surface temperature makes it possible for the camera to calculate and display the temperature of the observed area. Single shot images (160 × 120 pixels) can be stored, whereby the device stores each pixel of the image together with its corresponding temperature. Thus, when taking a thermographic image, the temperatures of all the pixels in the image area are registered. The images were later analyzed with a software program (ThermaCAM™ Researcher Basic 2.8 SR-1, FLIR Systems AB), which allows delineation of the wound cavity. After delineating the respective area, the program automatically calculated the average temperature for each area with its corresponding standard deviation. According to the manufacturer, the thermal sensitivity of the detector is 0.1°C at 30°C; the accuracy is ±

2°C. By standardizing the time the camera is switched on, by using internal calibration, an accuracy of 0.1°C for sequential images can be reached (ThermaCAM™ B2-operator´s Guide. FLIR Systems, *August, 2004*).

ETHICS

The local Ethical Committee approved all studies. Informed consent was obtained from involved patients when judged appropriate by the local Ethical committee. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

STATISTICAL ANALYSIS

Results were analyzed using SPSS for Windows version 15.0. Data are presented as means ± SD when normally distributed or otherwise as medians and 25th/75th percentiles. The Student *t*-test was used when variables were normally distributed. The $\chi 2$-test or the Fisher exact test was used for nominal data, and the Mann-Whitney U or the Wilcoxon's tests were used when variables were not normally distributed. Statistical significance was accepted for p values ≤ 0.05. All tests were two-sided.

Study IV

Survival in the CO_2 group and the treatment group, as well as final core temperature ≥ 36.0ºC, respectively, were analyzed using Kaplan-Meier curves. To identify variables associated with mortality, univariate Cox regression analysis was performed. The relationship between core and wound edge temperature differences at the end of surgery, and mortality, was analyzed using the Cox proportional hazards model adjusted for age and cancer. The *p*-values for the differences in patient characteristics were obtained by $\chi 2$ or *t*-tests. SPSS software (version 22, SPSS Inc, Chicago, Ill) was used for statistical analyses. All tests were two-sided. Statistical significance was accepted for p values ≤ 0.05.

RESULTS

STUDY I

Table 1 describes the median temperature at each measurement. Insufflation with CO_2 after pericardiotomy but before cannulation significantly increased the median temperature of Area IV (Area II+ Area III) with 1.2ºC and of Area V (I+II+III) with 0.5ºC, whereas the temperature of Area I, close to the gas diffuser, decreased significantly with 1.8ºC.

Table 1. *Median temperatures of the wound cavity area during the three time points of the study; 1=before; 2=during, 3=after insufflation of the wound cavity with CO_2*

Wound Area	Time	Median Temperature °C	25^{th} / 75^{th} percentile	Range	P
Area V (I+II+III = total wound)	1	32.5	31.9/32.7	31.4-33.7	$0.01^{\#}$
	2	33.0	32.4/33.5	31.3-34.2	0.01^{*}
	3	32.6	32.6/33.0	30.9-34.7	$0.67^{\&}$
Area IV (II+III = middle and cranial third)	1	32.5	32.1/32.9	31.4-33.8	$<0.01^{\#}$
	2	33.7	31.2/34.0	31.4-34.7	$<0.01^{*}$
	3	32.7	32.3/33.4	31.6-33.8	$0.09^{\&}$
Area I (caudal third excluding temperature of diffuser and tube)	1	32.2	31.3/32.7	30.5-33.8	$<0.01^{\#}$
	2	30.4	29.7/31.5	29.0-32.5	0.01^{*}
	3	31.6	30.6/32.5	29.3-33.6	$0.03^{\&}$

#=Time 1 vs. 2; *=Time 2 vs. 3; &=Time 1 vs. 3

The three graphs of Figure 5 illustrate the changes in median temperature, with corresponding 25th/75^{th} percentiles, of the areas V, IV, and I before, during, and after insufflation of CO_2. The wound temperature in Area IV and V almost completely regained their start temperatures after cessation of insufflation with CO_2, whereas area I remained significantly cooler, 1.2ºC, than its start temperature.

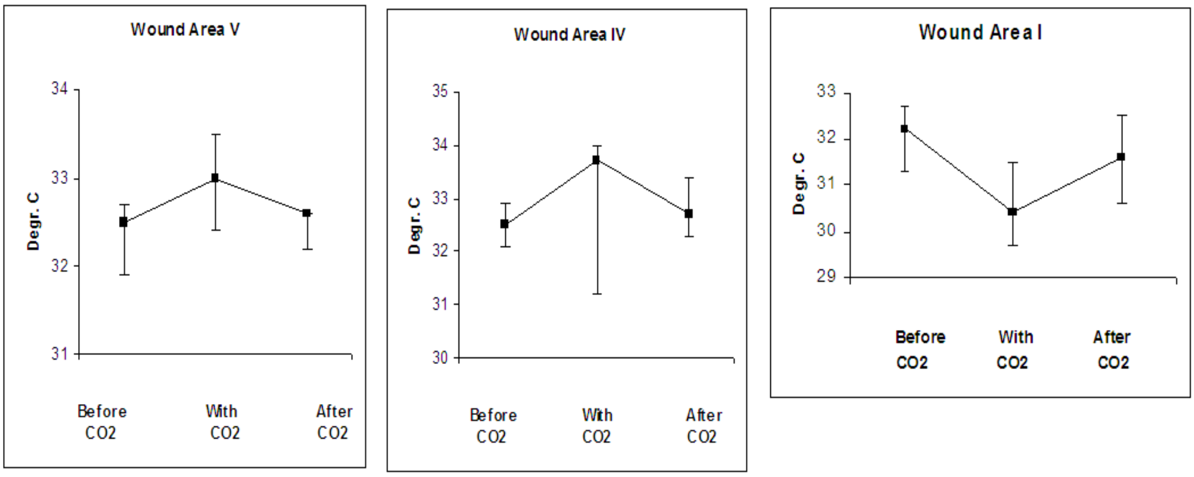


Figure 5. Median temperatures with corresponding 25th/75th percentiles of the cardiothoracic open wound Area V (Area I + II + III = total wound area excluding the diffuser and its tube), Area IV (Area II + III = middle and cranial third of the wound area), and Area I (Area I = caudal third of the wound area, excluding the diffuser and its tube)

Figure 6 depicts the thermal images during insufflation of the wound cavity with CO_2. These images easily allow the identification of the room tempered sternal retractor and the diffuser with its tube. As one can see, the wound edges and the wound area are warmer after 2 minutes of insufflation with dry CO_2. Besides, the temperature in close proximity to the diffuser was the lowest in the image, approximately 20ºC.

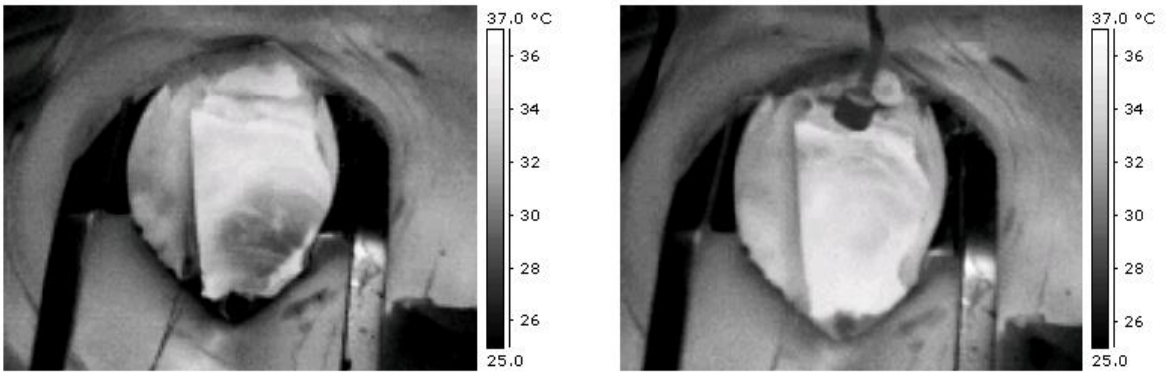


Figure 6: Infra-red pictures from an open cardiothoracic wound before (left) and 2 min after insufflation with dry CO_2 (right).

STUDY II

Initially eighty patients were randomized 41 in the CO_2 group, and 39 in the control group. Six patients were excluded before statistical analysis, three from the CO_2 group and three from the control group, leaving 74 to be analyzed. There were no significant differences between the two groups in age, gender, length, weight, or BMI (Table 2). Neither were there any significant differences between the groups regarding patients' diagnoses or operation codes.

Intraoperative variables are listed in Table 3. Forced air heating blankets covered the upper part of the body in all patients and the lower part of the body in 61-63% in both groups. The abdominal cavity was rinsed with a small amount of saline almost equally often in both groups. The mean operating time was similar in both groups, with 219 ± 104 minutes and 205 ± 85 minutes for the CO_2 group and the control group, respectively ($p=0.550$). Hospital stay and volume of intraoperative bleeding did not differ significantly between the groups. However, patients in the CO_2 group were extubated 8 minutes earlier compared to the control group ($p=0.035$).

Table 2. Group characteristics (mean ± SD, or number of patients and percentage)

	CO_2 group (n=38)	**Control group (n=36)**	**P**
Age (years)	61 ± 14	66 ± 19	0.222‡
Sex			
Male	21 (55%)	23 (64%)	0.486†
Female	17 (45%)	13 (36%)	
Length (m)	1.72 ± 0.1	1.68 ± 0.1	0.132‡
Weight (kg)	76 ± 14	73.6 ± 22	0.554‡
BMI (kg/m^2)	25.8 ± 4	25.7 ± 6	0.978‡
Diagnosis			
Cancer	25 (66%)	24 (67%)	0.936§
Diverticulitis	4 (11%)	2 (6%)	0.675†
Inflammatory bowel disease	8 (21%)	7 (19%)	0.863§
Other benign	2 (5%)	4 (11%)	0.424†
Operation codes			
Ileocolic resection	5 (13%)	1 (3%)	0.200†
Total colectomy	2 (5%)	3 (8%)	0.670†
Subtotal colectomy	0 (0%)	1 (3%)	0.486†
Right hemicolectomy	12 (32%)	7 (19%)	0.232§
Transverse colectomy	0 (0%)	1 (3%)	0.486†
Left hemicolectomy	1 (3%)	2 (6%)	0.610†
Sigmoid colectomy	6 (16%)	4 (11%)	0.737†
High anterior resection	3 (8%)	3 (8%)	1.000†
Proctocolectomy	2 (5%)	0 (0%)	0.494†
Rectal resection	3 (8%)	4 (11%)	0.707†
Rectal amputation	2 (5%)	3 (8%)	0.670†
Surgical treatment of intra-abdominal	2 (5%)	4 (11%)	0.424†
adherences	4 (11%)	2 (6%)	0.675†
Partial small bowel resection	3 (8%)	3 (8%)	1.000†
Colostomy	2 (5%)	3 (8%)	0.670†
Sigmoidostomy	1 (3%)	4 (11%)	0.194†
Ileostomy	2 (5%)	0 (0%)	0.494†
Colostomy closure	1 (3%)	0 (0%)	0.486†
Kock´s pouch	2 (5%)	3 (8%)	0.670†
Small pelvic reservoir	2 (5%)	0 (0%)	0.494†
Cholecystectomy	1 (3%)	0 (0%)	0.486†
Nephrectomy	3 (8%)	1 (3%)	0.615†
Partial liver resection	1 (3%)	1 (3%)	1.000†
Salpingo-oophorectomy	0 (0%)	1 (3%)	0.486†
Hysterectomy	1 (3%)	1 (3%)	1.000†
Closure of colostomy hernia	1 (3%)	1 (3%)	1.000†

* Mann-Whitney U-test, †Fisher exact test, ‡ *t* test, § χ^2 test. Please note that a patient undergoing a procedure may receive several operation codes.

The median wound area and the median wound edge temperatures were both significantly higher in the CO_2 group than in the control group (Table 4). The median core, wound area, and wound edge temperatures for the two groups during the first three hours of surgery are shown in Figure 7. Also, the mean core temperature after intubation was the same (35.9ºC) in both groups, but at end of surgery the two groups differed significantly with 36.2ºC in the CO_2 group and 35.8ºC in the control group (p=0.003). In accordance with this finding, the median core temperature was 0.5ºC higher in the CO_2 group during the operation.

Table 3. Intraoperative variables and hospital stay (mean ± SD, median with 25th/75th percentile, or n and percentage)

	CO_2 group (n=38)	**Control group (n=36)**	**P**
Forced-air heating blankets covering			
Upper part of the body	38 (100%)	36 (100%)	1.000†
Lower part of the body	24 (63%)	22 (61%)	0.856†
Abdominal cavity rinsing (n)	17 (45%)	17 (47%)	0.831†
Operating time (min)	219 ± 104	205 ± 85	0.550‡
Intraoperative Bleeding (ml)	325 (137.5/525)	300 (100/575)	0.992*
End of surgery to extubation (min)	18 ± 8	26 ± 21	0.035‡
Hospital stay (days)	9.5 (7/15)	9 (7/14)	0.895*

*Mann-Whitney U-test, †Fisher exact test, ‡ *t* test, $^{\S}\chi^2$ test

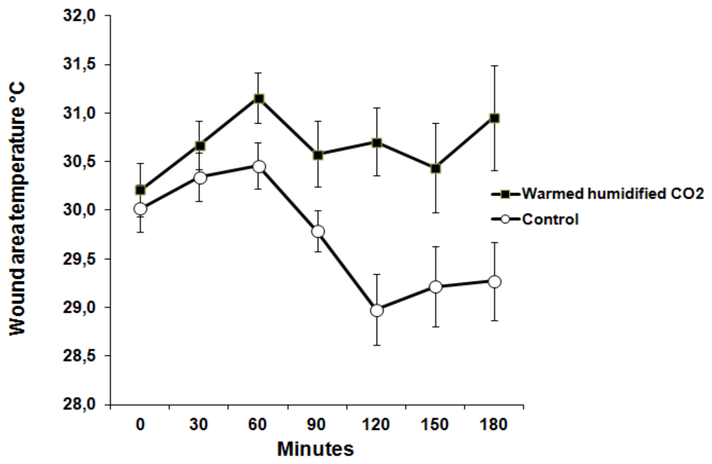
Table 4. Intraoperative temperatures (mean ± SD, or median with 25th/75th percentile)

	CO_2 group (n=38)	**Control group (n=36)**	**P**
Core temperature °C			
After intubation	35.9 ± 0.5	35.9 ± 0.4	0.877*
At start of surgery	35.6 ± 0.5	35.5 ± 0.6	0.252*
At end of surgery	36.2 ± 0.5	35.8 ± 0.5	0.003*
Core temperature during surgery °C (median AUC)	36.0 (35.6/36.2)	35.5 (35.4/36.0)	0.028†
Wound area temperature during surgery °C (median AUC)	31.1 (29.9/31.7)	29.9 (29.3/30.4)	<0.001†
Wound edge temperature during surgery °C (median AUC)	29.5 (28.4/30.5)	28.5 (27.7/29.3)	0.002†

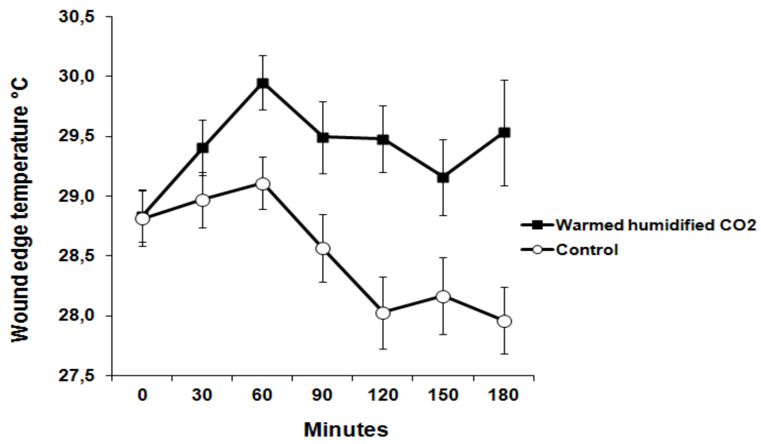
**t*- test, † Mann-Whitney U-test, AUC=area under the curve.

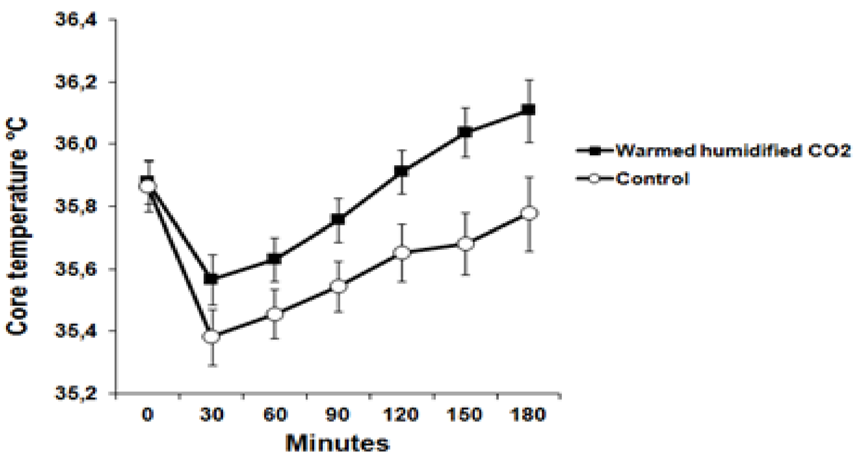
Figure 7. The mean wound area (a), and wound edge (b) and core (c) temperatures for the two groups during the first three hours of surgery

a)



b)





c)

STUDY III

Eighty three patients were initially randomized to either CO_2 or control. Four patients were excluded before statistical analysis, two from the warmed humidified CO_2 group and two from the control group, leaving 79 patients available for analysis. The two groups did not differ significantly in their clinical variables including age, gender, length, weight, and BMI. There were no significant differences between the groups regarding patients' diagnoses or operation codes (Table 5). Forced air heating blankets covered the upper part of the body in all patients and the lower part of the body in 85% of the patients. Median (25th/75th percentile) operating time did not differ significantly between the groups, with 181.5 (147.5/288) minutes in the warmed humidified CO_2 group versus 217 (149/288) minutes in the control group ($p=0.83$). Hospital stay, volume of intraoperative bleeding, and transfusion of red blood cells were also similar in both groups. There was a clear tendency towards more rinsing of the abdominal cavity in the control group (75%) versus the warmed humidified CO_2 group (56%, $p=0.058$). Patients in the warmed humidified CO_2 group had a small but non-significant tendency ($p=0.249$) to be extubated earlier compared with the control group, 11 and 21 minutes in median, respectively, after end of surgery (Table 6).

Table 5. *Group characteristics (mean ± SD, median with 25th/75th percentile, or number of patients and percentage)*

	Warmed humidified CO_2 group (n=40)	Control group (n=39)	P
Age (years)	63.5 (56/73)	63.5 (53/72)	0.965*
Sex			
Male	21 (53%)	23 (58%)	0.562§
Female	19 (47%)	16 (42%)	
Height (m)	1.71 ± 0.1	1.71 ± 0.1	0.844‡
Weight (kg)	72.5 ± 13	75.7 ± 15	0.299‡
BMI (kg/m^2)	25.0 ± 4.6	25.9 ± 4.7	0.378‡
Earlier abdominal surgery	22 (55%)	22 (56%)	0.900§
Diagnosis			
Cancer	22 (55%)	23 (59%)	0.721§
Diverticulitis	4 (10%)	2 (5%)	0.675†
Inflammatory bowel disease	10 (25%)	7 (18%)	0.446§
Other benign	4 (10%)	7 (18%)	0.348†
Operation codes			
Colon surgery	33 (83%)	34 (87%)	0.747†
Ileocolic resection	4 (10%)	2 (5%)	0.675†
Total colectomy	3 (8%)	0 (0%)	0.241†
Right hemicolectomy	8 (20%)	8 (21%)	0.955§
Left hemicolectomy	3 (8%)	4 (10%)	0.712†
Sigmoid colectomy	8 (20%)	6 (15%)	0.591§
High anterior resection	5 (13%)	5 (13%)	0.966§
Colostomy	3 (8%)	5 (13%)	0.481†
Rectal amputation	3 (8%)	6 (15%)	0.311†
Rectopexy	0 (0%)	1 (3%)	0.494†
Surgical treatment of intra-abdominal adherences	3 (8%)	2 (5%)	1.000†
Partial small bowel resection	1 (3%)	1 (3%)	1.000†
New ileostomy	2 (4%)	1 (3%)	1.000†
Closure of ileostomy	7 (18%)	4 (10%)	0.518†
Urinary bladder resection	1 (3%)	0 (0%)	1.000†
Ileal pouch-anal anastomosis	1 (3%)	1 (3%)	1.000†
Cholecystectomy	1 (3%)	0 (0%)	1.000†
Nephrectomy	1 (3%)	0 (0%)	1.000†
Partial liver resection	3 (8%)	0 (0%)	0.241†
Salpingo-oophorectomy	1 (3%)	1 (3%)	1.000†
Appendectomy	0 (0%)	1 (3%)	0.494†
Intestinal malrotation (LADDS)	0 (0%)	2 (5%)	0.241†
Incisional hernia	5 (13%)	1 (3%)	0.201†

*Mann-Whitney U-test, †Fisher exact test, ‡*t*-test, §χ^2 test. Please note that a patient under-going a procedure may receive several operation codes. The variables height, weight, and BMI were normally distributed according to the Lilliefors' one-sample test (all P > 0.067)

Table 6. Intraoperative variables and hospital stay (mean ± SD, median with 25th/75th percentile, or n and percentage)

	Warmed humidified CO_2 group (n=40)	**Control group (n=39)**	**P**
Forced-air heating blankets covering			
Upper part of the body	40 (100%)	39 (100%)	1.000†
Lower part of the body	37 (93%)	33 (85%)	0.311†
Abdominal cavity rinsing with in average 500 ml saline at 37 °C (n)	22 (56%)	30 (75%)	0.058†
Operating time (min)	181.5 (147.5/288)	217 (149/288)	0.312
Intraoperative Bleeding (ml)	300 (100/500)	250 (100/800)	0.424*
Transfusion of RBC units (n)	0.5 ± 1.4	1.3 ± 4	0.319*
End of surgery to extubation (min)	11 (8/15)	21 (15/26)	0.249*
Hospital stay (days)	7 (5/9)	7 (5/12)	0.818*

*Mann-Whitney U-test, †χ^2-test, ‡*t*-test, RBC=Red blood cells. None of the variables were normally distributed according to the Lilliefors' one-sample test.

As is depicted in Figure 8 the wound area and edge temperatures increased as soon as the skin was opened in both groups. After 20 minutes the groups started to separate significantly, and at 30 minutes the temperatures peaked in the control group compared with at 60 minutes in the warmed humidified CO_2 group. Thereafter, the wound area and edge temperatures tended to stabilize in the warmed humidified CO_2 group, whereas they started to drop in the control group. The mean wound area temperature during surgery was 31.3ºC in the warmed humidified CO_2 group compared with 29.6ºC in the control group ($p<0.001$). Also, the mean wound edge temperature during surgery was 30.1ºC compared with 28.5ºC in the control group ($p<0.001$). At end of surgery, before closure of the open wound, the mean wound area and edge temperatures were 32.1ºC and 30.5ºC in the warmed humidified CO_2 group compared with 30.3ºC and 28.8ºC, respectively, in the control group ($p<0.001$, Table 7). The mean wound area temperature was significantly higher than the mean wound edge temperature both in the warmed humidified CO_2 group ($p=0.001$) and in the control group ($p=0.001$).

Figure 8. Mean ± SEM wound area (a) wound edge (b), and core temperatures (c) during the first 4 hours of the operation. Also, included are fifth-degree polynomials, all with R2 close to 0.9.

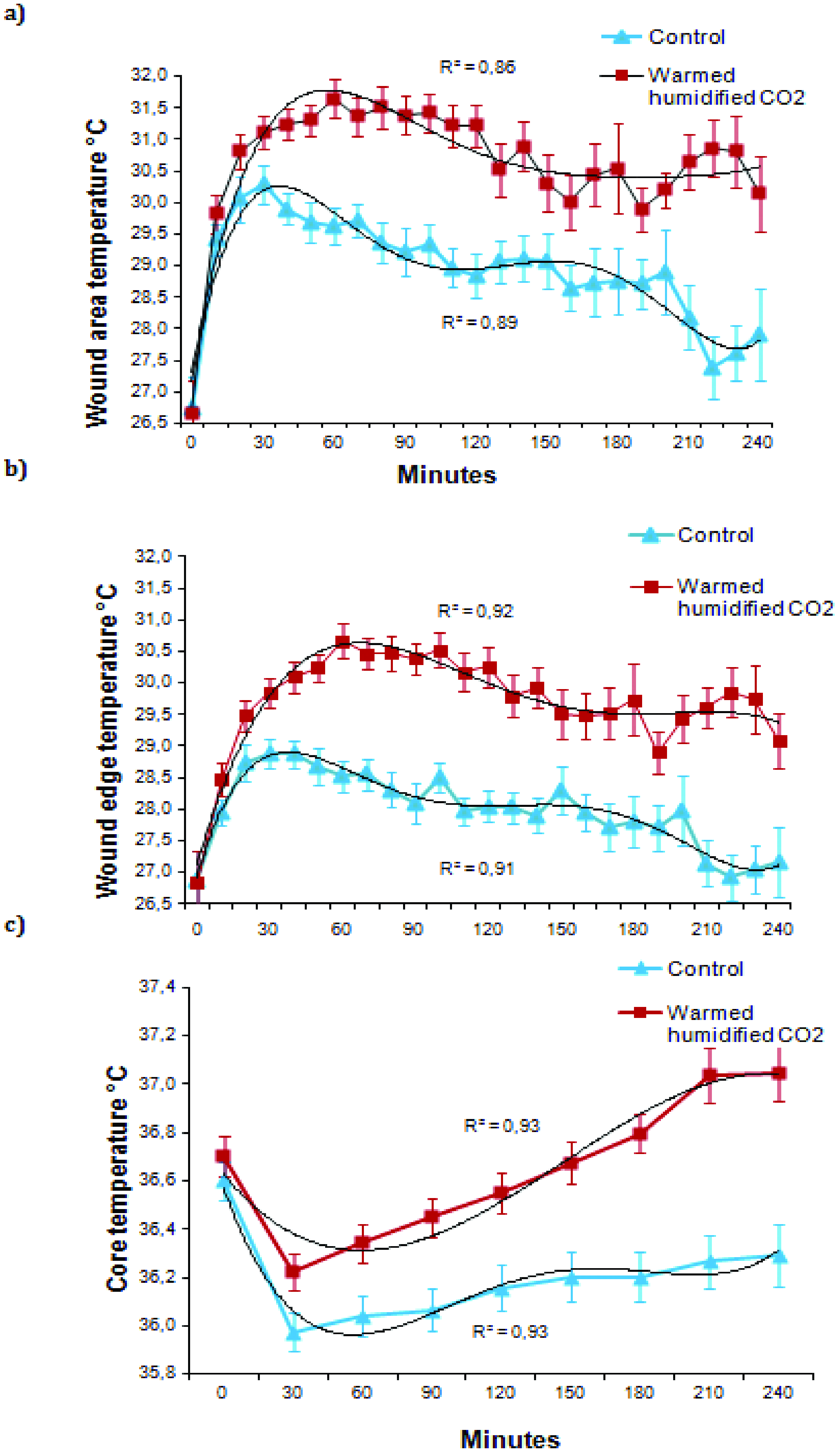


Table 7. *Perioperative temperatures (mean ± SD).*

	Warmed humidified CO_2 group (n=40)	Control group (n=39)	P	95% confidence interval
Wound area temperature during surgery °C (mean AUC)	31.3 ± 1.2	29.6 ± 1.3	<0.001	1.2 – 2.3
Wound edge temperature during surgery °C (mean AUC)	30.3 ± 1.1	28.5 ± 1.1	<0.001	1.3 – 2.3
Wound area temperature before closure of open wound °C	32.1 ± 1.6	30.3 ± 1.8	<0.001	1.0 - 2.5
Wound edge temperature before closure of open wound °C	30.5 ± 1.7	28.8 ± 1.7	<0.001	0.9 - 2.4
Core temperature °C				
Before start of surgery	36.7 ± 0.5	36.6 ± 0.5	0.179	0.4 - (-0.1)
At end of surgery	36.9 ± 0.5	36.3 ± 0.5	<0.001	0.38 - 0.82
Core temperature at end of surgery				
<36.5°C	8 (20%)	24 (62%)	0.001	
<36.0°C	0 (0%)	7 (18%)	0.005	
Core temperature during surgery °C (mean AUC)	36.5 ± 0.5	36.1 ± 0.5	0.001	0.2 – 0.7

t- Test, AUC=area under the curve.

The mean core temperature before start of surgery was similar with 36.7ºC in the warmed humidified CO_2 group and 36.6ºC in the control group. However, at end of surgery the two groups differed significantly with 36.9ºC in the warmed humidified CO_2 group and 36.3ºC in the control group ($p<0.001$). At end of surgery only 8 patients out of 40 in the warmed humidified CO_2 group had a core temperature< 36.5ºC (20%), while in the control group this was the case in 24 out of 39 (62%) patients ($p=0.001$). With a cut-off at <36.0ºC none of the patients in the warmed humidified CO_2 group compared with 7 patients (18%) in the control group were hypothermic at end of surgery ($p=0.005$). In accordance with this finding, the mean core temperature during surgery, calculated as area under the curve, was 36.5ºC in the warmed humidified CO_2 group compared with 36.1ºC in the control group ($p=0.001$). There was a significant positive correlation between duration of surgery and core temperature at end of surgery in the warmed humidified CO_2 group ($r=0.46$, $p=0.02$), whereas these factors did not significantly correlate in the control group ($r=0.03$, $p=0.84$).

STUDY IV

Median follow-up was 70.9 months and no patients were lost to follow up. Preoperative patient characteristics did not differ significantly between the treatment groups as described in Table 8. Peri- and postoperative end points are presented in Table 9. All temperatures at end of surgery as well as the temperature differences between core and wound were significantly higher in the CO_2 group. Mean operating time was 218 minutes in both groups and all remaining end points tended to be in favor of the CO_2 group.

Table 8. Demographic characteristics of the study cohort including comparisons between patients with and without humidified warmed CO_2

Clinical parameters	Humid warmed CO_2 (n=80)	Controls (n=78)	*P*-value
Age, years	62.9 ± 14.0	63.4 ± 17.7	0.833
Male gender	46 (57.5%)	45 (57.7%)	0.980
BMI, kg/m^2	25.5 ± 4.5	25.3 ± 4.4	0.787
Colon/rectal cancer	59 (73.8%)	58 (74.4%)	0.930
Primary surgery	72 (90.0%)	64 (82.1%)	0.149

*Data are presented as mean ± SD for quantitative variables, and as No. (%) for qualitative variables. BMI: body mass index

Figure 9 depicts the relationship between study groups and survival in all subjects. We could not show that patients receiving insufflation of warmed humidified CO_2 had a better overall survival compared with control patients (p=0.508). But as shown in Figure 10, patient with a core temperature ≥36.0ºC at end of surgery exhibited a better overall survival compared with to those who did not (OR 0.5, CI95% 0.26-096, p=0.035).

The effectiveness of the commercially available system used in **Study III** was compared with the noncommercial system used in **Study II**, analyzing the differences in temperatures between the CO_2 groups in both studies. Mean and final core, wound area and wound edge temperatures were all higher in the CO_2 group of **Study III** compared with the CO_2 group of **Study II** (Table 10), whereas other pre- and perioperative data did not differ significantly.

Table 9. End points

End point	**Humid warmed CO_2** (n=80)	**Controls** (n=78)	***P*-value**
Operation duration (min)	218.0 ± 97.2	218.0 ± 94.2	1.0
Anesthesia time (min)	297.3 ± 108.9	302.5 ± 104.9	0.775
Intra operative bleeding	473.7 ± 613.1	468.3 ± 537.3	0.954
Mean core temperature	36.2 ± 0.6	35.9 ± 0.5	0.005
Mean core temperature ≥36.0°C	51 (64.6%)	32 (42.7%)	0.006
Mean wound edge temperature	29.8 ± 1.2	28.5 ± 1.1	<0.001
Mean wound area temperature	31.0 ± 1.2	29.7 ± 1.1	<0.001
Final core temperature	36.5 ± 0.6	36.1 ± 0.6	<0.001
Final core temperature ≥36.0°C	66 (82.5%)	49 (65.3%)	0.015
Final wound edge temperature	29.7 ± 1.9	28.5 ± 1.7	<0.001
Final wound area temperature	31.2 ± 2.0	30.1 ± 1.9	0.001
Mean core – mean wound edge temp	6.4 ± 1.1	7.4 ± 1.1	<0.001
Mean core – mean wound area temp	5.2 ± 1.1	6.2 ± 1.2	<0.001
Final core – last wound edge temp	6.8 ± 1.8	7.7 ± 1.7	0.006
Final core – last wound area temp	5.3 ± 1.9	6.0 ± 1.9	0.023
Wound rupture	1 (1.3%)	3 (3.8%)	0.364
Re-operation	7 (8.8%)	7 (9.0%)	0.960
Surgical site infection <30 days	13 (16.3%)	13 (16.7%)	0.944
Mortality	19 (23.8%)	22 (28.2%)	0.519
Readmission <30 days	12 (15.0%)	13 (16.7%)	0.774
PRBC transfused (units)	0 [0-13]	0 [0-9]	0.738
Plasma transfusion	0 [0-12]	0 [0-5]	0.600
Platelet transfusion	0 [0-4]	0 [0-2]	0.992

*Data are presented as No. (%), mean ± standard deviation, or as median [range].

Overall univariate mortality predictions for all patients during elective major open colon cancer surgery are shown in Table 11. As expected, age and cancer showed a strong impact on mortality (p=<0.001 and p=0.004, respectively). Moreover, a final core temperature ≥36.0ºC (p=0.035) and a higher final wound edge temperature (p=0.011) were associated with lower mortality, as well as a smaller difference between final core and final wound edge temperature (p=0.017) improved survival. A multivariate analysis adjusted for age (p= 0.001) and cancer (p=0.165) showed that the temperature difference between final core and final wound edge temperature was associated with a better overall survival (p=0.050).

Table 10. Heating efficiency of the commercially available system used in study III compared with the noncommercial system by analyzing the differences in temperatures between the CO_2 groups in both studies.

End point	**Noncommercial** (n=37)	**HumiGard™** (n=43)	***P*-value**
Mean core temperature	35.9 ± 0.4	36.5 ± 0.5	**<0.001**
Mean core temperature ≥36.0°C	17 (45.9%)	34 (81.0%)	**0.001**
Mean wound edge temperature	29.5 ± 1.2	30.2 ± 1.0	**0.011**
Mean wound area temperature	30.7 ± 1.2	31.3 ± 1.1	**0.032**
Final core temperature	36.2 ± 0.5	36.9 ± 0.6	**<0.001**
Final core temperature ≥36.0°C	26 (70.3%)	40 (93.0%)	**0.008**
Final wound edge temperature	29.3 ± 2.1	30.3 ± 1.6	**0.024**
Final wound area temperature	30.6 ± 2.2	31.9 ± 1.5	**0.008**

Figure 9. Cumulative survival in the CO_2 and control group in all subjects after major open colon surgery (log rank p=0.508). Small vertical lines represent end of follow up.

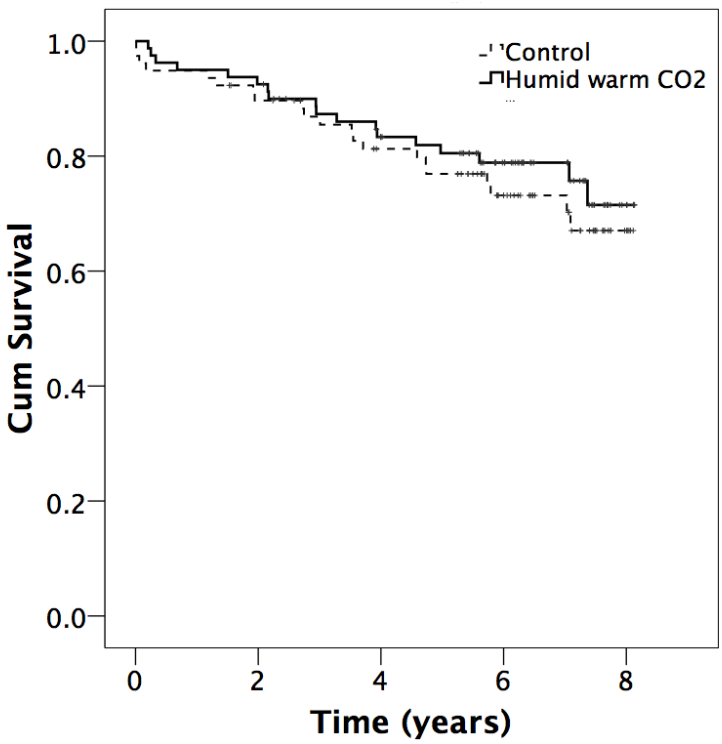


Figure 10. Cumulative survival in patients with a core temperature ≥36.0 °C and <36.0 °C at end of surgery in all subjects after major open colon surgery (log rank p=0.035). Small vertical lines represent end of follow up.

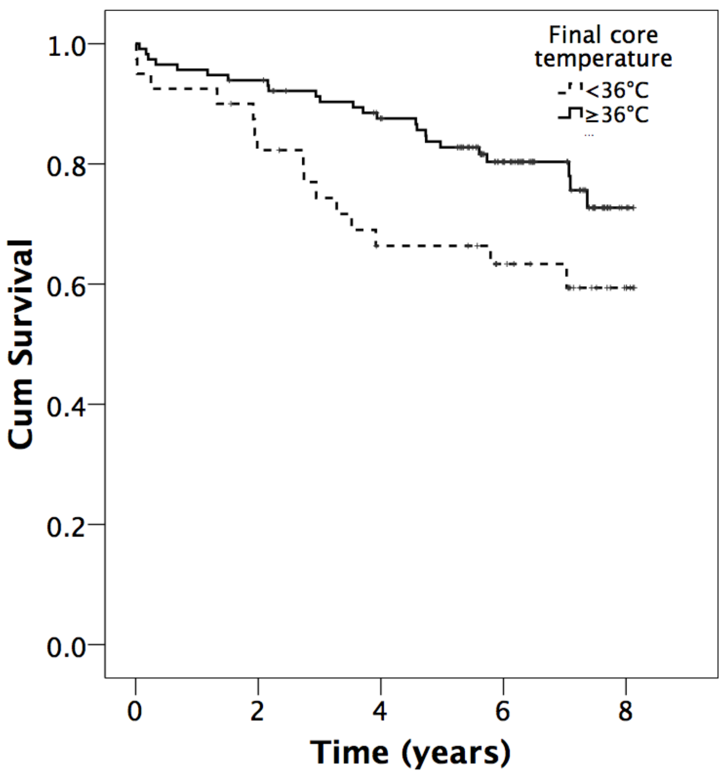


Table 11. Cox analysis for the prediction of mortality

Variable	**Univariate analysis**		**Multivariate analysis**	
	OR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Mean core – mean wound edge temp	1.24 (0.96 – 1.59)	0.097		
Mean core – mean wound area temp	1.15 (0.90 – 1.48)	0.256		
Final core – Final wound edge temp	1.24 (1.04 – 1.47)	0.017	1.20 (1.00-1.44)	0.050
Final core – Final wound area temp	1.13 (0.97 – 1.32)	0.125		
Age (10-year increase)	1.78 (1.37 – 2.33)	<0.001	1.05 (1.02-1.08)	0.001
Cancer	8.1 (1.95 – 33.7)	0.004	2.92 (0.64-13.3)	0.165
Final core temperature ≥36°C	0.50 (0.26 – 0.96)	0.035		
Mean core temperature	0.95 (0.54 – 1.69)	0.869		
Mean core temperature ≥36°C	0.93 (0.50 – 1.75)	0.821		
Mean wound area temperature	0.87 (0.68 – 1.10)	0.242		
Mean wound edge temperature	0.81 (0.63 – 1.03)	0.089		
Final core temperature	0.86 (0.51 – 1.43)	0.551		
Humidified warmed CO_2	0.80 (0.43 – 1.50)	0.490		
Final wound area temperature	0.88 (0.76 – 1.02)	0.095		
Final wound edge temperature	0.80 (0.68 – 0.95)	0.011		

GENERAL DISCUSSION

A major finding of this thesis was that insufflation of dry room-tempered CO_2 with a gas diffuser increases the average surface temperature in an open surgical wound cavity. Furthermore, when the CO_2 gas was warmed and humidified both the open wound temperature as well as the core temperature significantly increased. The effect was even stronger when the insufflated CO_2 gas was better warmed and fully humidified. This improved the maintenance of normothermia throughout the surgical procedure. Normothermia at end of surgery and a small end-of-operation temperature difference between final core and wound edge temperature were significantly associated with better patient survival in open colon surgery.

NEGATIVE CONSEQUENCES OF PERIOPERATIVE CORE HYPOTHERMIA

Perioperative core hypothermia is known to trigger thermoregulatory vasoconstriction, which decreases subcutaneous oxygen tension [22, 23]. Also, hypothermia shifts the oxygen saturation curve to the left, whereby tissue oxygen tension will be reduced (the Bohr Effect). Several clinical studies have found that the incidence of postoperative wound infections correlates with intraoperative subcutaneous oxygen tension [24, 25]. Subcutaneous tissue is particularly vulnerable to vasoconstriction, since there is little regulation of blood flow, except in response to locally applied heat [23, 26]. Inadvertent perioperative hypothermia, defined in our studies as <36.0 ºC core temperature, is well known to be associated with a multitude of poor outcomes. Large prospective, randomized clinical trials have shown that even mild hypothermia between 34-36 ºC causes numerous adverse outcomes in a variety of patient populations. These include increased risk for surgical wound infections[27-29], increased blood loss through impaired platelet function[30], prolonged duration of anesthetic drugs[3], delayed wound healing[27], delayed post anesthetic recovery[31], prolonged hospitalization[27], and postoperative shivering and thermal discomfort[3]. In a randomized study, patients undergoing colon surgery with an average 1.9 ºC fall in core temperature had a tripled risk of surgical wound infection and prolonged hospitalization compared with patients who were actively kept normothermic[27]. In high-risk heart patients a slight fall in core temperature by only 1.3ºC implied a three-fold higher risk for adverse myocardial outcomes [32]. Hypothermia also causes hypertension in elderly patients and individuals at high risk for cardiac complications [33]. A retrospective study from 2014 found that unintentional perioperative hypothermia during elective surgery was associated with a 4-fold increase in mortality and a doubled complication rate, in which sepsis and stroke increased the most [34].

THE SIGNIFICANCE OF THE OPEN WOUND TEMPERATURE

The significance of the temperature of the open wound and the possibility to warm it during surgery are almost unexplored in humans and very little is known about what happens when the internal tissues are abruptly exposed to the relatively cold and dry ambient air in the operating room. It is not known what the optimal wound temperature is during open surgery, however, normally the abdominal viscera is particle free, body tempered, and has tissue surfaces moist with peritoneal fluid. Thus, one may presume that during open surgery a local temperature increase towards normal and humidification is advantageous.

Insufflation of dry CO_2

In **Study I** we demonstrated that it is possible to rapidly increase the surface temperature in a thoracic surgical wound by insufflating the wound cavity with dry, room-tempered CO_2 via a gas diffuser for as short time as two minutes. The patients underwent open cardiac surgery, since it implicates a large, deep wound cavity. Besides, in our cardiothoracic department we routinely fill the open surgical wound cavity with CO_2 for the prevention of arterial air embolism in patients undergoing open heart surgery [15, 20, 21, 35]. The wound cavity was insufflated with CO_2 for 2 minutes, recognizing that at a flow rate of 5 L/minute about 1 minute is needed to fill up the wound cavity with CO_2 (>99% CO_2)[21]. The flow rate of 5 L/minute is the lower limit that counteracts the influence of diffusion during steady state in a cardiothoracic wound model[20]. We limited the insufflation period to 2 minutes, as we presumed that this short period would ensure an effect of CO_2 as well as a constant core temperature. The results confirmed that the total wound surface area temperature returned to start temperature 2 minutes after cessation of CO_2 insufflation. We did not humidify CO_2 since dry gas insufflation is currently used for de-airing in cardiac procedures.

The rapid increase in surface temperature can be explained by the green-house properties of CO_2, which provides an effective thermal insulation of the open wound as evaporation and convection are reduced[15]. It could also be that CO_2 induces a local vasodilatation within the wound. This might lead to a redistribution of body heat from deeper compartments into the surface of surgical wound.

The surface temperature increased significantly in the whole wound (Area V), and in the two cranial thirds proximal to the gas diffuser (Area IV). However, in the third area closest to the gas diffuser (Area I) we unexpectedly found a cooling effect, which according to the images was coolest in the proximity of the diffuser. This localized cooling effect was most probably caused by convection around the diffuser from the delivered laminar flow of room-tempered dry CO_2, although the outflow velocity was as low as 0.1 m/sec[20]. Moreover, already before insufflation the diaphragmal third of the wound area (Area I) was cooler than the other two thirds, indicating a lower tissue perfusion in this area. Its lower perfusion rate may have amplified the cooling effect of convection and this would explain why this area did not recover its original temperature 2 minutes after cessation of CO_2 insufflation. A possible way to overcome the problem of convection would be to lower the CO_2-flow. This would decrease the cooling effect, but at the same time, due to the impact of diffusion, increase the air content in the wound cavity. Thus, a reduced flow may interfere with the warming effect. Another solution would be to use polyurethane foam in the diffuser with more but smaller paths, which would lower the outflow velocity even more. However, this would increase the resistance of the foam and thus would build up a pressure gradient proximal to the diffuser. A larger diffuser would also reduce the local cooling effect by decreasing the flow velocity without interfering with the flow. The drawback of this alternative is that a larger diffuser may interfere with surgery in the limited space of the surgical field. Insufflation of CO_2 might alternatively be enhanced by using two or more diffusers. Again, this may interfere with surgery. Finally, the best solution in our view would be to use warmed humidified CO_2 that has a much higher heat capacity. This would promote heat transfer from the heated gas to the tissue as well as avoiding the cooling effect of evaporation. In addition, water vapor is known to be a greenhouse gas.

Study I may be criticized for lacking an important control with the exposure of the wound to some other gas that would be hypothesized not to produce a temperature raising effect. We used the wound temperature after the first two minute period as a control with the exposure of the wound to ambient air (mainly nitrogen and oxygen gas). Again, we analyzed the impact of exposing the wound to ambient air on wound temperature after the third two minute period (which followed the period of insufflation with CO_2). Thus, we used the patient's wound temperature during exposure to ambient air as his own control.

It may be argued that one should include a measure of core temperature and protected skin temperature to determine how the temperature changes related to changes occurring in body temperatures unrelated to experimental manipulation of the wound. We decided not to measure that in **Study I** because the two minute period of insufflation with CO_2 is far too short to affect core temperature and protected skin temperature. During the two minutes of insufflation we detected a significant rise in average wound temperature that we believe cannot be explained by anything else than CO_2 itself, since the patient was not warmed by any other arrangements.

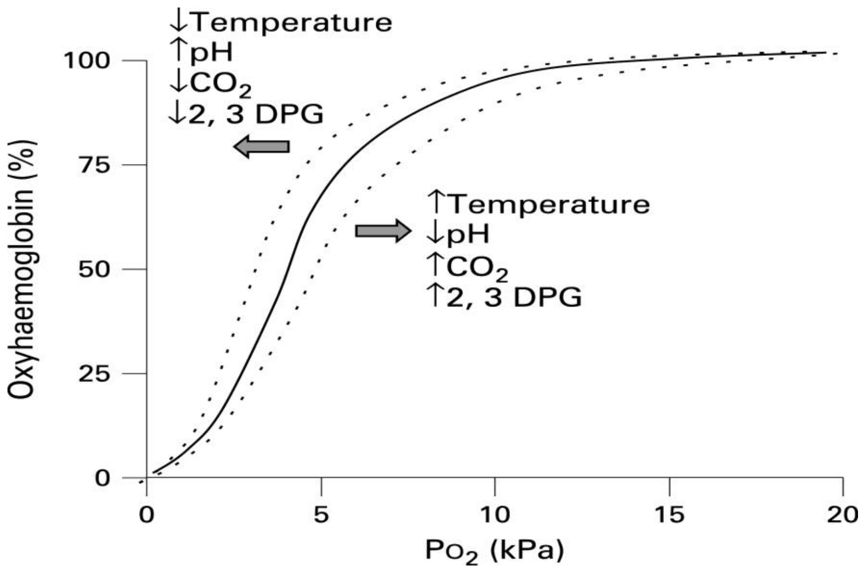
One may also ask if the drop in surface temperature in wound area I could be a reflection of the endothermic effect of gas expansion as it came out of the diffuser device and if so, could one use a heater on the diffuser to reduce this effect? The endothermic effect of gas expansion is restricted to the site of the pressure reducing port (down to 4.5-5.5 kPa), which is positioned at the CO_2 gas cylinder. Indeed, this metal port is cooled down to subzero (°C) temperatures during insufflation of CO_2 over hours. However, the dry CO_2 leaving the flow meter, which is connected distally to the port, will very quickly adopt room temperature when it travels along the 2.5 m PVC tube to and trough the gas diffuser.

Insufflation of warmed humidified CO_2

The technique of **Study II** and **III** with insufflation of warmed humidified CO_2 opens a new way to protect the tissues in open surgical wounds that are exposed to dry cold ambient air and airborne contamination. The local temperature of the open surgical wound and especially that of the open abdominal wound has until now hardly been studied in humans, although any change in local and indirectly in core temperature will affect local tissue perfusion and vice-versa. We do not yet know what the optimal local wound temperature is during surgery, but we do know that even a mild drop in core temperature especially in abdominal surgery affects patients' outcome including morbidity, mortality, and costs.

We used a continuous high inflow (10 L/min) of CO_2 to counteract the strong force of diffusion of surrounding air into the open wound cavity and to compensate for any turbulence caused by the surgeon's hands in the wound. A local atmosphere of CO_2 in the open wound has been shown to have an insulating effect, both experimentally [15, 16] and clinically [39]. Humidification of CO_2 with 93% rH at 30°C (**Study II**) and even better with 100% rH at 37°C (**Study III**) prevents evaporation from the open wound and the cooling effect that otherwise would have occurred[15, 16]. Warm humidified CO_2 has a much higher heat capacity than warm dry CO_2 and promotes heat transfer to tissues. In fact the heating capacity of the fully humidified gas increases logarithmically with temperature. In addition, water vapor itself is also a greenhouse gas. Furthermore, both a higher temperature and a higher CO_2 content will shift the oxyhemoglobin curve to the right, which will result in hemoglobin proteins releasing their load of oxygen. This improves local oxygen extraction at the tissue level (the Bohr Effect, Figure 11).

Figure 11. The oxygen-hemoglobin dissociation curve.



Since general anesthesia causes vasodilatation, heat is more easily redistributed from the core out to the wound surface. The lower the surface temperature the greater is the core-to-peripheral temperature gradient and thus the rate of heat redistribution and heat loss. Vice versa, if the wound surface temperature is warmed, the heat will redistribute to deeper departments within the body and core temperature increases and thus the possible risk of wound infection may be attenuated. A warm humidified CO_2 atmosphere provides an effective thermal insulation of the open surgical wound and the resulting increase in wound and core temperature may help to reduce the negative consequences of mild perioperative hypothermia.

More effective heating and humidifying system

In **Study II** our simple heating system did not deliver fully humidified CO_2 at body temperature (93% rH and 30°C) to the open surgical wound. This called for technical improvements of the heating system. Thus, in **Study III** the same flow rate was used but a more effective heating system delivered humidified CO_2 at 100% rH and 37 °C to the surgical wound. This corresponds to 43.9 mg of water per liter CO_2, which is 55% more than delivered with the noncommercial device used in **Study II**.

Why a clinical study in patients undergoing open colorectal surgery?

Over the last decades open abdominal surgery has lost ground to laparoscopic surgery. The latter technique has several advantages including that it prevents exposure of the internal tissues to ambient dry and cold air and airborne contamination. Instead, dry CO_2 is usually insufflated as a jet through a thin port into the peritoneal cavity, and CO_2's high solubility explains why CO_2 will cause much less harm than air if unintentionally introduced into the venous or arterial system. Many animal and clinical studies have been undertaken to investigate if the insufflated dry CO_2 jet causes local desiccation of the peritoneum and promotes local inflammation and whether or not this can be prevented by humidification. Based on the results of these studies many centers now use commercially available techniques to apply warmed and humidified CO_2 during laparoscopic surgery.

The positive effects of laparoscopically insufflated CO_2 have been evaluated both in animal and human studies. In rats CO_2 pneumoperitoneum has a protective effect against induced bacterial peritonitis. Furthermore, two recent meta-analyses demonstrated that insufflation of warmed humidified CO_2 compared with dry gas during laparoscopic surgery reduced the incidence of intraoperative hypothermia, postoperative pain, and opiate use [36, 37]. However, one must bear in mind the many basic differences between open and laparoscopic surgery, including the negative effects of an increased intraperitoneal pressure in the latter.

We chose to investigate the effects of warmed and humidified CO_2 during open abdominal surgery in patients because of many reasons. First, this type of surgery implicates a large, deep wound cavity, which exposes a very large wound surface to ambient air promoting evaporation, heat loss and the risk of intraoperative hypothermia. Second, the clinical application of warmed humidified gas is now common in laparoscopic surgery. Third, the technique of CO_2-insufflation via a diffuser is clinically used in cardiothoracic surgery. Forth, perioperative core hypothermia worsens clinical outcome i.e. increases the risk of surgical wound infection[16-18], morbid cardiac events[23], as well as prolongs hospital stay and increases costs. Fifth, despite growing awareness of the negative consequences of perioperative hypothermia more than one-third of general surgery patients undergoing abdominal operations will be hypothermic on arrival in the postoperative care unit [30, 32]. Sixth, since the rate of surgical site infection is 5-10 times higher in open colon surgery compared with cardiothoracic surgery, the potential impact of the technique is much larger in open colon surgery. Seventh, the technique opens a new way to protect exposed tissues in open surgical wounds, where such a vital parameter as local temperature has hardly been studied in humans.

Local wound temperatures during open colorectal surgery

Both in **Study II** and **III** the wound area and edge temperatures increased during the first hour of surgery in the groups with warmed humidified CO_2, after which these temperatures stabilized. Also in the control groups these temperatures increased at start, but peaked already after 30 minutes of surgery, although at lower values, after which there was a tendency to a steady decline (Figure 7 and 8).

In both **Study II** and **III**, as shown in Figure 7 and 8, the actual wound temperatures were surprisingly low. In **Study III** there was a peak wound area and edge temperature of 30.3ºC and 29.0ºC in the control group and 31.7ºC and 30.7º in the warmed humidified CO_2 group, respectively. It is not known what the optimal wound temperature is during open surgery, nor is it known if the 1.8ºC increase in wound area at end of surgery in the warmed humidified CO_2 group is of clinical importance. However, as the normal temperature of the abdominal viscera is close to the core temperature of 37ºC in a normothermic healthy adult,[38] one may presume that a temperature increase towards normal is advantageous. The initial wound temperature increase and the following stabilization in the treatment group both in **Study II** and **III** is most likely explained by the heating effects of warmed humidified CO_2. In the control group both in **Study II** and **III** where the open surgical wound is unprotected, there was in contrast a continuous drop in temperature most probably due to a loss of heat through evaporation throughout surgery. This heat loss seems to be the main reason why the core temperature of the control group was significantly lower than in the treatment group.

Further aspects of the local wound temperature measurements are also of interest. In both groups of **Study II** there were a significant difference between the wound area and edge temperatures with the area temperatures being ~ 1.5°C warmer than the edges (slightly smaller difference than in **Study III**). The reason for this is probably multifactorial. Normal skin temperature is lower than that of deeper tissues and the edges are also exposed to a greater tension by the circular abdominal wall retractor, which must restrict the local perfusion. When comparing **Study II** and **III**, the wound edge temperature in the CO_2 group was approx. 0.8°C higher in **Study III**. This was expected with the increased temperature and humidity of the insufflated CO_2. In **Study III**, the tendency to more frequent rinsing with saline of the abdominal cavity in the control group versus the warmed humidified CO_2 group at end of surgery cannot explain the drop in wound temperatures that were seen in the control group. On the contrary, this would rather have increased wound temperatures, as the saline was preheated to 37°C.

Core temperature during open colorectal surgery

Both in **Study II** and **III** there was a significant difference in core temperature between the two groups despite our efforts with conventional methods to avoid perioperative hypothermia in all patients. In **Study II** the warmed humidified CO_2 group had a median core temperature during surgery of 36.0°C, while the control group had 35.5°C. Both groups started at the same core temperature, 35.9°C, but at the end of surgery there was an increase with 0.3°C in the warmed humidified CO_2 group compared with a decrease of 0.1°C in the control group. In **Study III** the mean core temperature during surgery was 36.5°C and 36.1°C in the warmed humidified CO_2 group and in the control group, respectively. Also, both groups started at approximately the same core temperature, ~36.6°C. Thereafter, it increased with 0.2°C in the warmed humidified CO_2 group, but decreased with 0.3°C in the control group to end of surgery. Depending on where one wants to define the lower cut-off point for normothermia, one gets different percentages of patients that were hypothermic at end of surgery in each group, although the groups still differed significantly. When using the traditional definition of hypothermia, i.e. a core temperature of <36.0°C, in **Study II** 55% (20/36) of the patients in the control group were hypothermic at end of surgery compared with 24% (9/38) in the warmed humidified CO_2 group ($p<0.01$). In **Study III** 18% (7/39) in the control and none of the patients in the warmed humidified CO_2 group were hypothermic at end of surgery. With a core temperature cut off at <36.5ºC, 62% (24/39) of the patients in the control group were hypothermic, while this was the case in 20% (8/40) in the warmed humidified CO_2 group at end of surgery in **Study III**. It may still be argued that a difference in core temperature between the groups of about 0.5°C in both studies is rather modest, but some patients in the control group in **Study II** even dropped down to 34°C in core temperature. To what extent the attained 0.5°C difference in core temperature can be proven to make a difference in objective outcome data remains to be settled in a much larger study. However, in **Study IV**, the patients with a core temperature ≥36.0°C compared to the ones with a core temperature of < 36°C at end of surgery had a better overall survival ($p=0.035$). Besides, in **Study II** patients in the treatment group were extubated significantly earlier. This may possibly be explained by the higher core temperature and thereby a faster elimination of anesthetic drugs. However, patients in the treatment group of **Study III** were not extubated significantly earlier, indicating that other factors than warming could have been of importance, like when the anesthetic staff shifted in the afternoon, or that the anesthetic staff did not follow strict criteria i.e. normothermia for extubation. Moreover, any awake patient will feel distressed and be prone to shivering when subjected to mild core hypothermia (<36.0°C). Because of the recognized drawbacks of perioperative hypothermia, centers usually have to follow international guidelines [40, 41] and some centers do not even get reimbursement for patients if perioperative hypothermia occurs.

Relation of intraoperative temperature to postoperative mortality in open colon surgery

The retrospective Study IV was an almost 6-year follow up of all patients included in **Study II** and **III**. We looked at the potential ability of increased wound temperature to improve long term survival after major open colon surgery. This can be attributed to at least three different mechanisms.

First, perioperative hypothermia has been confirmed to increase cardiac demand and, consequently, to increase the risk of cardiac morbidity [[42]]. Those patients who survived a postoperative cardiac event continued to have a substantial risk of cardiac death, with a hazard ratio of 18 (95% CI, 6-57) in the first 6 month after discharge. In patients with cardiac risk factors who undergo non-cardiac surgery, perioperative maintenance of normothermia has been associated with a reduced rate of morbid cardiac events and ventricular tachycardia [39]. These data are consistent with our findings that patients with a core temperature ≥36.0ºC at end of surgery demonstrated a significantly better overall survival compared with those with core temperature <36.0ºC at end of surgery. Also, the treatment group with insufflation of warmed humidified CO_2 was inclined to have a better long time survival, although this did not reach significance, possibly due to a Type II error.

Second, insufflation of warmed humidified CO_2 in the open surgical wound increased core and wound temperatures and decreased the difference between core and wound temperatures. These changes may indicate an improved perfusion and a better oxygenation of the open surgical wound, where wound edge temperature is a more sensitive indicator of wound tissue perfusion than wound area, since the latter temperature is influenced by all exposed internal tissues. A recently published rat model showed that insufflation of warmed humidified CO_2 into the abdominal cavity during open abdominal surgery caused a rapid raise in wound tissue oxygen tension[29]. The humidification and warming to physiological temperature of the insufflated CO_2 decreases desiccation from the open wound and increases overall wound temperature thereby improving general wound perfusion and oxygenation.

Third, averting desiccation of the exposed peritoneal mesothelium by insufflation of warmed humidified CO_2 has been shown to reduce intra-peritoneal tumor dissemination in animal models, a finding which is consistent with maintaining the physiological integrity of the mesothelium as an intact barrier to tumor infiltration[3, 41]. The peritoneum was the only site of metastasis in > 50 % of patients with metastatic disease[23] and such metastasis remains fatal[43]. This could have influenced the long term survival in **Study IV**.

The strength of **Study IV** is that the long term effects of intraoperative wound area and wound edge temperatures have to our knowledge not been studied before. Importantly, patients were warmed with standard warming measures according to the NICE guidelines[2]. In addition, 94% of the patients received epidural analgesia together with general anesthesia and this combination has been shown to increase long-term survival after colon surgery in a retrospective study[43], which could be due to a reduction in neuroendocrine response and attenuated immunosuppression. Furthermore, none of our patients were lost to follow up.

SURGICAL SITE INFECTIONS

Wound healing and wound infection rates correlate with the perfusion and thus the oxygenation of the wound tissues and edges [24, 25]. Lowering of local wound and edge temperatures as well as a lowering core temperature will directly and indirectly cause vasoconstriction, hypoperfusion, local hypoxia, and hence increase the risk of surgical site infection[44]. A recent experimental study in rats has shown that insufflation of warmed, humidified CO_2 following open abdominal surgery increases intraoperative PtO_2 and decreases loss of peritoneal mesothelium[45]. In the former study insufflation of warmed, humidified CO_2 into the abdominal cavity during open surgery caused an immediate and clinically significant increase in local PtO_2 compared with exposure to ambient air. The increase in PtO_2 was an additive result of the delivery of CO_2 and avoidance of evaporative cooling via the delivery of gas humidified at body temperature. It is likely that an increase in local PtO_2 and protection against loss of peritoneal mesothelium will interact to prevent postoperative surgical site infections. The role of high PtO_2 in avoiding wound infections is well established[44], however, it is likely that maintenance of an intact mesothelial layer may further decrease the risk of infection, as the mesothelial layers provides both a physical barrier and also plays an active role in immune response[46].

Assuming a power of 80%, a type I error of 0.05, and that the method would half a surgical site infection rate from 5% to 2.5% or from 10% to 5%, 1806 and 864 patients, respectively, would have to be recruited to a large multicenter trial involving major colon surgery. Since the number of included patients in **Study II** and **III**, each represent less than 10% of the necessary number of patients needed we did not think it made sense to study SSI rate. In addition, the rate of postoperative SSI was not studied prospectively and not all patients underwent major colon surgery. Since we in none of our studies investigated the SSI rate prospectively, any retrospective review of data would have to be afflicted with uncertainty, especially when the standard definitions of SSI cannot be followed. However, when looking at a non-disputable variable such as wound ruptures that needed reoperation, we found in **Study IV** that this occurred twice in the control group only.

LIMITATIONS

In **Study II** and **III** the operating team was not blinded to type of treatment because insufflation of warmed humidified CO_2 into the surgical wound was immediately sensed by the surgical team and both seen and heard by all personnel in the operating room. However, none of the surgeons experienced the device getting in their way during surgery, given the small size of the gas diffuser.

Patients in **Study II** and **III** did not receive preoperative active warming and where only covered with preheated blankets when arriving at the pre-anesthetic room according to the clinical routine.

Inhalation of high concentrations of CO_2 may increase the breathing rate or even cause fainting-fits. However, due to the high density of CO_2, surplus CO_2 will drain away quickly from the surgical wound to the floor. The gas finally escapes via the extraction system as do anesthetic gases. Besides, when CO_2 is insufflated at a flowrate of 10L/min via the gas diffuser, the CO_2 concentration at 10 cm above the wound opening is less than 1%. Furthermore, CO_2 has been used for about 50 years now and there have not been any reports of such side-effects. When considering a method that is based on a continuous outflow of greenhouse gas, such as CO_2, we need to understand to what extent this will contribute to the worldwide CO_2 discharge. Continuous wound ventilation during a 3-hour operation will use about 3 kg CO_2. This corresponds to the discharge from a regular car during 15 minutes 'drive. It should also be noted that medical CO_2 is a recycled by-product from beer, spirits and fertilizer production. If not recovered, it would be vented to the atmosphere. Thus, the CO_2 used does not contribute to extra CO_2 being released into the earth's atmosphere.

Potential limitations of **Study IV** are that this was a retrospective post hoc study of postoperative morbidity and mortality, although patients had been randomized before surgery. Also, two different heating systems were used in **Study IV**. Moreover, **Study IV** was relatively small with a concurrent probability of Type II errors.

FUTURE STUDIES

As with any technology the new method will most likely be improved over time. Originally, we showed that insufflation of cold and dry CO_2 through a gas diffuser into the cardiothoracic wound cavity during open heart surgery could prevent arterial air embolism[35]. Since we became concerned that the use of dry CO_2 might cause desiccation and cooling of exposed tissue surfaces within the open wound cavity we have improved the technique by warming and humidifying the delivered gas. By doing so we have experimentally shown that this improvement could prevent evaporation, cooling, and desiccation of exposed surfaces.

Also, CO_2 itself is a bacteriostatic drug. For this thesis we have mainly studied patients undergoing open colon surgery. The method can, however, be used in more or less any open surgical procedure, provided that a cavity is created around the open wound surface e.g. by placing a brim around the wound edges.

There is need for larger, randomized studies investigating the influence of intraoperative insufflation of warm humidified CO_2 on postoperative complications like surgical site infections, adhesion, and cancer recurrence. A recently published study [47] showed that a low maximal body temperature (<37.4°C) after rectal surgery was an independent predictor of recurrence and survival in rectal cancer patients. The authors suggested that these patients might gain a survival benefit from warming strategies during the perioperative treatment.

It would also be of interest to look at biopsies from the peritoneum and determine if intraoperative insufflation of warm humidified CO_2 gas can prevent local desiccation and damage of the mesothelium. However, as these changes are most likely to appear 12 hours postoperative, this would be difficult to study in humans.

One possible further alternative would be to warm the humidified CO_2 gas to > 37°C to achieve more effective heating. However, this temperature could also have negative consequences on local tissue and thus has first to be tested in comprehensive animal studies.

Another interesting thought would be to use the CO_2 gas as a carrier for delivery of local antibiotics or alcohol to the open wound. The latter possibility is bacteriostatic in an experimental model and the alcohol vapor becomes non-explosive when combined with the fire extinguisher CO_2 [48].

CONCLUSIONS

This thesis concludes that

- Shortterm insufflation of dry room-tempered CO_2 gas in an open wound increases the surface temperature significantly during open cardiac surgery.

- Insufflation of warmed (30°C) humidified (93% rH) CO_2 in an open surgical wound cavity increases both the surgical wound temperature as well as the core temperature significantly during open abdominal procedures.

- Insufflation of warmed (37°C) humidified (100% rH) CO_2 increases both wound and core temperature during open abdominal procedures and helps to maintain normothermia.

- Normothermia at end of surgery and a small end-of-operation temperature difference between final core and wound edge temperature were significantly associated with better patient survival in open colon surgery.

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