The peritoneal response to injury and implications for laparoscopic insufflation

Dr Tarik Sammour

Aim To elucidate the effect of the cold, dry pneumoperitoneum used in laparoscopic surgery on peritoneal inflammatory response, and determine its impact on clinical outcome.

Methods The literature on peritoneal physiology was reviewed, focusing on the peritoneal inflammatory response and methods that should be investigated to attenuate this, including minimally invasive surgery. A meta-analysis and followup case-control study were undertaken comparing the systemic and peritoneal cytokine response in laparoscopic versus open colorectal surgery. A systematic review and follow-up double-blinded, multi-centre, randomised, controlled trial were performed evaluating the impact of warmed and humidified insufflation on postoperative pain and inflammation after laparoscopy. An experimental study was conducted in an appropriate rat model of pneumoperitoneum, investigating the impact of warming and humidification on the pneumoperitoneum associated oxidative stress response.

Findings Laparoscopic surgery is associated with a local and systemic oxidative stress and inflammatory cytokine response that is similar to that seen in equivalent open surgery. This effect is over and above that of increased intra-abdominal pressure alone. Review of the literature indicates that this inflammatory response may be caused by peritoneal desiccation due to insufflation of cold, dry carbon dioxide. However, these findings are limited by the quality of the published studies reviewed. The results of the investigations conducted in this thesis indicate that warming and humidification of insufflation gases do not reduce oxidative stress marker or cytokine levels, and do not confer any clinical benefit in prolonged laparoscopy.

Conclusions Dessication caused by cold, dry gas insufflation does not contribute significantly to the inflammatory response associated with pneumoperitoneum. Future research should re-assess the aetiology and occurrence of this response using more accurate and clinically relevant models of laparoscopic surgery.
To my father and mentor Mr Khalil Y Sammour,

whose life story continues to inspire achievement in mine.
The author wishes to thank Associate Professor Andrew G Hill first and foremost, for his tireless supervision, guidance, and teaching by example.

This work would not have been possible without the comradery of Dr Arman Kahokehr, who far more than providing comic relief, served as a beacon of youthful enthusiasm and dedication to surgery and research.

While I have chosen to dedicate this work to my father, I would also like to thank my mother, Samira Kobti, my brother and sister, Youssef Sammour and Samar Bourke, and my fiancé Shireen Goodchild. Their belief in me has sustained me throughout, and will continue to do so in years to come.

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I would like to gratefully acknowledge the Royal Australasian College of Surgeons, who granted me deferral from surgical training, and provided a very generous salary through scholarship for the duration of my research.

Finally, I want to give heart-felt thanks to all the patients who consented to take part in my research for graciously volunteering their time and effort, and to those who did not for allowing me the privilege of meeting them.


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GLOSSARY

Symbols

%  Percentage
°C  Celsius
χ²  Chi squared (Chi ²) test

Numbers

8OHdG  8-Hydroxydeoxyguanosine

A

Abs  Absorbance
AH  Absolute Humidity
ANOVA  Analysis of Variance
AOA  Antioxidant Activity
AOPP  Advanced Oxidation Protein Products
ASA  American Society of Anaesthesia Score
AUC  Area Under Curve

B

bpm  Beats Per Minute

C

Ca  Cancer
CASP  Colon Ascending Stent Peritonitis
Chole  Cholecystectomy
CI  Confidence Interval
cm  Centimeters
Cr-POSSUM  Colorectal Physiological and Operative Severity Score
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<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
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<td>CV</td>
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<td>E</td>
<td>Epidermal Growth Factor</td>
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<td>EP</td>
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<tr>
<td>EtCO₂</td>
<td>End Tidal CO₂</td>
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<tr>
<td>EW</td>
<td>External Warming Device</td>
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<tr>
<td>F</td>
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<td>HPLC</td>
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<td>MDA</td>
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<td>MDC</td>
<td>Minimum Detectable Concentration</td>
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<tr>
<td>MEDDD</td>
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mg  Milligrams
Midop  Mid-operation
Min  Minutes
Mini-lap  Mini-laparotomy
µL  Microlitres
mL  Millilitres
µM  Micromoles
mm  Milimeters
Morph  Morphine Equivalent
µPA  Urokinase Plasminogen Activator

N
n  Number
N  No
NA  Not Applicable
nm  Nanometers
nM  Nanomoles
NO  Nitric Oxide

O
oLab  Oxidized Low Density Lipoprotein Autoantibodies
oLDL  Oxidized Low Density Lipoprotein
OT  Operation / Operating Theatre

P
PACU  Post-Anaesthesia Care Unit
PAI  Plasminogen Activator Inhibitor 1
PC  Protein Carbonyls
PCA  Patient Controlled Analgesia
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<tr>
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<tr>
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<td>Postoperative</td>
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<td>Control</td>
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<td>Standard Error</td>
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<td>Standardised Mean Difference</td>
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<td>Surgical Recovery Score</td>
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<td>Tissue Plasminogen Activator</td>
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CHAPTER 1: INTRODUCTION
The peritoneum is a bi-layer serous membrane that lines the abdominal cavity and its organs, providing a frictionless environment for the movement of these organs. (1, 2) Classical descriptions of peritoneal function have been limited to this “Teflon” characteristic. This role, while seemingly important, is not vital to organ function and preservation, and as a result, surgical techniques have developed where the peritoneum is traversed, resected, biopsied, diathermied, and desiccated with perceived impunity. However, there is now a considerable body of evidence that peritoneal integrity is far more important than previously thought. It would appear that a large multimodal, systemic burden results from peritoneal damage. The following sections present an account of current knowledge on peritoneal structure and function, with a focus on the peritoneal response to injury, and the clinical implications of peritoneal damage.

1.1 ANATOMY

1.1.1 Compartments

Embryological growth, movement, and rotation of intra-abdominal organs make the peritoneum the largest and most complexly arranged serous membrane in the body. Of mesodermal origin, it grows to a total area of approximately 1.8 m² in an adult (almost equal to the body surface area of the skin). (1) Various reflections of the peritoneum connect the viscera to each other or to the abdominal wall. These take the form of the small bowel mesentery, mesoappendix, transverse mesocolon, sigmoid mesocolon, lesser omentum (connecting the stomach to the liver), greater omentum, and the various ligaments associated with the liver, stomach, and spleen. (2) The peritoneal cavity is a potential space between the parietal peritoneum, which lines the abdominal wall and retro-peritoneum, and the visceral peritoneum, which envelopes the abdominal organs. In between is a thin film of serous fluid. (3) The cavity is closed, except at the communication with the outside world in the female via the two tiny openings of the fallopian tubes. (1)

Most of the peritoneal cavity consists of the greater sac, with the remainder forming the small omental bursa (or lesser sac) behind the stomach. These two regions communicate at the epiploic foramen (of Winslow). (4) More detailed description of the cavity sees it divided it into supra-colic and infra-colic compartments by the transverse mesocolon. The supra-colic compartment contains
the omental bursa, right and left subphrenic spaces, right sub-hepatic space, and left peri-hepatic space. The infra-colic compartment is further divided into the right and left infra-colic spaces, and the right and left para-colic gutters. (4) There is free communication between these compartments.

1.1.2 Blood supply
The parietal layer of the peritoneum receives its blood supply from the corresponding arteries and veins of the abdominal wall, while the visceral peritoneum, forming the larger portion of the surface, is supplied primarily by branches of superior mesenteric artery and vein. (1) Blood vessels within the peritoneum run in the deep areolar layer of the mesothelium (see section 1.1.4.1 Mesothelium). (5)

1.1.3 Nerve supply
The parietal peritoneum is innervated segmentally by the spinal nerves which supply the overlying muscles. (2) Thus the diaphragmatic peritoneum is supplied by the phrenic and intercostal nerves, which is why pain from this area is referred to the base of the neck or shoulder (C3, C4, C5). The rest of the parietal peritoneum is supplied by the corresponding intercostal, lumbar, and obturator nerves. When there is peritoneal involvement in visceral disease, most pain sensation is transmitted through the lower intercostal nerves. (5)

Traditionally it was thought that the visceral peritoneum had no afferent supply, as pain fibres were not clearly demonstrated. Therefore, pain from viscera was attributed to spasm of the visceral smooth muscle or tension on the mesenteric folds. (2) More recently, however, a distinct neuro-immuno-humoral axis has been identified, projecting via the paracrine action of local cytokines on afferent nerve endings (further described in section 1.2.3 The Neuro-immuno-humoral axis). (6)

1.1.4 Histology
1.1.4.1 Mesothelium
Bichat, in 1827, observed for the first time that the surface of the peritoneum was covered by a layer of flattened cells. (7) This was termed the mesothelium, due to its origin from mesoderm, and
its resemblance to epithelium. The mesothelium has a surface film of hyaluronic acid-rich glycoprotein (glycocalyx), and an underlying basal lamina which covers a layer of loose areolar tissue. This areolar tissue is loose and cellular over expansile parts, while very dense and thick over non-expansile parts. It has a high density of anionic sites making it negatively charged.

Some mesothelial cells appear to be more metabolically active. These are termed cubic (small, high) mesothelial cells and, while rare, are typically found on the peritoneal surface of the diaphragm, over organs with hollow reservoir function (stomach, rectum, urinary bladder, uterus and distal portion of the uterine tube) and organs that change their volume considerably (spleen, areas of the liver, ovary). These organelle rich cells have a network of intercellular contacts and a relatively dense coat of microvilli. They are thought to promote the flow of fluid within the peritoneal cavity, facilitating clearance of pathogenic contamination.

Interestingly, cells that are morphologically similar to the cubic mesothelial cells may also be identified in other sites after injury.

### 1.1.4.2 Microvilli

The mesothelial apical membrane bears a significant number of microvilli. The fact that omental mesothelial cells can transiently increase their population of microvilli up to sevenfold suggests that under physiological conditions, their concentration in any given area could reflect functional adaptation rather than static structural variation.

### 1.1.4.3 Lamellar bodies

Lamellar bodies are fine, densely packed, membrane structures that store surfactant. First described in the pleura, it is now known that the peritoneal mesothelium as a structure releases lamellar material (surfactant) in amounts similar to those produced by the lung. This surfactant is unique to the peritoneum, having distinct properties that minimise friction between adjacent organs.
1.1.4.4 Stomata

Stomata are micrometer sized openings between junctions of mesothelial cells, which are able to absorb large particles or whole cells, and form an important channel of communication via the diaphragm from the peritoneal to the pleural cavity. (1) The local absence of a basement membrane allows the stomata to communicate with underlying terminal lymphatic lacunae. These lacunae contain valves and run parallel to the muscular fibres of the diaphragm. They drain to the main lymphatic ducts via mediastinal lymph nodes assisted by the action of the “diaphragmatic pump” (see section 1.2.3.2 Immune Response). (13, 14)

1.1.4.5 Milky Spots

Small white spots over the serosa of the greater omentum representing accumulations of lymphoid tissue are called milky spots. Their finding and protective role led Morrison to call the greater omentum the ‘policeman of the abdomen’ in 1906. (1) More recently, milky spots have been identified in multiple other areas of the peritoneum. (15) Milky spots in an un-stimulated state in adults have a cellular composition of about 70% macrophages, 10% B lymphocytes, 10% T lymphocytes, and 10% mast cells. (16)
1.2 PHYSIOLOGY

1.2.1 Transport

Movement of isotonic solution across the peritoneal capillary walls depends on transcapillary pressure, which in turn is modulated by two opposing forces: capillary hydrostatic pressure directed out of the capillaries, and osmotic (oncotic) pressure of the plasma proteins directed into the capillaries. Further transport requirements are met by various surface modifications and both inter- and intra-cellular mechanisms.(7) Liquid enters the peritoneal space through the parietal peritoneum down a net filtering pressure gradient, and liquid removal is provided by an absorptive pressure gradient through the visceral peritoneum, by lymphatic drainage through the stomata of the parietal peritoneum, and by cellular mechanisms.(1) The normal peritoneal cavity contains less than 100 ml of serous fluid, which is essentially an ultrafiltrate of plasma with a protein concentration lower than 3 g/dl.(13) Little is known about the rate of turn-over of this fluid in the resting state.

1.2.2 Secretion

Mesothelial cells synthesize and secrete glycosaminoglycans, lubricant surfactant,(17) and a diverse array of inflammatory mediators and cytokines, growth factors, products of the coagulation cascade, and fibrinolytic agents (described in further detail in section 1.2.3.3 Humoral Response).

1.2.3 The Neuro-immuno-humoral axis

The peritoneum exhibits a profound generalised response to injury. This is exemplified in the significantly higher stress response in patients undergoing abdominal surgery than in those undergoing limb surgery (despite similar operating times).(18) Because the entire peritoneal cavity is linked via trans-coelomic spread of immuno-humoral factors in the peritoneal fluid, it exhibits a coordinated response to injury which is generalised and not limited to the localised area of insult.(19, 20)

There is a much higher cytokine concentration in peritoneal fluid than in plasma after gastrointestinal surgery suggesting that cytokine production occurs in a compartmentalized fashion.
within the abdominal cavity. (21-23) Furthermore, peritoneal cytokines are incompletely absorbed into the blood stream, and what is absorbed is degraded by the liver and diluted in the plasma. (24) The different responses of the same cytokines further illustrates the apparent independence of the peritoneal response from that in plasma. (25) The local effect of these cytokines must not be underestimated. The following discussion outlines the presence of a direct neural communication between the peritoneum and the brain, the various local inflammatory mediators that influence this, and their clinical implications.

1.2.3.1 Neural Innervation

The parietal peritoneum is innervated segmentally by the spinal nerves which supply the overlying muscles. (2) Thus the diaphragmatic peritoneum is supplied by the phrenic and intercostal nerves, and the rest is supplied by the corresponding intercostal, lumbar, and obturator nerves. (5) Traditionally it was thought that the visceral peritoneum had no afferent supply, as pain fibres were not clearly demonstrated. Therefore, pain from viscera was attributed to spasm of the visceral smooth muscle or tension on the mesenteric folds. (2)

More recently, a distinct neuro-immuno-humoral axis has been identified, projecting via the paracrine action of local cytokines on afferent nerve endings. (6, 26) There are two types of afferent endings: vagal afferents and spinal afferents which transmit sensory information from the gastrointestinal tract to the central nervous system. (27) Vagal afferents are either intramuscular or intraganglionic, and have cell bodies in nodose ganglia entering via the brainstem. Spinal afferents, on the other hand, are located in the serosa, submucosa and mesentery of the gastrointestinal tract, and have cell bodies located in the dorsal root ganglion projecting to the dorsal horn of the spinal cord. They follow the paths of the sympathetic and parasympathetic efferents to the gut wall. (27)

In contrast to the systemic activation, the role of peritoneal pathways in the post-operative stress and fatigue response remain poorly understood. It is likely that both systems are contributory (see Figure 1). (28) In the rat, sub diaphragmatic vagotomy blocks the effects of intraperitoneal Interleukin 1β (IL-1 β) administration, preventing increases in body temperature, and blunting
increases of brain and plasma catecholamines. (29, 30) Fleshner et al. have also shown that vagotomy reduces expression of Tumour Necrosis Factor alpha (TNFα mRNA in the brain of rats after the administration of lipopolysaccharide to the abdominal cavity. (26) Vagotomy is therefore thought to reduce the “sickness response” to intraperitoneal stimuli, with the vagus playing a major role in the surgical stress response. (31)
Figure 1. Schematic Description of the Response to Injury.
The clinical implications of the existence of a neuro-immuno-humoral axis are far-reaching and multifaceted. The degree of activation of the neuro-immuno-humoral axis is likely to depend on the duration and degree of the surgical challenge. A pro-inflammatory cytokine cascade after abdominal surgery, with direct action on the vagus nerve as a major vehicle, is a feasible contributor to post-operative pain perception, fatigue response, and ileus. Levels of peritoneal cytokines have been directly related to post-surgical complications,(21, 32, 33) and fatigue experiences after surgery.(34) Zargar-Shoshtari et al used a single dose of pre-operatively administered intravenous dexamethasone, effectively dampening the local peritoneal response after colonic surgery (as measured by local cytokine concentrations).(35) This double-blinded, randomised trial demonstrated a significant reduction in post-operative fatigue, pain by visual analogue score (once the epidural was removed), and nausea and vomiting in the treatment group. It is unclear whether the effects observed were due to effects at a local or systemic level, but the significant reduction seen in peritoneal inflammatory cytokine levels would suggest that a local response was at least a contributing factor.

The instillation of local anaesthetic agents into the intraperitoneal cavity to block the neuro-immuno-humoral axis is also under investigation (note that the axis escapes epidural blockade).(36) So far, intra-peritoneal local anaesthetics have been convincingly shown to reduce post operative pain,(37-44) but other benefits of blocking neuro-immuno-humoral activation with local anaesthetic are not fully realised yet. There have been some clinical reports to indicate that bowel motility is improved,(45, 46) and the metabolic response reduced,(47) after abdominal surgery. Early literature from Russia would also suggest a reduction in adhesion formation using intra-abdominal local anaesthetic.(48, 49) Further clinical investigations are necessary in order to further define the role of vagal neuro-immuno-humoral axis blockade.
1.2.3.2 Immune Response

The immuno-modulating effects of the peritoneal cavity were first noted by MacCallam (1903) who stressed the importance of phagocytosis and intercellular passage of particulate matter across the mesothelium.(1, 50, 51) The peritoneum has now established itself as an integral part of the human immune system. It is evident that peritoneum-associated lymphoid tissue (PALT) enjoys an intriguing relationship with both the innate immune mechanism and the gut-associated lymphoid tissue (GALT) system. Peritoneal defences are dampened by surgical destruction, leaving the patient subject to infectious complications. The peritoneum deals with infection in four general ways.(16, 52)

Step 1: The diaphragmatic pump

Bacteria injected into the peritoneal cavity can be detected in the lymphatics after 6 minutes, even before an influx of phagocytotic cells has taken place.(53) Absorption occurs through the stomata in the diaphragmatic peritoneum (described in section 1.1.4.4 Stomata), and then the lymph with its bacterial load enters the thoracic lymphatic network. Diaphragmatic movement during exhalation causes rapid inflow into the lacuna, while contraction on inhalation pushes the contents of the lacuna into the efferent system, with reverse flow prevented by valves within the thoracic lymphatics.(14)

This process is affected by several factors. Disruption to breathing patterns by general anaesthesia or abdominal wound pain decreases peritoneal clearance and, conversely, positive pressure ventilation accelerates the outflow.(54, 55)

Step 2: Innate immune system

Complement

The complement system consists of approximately 20 serum proteins. Defence of the abdominal cavity against major contamination is triggered by C3a and C5a, which are induced by products of
bacteria and inflammation. They in turn stimulate the chemotaxis of neutrophils, and degradation of basophils and mast cells. In patients with cirrhosis and ascites, the peritoneal concentration of C3 has been found to be an independent predictor of the occurrence of spontaneous bacterial peritonitis. (16)

**Neutrophils**

The peritoneum usually contains fewer than 300 cells per mm. This consists mostly of macrophages plus some desquamated mesothelial cells and lymphocytes. Abdominal surgery, contamination, or infection, elicit a rapid influx of leukocytes which may raise their numbers to more than 3000 per mm. (16, 56-58) The cytokine interferon gamma (IFN-g) may play a role in controlling the type of infiltrating leukocyte during the course of an inflammatory response, in part via regulation of resident cell chemokine synthesis. (59)

The migration of neutrophils into the peritoneum is dependent on adhesion molecules which are responsible for contact formation to the blood vessel wall and subsequent neutrophil extravasation. (60, 61) This process appears to be P-selectin (and perhaps L-selectin) dependent, but appears to be E-selectin independent. (60, 62, 63) Peritoneal mesothelial cells and microvilli constitutively express Intra-Cellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), and Platelet-endothelial Cell Adhesion Molecule-1 (PCAM-1). These are up-regulated after inflammation or cytokine stimulation (ICAM-1 expression increases approximately eightfold, 24 hours after stimulation) and appear to play a functional role in leukocyte adherence to and migration across the mesothelium. Chemokines such as Leukotriene B4 (LTB4) and Interleukin-8 (IL-8), (64, 65) produced by cytokine (TNFα and C5a) (65) activated mesothelial and peritoneal endothelial cells also play a central role in the process of leukocyte recruitment. (66-68) The creation of a chemotactic gradient across the mesothelium appears to be the primary determinant to the degree and direction of cell trafficking. (69)
**Macrophages**

Resident peritoneal macrophages are drawn to bacteria by chemotaxis. They are activated by IFN-γ, Interleukin-2 and Interleukin-12 (IL-2 and IL12), and appear to display good effector mechanisms against microbes, such as Leishmania and Candida. IFN-γ also up-regulates major histocompatibility complex (MHC) class II molecule expression on the surface of macrophages and the mesothelial cells themselves. These molecules have an antigen presenting function. In this sense, the peritoneum functions as a lymphoid organ involved in enhancement of effector cell function.

**Mast cells**

Degranulation of peritoneal mast cells releases vasoactive substances (increasing vascular permeability), complement (components of which are chemotactic for macrophages), and opsins, which coat bacteria and promote phagocytosis.

**Monocytes**

Monocyte migration across endothelial mono-layers seems to occur much like the neutrophil transmigration, although this occurs later in the piece (within 24 hours). While the process does require the interaction of specific adhesion molecules such as Monocyte Chemoattractant Protein-1 (MCP-1), in contrast to neutrophil migration, the mesothelium itself has little demonstrable effect on the process of trans-mesothelial cell migration of monocytes.

**Step 3: Specific immune system**

**T Cells**

Peritoneal involvement in the specific immune system can be traced back to the initial steps of T cell lymphopoesis. Adult human peritoneum provides a microenvironment capable of supporting a thymus-independent differentiation of T lymphocytes, and extra-thymic pathways of T lymphocyte differentiation have been demonstrated to occur in multiple sites in mice. Such
sites include the sinusoids of the liver, the intra-epithelial region of the intestine, and the omentum of the peritoneal cavity. Although these extra-thymic pathways are minimal in the young, they become predominant with age. T cells differentiated in the peritoneum display many properties distinct from those of intra-thymically differentiated T cells, (e.g. double-negative CD4- CD8-cells).(16) VCAM-1, expressed on mesothelial cells, is a ligand for adhesion molecules expressed by T lymphocytes.(77)

T cells are categorized into subsets of T helper (Th) cells according to their secretion patterns. Those secreting IL-2, IFN-g and Tumour Necrosis Factor alpha (TNFα) are designated Th1 cells, and direct cell-mediated immunity. T cells producing other interleukins (IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13), termed Th2 cells, regulate the humoral immune response. Consequently, Th1 and Th2 cells mediate distinct effector functions, some of which are cross-inhibitory.(78) This shapes the normal balance between inflammation and anti-inflammation in bacterial infection, and an exaggeration of either of these converse actions can be beneficial or detrimental for the host. The need for suppressive activity and a fine balance is highlighted by the need for lifelong tolerance against commensal enteric bacteria, while still maintaining the ability to combat infective ones.(79-81)

Studies have been performed on a highly reproducible murine model of abdominal sepsis, termed Colon Ascending Stent Peritonitis (CASP), where an open stent is inserted into the ascending colon of mice allowing transmigration of bacterial flora into the peritoneal cavity resulting in endotoxemia.(82) It has been shown that suppressive activity dominates the T helper cell response against enteric bacteria.(74, 83) CD4+ T cells in particular have a large part to play in this response, and experimental elimination of the CD4+ T cells in CASP mice improved survival from 25% in controls, to 75% after CD4+ T cell depletion.(84) There was demonstrable improved local bacterial clearance, and reduced systemic bacterial dissemination with significantly lower numbers of colony-forming units detected 20 h after CASP surgery in the peritoneal cavity, blood, and all
investigated tissues (liver, spleen, kidney and lung).(84) The blunted peritoneal T cell response in normal mice (and by extension humans) is thought to account for the extreme manifestations of peritoneal sepsis when compared with other organ compartments, and current research efforts are ongoing for methods to de-inhibit the immune response in patients with intra-peritoneal sepsis.

B Cells
In the human foetal omentum the pre-B cell progenitors can be detected from the 8th until to the 23rd week of gestation, supporting the idea that a transitory development of B cells occurs in the foetal omentum. Nevertheless, B cells represent only a minor proportion of lymphocytes (<5%) in the adult peritoneum. The human B lymphocyte repertoire includes at least three distinct B lymphocyte subsets: 1. B1a cells that develop from progenitors in the omentum and are self-replenishing in nature; 2. B1b cells, progenitors of which can be found in the splanchnic area and bone marrow, and 3. B-2 cells, which arise in the foetal liver and are replenished in adult life by progenitors in the bone.(16)

There is an equal absolute number of B1a cells in the peritoneum and the spleen, although they represent a minor proportion of the splenic B cell compartment. Little exchange between spleen and peritoneum has been observed, although splenectomy severely decreases the number and the repertoire of peritoneal B-1 cells.(79)

B2 cells migrate into the peritoneum from the circulation by two pathways. The direct pathway involves specific integrins (a4B1) that direct entry of B2 cells into the peritoneal cavity and their retention at that site. The second pathway is via the omental milky spots and involves a different set of integrins (a4B7). Surgical removal of the omentum results in a 40% reduction in the immigration of B2 cells from the circulation into the peritoneum but, interestingly, does not impair B cell exit from this compartment.(85)
Traditionally peritoneal B cells are described to produce predominantly immunoglobulin (Ig) M. More recent studies reveal that high frequencies of specialised CD8+ T cells occur in normal human peritoneum. These exhibit reduced cytolytic activity and provided B cell helper function for IgG and IgA synthesis.

**NK Cells**

NK (or natural killer cells) are large granular lymphocytes capable of destroying virally infected cells and tumour cells extracellularly by inducing apoptosis or programmed cell death. These cells are present in the peritoneum and their numbers can be increased by stimulation with IL-2 or IFN-g.

**Step 4: Abscess formation**

Abscess formation in the peritoneal cavity is the result of fibrin production by the action of vasoactive and pro-coagulant substances released during peritoneal inflammation. Normal fibrinolytic pathways are diminished during peritonitis (see section 1.2.3.3 Humoral Response). The formed fibrin clot entraps bacteria which liquefy its central core, while the external perimeter is surrounded by phagocytes (to which it is impermeable). This process serves to limit the spread of peritoneal sepsis. However once an abscess is established, it only resolves after drainage (either spontaneous or controlled).
1.2.3.3 Humoral Response

Mesothelial cells and local peritoneal immune cells respond to peritoneal injury by secreting various mediators (see Table 1 for a summary and abbreviations). This local milieu is responsible for the local and systemic inflammatory and repair processes that define the injury response and its clinical implications (Figure 2). (21, 22)

<table>
<thead>
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</tbody>
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**Table 1: Classification of Mediators Produced by Mesothelial Cells with Examples.**

Figure 2: Schematic Description of Local and Systemic Peritoneal Inflammatory Processes and their Clinical Implications.

Nitric Oxide

NO is a gas generated in the endothelium of peritoneal vessels by endothelial nitric oxide synthase (eNOS). Loss of eNOS prevents NO production, resulting in the vascular effects of inflammation (vasoconstriction, smooth muscle proliferation, and activation of coagulation elements).(90) Cytokines and bacterial products such as lipopolysaccharide (LPS) are known to down regulate eNOS.(90, 91) NO can also be produced through the activity of constitutive nitric oxide synthase within the enteric nervous system (iNOS), and is the main inhibitory neurotransmitter regulating intestinal motility.(92) iNOS expression is increased by intestinal manipulation during surgery.(92)

Chemokines

Interleukin-8 and Leukotriene-B4

As mentioned above, stimulation of mesothelial cells with either C5a or TNFα results in a significant, time-dependent release of IL-8 and LTB4 which directs the transmigration of neutrophils.(65) There is a distinct possibility that IL-8 may play a significant role at the site of resection and anastomosis in abdominal surgery. Human studies have demonstrated that the concentration of IL-8 in the region of the anastomosis of patients who have undergone rectal surgery is much higher than those who have undergone colonic surgery.(21) Whether this relates to the higher anastomotic leak and local recurrence rates with rectal resections is uncertain. Interleukin-8 has also been linked to tumour development, promoting tumour cell growth in colon cancer cells after binding to its receptors.(93)

Monocyte Chemoattractant Protein-1

MCP-1 is produced by peritoneal cells in response to a surgical insult, and is a monocyte chemotactic factor as its name would suggest. Interestingly, the capacity of the peritoneum to produce MCP-1 in response to stimulation by sepsis is substantially reduced in patients with peritonitis.(25) The transient nature of the increased production of MCP-1 may be attributed to the release of endogenous inhibitors which switch off synthesis of the chemokine. Interestingly,
production of MCP-1 in the *blood* of patients with peritonitis is *not* reduced. This limits migration of activated leukocytes into the inflamed tissue and the peritoneal cavity by reducing or even reversing the chemotactic gradient. (25) The significance of this compartmentalisation is uncertain.

**Cytokines**

Cytokines are polypeptides or glycoproteins produced by diverse cell types at the site of injury as well as by systemic immune cells. (22) Cytokines differ from classic hormones (those produced by specialized tissues and act predominantly by endocrine routes) in that they are not stored as preformed molecules and function by means of paracrine and autocrine mechanisms. (94) There is a sequential, high concentration peritoneal cytokine response after peritoneal injury (Figure 3). (24, 73, 95) These cytokines play a pivotal role in acute phase responses and their production increases with the severity and duration of the surgical insult, as well as the need for blood transfusion. (18, 21, 22)

*Tumour Necrosis Factor – alpha*

TNFα is one of the first cytokines to be produced in the peritoneum after injury. Despite a half-life of less than 20 minutes, a brief increase in TNFα is sufficient to evoke marked metabolic changes and activate mediators distally in the cytokine cascade. (94) There is evidence in the literature that the increase in plasma TNFα is deleterious in septicaemia, as demonstrated by the finding that passive immunization against this cytokine prevents lethality. (96, 97) Conversely, however, local production of TNFα in the abdominal cavity during peritonitis may have a protective function. Anti-TNFα has been found to convert a sub-lethal course into a lethal course in CASP mice. (98) Furthermore, treatment with recombinant TNFα has been reported to protect rats in the same model. (99)
Figure 3: Concentration of Cytokines in the Peritoneum after Major Abdominal Surgery.
(schematic diagram modified from Badia et al 1996)
TNF: Tumour Necrosis Factor α, IL1: Interleukin 1 β, IL6: Interleukin 6.
Studies in humans undergoing major abdominal surgery have shown that TNFα levels decreased during follow-up in patients with an unremarkable postoperative course, while in all patients with postoperative complications, peritoneal TNFα concentrations increased in advance of clinical manifestation of a complication.(100) This suggests that sequential measurements of peritoneal TNFα concentrations might be helpful in the process of early identification of patients who develop postoperative complications.

TNFα also appears to have angiogenic properties, leading to up-regulation of Vascular Endothelial Growth Factor (VEGF) in cell culture.(101)

*Interleukin - 1*

There are two known species of IL-1: IL-1α and IL-1β. IL-1α is predominantly associated with cell membranes, but IL-1β is more readily detectable in the circulation and is capable of eliciting physiologic and metabolic alterations similar to those elicited by TNF-α.(94) In fact, IL-1β is produced and released soon after TNFα. Peritoneal macrophages are the source of IL-1β, and this then reaches the lung via plasma or by direct transudation, playing a specific role in injury mechanisms at this site.(102) It thus has a significant role in the pathogenesis of acute pancreatitis-associated lung injury. IL-1β has also been identified in vivo and in vitro as having strong fibrogenic (and angiogenic) properties through up-regulation of Tumour Growth Factor Beta (TGF-β).(101)

Experimental treatment of human mesothelium with IL-1β or TNFα results in a rapid change in cell morphology and a disruption of barrier integrity in a time- and dose dependent fashion.(103) This phenomenon may be of critical importance for the initiation and progression of inflammatory and neoplastic peritoneal disease processes.(104)
Interleukin - 6

The IL-1β peak precedes a more marked IL-6 response - a timeline consistent with the observation that IL-1β induces IL-6 synthesis.(22) IL-6 is considered to be a major mediator of the acute phase protein response following injury. In comparison with other cytokines, the concentration of IL-6 has been reported to be most consistently increased in the circulation of injured patients,(22, 23) and levels appear to be proportional to the extent of tissue injury.(94) The main producers of IL-6 are endothelial cells of the peritoneal capillaries.(105) Peritoneal IL-6 production occurs within the first hour of surgery and significantly increases after 4 h, with a significant rise in serum detected after 6 h,(73) persisting for as long as 10 days.(94) Post-operatively, an early exaggerated IL-6 response precedes the clinical onset of major complications by 12-48 h,(22, 100) and like TNFα, routine measurement of local and/or plasma IL-6 after major surgery may have a valuable role in identifying patients in need of careful postoperative monitoring.(22, 100) In experimental animal studies, systemic IL-6 administration has been shown to have a direct detrimental effect on the healing of colonic anastomoses.(106)

Interleukin-10

IL-10 is considered to be an anti-inflammatory cytokine by virtue of its capacity to inhibit the production of several of the pro-inflammatory cytokines outlined above, and to down-regulate the functional properties of immunocompetent cells.(33) This serves to keep the balance of the inflammatory process, and may offer a survival advantage in that respect. Treatment with anti-IL-10 antibody increases mortality in a murine model of peritonitis.(107, 108) Levels of intraperitoneal IL-10 have also been shown to rise in advance of clinical development of major complications after rectal surgery.(100)

In conjunction with the actions of IL-10, the anti-inflammatory process is also modulated by mesothelial cell glucocorticoid receptors, and endogenous TNFα / IL-1 antagonists.(101, 109)
Growth factors

Repair of fascial / peritoneal injury involves several coordinated phases. Growth factors such as TGF-α, TGF-β and Platelet Derived Growth Factor (PDGF) have been shown to play an important role in normal peritoneal healing.(110) Mesothelial cells proliferate with a limited speed under normal homeostasis, with less than 0.5% of mesothelial cells in mitosis at any one time. When the peritoneum is injured (such as during surgery) most of the mesothelial lining is shed.(105) In response to this, the rate of mitosis increases to 30–60% of cells, stimulated by increased levels of growth factors.(111, 112)

After injury, growth factors also exert a mitogenic action on peritoneal fibroblasts, which then exhibit a significant increase in the basal mRNA levels for TGF-β and IL-10.(113, 114) The effect is increased interstitial collagen deposition, and adhesion formation.(115, 116) Peritoneal adhesions are macroscopically fibrous connections which form between organs, and from organs to the abdominal wall. The potential to form adhesions is significantly higher in visceral than in parietal peritoneal lesions.(117) Microscopically these bands are dynamic structures undergoing active remodelling.(118) Adhesions which appear dense have increased total collagen contents, and a higher collagen type I/III ratio, (119, 120) which is further accentuated by hypoxia.(121, 122) Older, “mature” adhesions tend to be cell rich, consisting of mononuclear round cell infiltrates, fibroblasts, and vascular endothelial cells. They reveal less collagen bundles than younger adhesions, and relatively more adipose tissue.(123-125)

Coagulation Cascade

The significance of the coagulation cascade in the human peritoneum is largely limited to its fibrinolytic activity, which is important in the lysis of fibrinous deposits which make up adhesions.(126) Fibrinolysis is driven by the enzyme plasmin, produced by macrophages and mesothelial cells.(127, 128) Plasmin is derived from its inactive substrate plasminogen by Tissue
Plasminogen Activator (tPA) and Urokinase Plasminogen Activator (uPA). In turn, tPA is inhibited by Plasminogen Activator Inhibitor 1 (PAI-1), to keep the balance.

Fibrinolytic activity of undamaged mesothelial cells may be important in preventing adhesions after surgery.(7, 129) If there is insufficient fibrinolytic activity, there is organisation of the fibrin matrix, replacement of adipose tissue by extracellular matrix,(118) and cellular and vascular ingrowth, leading to adhesions.(111) The fibrinolytic activity of inflamed or damaged peritoneum is markedly lower than that of normal tissue. Increased mesothelial and endothelial cell production of PAI-1 stimulated by TNFα is thought to be partially responsible for this reduction.(130, 131) However, experimental studies in abdominal surgery demonstrate higher intra-operative levels of tPA in peritoneal fluid than in plasma, but similar levels of PAI-1 and uPA, suggesting a specific local response.(110)

Matrix metalloproteinases
The matrix metalloproteinases (MMPs) are the primary enzymes responsible for extra-cellular matrix (ECM) turnover and remodelling.(132) The MMPs are a family of 28 zinc-dependent, tightly regulated endopeptidases that can collectively degrade all components of the ECM. The enzymes are secreted in zymogen form and require proteolytic cleavage of a portion of the pro-peptide for activation. MMPs are inhibited by four specific inhibitors (TIMPs 1–4), and the balance between TIMPs and activated MMPs defines the total MMP activity. In normal tissue, MMP expression is minimal. However, under circumstances of extensive remodelling, such as during tissue repair or under inflammatory conditions, MMP expression is markedly induced.(132)
1.3 CLINICAL IMPLICATIONS OF PERITONEAL DAMAGE

Abdominal surgery results in peritoneal damage. The resulting peritoneal inflammatory response has several significant and clinically relevant consequences, which can be grouped into the broad categories of local and systemic.

1.3.1 Local

1.3.1.1 Adhesions

Abdominal adhesions account for up to 75% of all small bowel obstructions, the most serious adhesion-related complication, with a 10% risk of mortality if not diagnosed and treated expeditiously.\(^{(133)}\) Colorectal procedures in particular are associated with a near 30% risk of adhesion-related complications over 4 years.\(^{(134)}\)

As described above, the interplay between growth factors (TGF-\(\alpha\), TGF-\(\beta\), PDGF) and fibrinolytic pathways drives peritoneal adhesion formation.\(^{(110, 127, 128)}\) After peritoneal injury, growth factor levels increase substantially and fibrinolytic activity is markedly reduced.\(^{(7, 111, 112, 118, 129)}\) This imbalance results in increased interstitial collagen deposition by fibroblasts, and reduced lysis by plasmin. The result is the formation of adhesions.\(^{(115, 116)}\)

1.3.1.2 Ileus

Postoperative ileus is defined as transient bowel dysmotility following gastrointestinal surgery causing nausea, vomiting, and reduced passage of bowel content.\(^{(135, 136)}\) It occurs to some extent after almost all major abdominal operations\(^{(137)}\) and has several causes, including the effects of surgical trauma, anaesthetic agents, and opioids in the peri-operative period.\(^{(138)}\)

There is clear evidence that specific inflammatory transcription factors, cytokine and chemokine up-regulation, and leukocyte recruitment have a very significant part to play, with a direct inhibitory effect on the muscularis externa of the bowel.\(^{(92, 139-141)}\) Furthermore, this affect appears to be
age-dependent, with an increase in the pro-inflammatory mediator expression with increasing age.(141) This may be the basis for the increased severity and duration of post-operative ileus observed in the elderly.

Numerous studies have also demonstrated the importance of neural mechanisms,(139) including activation of the somatic pathway,(142) and an increased sensitization of the neuro-immuno-humoral axis by the local inflammatory response.(139) The somatic pathway can be effectively blocked by utilisation of a thoracic epidural, which reduces post-operative ileus after major abdominal surgery.(143, 144)

Rodent studies directly measuring whole gastro-intestinal transit time after bowel manipulation have demonstrated a significant reduction in bowel dysmotility if the peritoneal inflammatory response is ameliorated by gene knockout, COX-2 inhibition, adrenergic inhibition, and celiac ganglionectomy.(92, 135, 145) As mentioned above, there have also been some clinical reports to indicate that bowel motility is improved in humans after peritoneal instillation of local anaesthetic solutions to block the neuro-immuno-humoral axis.(45, 46)

1.3.1.3 Tumour progression

A significant proportion of abdominal operations are performed for treatment of neoplastic disease. Despite improvement in surgical outcomes, interval loco-regional recurrence and metastatic progression remain significant challenges to overcome.(146)

Metastatic progression is not strictly regulated by genetic changes intrinsic to cancer cells. The tumour microenvironment at the site of metastasis is important in promoting tumour cell invasion.(147) Cancer cells exfoliated from the primary lesion, or leaked out of lymphatics, can bind to the endothelium and mesothelium through interaction between ligands induced by epidermal growth factor, interleukin 1β, and other humoral factors.(19, 146)
Experimental and clinical studies suggest that surgical trauma promotes tumour progression.\(^{20, 148, 149}\) It is hypothesised that this process is biphasic.\(^{19}\) First, the inflammatory response post-surgery leads to up-regulation of adhesion molecules thus promoting the anchoring of tumour cells, with preferential implantation on damaged surfaces.\(^{146, 150, 151}\) Secondly, subsequent peritoneal healing leads to growth promotion through the action of locally produced growth factors.\(^{19, 152}\) The enhancing effect of trauma is not restricted to the inflicted site but rather has a generalized character, promoting tumour growth in non-traumatised, as well as traumatised peritoneum.\(^{19, 20}\)

Some authorities suggest that the peritoneum represents the first line of defence in host resistance to peritoneal cancer spread,\(^{153}\) citing clinical evidence of trocar site recurrence from laparoscopic biopsy of peritoneal mesothelioma, ovarian cancer incisional and vaginal-cuff recurrence post-hysterectomy, and suture line recurrence in low colorectal anastomosis.

1.3.2 Systemic

1.3.2.1 Metabolic derangement

The exaggerated production of pro-inflammatory cytokines as part of the acute phase response manifests systemically as hemodynamic instability and metabolic derangement.\(^{154}\) This, in conjunction with the profound endocrine responses exhibited by patients undergoing major surgery,\(^{143}\) results in the classical pattern of reduced metabolism for approximately 24 hours postoperatively, followed by a catabolic phase of up to 2 weeks, before recovery begins to occur.\(^{154}\) This is associated with muscular protein degradation, lipolysis, disturbances in glucose metabolism, and systemic immunosuppression.\(^{155, 156}\)

The specific role of local peritoneal inflammation in metabolic derangement after abdominal surgery, as distinct from the systemic inflammatory response, has not been fully elucidated.
However, the two systems are inextricably linked, with the peritoneum being the primary site of injury and inflammatory mediator production.

1.3.2.2 Pain

The subjective sensation of abdominal pain is classically described to be a result of noxious stimulation of somatic afferents, with nerve endings in the parietal peritoneum, overlying fascia, muscle and skin.(5) This is distinct from visceral pain, which is transmitted by vagal and spinal afferents innervating abdominal organs.(157) As discussed above (section 1.2.3.1 Neural Innervation), it is theorised that a pro-inflammatory cytokine cascade after abdominal surgery, with direct action on the vagus nerve contributes to post-operative pain perception.(6, 26, 37-44) It is also thought that approximately half of these afferent fibres are “silent nociceptors” that are normally unresponsive to stimuli and only become activated in the presence of inflammation.(158)

1.3.2.3 Post-operative Fatigue

Postoperative fatigue is a significant issue following surgery, persisting for up to 3 months after major uncomplicated gastrointestinal operations, adversely affecting patients’ quality of life, and preventing them from returning to normal function.(159, 160) Research studies suggest a complex bio-psycho-social etiology,(161) but there is recent evidence that locally occurring peritoneal inflammatory responses may influence development of fatigue via the neuro-immuno-humoral axis.(34) Paddison et al measured peritoneal fluid cytokine concentrations in patients 24 hours following colorectal surgery, and found a significant positive correlation between peritoneal IL-6, IL-10 and TNFα and fatigue scores after controlling for age, gender, co-morbidity, and pre-surgery fatigue using linear mixed modelling.(34) Furthermore, the double-blinded, randomised controlled trial of preoperative dexamethasone mentioned above demonstrated a reduction in post-operative fatigue after colonic surgery correlating with a corresponding significant reduction in local and systemic cytokine levels.(35)
1.4 REDUCING PERITONEAL DAMAGE

The literature is replete with publications and research on methods to reduce peritoneal damage during surgery. These fall into three broad categories: basic surgical principles, adhesion reduction techniques, and minimally invasive surgery.

1.4.1 Basic surgical principles

There are several intra-operative strategies to reduce peritoneal injury. Many of these follow time-honoured surgical methods, and can be summarised as follows:(162, 163)

1. Reducing unnecessary handling of tissues.
3. Minimizing contamination with pus / bowel content.
4. Avoidance of tissue exposure to a dry environment.
5. Judicious use of coagulating devices to avoid thermal injury.
6. Avoidance of the introduction of foreign material into the peritoneal cavity (starch from gloves, talc, lint from gauze swabs, free ends of non-absorbable ligatures).
7. Covering raw serosal areas with a vascular graft such as omentum, broad ligament, falciform ligament, or peritoneal flap.
8. Placing the omentum behind the abdominal wound so that it lies between intestine and wound.

1.4.2 Adhesion reduction techniques

A number of agents and methods have been employed to minimise adhesion formation with varying degrees of success, including the use of corticosteroids,(164) non-steroidal anti-inflammatory drugs,(165) calcium channel blockers,(166) plasminogen activators,(167) liquids(168, 169) and, more recently, 'bio-absorbable' mechanical barriers.(170-173) There is currently no accepted standard treatment for adhesion reduction, largely because the efficacy of these techniques has been marginal.(168)
Adhesion prevention in the future is likely to require a multimodal approach perhaps incorporating anti-cytokine agents targeting specific fibrogenic pathways, including the neuro-immuno-humoral axis,(35, 174-178) with the aim of preventing inappropriate adhesion formation while otherwise preserving the normal host response to injury.

1.4.3 Minimally invasive surgery
Laparoscopy was first described in 1901 by Kelling.(179) In 1960 Kurst Semm, a German gynaecologist, developed an automatic insufflator to establish pneumoperitoneum, and in 1985 Erich Muhe reported the first successful laparoscopic cholecystectomy.(180) Laparoscopic surgery is now common place.(181) There is evidence from animal and clinical studies that the systemic stress response may be reduced after laparoscopic surgery compared to equivalent open operations for some indications.(182, 183) Furthermore, it has been argued that a reduction in plasma cytokines after laparoscopic surgery when compared to open surgery is partly responsible for improved patient recovery. (77, 182-190) However, a difference in levels of local intraperitoneal cytokines has not been convincingly demonstrated, particularly in prolonged laparoscopic procedures such as laparoscopic colectomy.(183, 191-194)

The reduction of peritoneal adhesions by performing surgery laparoscopically is not clearly defined.(195) Gutt et al. reviewed 15 studies from 1987 to 2001, revealing that there were fewer adhesions after laparoscopic procedures than after open surgery.(196) However, the authors cautiously pointed out that very few clinical studies existed, with most of the available data from very heterogeneous experimental animal studies, making a systematic analysis impossible. When epidemiological data from the SCAR-2 study on adhesion-related outcomes are considered,(197) it is apparent that the burden of adhesion-related outcome readmissions following most gynaecological procedures undertaken laparoscopically were similar to those following comparative open procedures. Similarly, in colorectal surgery, despite reports of reduced
adhesions in observational studies of laparoscopic resections. (198) A long term difference in re-operation rates for adhesions has not been demonstrated. (199, 200)

It is known that the intraperitoneal pressure associated with prolonged pneumoperitoneum induces adverse effects on the microcirculation (133, 201-203) causing hypoxia which could potentially lead to oxidative stress. (195, 204-212) This may in turn stimulate production of inflammatory cytokines, and expression of growth factors such as VEGF, resulting in an increase in adhesion formation. (213) There is also rising concern that the properties of the gas used for insufflation may be important adhesiogenic factor in themselves. The most widely used gas in laparoscopic surgery is carbon dioxide (CO\textsubscript{2}). Local acidosis caused by the CO\textsubscript{2} being converted to carbonic acid in the peritoneal fluid, results in histological damage to the mesothelial ultra-structure, which is more pronounced than when peritoneum is exposed to room air. (214-217) Yilmaz et al. also showed a significantly higher increase in peritoneal oxidative stress markers when CO\textsubscript{2} was used compared with helium for a given insufflation pressure in a rat model. (218) Another potential problem is that the gas used in laparoscopy is typically cold and dry, at 21\textdegree C and 0% relative humidity. (205) Large volumes of gas may be required for a single case (up to 500L), owing to the imperfect seal of the laparoscopic ports, and peritoneal carbon dioxide absorption. The effect of this gas flow on the peritoneal environment has probably been underestimated, with the desiccating and cooling effect producing visible structural, morphological, and biochemical changes in the peritoneal mesothelial surface layer. (205, 217, 219-224) As a result of this, active research for damage-reduction strategies involving insufflators has begun, with some promising results in animal studies. (174, 217, 224-226)
1.5 SUMMARY

The peritoneum is a metabolically active organ, responding to insult through a complex array of immunologic and inflammatory cascades. This response increases with the duration and extent of injury and is central to the concept of surgical stress, manifesting via a combination of systemic effects, and local neural pathways via the neuro-immuno-humoral axis. Peritoneal disruption by surgery contributes to post-operative adhesions, ileus, tumour progression, metabolic derangement, pain perception, and fatigue. Interventions that reduce the peritoneal inflammatory response and/or block the neuro-immuno-humoral pathway should be investigated as possible avenues of enhancing recovery after surgery, and reducing post-operative complications.

There may be a decreased systemic inflammatory response after minimally invasive surgery; however, it is unclear whether this is due to a reduced local peritoneal reaction. The following chapters outline an investigation of the local peritoneal reaction after laparoscopy through systematic literature review and original experiments. The specific questions to be answered are: Is the local inflammatory reaction significantly reduced after prolonged laparoscopic surgery compared with equivalent open surgery? Does the pneumoperitoneum itself contribute to the local peritoneal inflammatory response? If so, can this response be attenuated by conditioning the insufflation gas to improve post-operative clinical outcomes after prolonged laparoscopic surgery?
The following chapters present an account of the effects of pneumoperitoneum on the local and systemic inflammatory response after surgery, followed by an investigation of warming and humidification of insufflation gas as a means of attenuating this response.

The first chapter (Chapter 4) is a systematic review of the literature investigating the humoral inflammatory response after laparoscopic and equivalent open colorectal surgery. This indication is discussed as a model of prolonged laparoscopy, where the effects of pneumoperitoneum are relatively pronounced. Thirteen studies are systematically reviewed and 8 of these are included in meta-analysis demonstrating a significantly higher serum IL-6 on day 1 after open colorectal resection compared with laparoscopic resection. No correlations with other cytokines or clinical outcomes could be derived from the published literature. In particular, the local peritoneal humoral response was largely un-investigated.

The second chapter presents a prospective case-control clinical study designed to answer the questions posed by the previous systematic review. Fifty patients undergoing laparoscopic colectomy are compared with 25 patients undergoing open colectomy. Patients were well matched at baseline. Plasma and peritoneal fluid concentrations of IL-6, IL-8, IL-10, and TNFα were measured at 20 – 24 hours after surgery and detailed data on clinical outcome were collected. With the exception of a marginally lower peritoneal IL-6 level, the systemic and peritoneal cytokine response at 20-24 hours was found to be similar after laparoscopic versus open colonic resection, with corresponding equivalent rates of post-operative recovery. Significant correlations were found between other cytokine levels (which were similar in the two groups) and clinical outcomes.

The inflammatory humoral response is only marginally attenuated after laparoscopy compared with equivalent open surgery in the colectomy model, with the difference only demonstrable for a single cytokine and not corroborated by levels of other cytokines or correlated with clinical outcome. Therefore in Chapter 6, oxidative stress associated with pneumoperitoneum is reviewed as a
possible reason for the significant inflammatory response after laparoscopy. A systematic review of 73 relevant papers is presented including animal studies, human clinical trials, and reviews. The published data indicate that pneumoperitoneum causes a reduction in splanchnic blood flow resulting in biochemical evidence of oxidative stress in a pressure and time dependent manner. There is evidence that the use of cold, dry carbon dioxide for insufflation may be contributory, but there have not been any animal or human studies investigating whether warming and humidification of insufflation gas is beneficial in this respect.

Several clinical trials have investigated warmed and humidified insufflation in laparoscopic abdominal procedures, using pain and opiate usage as a proxy for peritoneal inflammation. These studies have been largely inconclusive due to small sample sizes. Chapter 7 presents a meta-analysis of published randomised controlled trials investigating the effect of warming and humidification on post-operative pain as determined by visual analogue score or morphine usage. Seven trials were included. There was a significant reduction in pain scores at 6 hours, day 1, and day 3; and in morphine equivalent usage on day 2 in the group receiving warmed and humidified gas. However, conclusions were limited by the poor quality of the included trials, the lack of standardisation of warming measures, and the fact that none of the studies investigated warming and humidification in prolonged laparoscopy.

The following chapter presents the results of independent testing of a commercial device specifically designed for warming and humidification of insufflation carbon dioxide. Testing is undertaken using the gravimetric method and at variable flow rates, and demonstrates that the humidifier is able to saturate 50.0L of carbon dioxide to close to saturation humidity at every flow rate tested.

After confirmation of the effectiveness of the device, a multi-centre, double blinded, randomised controlled trial investigating warming and humidification conducted in laparoscopic colonic surgery
is undertaken and presented in Chapter 9. The study group received warm, humidified insufflation carbon dioxide and the control group standard cold, dry carbon dioxide. The primary outcome was morphine equivalent use, and secondary outcomes included peritoneal and plasma cytokine concentrations at 20h post-operatively, pain by visual analogue score, intra-operative core temperature, camera fogging as rated by the surgeon on a Likert scale, and post-operative recovery using defined discharge and complication criteria, and the Surgical Recovery Score. Eighty two patients were randomised, with 41 in each arm. Groups were well matched at baseline. Intra-operative core temperature was similar in both groups. Median camera fogging score was significantly worse in the Study Group. There were marginal differences in pain scores, but no significant differences were detected in opiate usage, cytokine concentrations or any recovery parameter measured. The conclusion from these results is that warming and humidification of insufflation carbon dioxide does not attenuate the early inflammatory cytokine response, and confers no clinically significant benefit in laparoscopic colonic surgery.

The final chapter describes a re-assessment of the effect of warming and humidification in an accurate animal model of pneumoperitoneum (without further intra-peritoneal surgery). Insufflation parameters were derived from clinical trial data and scaled down with corrections for animal size or physiology. Ten male Wistar rats were included in each arm, with the study group receiving warm, humidified insufflation carbon dioxide and the control group standard cold, dry carbon dioxide for insufflation. The primary outcome was the Malondialdehyde (MDA) level in plasma and homogenates of liver, kidney, jejunum and lung. Secondary outcomes measured included Protein Carbonyl levels in plasma and tissue homogenates, histology by light microscopy, and rectal / intra-abdominal temperature measurements. Animals who received warmed, humidified insufflation had a significantly more pronounced increase in rectal and intra-abdominal temperature during the surgery. Despite this, there were no detectable differences in levels of oxidative stress markers, or histology on light microscopy between the two groups.
The main conclusion from these investigations is that the dessication caused by cold, dry gas insufflation does not contribute significantly to the inflammatory response associated with pneumoperitoneum, and that future research should re-assess the aetiology and occurrence of this response using more accurate and clinically relevant models of laparoscopic surgery.
CHAPTER 3: METHODS
The methods utilised in the experiments that follow are presented below. A detailed list of materials is presented in Appendix A. Individual variations on experimental methods and details of data analysis are presented separately in the appropriate sections.

3.1 META-ANALYSIS

3.1.1 Study question

Meta-analyses were centred on a principal study question which outlined intervention and comparison groups within a specified population, a primary outcome, and a timeline over which this outcome would be measured. This question is stated in the appropriate methods section of the meta-analysis chapter.

3.1.2 Systematic literature search

A high-sensitivity, low precision search was conducted with specified search terms that were in-line with the validated methods of the Quality of Reporting of Meta-Analyses (QUORUM) statement.(227) The search was run independently by two investigators with no restrictions on language. Foreign language studies were translated into English with the assistance of faculty at the University of Auckland. Relevant primary studies were identified from the Cochrane Central Register of Controlled Trials (CENTRAL/CCTR), the Cochrane Library, Medline including in-process and non-indexed citations, PubMed and Embase. Relevant scientific meeting abstracts and reference lists of included papers were manually searched to identify further relevant publications. The time period over which the search was conducted is stated in the appropriate methods section of the meta-analysis chapter.

3.1.3 Study selection

All search results were entered into a unified database, after which duplicate results were removed. The titles and abstracts of all the studies were screened. Published and unpublished papers that fulfilled certain inclusion criteria were retained and studies that did not were excluded.
from further review. Inclusion and exclusion criteria were stated in the appropriate methods section of the meta-analysis chapter. After application of these criteria potentially relevant publications were retrieved for more detailed evaluation. These were scrutinised for inclusion independently by two investigators, with disagreement resolved by consensus, and consultation with the senior doctoral supervisor if consensus could not be reached. A QUORUM diagram outlining every step of the above process is presented in the appropriate methods section of the meta-analysis chapter. (227)

3.1.4 Validity assessment

Assessment was performed by a single investigator who was blinded to the journal title, article title and authors of the publications. The Jadad score was used for this purpose. (228) This is a simple, validated measure of the methodological quality of randomised controlled trials. (229) It is the most widely used instrument of its kind, with its seminal publication cited in over 3000 scientific works since 1996. (229, 230) It consists of a three-point questionnaire, with each question answered with either a “yes” or a “no”. Each “yes” scores a single point, and each “no” zero points, with no fractional points.

The questions are as follows:

1. **Was the study described as randomized?**
   This includes the use of words such as randomly, random, and randomization.

2. **Was the study described as double blind?**
   A study is regarded as double blind if the word “double blind” is used.

3. **Was there a description of withdrawals and dropouts?**
   Participants who were included in the study but did not complete the observation period or who were not included in the analysis must be described. The number and the reasons for withdrawal in each group must be stated. If there were no withdrawals, it should be stated in the article. If there is no statement on withdrawals, this item must be given no points.
An additional point is given for each of the following:

1. The method of randomisation is described and is appropriate.
   A method to generate the sequence of randomization is regarded as appropriate if it allows each study participant to have the same chance of receiving each intervention and the investigators could not predict which treatment was next. Methods of allocation using date of birth, date of admission, hospital numbers, or alternation are not regarded as appropriate (table of random numbers, or computer generated random numbers are appropriate).

2. The method of blinding is described and is appropriate.
   The method is regarded as appropriate if it is stated that neither the person doing the assessments nor the study participant could identify the intervention being assessed, or if in the absence of such a statement the use of active placebos, identical placebos, or dummies is mentioned.

A point is deducted for each of the following:

1. The method of randomisation was described, but was inappropriate.

2. The method of blinding was described, but was inappropriate.

A paper reporting a clinical trial could therefore receive a Jadad score of between 0 and 5 points, with 2 or less indicating low quality, and 3 or more indicating high quality.

3.1.5 Data abstraction
Data were gathered and the results summarized on an intention-to-treat basis in prospectively designed 2 x 2 tables. For the purposes of meta-analysis, standard deviation of the outcome data was used if provided by the authors, or calculated from $P$ values, confidence intervals, or data ranges if they were not. The corresponding author for each publication was contacted if information was missing or unclear to obtain as much raw data as possible.
3.1.6 Statistical analysis

Analysis of combined data was performed using Review Manager Version 5.0 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008). Results of the meta-analysis were assessed by graphical presentations of standardised mean difference with 95 per cent confidence intervals on forest plots using the fixed or random effects model for more conservative estimates; (231) \( P < 0.050 \) was considered statistically significant. Statistical heterogeneity was evaluated using \( I^2 \) statistics, with values up to 25 per cent, up to 50 per cent, and above 50 per cent indicating low, moderate, and high levels of heterogeneity. Alternatively (or in addition) a \( \chi^2 \) test for heterogeneity was performed, in which \( P < 0.100 \) was regarded as significant. Funnel plots were used to screen for publication bias. An *a priori* sensitivity analysis was performed where possible to assess the effect of various variables on data heterogeneity.
3.2 HUMIDIFIER

The humidifier used in human and laboratory experiments was a Fisher & Paykel MR860 heated humidifier (Figure 4) specifically designed for use in laparoscopic surgery (Fisher & Paykel MR860 Laparoscopic Humidification System, Fisher & Paykel Healthcare, Auckland, New Zealand). It is used to condition the insufflation gas during surgery as the gas is delivered to the patient.(232) The following technical information is from the MR860 humidifier technical manual (Revision D) supplied by Fisher & Paykel Healthcare.(232)

Figure 4: Fisher & Paykel MR860 Laparoscopic Humidifier
(Fisher & Paykel Healthcare, Auckland, New Zealand)(232)
3.2.1 Humidifier specifications

3.2.1.1 Mechanical
The humidifier dimensions are 140 mm x 173 mm x 135 mm, and it weighs 2.54 kg without the chamber fitted (2.62kg with the chamber fitted and filled with 30 mL of water).

3.2.1.2 Electrical
The humidifier operates with a supply voltage of 230 V, a supply current of 1.0 A Max, and a supply frequency of 50 / 60 Hz. The heater plate capacity is 150 W at nominal mains voltage. The heater wire operates at 22 ± 5 V, 2.7 A Max, 60 W Max, and 50 Hz. The heater plate high-temperature cut-out is at 118 ± 6 °C.

3.2.1.3 Temperature and humidity parameters
The humidifier requires a warm up time of less than 15 minutes. Recommended ambient temperature for operation is 18 to 24 °C and for transport / storage is -10 °C to 50 °C. During normal operation the chamber set point is 37 °C. The temperature is read out via a 3 digit, 14mm, 7 segment LED with a range of 10 - 70 °C, and an accuracy of ± 0.3 °C in the 25 to 45 °C range. A high temperature visual alarm is activated at a chamber temperature of 43 °C. A low temperature audible alarm (exceeding 50 dBA at 1 m) activates after 10 min at less than 29.5 °C or 60 min at less than 35 °C. The humidifier achieves an absolute humidity of > 33 mg / mL at a gas flow rate of up to 10 L / min.

3.2.1.4 Standards and approvals
The Fisher & Paykel MR860 humidifier fulfils the standards and has received approvals of IEC 60601-1, IEC 60601-1-2, EN 60601-1, and ISO 8185.
3.2.2 Humidifier setup

3.2.2.1 Physical setup

The humidifier system is set up to deliver warm, humidified CO\textsubscript{2} to patients undergoing laparoscopic surgery. The gas is passed from the insufflator through a water chamber which is filled with 30 mL of sterile water and sits on a heater plate. Water evaporates from the chamber into the gas which flows over it. The temperature of the gas is then maintained as it travels along a heated tube to the laparoscopic port and the patient. A diagram of equipment setup is shown in Figure 5.

![Diagram of equipment setup](image)

Figure 5: Fisher & Paykel MR860 Laparoscopic Humidification System
(Fisher & Paykel Healthcare, Auckland, New Zealand)(232)
3.2.2.2 Heating circuits

The MR860 has, in effect, two heating systems. The first is the heater plate, which heats the water in the humidification chamber, humidifying the gas passing through it. The humidifier monitors the temperature of the gas at the chamber outlet with the chamber flow probe attachment of the 4-in-1 probe (see Figure 6), and controls the amount of power delivered to the heater plate to maintain the chamber set point. The second heating system is the heater wire encapsulated within the heated tube. Humidified gas from the chamber travels through the heated tube where its temperature must be maintained in order to prevent the generated humidity from condensing. The humidifier maintains the temperature along the heated tube by controlling the power delivered to the heater wire via the heater wire connector of the 4-in-1 probe.

![Figure 6: 4-in-1 Probe of the Fisher & Paykel MR860 Humidifier](image)

(Fisher & Paykel Healthcare, Auckland, New Zealand)(232)
3.2.3 Humidifier testing

A 2.5L insulated plastic chamber was constructed from polyethylene terephthalate with an inlet and outlet port for gas flow (Figure 7). The inlet port was connected to a laparoscopic insufflator (Karl Storz – Endoskope, electronic endoflator, Medipak Surgical, Auckland, NZ) via the humidifier (Fisher & Paykel MR860 Laparoscopic Humidification System, Fisher & Paykel Healthcare, Auckland, New Zealand). The outlet port was open to the external environment. A highly sensitive electronic mass flow meter and temperature sensor (calibrated for CO₂) was incorporated into the setup proximal to the humidifier to allow accurate inlet temperature and flow readings (Red-y Smart Meter, Vogtlin Instruments AG, Switzerland, Figure 8). This meter was connected to a computer for real-time data collection with a response time of 50msec and accuracy of ± 1.5% for flow and temperature across the range (Get Red-y v 4.8.0.0, Get Red-y Flow Measuring and Control Software, Vogtlin Instruments AG, Switzerland, Figure 9).

Figure 7: Schematic Diagram of Apparatus Setup.
Figure 8: Flow Meter.

Figure 9: Screenshot of Flow Meter Readout.
A digital thermometer and hygrometer was placed inside the chamber (ITW JT-07CRL Humidity & Temperature Meter, Shenzhen Jingtengwei Industry Co. Ltd, Guangdong, China). This device has a relative humidity (RH) reading range of 5-98%, with ± 3.5% accuracy, and a temperature reading range of -10ºC to 50ºC, with ± 1ºC accuracy. Ambient room temperature was kept at 22.0 ± 0.5 ºC throughout the study period.

The humidifier water vessel was weighed using an electronic scale accurate to 0.001g (Mettler PE360 weight scale, Mettler instruments AG, Greifensee, Zurich) and exactly 30.0g of water poured in. The humidifier was switched on and allowed to warm up to 37.0ºC. At this point the water vessel was weighed again, and the scale tiered at this weight. 50.0L of CO₂ was then insufflated into the chamber via the humidifier at 2.0L/min and 0mmHg (chamber open to atmosphere). The flow rate was adjusted at the insufflator to maintain the desired rate as measured by the flow meter. The temperature of the gas inflow (measured by the flow meter) and the gas outflow (displayed on the humidifier digital readout) were recorded at 30 second intervals. Measurements of temperature and humidity in the chamber were also taken at 30 second intervals. After 50.0L of gas was insufflated the water left in the humidifier was weighed, and the weight of the water that had evaporated derived. This was used to calculate the mean absolute humidity (AH) of the gas that was insufflated in mg/L by the gravimetric method using the formula:(233, 234)

\[
AH = \frac{m \times 1000}{[(50 \times To / Ta) + (1.4081 \times m)]}
\]

Where m = mass of evaporated water (grams), To = mean temperature of outflow gas (Kelvin), Ta = mean temperature of inflow gas (Kelvin)

The absolute humidity at saturation (AHₛ) was derived using the Vaisala Humidity Calculator for CO₂ (the mean temperature of the outflow gas was used to derive this).(235) The procedure was repeated at flow rates of 4.0L/min, 6.0L/min, 8.0L/min, and 10.0L/min representing a wide range of clinically relevant insufflation rates.
3.3 HUMAN CLINICAL TRIAL

3.3.1 Participants

3.3.1.1 Patient population

The study population included all New Zealand citizens and permanent residents residing within the catchment area of the three District Health Boards serving Auckland city. These were Auckland District Health Board (DHB), Waitemata DHB, and Counties Manukau DHB (see Figure 10).

Figure 10: Map of Area Covered by the Three Auckland District Health Boards.
3.3.1.2 Inclusion Criteria

All patients undergoing elective laparoscopic colonic resection for any indication and at any of the three public hospitals between April 2008 and June 2009 were screened for inclusion.

3.3.1.3 Exclusion Criteria

Exclusion criteria were: Patients under 15 years of age, acute colonic resection, hand-assisted colonic resection, decision to perform open surgery pre-operatively (intra-operative conversions were included as intention to treat), surgery for rectal lesions defined as within 15 cm of the anal verge on sigmoidoscopy / colonoscopy, stoma formation (preoperative or intra-operative decision), patients who did not have colon resected despite initial surgical plan, pre-operative steroid dependence, inability to consent or complete visual analogue scores in study questionnaires due to cognitive impairment or language barrier (see section 3.3.4 Outcome), patients with American Society of Anesthesiologists (ASA) score \( \geq 4 \), and deviation from anaesthetic protocol (see section 3.3.2.2 Anaesthesia). Conversion to open colectomy was at the discretion of the individual surgeon for concerns of patient safety, technical difficulties, or associated unexpected conditions requiring treatment by laparotomy. Conversions were recorded and analyzed in the allocated group on an intention to treat basis.

3.3.1.3 Recruitment and consent

Patients were recruited on the day of surgery. All patients were seen on a one to one basis pre-operatively by the research fellow (Tarik Sammour), and trial rationale and procedure was explained verbally. Patients were also given a participant information sheet (page 26 – 28 of the patient booklet in Appendix C: Patient booklet). Written informed consent was thus obtained prior to randomisation (page 24 – 25 of the patient booklet in Appendix C: Patient booklet).
3.3.2 Interventions

3.3.2.1 Surgery

All patients underwent routine laparoscopic colonic resection either by, or under the supervision of, consultant colorectal specialists employed by the three Auckland District Health Boards (Figure 11). Technical aspects of the surgical procedure, and post-operative care not related to the analgesia protocol (see next section) were left up to the discretion of the surgical team. Typical surgical techniques for the colectomies most commonly performed in this study are described below.

**Figure 11**: A Patient Undergoing a Laparoscopically – assisted Anterior Resection at Auckland City Hospital.
Laparoscopically – assisted right hemicolectomy

The patient was positioned supine and secured on a pneumatic mattress or “bean-bag”. The abdomen was shaved, prepared with antiseptic solution, and draped to display a sterile operative field. An umbilical port was inserted by the open Hassan technique and pneumoperitoneum established. A 30° laparoscopic camera was inserted into the umbilical port and the liver and peritoneal cavity inspected for evidence of metastatic disease. Three to four other ports, including one 12mm port, were then positioned under direct vision to allow insertion of laparoscopic instruments. The liver and peritoneal cavity were inspected for evidence of metastatic disease.

In the medial to lateral approach, the vessels supplying the area of colon to be removed are exposed first. The mesentery adjacent to the ileocaecal junction was grasped and placed under traction, tenting up the ileocolic vascular pedicle. The avascular mesenteric windows on either side of the base of the ileocolic pedicle were opened and the vessels skeletonised. The vessels were clipped and divided with scissors, a bipolar device, or a vascular linear stapler. The caecum and appendix were then elevated cranially exposing the junction of the mesentery with the retroperitoneum. The peritoneum around the caecum and terminal ileum was scored along this line to enter the retroperitoneal plane. Dissection was continued lateral to the caecum and proximal ascending colon which were mobilized fully toward the midline. The medial peritoneal attachments of the terminal ileum were divided toward the level of the duodenum. The hepatic flexure was mobilised by grasping the gastrocolic attachments and elevating them allowing adequate exposure for dissection. At this point, the bowel was sufficiently mobilized to permit exteriorization. Mesenteric division, bowel resection, and anastomosis were performed extracorporeally and the bowel returned to the abdominal cavity. The fascia of the periumbilical and exteriorization incisions was closed with non-absorbable continuous sutures. All skin incisions were closed with subcuticular absorbable sutures after infiltration with local anaesthetic, and covered with water-proof dressings.
Laparoscopically – assisted anterior resection

The patient was positioned in modified Lloyd-Davis position and secured on a pneumatic mattress or “bean-bag”. The patient’s legs were secured in adjustable stirrups. The abdomen was shaved, prepared with antiseptic solution, and draped to display a sterile operative field. An umbilical port was inserted by the open Hassan technique and pneumoperitoneum established. A 30° laparoscopic camera was inserted into the umbilical port and the liver and peritoneal cavity inspected for evidence of metastatic disease. Three to four other ports, including one 12mm port, were then positioned under direct vision to allow insertion of laparoscopic instruments.

In the medial to lateral approach, the vessels supplying the area of colon to be removed are exposed first. The sigmoid colon was grasped at the pelvic brim and mobilised superiorly to locate the inferior mesenteric vessels. Care was taken to ensure that the left ureter and gonadal vessels were identified clearly before vascular division. The avascular mesenteric windows on either side of the proximal aspect of the vascular pedicle were opened and the vessels skeletonised. The vessels were clipped and divided with scissors, a bipolar device, or a vascular linear stapler.

Further dissection was undertaken to divide the adhesions between the colonic mesentery and the duodenum on the left hand side. Dissection was then taken laterally along the left paracolic gutter, and around the splenic flexure. The omentum was placed under traction and dissection conducted at the upper border of the transverse colon to enter the lesser sac. The pancreas and posterior wall of the stomach were identified. Dissection was continued down to the upper rectum, with the upper part of the mesocolon taken to the sacral promontory.

A laparoscopic reticulating stapler was inserted through the left iliac fossa port and the staple line taken through the upper rectum. At this point, the bowel was sufficiently mobilized to permit exteriorization. Mesenteric division and bowel resection were performed extracorporeally and the bowel returned to the abdominal cavity after securing the anvil of a circular stapler to the distal end.
with non-absorbable purse-string suture. A circular staple gun was passed per rectum, and a circular stapled anastomosis conducted. Anastomotic integrity was air-tested, and correct bowel orientation confirmed. The fascia of the periumbilical and exteriorization incisions was closed with non-absorbable continuous sutures. All skin incisions were closed with sub-cuticular absorbable sutures after infiltration with local anaesthetic, and covered with water-proof dressings.

### 3.3.2.2 Anaesthesia and Analgesia

All patients were administered standardised premedication and peri-operative analgesia as per a protocol designed in conjunction with the Department of Anaesthesia at Auckland City Hospital. This was agreed upon by each individual anaesthetist prior to commencement of the operation. All patients received paracetamol 1g orally as pre-medication (Panadol tablets, GlaxoSmithKline Consumer, Auckland, NZ). No sedation or non-steroidal anti-inflammatories were given. All patients received dexamethasone 8mg intravenously after induction (DBL dexamethasone sodium phosphate injection, Hospira NZ Limited, Wellington, NZ), and morphine / fentanyl intravenously as required for analgesia during the procedure (morphine sulphate injection, Biomed Ltd, Auckland, NZ; fentanyl injection, AstraZeneca Ltd, Auckland, NZ). Choice of induction agent and muscle relaxant was left to the discretion of the theatre anaesthetic team. Epidural, spinal, and intra-thecal analgesia / anaesthesia were not used. Room temperature was set at 19°C before the start of the case, and all patients were covered with a forced-air-rewarming blanket (The Original Bair Hugger Forced Air Warming Temperature Management Units, Arizant Healthcare Inc, Eden Prairie, MN 55344, USA). Choice, volume, and temperature of intra-venous fluid given intra-operatively were left up to the discretion of the anaesthetic team. Prior to incision, all laparoscopic port sites were infiltrated with 0.25% bupivacaine with adrenaline (Marcain 0.25% with adrenaline 1:400,000 injection, AstraZeneca Ltd, Auckland, NZ), and at the end of the procedure all skin wounds were infiltrated with 0.25% bupivacaine with adrenaline (up to a maximum total dose of 2 mg/kg).
Post-operatively all patients received regular paracetamol 1g orally until discharge, and morphine / fentanyl intravenously via patient controlled analgesia until pain could be satisfactorily controlled using oral opiates. Oral opiates used were tramadol 100mg orally as first line (tramadol hydrochloride, AFT Pharmaceuticals Ltd, Auckland, NZ), and morphine / oxycodone orally as second line (morphine sulphate, Douglas Pharmaceuticals Ltd, Auckland, NZ; oxycodone hydrochloride, Mundipharma NZ Ltd, Auckland, NZ).

3.3.2.3 Study Group
The Study Group received warm, humidified insufflation gas. Insufflation pressure was set at 12 – 15 mmHg with a variable flow rate (Figure 12). The gas used was carbon dioxide (Carbon dioxide medical gas, BOC Ltd, Auckland, NZ), and this was warmed to 37°C and humidified to 98% RH using a Fisher & Paykel laparoscopic humidifier (Fisher & Paykel MR860 Laparoscopic Humidification System, Fisher & Paykel Healthcare, Auckland, New Zealand). This humidification system is specifically designed to deliver warm, humidified CO₂ to patients undergoing laparoscopic surgery, and had undergone independent testing by our group to confirm the effectiveness of gas conditioning (see Chapter 8). The gas is passed from the insufflator through a chamber which is filled with 30 mL of sterile water and sits on a heater plate. Water evaporates from the chamber into the gas which flows over it. The temperature of the gas is maintained as it travels along a heated tube to the laparoscopic port and into the patient’s abdomen. The humidifier monitors the temperature and flow rate of the gas at the chamber outlet with a probe attachment, controlling the amount of power delivered to the heater plate to maintain the chamber set point temperature. (232)

3.3.2.4 Control Group
The Control Group received standard dry carbon dioxide for insufflation (Carbon dioxide medical gas, BOC Ltd, Auckland, NZ), delivered at room temperature (19 °C) and 0% RH. Insufflation pressure was set at 12 – 15 mmHg with a variable flow rate.
3.3.3 Objectives

The objective of this study was to test the hypothesis that warming and humidification of insufflation gas during laparoscopic colectomy would lead to reduced post-operative pain and improved recovery by reducing peritoneal inflammation.

3.3.4 Outcomes

All data were collected by a single, blinded investigator (Tarik Sammour) to ensure standardisation of data collection. Intra- and post-operative data were collected using a standardised data collection form (Appendix B: Clinical trial data collection form), patient booklet (Appendix C: Patient booklet), and patient questionnaire which was mailed to them after discharge from hospital.
(Appendix D: Patient questionnaire). All forms were designed *a priori*. Total postoperative opiate use forms the primary outcome of this study. Secondary outcomes include post-operative pain, intra-operative heat loss, and the post-operative plasma and peritoneal cytokine response. All variables measured are described in detail in the following sections.

### 3.3.4.1 Baseline Data

**Patient data**

Baseline patient data recorded included: National Health Index (NHI) number, the hospital that the patient was treated at, patient surname, age, sex, ethnicity as coded in hospital electronic records (self-identified), weight in kilograms, height in centimetres, past medical history, past surgical history, ASA score, and Colorectal Physiological and Operative Severity Score (Cr Possum). (237)

**Operative data**

Operative data recorded included: pre-operative diagnosis, post-operative diagnosis on histology, lesion location, date of surgery, operation performed, approach (laparoscopic, laparoscopic-assisted, conversion to open), operation start time (scalpel to skin), gas insufflation start time, gas insufflation end time, operation end time (all wound dressings applied), volume of gas used for insufflation, use of intra-peritoneal fluid washout, contamination with pus or faeces, dose of morphine / fentanyl used intra-operatively, dose of any anti-emetics used intra-operatively, and type and volume of intravenous fluid used intra-operatively. At the end of the surgery the operating surgeon was asked to rate the camera fogging on a Likert scale from 1 to 10 (1 = perfect image, 10 very poor quality image).

**Other data**

Other relevant data recorded post-operatively were: volume of fluid in the intra-peritoneal drain at the time of sample collection, total wound size on day 2 in cm (all wounds measured with a ruler and lengths added), and specimen histology including full TNM staging for neoplastic lesions.
3.3.4.2 Primary outcome

The primary outcome was total opiate analgesia use during the index inpatient stay. This was calculated using the Mean Equivalent Daily Dose (MEDD) method. All opiates administered to the patient via patient controlled analgesia, intravenous, and oral routes were converted to an equivalent MEDD dose and collated as defined by the MEDD chart seen in Figure 13. These data were collected in the post-operative recovery unit, day 0 (day of operation), day 1, day 2, day 3, and day 4 until discharge (see data collection form in Appendix B: Clinical trial data collection form). Days 1 to 3 were defined as a 24 hour period from midnight to midnight. Absolute MEDD dose, and MEDD dose per kilogram of patient weight were determined.

3.3.4.3 Secondary outcomes

Pain

Pain at rest, on moving, and on coughing was assessed by means of three 10 point Visual Analogue Scales (VAS) which were included in the patient booklet and questionnaire (Appendix C: Patient booklet; Appendix D: Patient questionnaire). The patient was asked to fill out the VAS questions pre-operatively (baseline), and at 2 h, 4 h, 8 h, 12 h, day 1, day 2, day 3, day 7, day 14, day 30, and day 60 post-operatively. If patients were discharged from hospital, a questionnaire with specific instructions was mailed to them with a pre-paid return envelope.
Table 1: MEDD Calculation

<table>
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<th>MEDD-Factor</th>
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<th>Route</th>
<th>MEDD-Factor</th>
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Figure 13: Mean Equivalent Daily Dose (MEDD) Chart. (238, 239)
MEDD calculation = (Dose) x (MEDD-Factor)


Intra-operative core temperature

Intra-operative core temperature was measured at 15 min intervals using an oesophageal probe (see data collection form in Appendix B: Clinical trial data collection form). The change in temperature between the start and end of the procedure, as well as the minimum, maximum, and mean / median temperatures were compared between groups.
Cytokine response

Patients had a size 15F Blakes drain (Blake Silicone Drain, Ethicon inc, Somerville, NJ 08876-0151, USA) inserted into the peritoneal cavity at the conclusion of surgery. This was brought out through the abdominal wall, and sutured to the skin. The drain was attached to a low vacuum drain bottle (Low vacuum wound drainage system 80kPa / neg 150mmHg, Leur-lock replacement bottle, Van Straten Medinorm, 66583 Spiesen, Germany). At 20 hours post-operatively, 4 mL of fluid from the drain bottle as well as a simultaneous sample of 4 mL of venous blood were collected into two separate vacutainer tubes containing ethylenediamine tetra-acetic acid (K2 EDTA BD Vacutainer, BD Diagnostics Franklin Lakes, NJ 07417, USA). The timing of sample collection was chosen because peritoneal IL-6 levels peak at 20 – 24 hours after abdominal surgery.(24) These samples were then sent to the Middlemore Hospital Laboratory for processing and storage for cytokine analysis (see section 3.3.5 Cytokine analysis). Median concentrations of IL-1α, IL-6, IL-8, IL-10, and TNFα were compared between groups. Final peritoneal fluid concentrations were corrected for dilution using the urea method. Since urea readily diffuses throughout the body, the plasma and peritoneal fluid urea concentrations are the same. Consequently, when the urea concentrations in plasma and peritoneal samples are known, the dilution of the initial volume of peritoneal fluid obtained can be calculated.(240)

3.3.4.4 Other variables recorded

Nausea and Vomiting

Nausea was assessed by means of a 10 point VAS which was included in the patient booklet and questionnaire (Appendix C: Patient booklet; Appendix D: Patient questionnaire). The patient was also asked the number of vomiting episodes they had in the 6 hour period prior to filling out the booklet. The patient filled these items out pre-operatively (baseline), day 1, day 2, day 3, day 7, day 30, and day 60 post-operatively.
Antiemetic and intravenous fluid use

All anti-emetic use was documented and data on this collected in the post-operative recovery unit, day 0 (day of operation), day 1, day 2, day 3, day 4 until discharge (see data collection form in Appendix B: Clinical trial data collection form). Days were defined as a 24 hour period from midnight to midnight. The total use was then calculated and expressed in the form of total standard doses during the hospital stay. Standard doses of the various anti-emetics used are shown in Table 2. Volume of post-operative intravenous fluid use was also recorded.

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<td>Stemetil</td>
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Table 2: Anti-emetic Standard Doses.

Return of bowel function

This was defined as days to achieving passage of flatus or bowel motion, resumption of full solid oral diet, and the absence of nausea and vomiting. Passage of flatus / bowel motion and oral intake were determined from the patient clinical notes as well as using questions included in the patient booklet (Appendix C: Patient booklet).

Discharge parameters

Day that discharge criteria were achieved and actual day of discharge were recorded. Discharge criteria were defined as return of bowel function (see above), independent mobilisation (or mobilisation back to baseline function), and adequate pain relief using oral analgesia only. Readmission was defined as return to hospital within 30 days post-discharge requiring hospital stay of 24 hours or more. Total hospital stay (day stay on index admission plus hospital stay on readmission) was derived and recorded.
Functional recovery

Functional recovery was measured using the Surgical Recovery Score (SRS), a comprehensive recovery assessment questionnaire derived from the previously validated multi-dimensional Identity-Consequence Fatigue Scale questionnaire (Appendix E: Surgical Recovery Score). This score assesses 5 categories of post-operative recovery: Fatigue, Vigor, Mental Function, Impact on Patient Activity, and Impact on Activities of Daily Living and is expressed as a percentage of maximum possible score. The SRS questionnaire was included in the patient booklet and questionnaire (Appendix C: Patient booklet; Appendix D: Patient questionnaire) and filled out by all patients pre-operatively (baseline) and at 1, 3, 7, 30, and 60 days post-operatively.

Post-operative complications

Post-operative complications up to 30 days after surgery were recorded prospectively using pre-defined criteria. Complications were defined as per the standardised “definitions of operation and/or disease related complications” proposed by Buzby et al. In addition to this, ileus was defined as post-operative obstipation and vomiting requiring nasogastric tube insertion, but without radiological evidence of bowel obstruction. All complications were recorded per patient and graded by the Clavien-Dindo classification.

3.3.5 Cytokine analysis

3.3.5.1 Sample processing and storage

Plasma and peritoneal drain samples for cytokine analysis (see section 3.3.4.3 Secondary outcomes) were collected into vacutainer tubes containing ethylenediamine tetra-acetic acid (K2 EDTA BD Vacutainer, BD Diagnostics Franklin Lakes, NJ 07417 USA). These samples were then sent on ice to the Middlemore Hospital Laboratory. All samples were centrifuged for 10 minutes at 1000 x g, after which the supernatant was removed. Samples were then aliquotted and stored in polypropylene tubes at -80 °C.
3.3.5.2 Preparation of reagents for immuno-assay

Cytokine assays were performed using Milliplex™ human cytokine kit (#MPXHCYTO-60K 96 well plate assay, Millipore Corporation, Billerica, MA 01821, USA). All reagents were allowed to warm to room temperature (20-25°C) before use. The following describes the procedure for reagent preparation in detail as per the manual supplied with the cytokine kit.

Preparation of Antibody-immobilized beads
Antibody-immobilized beads were prepared by sonicating each antibody-bead vial for 30 seconds, vortexing for 1 minute, then adding 60 μL from each antibody bead vial to the mixing bottle. The final volume was brought to 3.0 mL with bead diluent. The mixture was then vortexed again.

Preparation of Quality Controls
Before use, Quality Control 1 and Quality Control 2 were reconstituted with 250 μL de-ionized water. The vial was inverted several times to mix and vortexed. The vial was allowed to sit for 10 minutes and then the controls transferred to appropriately labelled polypropylene microfuge tubes.

Preparation of Wash Buffer
The 10X Wash Buffer was brought to room temperature and mixed to bring all salts into solution. 30 mL of 10X Wash Buffer was diluted with 270 mL de-ionized water.

Preparation of Serum Matrix
1.0 mL de-ionized water was added to the bottle containing lyophilized Serum Matrix, and mixed well, allowing 10 minutes for complete reconstitution.

Preparation of Human Cytokine Standards
Prior to use, the Human Cytokine Standard was reconstituted with 250 μL de-ionized water to give a 10,000 pg / mL concentration of standard for all analytes. The vial was inverted several times to
mix, vortexed for 10 seconds, allowed to sit for 10 minutes, and then transferred to an appropriately labelled polypropylene microfuge tube. This was used as the 10,000 pg / mL standard. Five polypropylene microfuge tubes were labelled 2000, 400, 80, 16, and 3.2 pg / mL. 200 μL of Assay Buffer was added to each of the five tubes. Serial dilutions were prepared (see Figure 14) by adding 50 μL of the 10,000 pg / mL reconstituted standard to the 2000 pg / mL tube and mixing, then transferring 50 μL of the 2000 pg / mL standard to the 400 pg / mL tube and mixing, then transferring 50 μL of the 400 pg / mL standard to the 80 pg / mL tube and mixing, then transferring 50 μL of the 80 pg / mL standard to 16 pg/mL tube and mixing, then transferring 50 μL of the 16 pg / mL standard to the 3.2 pg / mL tube and mixing. The 0 pg / mL standard (Background) was Assay Buffer.

<table>
<thead>
<tr>
<th>Standard Concentration (pg/mL)</th>
<th>Volume of Deionized Water to Add</th>
<th>Volume of Standard to Add</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000</td>
<td>250 μL</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard Concentration (pg/mL)</th>
<th>Volume of Assay Buffer to Add</th>
<th>Volume of Standard to Add</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>200 μL</td>
<td>50 μL of 10,000 pg/mL</td>
</tr>
<tr>
<td>400</td>
<td>200 μL</td>
<td>50 μL of 2000 pg/mL</td>
</tr>
<tr>
<td>80</td>
<td>200 μL</td>
<td>50 μL of 400 pg/mL</td>
</tr>
<tr>
<td>16</td>
<td>200 μL</td>
<td>50 μL of 80 pg/mL</td>
</tr>
<tr>
<td>3.2</td>
<td>200 μL</td>
<td>50 μL of 16 pg/mL</td>
</tr>
</tbody>
</table>

Figure 14: Serial Dilution Technique Used to Prepare Human Cytokine Standards.

pg: Picograms, mL: Millilitre, μL: Microlitre
3.3.5.3 Immuno-assay Procedure

The following describes the procedure for immuno-assay as per the manual supplied with the Milliplex™ human cytokine kit (#MPXHCYTO-60K 96 well assay, Millipore Corporation, Billerica, MA 01821, USA). The steps are summarised in Figure 15. Prior to use in the assay stored plasma and drain samples from both groups were retrieved together, thawed completely, and mixed by vortexing and centrifuge to remove particulates. All samples were assayed in duplicate, and peritoneal fluid samples were re-assayed for IL-6 in 1:5 dilution, as peritoneal fluid levels of this cytokine are frequently above the maximum detection limit of the assay (> 10000 pg/ml).

Figure 15: Immuno-assay Procedure Flowchart.
µL: Micro litre, RT: Room Temperature.
The placement of Standards [0 (Background), 3.2, 16, 80, 400, 2000, and 10,000 pg/mL], Controls 1 and 2, and Samples were diagrammed on a Well Map Worksheet in a vertical configuration (see Figure 16).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 pg/mL Standard (Background)</td>
<td>400 pg/mL Standard</td>
<td>QC-2 Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0 pg/mL Standard (Background)</td>
<td>400 pg/mL Standard</td>
<td>QC-2 Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.2 pg/mL Standard</td>
<td>2000 pg/mL Standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>3.2 pg/mL Standard</td>
<td>2000 pg/mL Standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>16 pg/mL Standard</td>
<td>10,000 pg/mL Standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>16 pg/mL Standard</td>
<td>10,000 pg/mL Standard</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>80 pg/mL Standard</td>
<td>QC-1 Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>80 pg/mL Standard</td>
<td>QC-1 Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 16: Well Map.**
pg/mL: Picogram Per Millilitre, QC: Control

The assay was run in duplicate. The filter plate was set on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate did not touch any surface. The filter plate was pre-wet by pipetting 200 μL of Assay Buffer into each well of the Microtiter Filter Plate, after which this was sealed and mixed on a plate shaker for 10 minutes at room temperature (20-25°C). The Assay Buffer removed by vacuum, and the excess blotted from the bottom of the plate with an absorbent pad. 25 μL of each Standard or Control was added into the appropriate wells. 25 μL of Assay Buffer was added to the sample wells. 25 μL of Serum Matrix solution was added to the background, standards, and control wells. 25 μL of sample was added into the appropriate wells. The mixing bottle was vortexed and 25 μL of Antibody-immobilized beads was added to
each well, shaking the bottle intermittently to avoid settling. The plate was sealed with a plate sealer, and covered with a lid. A rubber band was wrapped around the plate holder, plate and lid, which were then wrapped in aluminium foil. The plate was incubated with agitation on a plate shaker overnight at 4°C (see Figure 17).

![Covered Immunoassay Plate on a Plate Shaker.](image)

Figure 17: Covered Immunoassay Plate on a Plate Shaker.

The next day (after 16 – 18 hours) the plate was opened and fluid gently removed by vacuum and washed 2 times with 200 μL / well of Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Excess fluid was blotted from the bottom the plate by with an absorbent pad.
25 μL of Detection Antibodies (which has been allowed to warm to room temperature) were added into each well. The plate was sealed, covered with a lid, and incubated with agitation on a plate shaker for 1 hour at room temperature (20 – 25°C). 25 μL of Streptavidin-Phycoerythrin was added to each well. The plate was sealed, covered with a lid, and incubated with agitation on a plate shaker for 30 minutes at room temperature (20 – 25°C). All contents were gently removed by vacuum, and plate washed 2 times with 200 μL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Excess buffer on the bottom of the plate was wiped with a tissue. 150 μL of Sheath Fluid was added to all wells. The beads were re-suspended on a plate shaker for 5 minutes. The plate was then run on Luminex 100™ laser-based fluorescent analytical test instrumentation (Luminex 100™ IS, Luminex Corporation, Austin, TX 78727, USA). The Median Fluorescent Intensity (MFI) data obtained using Luminex IS 2.3 software was saved and analysed using a weighted 5-parameter logistic curve-fitting method for calculating cytokine / chemokine concentrations in samples (see Figure 18).

Figure 18: Luminex 100 IS Plate Analyser, with Computer Running IS 2.3 software. (Luminex Corporation, Austin, TX 78727, USA)
3.3.5.4 Assay Sensitivity

Minimum Detectable Concentration (MDC) was obtained from the manual supplied with the Milliplex™ human cytokine kit (#MPXHCYTO-60K 96 well plate assay, Millipore Corporation, Billerica, MA 01821, USA). MDC measures the true limits of detection for an assay by mathematically determining what the empirical MDC would be if an infinite number of standard concentrations were run for the assay under the same conditions. Values for assay sensitivity are shown in Table 3.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Mean MDC (pg/ml)</th>
<th>Mean + 2SD (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>IL-1a</td>
<td>3.5</td>
<td>6.4</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**Table 3: Cytokine Kit Assay Sensitivity.**

TNFα: Tumour Necrosis Factor α, IL-1β: Interleukin 1β, IL-6: Interleukin 6, IL-8: Interleukin 8, IL-10: Interleukin 10, IL-13: Interleukin 13, MDC: Minimum Detectable Concentration, SD: Standard Deviation, pg: Picograms, ml: Millilitres.
3.3.5.5 Assay Precision

Intra- and inter-assay precision was obtained from the manual supplied with the Milliplex™ human cytokine kit (#MPXHCYTO-60K 96 well plate assay, Millipore Corporation, Billerica, MA 01821, USA). Intra-assay precision is generated from the mean of the percent coefficient of variations from 8 reportable results across two different concentrations of cytokines in one experiment. Inter-assay precision is generated from the mean of the percent coefficient of variations from two reportable results each for two different concentrations of cytokine across 4 different experiments (Table 4).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Intra-assay precision (% CV)</th>
<th>Inter-assay precision (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>10.5</td>
<td>15.9</td>
</tr>
<tr>
<td>IL-1α</td>
<td>8.2</td>
<td>16.8</td>
</tr>
<tr>
<td>IL-6</td>
<td>8.1</td>
<td>11.6</td>
</tr>
<tr>
<td>IL-8</td>
<td>7.1</td>
<td>11.6</td>
</tr>
<tr>
<td>IL-10</td>
<td>5.2</td>
<td>9.5</td>
</tr>
<tr>
<td>IL-13</td>
<td>4.8</td>
<td>8.9</td>
</tr>
</tbody>
</table>

Table 4: Cytokine Kit Assay Precision.

TNFα: Tumour Necrosis Factor α, IL-1β: Interleukin 1β, IL-6: Interleukin 6, IL-8: Interleukin 8, IL-10: Interleukin 10, IL-13: Interleukin 13, % CV: Percent Coefficient of Variation.
**3.3.5.6 Assay Accuracy**

Assay accuracy data was obtained from the manual supplied with the Milliplex™ human cytokine kit (#MPXHCYTO-60K 96 well plate assay, Millipore Corporation, Billerica, MA 01821, USA). The data in Table 5 represent spike recovery, which is the mean percent recovery of 6 levels of spiked standards ranging from 3 to 10,000 pg / mL in serum matrices of 8 independent experiments.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Spike recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>98.7</td>
</tr>
<tr>
<td>IL-1α</td>
<td>103.0</td>
</tr>
<tr>
<td>IL-6</td>
<td>100.0</td>
</tr>
<tr>
<td>IL-8</td>
<td>101.9</td>
</tr>
<tr>
<td>IL-10</td>
<td>99.7</td>
</tr>
<tr>
<td>IL-13</td>
<td>100.2</td>
</tr>
</tbody>
</table>

**Table 5: Cytokine Kit Assay Accuracy.**

TNFα: Tumour Necrosis Factor α, IL-1β: Interleukin 1β, IL-6: Interleukin 6, IL-8: Interleukin 8, IL-10: Interleukin 10, IL-13: Interleukin 13, %: percentage.
3.3.6 Sample Size

A priori power calculation was based on the primary outcome which was reduction in total opiate analgesia use (measured in MEDD) during the index inpatient stay. Data from three previously published studies (246-248) that measured opiate use after laparoscopic colectomy were used to calculate mean MEDD dose expected. Intravenous morphine use in the control group of the study by Senagore et al was 64 mg with a standard deviation (SD) of 41 mg,(248) in that of Schlachta et al this was 62 mg (SD 41 mg),(246) and in that of Schwenk et al this was 59 mg (SD 41 mg).(247) Averaging these out gives a dose of 62 mg (SD 41 mg) of intravenous morphine during the hospital stay which is equivalent to 62 mg (SD 41 mg) MEDD. However, in all three studies patients were also administered some form of regular non-steroidal or opiate analgesia as baseline with the aforementioned morphine dose extra to this. Schwenk et al used regular Tramadol (300 mg orally daily for an average of 5 days), which is the equivalent to 75 mg MEDD. The other two studies used non-steroidal analgesia in their baseline medication which cannot be converted to an equivalent MEDD dose (and was not used in our study for this reason). Therefore the total MEDD dose during the hospital stay was estimated at 137 mg (SD 41 mg). For the power calculation, we used a conservative rounded figure of 150 mg (SD 50 mg) MEDD. Using a two tailed Mann–Whitney U test for difference between two independent groups, 37 patients would be required in each arm to detect a difference of 20% between groups with an alpha of 0.05 and power of 0.8.(249) The protocol and distribution plot for this power analysis are shown in Figure 19.
Input:

- Tails = Two
- Effect size d = 0.6625892
- \( \alpha \) err prob = 0.05
- Power (1–\( \beta \) err prob) = 0.8
- Allocation ratio N2/N1 = 1

Output:

- Noncentrality parameter \( \delta \) = 2.849904
- Critical t = 1.993464
- Df = 72
- Sample size group 1 = 37
- Sample size group 2 = 37
- Total sample size = 74
- Actual power = 0.802820

Figure 19: Protocol and Distribution Plot for Power Analysis

3.3.7 Randomisation

3.3.7.1 Sequence generation

Randomisation was conducted using random numbers obtained from an open source computer-based random number generator (www.random.org). Randomisation was stratified by hospital to ensure equal distribution of intervention and control group patients between these, and minimise bias due to differences in pre, intra and post-operative protocols between sites.
3.3.7.2 Allocation concealment

Allocations were concealed in opaque numbered envelopes. There were kept in a central location and not used until interventions were assigned on the day of surgery.

3.3.7.3 Implementation

The randomisation sequence was generated by a third party not involved in the study. Patients were recruited on the day of surgery. All patients were seen on a one to one basis pre-operatively by Tarik Sammour, and trial rationale and procedure were explained verbally. Patients were then given a participant information sheet, after which written informed consent was obtained prior to randomisation. Allocation of each individual patient into either Study or Control Group was performed intra-operatively by an unblinded research assistant (see below), after the patient was anaesthetised and before the insufflation was started.

3.3.8 Blinding

The patient, study investigators, surgeon, and medical staff responsible for patient care were all blinded to patient allocation. This was achieved by having the humidifier connected to the insufflation apparatus and power supply regardless of allocation, and covered with a specially designed plastic casing which concealed its LCD screen and water chamber (Figure 20). This was to ensure that none of the theatre occupants were able to tell if the humidifier was switched on or not. A research assistant not involved in patient management, study design, data collection, data analysis or results write-up was responsible for setting up the humidifier. After the patient was anaesthetised, the assistant opened an opaque envelope with allocation instructions and set-up the humidifier away from view of the theatre staff and investigator. If the patient was in the Study Group, 30ml of sterile water was added to the chamber and the humidifier was switched on and muted so that it did not make any noise. If the patient was in the Control Group water was not added and humidifier was not switched on.
The blinding protocol was practised in simulation several times and tested in March 2008 (prior to study commencement) on a consented patient undergoing a laparoscopic colonic resection at Counties Manukau DHB. The patient, study investigators, surgeon, and all medical staff responsible for patient care were blinded successfully. Data analysis was also blinded, with the investigator undertaking statistical analysis on study completion (Tarik Sammour) only allowed access to modified data tables with the allocation concealed. These specified patients as being allocated to “Group 1” and “Group 2”.

3.3.7 Statistical analysis

Results were analysed using SPSS® for Windows® version 17.0 (Lead Technologies Inc, Chicago, Illinois, USA). Continuous variable parametricity was tested using the Shapiro-Wilk test. Results were presented as Mean (Standard Deviation) for parametric data and Median (Inter-quartile Range) for non-parametric data. Groups were compared using the Fisher’s Exact or $\chi^2$ test for categorical variables, the Mann–Whitney U test for non-parametric continuous variables, and the t test for parametric continuous variables. Statistical significance was accepted at the 0.050 level. No subgroup analyses were planned or undertaken.
3.3.8 Ethical approval and trial registration

Ethical approval was granted by the Ministry of Health, Northern X Regional Ethics Committee in March 2008 (trial number NTX/08/02/010). Approval was also granted from the Maori Research Review Committees at Auckland and Counties Manukau DHB’s and the Maori Research Advisory Group at Waitemata DHB. Clinical board approval was obtained from Auckland DHB (trial number A+3967), Counties Manukau DHB (trial number NTX/08/02/010), and Waitemata DHB (trial number RM10130). The trial was also prospectively registered with ClinicalTrials.gov (trial identifier: NCT00642005, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894).

3.3.9 Funding sources

The following sources were used to fund the human clinical trial:

3. Project and Equipment Grant, Lottery Health Research.
4. Performance Based Research Fund, University of Auckland Research Committee.
3.4 ANIMAL LABORATORY STUDY

3.4.1 Animal preparation and handling

Adult inbred male Wistar rats were housed in standard plastic boxes with a mesh stainless steel lid (2 rats per box). The boxes were floored with fresh pine wood shavings (Figure 21). The animals were subjected to a 12 hour day / night cycle and were maintained in a controlled room at constant room temperature of 25 ± 2 °C and humidity of 30 – 70%. They were fed standard rodent chow (Teklad 2018, Madison, WI, USA) and had ad libitum access to tap water. Food was withdrawn 6 hours prior to surgery.

Figure 21: Rodent Housing in a Standard Plastic Box with Food and Water Accessible.
3.4.2 Anaesthesia

All experiment animals were ventilated in a stable anaesthetised state as described below.

3.4.2.1 Isoflurane anaesthesia

The rats were anaesthetised in a standard Perspex induction chamber using Isoflurane 5% + 5 L / min oxygen (Isoflurane, Luman Better Pharmaceutical Co. Ltd, Shandong 276006, China). The Perspex induction chamber was placed in a fume hood and all excess Isoflurane vapours were thus scavenged (Figure 22).

Figure 22: Fume Hood for Induction of Anaesthesia.
Once anaesthetised, the animal was weighed on an electronic scale and and placed supine onto a heated pad under the operating microscope (Figure 23). Anaesthesia was initially maintained by nose-cone ventilation of Isoflurane 3% + 2 L / min inspired 40% oxygen. The exhaled Isoflurane vapours were scavenged by a vacuum scavenger attached to the outside of the nose-cone (VetTech vacuum scavenger, Independent Vacuum Services, Auckland, NZ). Slowing of respiration and loss of pedal withdrawal reflex was used to assess the depth of anaesthesia. This latter test involves extension of a hind limb and pinching of the skin between the digits; anaesthesia is deemed sufficient if there is no attempt to withdraw the limb. (250)

![Figure 23: Animal Laboratory Setup with the Operating Microscope](Leica Microsystems Pty. Ltd., Gladesville, NSW, Australia).

### 3.4.2.2 Analgesia

After deep anaesthesia was confirmed, a single subcutaneous dose of buprenorphine 0.05 mg / kg in 0.2 ml of 0.9% Sodium Chloride was administered.
### 3.4.2.3 Ventilation

All animals had a tracheostomy performed after deep anaesthesia was confirmed and analgesia was administered. A 2 cm midline neck incision was made, and the trachea exposed by blunt dissection of the strap muscles of the neck. A tracheostomy was made 0.5 cm below the larynx using micro-scissors and immediately intubated with a modified 14G cannula (cut to 2 cm length). The cannula was secured in place with a 3/0 silk ligature around the trachea (Figure 24) and tissue glue (Histoacryl glue 0.5 mL, Aesculap AG & Co., Tuttingen 78532, Germany). The cannula was then attached to a small animal ventilator (Pressure controlled ventilator, Kent Scientific Corporation, Torrington, CT, USA), and checked to ensure it was air tight. The fraction of inspired oxygen was kept at 40%; the respiratory rate (RR) was maintained at 40 – 60 breaths per minute; and the peak inspiratory pressure (PIP) at 15 – 25 cm H₂O. This maintained the expired CO₂ (EtCO₂) at 35 – 55 mL/L as measured by a respiratory profile monitor and capnograph (CO₂SMO+ respiratory profile monitor, Novametrix Medical Systems Inc, Wallingford, CT, USA). The anaesthetic setup is shown in Figure 25.

![Figure 24: Tracheostomy Secured with Silk Ligature.](image_url)
3.4.2.4 Venous and arterial access

The intra-venous line comprised of a 15 cm length of PE55 clear polyethylene tubing with a 21G needle inserted at one end (bevelled end cut off). The line was soaked in 70% ethanol for at least 24 hours prior to use. It was primed with 0.9% Sodium Chloride. The arterial line used was a size 2F, 140 cm long Millar Mikro-Tip catheter with a solid state pressure transducer at the tip (Millar Instruments Incorporated, Houston, Texas, USA).
The right femoral neurovascular bundle was exposed via a 3 cm right groin incision. A self-retainer was used to spread the tissues and provide exposure. The right femoral artery and femoral vein were visualised using the operating microscope and skeletonised using combination of blunt and sharp dissection.

The right superficial femoral vein and profunda femoral vein were ligated and divided. The distal end of the right femoral vein was ligated, and an un-tightened 3/0 silk loop placed at the proximal end. A Biemer clip temporarily occluded the proximal end. The vein was then opened transversely with microscopic scissors. The femoral line was inserted and secured with the 3/0 silk loop. Finally, the Biemer clip was removed. The distal right femoral artery was ligated. An un-tightened 3/0 silk loop was placed at the proximal end. A Biemer clip temporarily occluded the proximal end. The artery was then opened transversely with microscopic scissors. The arterial line was inserted and secured with the 3/0 silk loop. The Biemer clip was removed. A photograph post insertion and securing of both lines is shown in Figure 26.

Figure 26: Right Femoral Arterial and Venous Lines.
3.4.2.5 Temperature

Rectal temperature was measured using a calibrated digital rectal thermometer. A second calibrated thermometer was inserted through a 1 cm midline incision in the abdominal wall and secured with an air tight purse string suture.

Body temperature was maintained using a warming plate set at 39 °C. A double layer of aluminium foil was also placed on top of the animal once pneumoperitoneum was started (see below), simulating a warming blanket.

3.4.2.6 Monitoring and recording

Continuous invasive heart rate (HR), and mean arterial pressure (MAP) was measured via the 2F Millar Mikro-Tip catheter inserted into the right femoral artery and connected to a PowerLab data acquisition system (ADInstruments, Dunedin, New Zealand, Figure 27). The HR was maintained at 280 – 340 bpm, and the MAP at > 70 mmHg.

Intra-abdominal and rectal temperatures were also continuously measured using PowerLab. All data was monitored and recorded using LabChart 5 (ADInstruments, Dunedin, New Zealand), see Figure 28.

In addition to the LabChart record, written recordings of the HR, MAP, rectal temperature, intra-abdominal temperature, Respiratory Rate (RR), Peak Inspiratory Pressure (PIP) and End Tidal CO₂ (EtCO₂) were made every 10min for the duration of pneumoperitoneum (see Appendix F: Animal study proforma). The study was terminated if any animal experienced 10 continuous minutes of: MAP < 65 mmHg, HR > 420 bpm, RR > 80 bpm, PEP > 25 mmH2O, EtCO2 > 70, or evidence / suspicion of lightening of anaesthesia which could not be deepened.
Figure 27: The PowerLab Data Acquisition System
(ADInstruments, Dunedin, New Zealand).
Figure 28: Real-time Intra-operative Recording of Rectal Temperature, Intra-abdominal Temperature, Heart Rate, Mean Arterial Pressure, Systolic / Diastolic Blood Pressure, and Gas Flow Rate.
LabChart 5 software (ADIInstruments, Dunedin, New Zealand), F&P Healthcare Flow Monitor (Fisher & Paykel Healthcare, Auckland, NZ)
3.4.2.7 Intravenous fluid administration

A continuous intravenous fluid infusion of 0.9% NaCl was administered via the femoral venous line using an automatic syringe driver (Genie precision programmable syringe driver, Kent Scientific Corporation, Torrington, CT, USA, Figure 29). The fluid flow rate corrected for weight was determined individually for each rat based on data from 74 human laparoscopic colonic resections in Auckland, North Shore, and Middlemore Hospitals from May 2008 to June 2009. (251) The median volume of intravenous fluid given to these patients intra-operatively was 2000 mL, the mean duration of surgery was 180.70 min, and the mean patient weight was 72.41 kg. This gives a flow rate per kilogram of weight of 9.17 mL/hour.

Figure 29: Programmable Syringe Driver.

3.4.2.8 Paralysis

After successful ventilation and line insertion, a repeat assessment was performed to confirm deep anaesthesia, and stable vital signs. Providing these conditions were satisfied, an intravenous injection of pancuronium (0.2 mL of 4mg / 2 mL solution) was administered via the femoral line. All vital signs were closely monitored for five minutes after administration.
3.4.3 Pneumoperitoneum

A 16G cannula was inserted in the midline of the abdomen inferior to the umbilicus and served as the gas inflow port. This was connected to the calibrated electronic insufflator (CO₂-OP-Pneu insufflator, Wisap, Munich, Germany) which was set at 5 mmHg and 1 L / min. Carbon dioxide gas was used for insufflation (Carbon dioxide medical gas, BOC Ltd, Auckland, NZ).

Rats were alternately assigned to the Study and Control Groups. The humidifier (Fisher & Paykel MR860 Laparoscopic Humidification System, Fisher & Paykel Healthcare, Auckland, New Zealand) was connected in all cases, but only filled with water and activated in the Study Group. Thus the Study Group received warmed (37°C) and humidified (98% RH) carbon dioxide and the Control Group received standard dry carbon dioxide at room temperature (19°C) and 0% RH.

Insufflation was started, and once the abdomen distended sufficiently (5 mmHg; calibrated electronic insufflator), 3 x 24G outflow cannulas were inserted through abdominal wall in the right iliac fossa via the incision previously made for femoral access (Figure 30 and Figure 31). This mimicked laparoscopic surgery working ports. Gas escape was adjusted by inserting or removing cannulas to maintain an intra-abdominal pressure of 5 mmHg at the desired flow rate (see below). The insufflation was continued for 110 minutes.

The flow rate was precisely controlled by means of an adjustable mechanical flow limiter (Precision Flow Control Valve, GRPO-10-PK-3, Esslingen, Germany) located downstream from a reservoir which served to blunt the pulsed flow from the insufflator. A flow meter (TSI model 4140, TSI Incorporated, MN, USA) was incorporated into the apparatus. This allowed real-time monitoring and recording of the insufflation rate during the experiment (Figure 28). A schematic diagram of the experiment setup is shown in Figure 32.
Figure 30: Animal Study Setup: Side View.
Figure 31: Animal Study Setup: Top View.
Figure 32: Schematic Diagram of Animal Study Setup.
The insufflation flow rate was determined individually and corrected for the peritoneal surface area of each rat based on data from 74 human laparoscopic colonic resections. (251-253) The insufflation was continued for a total of 110 minutes (based on the median pneumoperitoneum time from the same clinical trial data). (251)

Mean carbon dioxide volume used = 148.7 L
Mean duration of pneumoperitoneum = 109.9 min
Mean patient height = 167.4 cm
Mean patient weight = 72.4 kg

⇒ Human Body Surface Area (BSA as estimated by the method of Dubois and Dubois) (252)

\[ \text{BSA} = 0.007184 \times \text{Weight(kg)}^{0.425} \times \text{Ht(cm)}^{0.725} \]

\[ = 1.82 \text{ m}^2 \]

⇒ Human Peritoneal Surface Area (PSA as estimated by the method Pawlaczyk et al.) (253)

\[ \text{PSA} = 2.502 \times \text{BSA (cm}^2\) – 30373 \]

\[ = 1.52 \text{ m}^2 \]

⇒ Gas flow rate in humans per unit PSA (L/min/m\(^2\))

\[ = \frac{(148.7 / 109.9)}{1.52} \]

\[ = 0.8898 \text{ L/min/m}^2 \]

⇒ Estimated male Wistar rat PSA (estimated by the method Pawlaczyk et al.) (253)

\[ = \frac{(1.3503 \times \text{Weight(g)} + 84.294)}{10000} \]

⇒ Equivalent gas flow rate in male Wistar rat (L/min)

\[ = 0.8898 \times \frac{(1.3503 \times \text{Weight(g)} + 84.294)}{10000} \]

\[ = \frac{(1.20149694 \times \text{Weight(g)} + 75.0048012)}{10000} \]
3.4.4 Specimen collection

All specimen collection was performed at the end of the period of pneumoperitoneum. A laparotomy was performed, and the sampling technique was standardized as described below.

3.4.4.1 Blood

Blood was collected by a euthanizing cardiac puncture via the diaphragm using a 21G needle and a 10 mL syringe (Figure 33). The blood was immediately distributed between microtainers containing Ethylenediamine Tetra-acetic Acid (EDTA), Lithium Heparin, and SST Serum Separator (BD Diagnostics, Franklin Lakes, NJ 07417, USA). The heparin and EDTA tubes were then centrifuged for 15 min at 3000 rpm and 4°C (Eppendorf centrifuge, Hamburg, Germany). The supernatant was aliquoted into labelled 1.5 mL Eppendorf tubes. The SST tubes were stood vertically at room temperature for 30 min to allow the blood to clot. They were then were centrifuged for 15 min at 3000 rpm and 20 °C, and the supernatant aliquoted into labeled 1.5 mL Eppendorf tubes. Samples were stored at -80°C until further analysis.

Figure 33: Collection of Blood by Cardiac Puncture.
3.4.4.2 Organs

Liver and Kidney
The left lobe of the liver and both kidneys were excised in their entirety, after division of their blood supply and peritoneal attachments. Approximately 1 cm³ of the tissue specimens were divided into Eppendorf tubes, snap frozen in liquid nitrogen, and stored at -80°C for future analysis. The remaining specimens were fixed in 10% neutral buffered formalin (and stored in the dark at room temperature).

Pancreas
The whole pancreas was mobilised by dividing the gastric, hepatic, renal and colonic adhesions. The duodenum was divided at the pylorus and the duodenal-jejunal flexure, enabling removal of the whole pancreas (Figure 34). The duodenum was then excised from the head of the pancreas the spleen was excised from the tail. Approximately 1 cm³ of the tissue specimens were divided into Eppendorf tubes, snap frozen in liquid nitrogen, and stored at -80°C for future analysis. The remaining pancreas was fixed in 10% neutral buffered formalin (and stored in the dark at room temperature).

Figure 34: Harvesting the Pancreas for Histology.
Small intestine

5 cm pieces of mid jejunum were harvested (Figure 35). Some of these were placed in Eppendorf tubes, snap frozen in liquid nitrogen, and stored at -80° C for future analysis. The remaining jejunum was fixed in 10% neutral buffered formalin (and stored in the dark at room temperature).

Lung

A median sternotomy was performed and both lungs excised in their entirety. Approximately 1 cm³ of the tissue specimens were divided into Eppendorf tubes, snap frozen in liquid nitrogen, and stored at -80° C for future analysis. The remaining specimen was fixed in 10% neutral buffered formalin (and stored in the dark at room temperature).
3.4.5 Outcomes

3.4.5.1 Baseline data
Baseline data recorded included rat age in weeks, weight in g, nose to base of tail length in cm, total surgical time in min (from induction of anaesthesia to termination of pneumoperitoneum), volume of gas used for pneumoperitoneum in L, volume of intravenous fluid given in mls, and mean and median gas flow rates in L / min. The mean HR, MAP, RR, PIP and EtCO$_2$ served as a baseline comparison of haemodynamic and respiratory stability.

3.4.5.2 Primary outcome
The primary outcome was oxidative stress as measured by levels of malondialdehyde (MDA) and protein carbonyls (PC) at the conclusion of surgery. MDA and PC levels were measured in plasma and tissue as described in detail below. Results were corrected for protein concentrations determined using a Biuret assay and expressed as nM / g of protein.

3.4.5.3 Secondary outcomes
Organ histology
Light microscopy was performed by a blinded consultant histopathologist (B.D.) on 5 μm thick longitudinal paraffin sections using haematoxylin and eosin staining (Figure 36). A previously published standardised scoring system for organ inflammation was used.(254)

Temperature parameters
Continuous recording of intra-abdominal and rectal temperature was undertaken (mean values, as well the change from baseline were calculated).
Figure 36: Samples of Rat Organ Haematoxylin and Eosin Stains Used for Light Microscopy.
3.4.6 Oxidative stress analysis

3.4.6.1 Malondialdehyde

MDA levels in plasma, liver, kidney, pancreas, jejunum, and lung were measured using a published Thiobarbituric Acid Reactive Substances (TBARS) method,(255) based on the Animal Models of Diabetic Complications Consortium Protocol.(256)

Tissue homogenisation

Tissue samples liver, kidney, pancreas, jejunum, and lung (each weighing 40 – 80 mg) were placed into 2 separate 2 mL eppendorf tubes. RIPA buffer (pH 7.6 at room temperature, containing 25mM Tris stock, 150mM Sodium Chloride, 1% Sodium Deoxycholate, 1% Triton-x 100, 0.1% SDS) containing 1 complete protease inhibitor tablet (Roche complete mini tablet, Roche Applied Science, Mannheim 68298, Germany) was added to the eppendorf tubes in a ratio of 5 : 1 of tissue. A steel ball bearing was added to each tube, and the tissue homogenized for 3 minutes at 25 / s in a tissue lyser (TissueLyser II, Qiagen GmbH, Hilden, Germany) Samples were then centrifuged at 3000 rpm for 10 minutes at 4°C. 10 uL of lysate was used for the BIURET assay, and 100 uL was extracted for use in the TBARS assay.

BIURET assay (protein determination)

10 uL of tissue lysate or serum was pipetted into a 96-well microplate on ice. 10 uL of Bovine Serum Albumin (BSA) standard (10 mg / mL) was pipetted into 3 empty wells. 200 uL BIURET dye was then added to each well containing lysate, serum, or BSA standard, and to 3 empty wells which served as buffer blanks. The plate was incubated for 20 min at room temperature, and absorbance read at 540nm in a spectrophotometric plate reader (Spectramax 340PC384, MDS Analytical Technologies, CA, USA). Protein concentration was determined as follows:

\[
[(\text{Absorbance} – \text{buffer blank}) / (\text{BSA absorbance} – \text{buffer blank})] \times 10\text{mg/ml}
\]
TBARS assay

MDA standards were prepared using a dilution series of 20 uM 1, 1, 3, 3 tetramethoxypropane solution as outlined in Table 6.

<table>
<thead>
<tr>
<th>Tube number</th>
<th>MDA concentration (uM)</th>
<th>H₂O volume (uL)</th>
<th>Volume transferred (uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>500</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.15625</td>
<td>500</td>
<td>500 from tube 3</td>
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<td>0.3125</td>
<td>500</td>
<td>500 from tube 4</td>
</tr>
<tr>
<td>4</td>
<td>0.625</td>
<td>500</td>
<td>500 from tube 5</td>
</tr>
<tr>
<td>5</td>
<td>1.25</td>
<td>500</td>
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</tr>
<tr>
<td>6</td>
<td>2.5</td>
<td>500</td>
<td>500 from tube 7</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>500</td>
<td>500 from tube 8</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>500</td>
<td>500 from 20uM stock</td>
</tr>
</tbody>
</table>

Table 6: Preparation of MDA Standard Solutions.
MDA: Malondialdehyde, uM: Micromoles, uL: Microlitres

100 uL of tissue lysate or serum were pipetted into labeled 1.6 mL eppendorf tubes. 200 uL of ice cold 10% Trichloroacetic Acid (TCA) was added to each sample. Samples were incubated for 15 minutes on ice, and then centrifuged at 2200 rpm for 15 min at 4°C. 200 uL supernatant and standards were pipetted into new 1.6 mL eppendorfs and 200 uL of 0.67% Thiobarbituric Acid (prepared using 67mg TBA in 1ml DMSO and 9ml H₂O) added to these. Samples and standards were incubated at 99°C for 10 minutes. On cooling, 200 uL of standards and samples were pipetted into a 96-well microplate and absorbance read at 532 nm using a spectrophotometric plate reader (Spectramax 340PC³⁸⁴, MDS Analytical Technologies, CA, USA). The MDA standard readings were used to construct a standard curve by plotting the concentration of MDA standards against their absorbances (Figure 37). This was then used to calculate the MDA concentrations in the samples of tissue lysate and serum. Results were corrected for protein concentrations determined by the BIURET assay and expressed as nM / g of protein.
Figure 37: Example of MDA Standard Curve

MDA: Malondialdehyde, uM: Micromoles, nm: Nanometers, Abs: Absorbance

<table>
<thead>
<tr>
<th>MDA concentration (uM)</th>
<th>Absorbance at 532nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>0.15625</td>
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<tr>
<td>0.3125</td>
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<td>5</td>
<td>0.242</td>
</tr>
<tr>
<td>10</td>
<td>0.447</td>
</tr>
</tbody>
</table>

\[ y = 0.041x + 0.033 \]
\[ R^2 = 0.999 \]
3.4.6.2 Protein carbonyls

PC levels in serum, liver, kidney, pancreas, jejunum, and lung were measured using an ELISA test kit (BioCell PC Test kit, BioCell Corporation Ltd, Manukau, New Zealand) using published methodology.(257) The samples containing protein are derivatised with dinitrophenylhydrazine (DNP), and the protein is adsorbed to an ELISA plate. Unconjugated DNP and non-protein constituents are washed away. The adsorbed protein is probed with biotinylated anti-DNP antibody followed by streptavidin-linked horseradish peroxidise. Absorbances at 450 nm are related to a standard curve prepared for serum albumin containing increasing proportions of hypochlorous acid-oxidized protein that has been calibrated colourimetrically.

Tissue homogenisation

Tissue samples liver, kidney, pancreas, jejunum, and lung (each weighing 80 – 120 mg) were placed into 2 mL eppendorf tubes. Ice cold chloride free phosphate buffer (containing 20 mM KH₂PO₄ and 20 mM Na₂HPO₄) was added to the eppendorf tubes in a ratio of 10 : 1 of tissue. A steel ball bearing was added to each tube, and the tissue homogenized for 3 minutes at 25 / s in a tissue lyser (TissueLyser II, Qiagen GmbH, Hilden, Germany). Samples were then centrifuged at 3000 rpm for 10 minutes at 4°C. 10 uL of lysate was used for the BIURET assay, and 500 uL was extracted for use in the Protein Carbonyl assay.

BIURET assay (protein determination)

10 uL of tissue lysate or serum was pipetted into a 96-well microplate on ice. 10 uL of Bovine Serum Albumin (BSA) standard (10 mg / mL) was pipetted into 3 empty wells. 200 uL BIURET dye was then added to each well containing lysate, serum, or BSA standard, and to 3 empty wells which served as buffer blanks. The plate was incubated for 20 min at room temperature, and absorbance read at 540nm in spectrophotometric plate reader (Spectramax 340PC³⁸⁴, MDS Analytical Technologies, CA, USA). Protein concentration was determined as follows:

\[ \text{[(Absorbance – buffer blank) / (BSA absorbance – buffer blank)] x 10mg/ml} \]
Reagent preparation

The following describes the reagent preparation procedure in detail as per the instruction manual supplied with the test kit. All test kit reagents were brought to room temperature. Ready to use Chromatin and Stopping reagents are supplied in the kit.

EIA buffer was prepared by dissolving EIA buffer powder in 1 L of de-ionised H2O.

Diluted blocking solution was prepared by adding 2 mL of the EIA buffer to the blocking reagent container and mixing well. This was transferred to a 100 mL container, and more EIA buffer added to bring the final volume to 75 mL.

Diluted DNP solution was prepared by adding 1 mL of DNP reagent to 9 mL of Guanidine Hydrochloride.

Anti-DNP-biotin-antibody was made by adding 0.5 mL of diluted blocking solution to the Anti-DNP-biotin-antibody microvial. This was mixed well and made up to 20 mL with diluted blocking solution.

Streptavidin-horseradish-peroxidase was made by adding 0.5 mL of diluted blocking solution to the streptavidin-horseradish-peroxidase microvial. This was mixed well and made up to 20 mL with diluted blocking solution.

Oxidized protein standards (x 6) were made by adding 25 uL of deionised H2O to each of the 6 oxidized protein samples. The samples were vortexed, stood at 37°C for atleast 30 min, and then vortexed again to ensure all the samples had dissolved. The carbonyl control sample was similarly made by adding 25 uL of deionised H2O to the carbonyl control sample vial. The sample was vortexed, stood at 37 °C for atleast 30 min, and then vortexed again to ensure all the sample had dissolved.
Assay procedure

All samples were analysed in duplicate using the standard assay procedure described in the instruction manual supplied with the test kit. The following describes the procedure in detail as per the instruction manual supplied with the test kit.

The assay is setup so that approximately 1 ug of derivatised protein is applied to each well of the ELISA plate. At this level saturation is achieved, so that some variation in the amount of protein applied to the plate will not affect the response. 5 uL of each sample of lysate / serum, standard and control were added to 1.5 mL Eppendorf tubes containing 200 uL of diluted DNP solution. All samples were mixed and incubated for 45 min, taking care to ensure that the incubation time was similar for all samples. 5 uL of each DNP treated sample were then added to new 1.5 mL Eppendorf tubes containing 1 mL EIA buffer. The tubes were mixed well.

200 uL of each sample were pipetted into 96 well plates, and the plate covered with sealing tape. The plate was incubated overnight at 4 °C. The plate was washed with 5 x 300 uL EIA buffer per well. 250 uL of diluted blocking solution was added into each well and the plate incubated for 30 min at room temperature. The plate was washed with 5 x 300 uL EIA buffer per well. 250 uL of diluted anti-DNP-biotin-antibody solution was added into each well and the plate incubated for 1 h at 37 °C. The plate was washed with 5 x 300 uL EIA buffer per well. 250 uL of diluted streptavidin-horseradish-peroxidase solution was added into each well and the plate incubated for 1 h at room temperature. The plate was washed with 5 x 300 uL EIA buffer per well.

200 uL of chromatin reagent was added into each well, and the colour allowed to develop for 6 min. After this time the reaction was stopped by adding 100 uL of acidic stopping reagent to each well, taking care to ensure the all the wells were exposed to the chromatin reagent for the same length of time. The plate was shaken gently to mix reagents.
Absorbances were read at 450 nm using a spectrophotometric plate reader (Spectramax 340PC, MDS Analytical Technologies, CA, USA) directly after stopping the reaction. The absorbance of the carbonyl control sample was checked to ensure that this was similar to that of the 4th oxidized protein standard (i.e. falling within the quoted intra- and inter-assay variation of 5%). This is done to provide re-assurance that the assay is in fact detecting protein carbonyls. As for the MDA analysis, a standard curve was constructed by plotting the protein carbonyl concentration of the oxidized protein standards against their absorbances (Figure 37). This was then used to calculate the protein carbonyl concentrations in the samples of tissue lysate and serum. Results were corrected for protein concentrations determined by the BIURET assay and expressed as nM / g of protein.

Variation
The Intra- and inter-assay variation (for assays performed on the same or on different days) of samples with high protein carbonyls (> 0.1 nM) is expected to be around 5%, and for samples with low protein carbonyls (< 0.1 nM) is expected to be higher at 15% as they are closer to the lower end of the standard curve.

3.4.7 Statistical analysis
Results were analysed using SPSS® for Windows® version 17.0 (Lead Technologies Inc, Chicago, Illinois, USA). Continuous variables were tested using the Shapiro-Wilk test for normality. Groups were compared using the Mann–Whitney U test for non-parametric continuous variables, and the t test / ANOVA for parametric continuous variables (ANOVA post-hoc analysis was performed using t tests with Bonferroni correction). Statistical significance was accepted at the 0.050 level. Results are presented as Mean (Standard Deviation) for parametric data and Median (Inter-Quartile Range) for non-parametric data.
3.4.8 Ethical approval

The Animal Ethics Committee of the University of Auckland approved the protocol and experiment as outlined above (Protocol approval number R533).

3.4.9 Funding sources

The following sources were used to fund the animal laboratory study:

2. University of Auckland Research Committee.
3. Maurice & Phyllis Paykel Trust.
4. Auckland Medical Research Council.
5. Lottery Health New Zealand.
CHAPTER 4: SYSTEMATIC REVIEW AND META-ANALYSIS – THE HUMORAL RESPONSE AFTER LAPAROSCOPIC VERSUS OPEN COLORECTAL SURGERY
4.1 BACKGROUND

Major abdominal surgery evokes an intense local and systemic inflammatory reaction that has important immunological consequences. Peritoneal mesothelial cells and resident leukocytes, activated by injury, secrete various mediators (see Table 1) creating a local milieu that is directly responsible for the inflammatory and repair processes that contribute to the injury response. (21, 22, 131, 258) The importance of this phenomenon lies in its association with several clinical outcomes. The exaggerated production of pro-inflammatory cytokines such as interleukin 6 (IL-6) and tumour necrosis factor (TNF-α) in the acute phase manifests systemically as hemodynamic instability and metabolic derangement. (154) This, in conjunction with the endocrine responses exhibited by patients undergoing major surgery, (143) results in the classical pattern of reduced metabolism for approximately 24 hours postoperatively, followed by a catabolic phase of at least 2 weeks duration. (154) Associated with muscle protein degradation, lipolysis and disturbances in glucose metabolism. (155, 156) Even after these physiological parameters have seemingly recovered, post-operative patient fatigue can persist, lasting for up to 3 months after major uncomplicated gastrointestinal operations. (35, 160) Research studies into fatigue suggest a complex bio-psycho-social etiology, (161) but there is recent evidence that post-surgical inflammatory responses may directly influence its development. (34)

The local humoral environment after surgery also has direct and specific consequences. For example, after peritoneal injury, levels of local growth factors increase substantially, and fibrinolytic activity is markedly reduced. (7, 111, 112, 118, 129) with the imbalance resulting in increased interstitial collagen deposition and peritoneal adhesion formation. (110, 127, 128, 259) Also, there is evidence that cytokine and chemokine up-regulation has a direct inhibitory effect on the muscularis externa of the bowel, contributing to post-operative ileus. (92, 139-141) While these observations suggest a significant role for the post-operative inflammatory response in recovery after surgery, perhaps the most important consequence is the detrimental impact that this response may have on oncological outcome. (147) During and after surgery for neoplasia, cells exfoliated from the primary
lesion (or leaked out of lymphatics) can bind to the endothelium and mesothelium facilitated by interactions between ligands induced by epidermal growth factor, interleukin 1β, and other humoral factors. (19, 146) Peritoneal disruption during surgery dramatically increases levels of these local mediators, leading to a tumour promoting effect that is not restricted to the inflicted site but rather has a generalized character, promoting tumour growth in non-traumatised, as well as traumatised peritoneum. (19, 20)

Thus, the amelioration of the post-operative inflammatory response is, and has been, the subject of intense investigation. One avenue of research has focussed on minimally invasive techniques to reduce the physiological impact of surgery. Indeed, there is some evidence from animal and clinical studies that the systemic humoral response is reduced after laparoscopic surgery compared with equivalent open procedures, and it has been argued in several review articles that this may lead to improved patient recovery. (182, 184, 260, 261) Some have even hypothesised that oncological outcomes may be improved by laparoscopic techniques. (262, 263) Conclusions have tended to be cautious however, as many of the studies are non-randomised, (92) and focus on systemic levels of a heterogeneous group of humoral factors, with relatively little attention paid to the local peritoneal response which may be the more important determinant of outcome. (192, 264) Furthermore, while the immunological benefits may be expected to be pronounced in procedures where the access incision constitutes a proportionally large burden of injury, such as in laparoscopic cholecystectomy; in other procedures, where the intra-abdominal insult is the more major, the evidence remains unclear. (21, 183, 265, 266) Colorectal surgery serves as a case in point. Despite a large number of trials comparing the post-operative humoral response between laparoscopic and open surgery for this indication, results are conflicting and opinion remains divided. (92)

The aim of this study is therefore is to systematically review the results from randomised controlled clinical trials comparing the humoral response in laparoscopic and open colorectal surgery.
4.2 METHODS

The principal study question is whether or not patients undergoing elective laparoscopic colorectal surgery exhibit a reduced inflammatory response as measured by local and systemic levels of humoral markers. The comparison group is formed by patients undergoing equivalent open colorectal surgery. The following is a summary of the methods used in this study. Further details can be found in CHAPTER 3: Methods.

4.2.1 Systematic literature search

A high-sensitivity, low precision search was conducted with the search terms outlined in Table 7, and in-line with the validated methods of the QUORUM statement. (227) The search was run independently by two investigators (Tarik Sammour, Arman Kahokehr), with no restrictions on language. Relevant primary studies were identified from the Cochrane Central Register of Controlled Trials (CENTRAL/CCTR), the Cochrane Library, Medline including in-process and non-indexed citations (from January 1966 to January 2009), PubMed (from 1950 to January 2009) and Embase (from 1947 to January 2009). Relevant scientific meeting abstracts and reference lists of included papers were also manually searched.

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<thead>
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<th>Search terms</th>
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<tbody>
<tr>
<td>CENTRAL (15 results)</td>
<td>(laparoscopy/ or pneumoperitoneum,artificial/ or surgical procedures, minimally invasive/)</td>
</tr>
<tr>
<td>Medline (72 results)</td>
<td>AND</td>
</tr>
<tr>
<td>Cochrane library (120 results)</td>
<td>(adipokines/ or angiogenic proteins/ or cytokines/ or endothelial growth factors/ or endothelins/ or epidermal growth factor/ or fibroblast growth factors/ or interferons/ or kinins/ or transforming growth factors/ or tumor necrosis factors/)</td>
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<tr>
<td>Pubmed (1256 results)</td>
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</tr>
<tr>
<td>Embase (153 results)</td>
<td></td>
</tr>
</tbody>
</table>

Table 7: Search Terms Used in Different Databases.
4.2.2 Study selection

Search results were entered into a unified database and duplicate results removed. The titles and abstracts of all the studies were screened. Published and unpublished randomised controlled clinical trials that evaluated laparoscopic versus open surgery for any colorectal indication were included. Exclusion criteria were: animal studies, trials for non-colorectal indications, non-randomised trials (no restriction on randomisation method), and studies that did not measure plasma, serum, or peritoneal levels of chemokines, cytokines or growth factors as defined by Table 1. Studies that only measured other markers of inflammation such as serum / plasma C-reactive protein, erythrocyte sedimentation rate, leukocyte count, immunoglobulin levels, and human leukocyte antigen expression were not included.

All papers were scrutinised for inclusion independently by two investigators (Tarik Sammour, Arman Kahokehr), with disagreement resolved by consensus, and consultation with the senior doctoral supervisor (Andrew G. Hill) if consensus could not be reached.

4.2.3 Validity assessment

Assessment was performed by a single investigator (Tarik Sammour) who was blinded to the journal title, article title and authors of the publications. Methodological quality was determined using the Jadad score.(228) This score depends on three items: random allocation, masking of patients, and dropouts and withdrawals. The scale ranges from 0 to 5 points, with 2 or less indicating low quality and 3 or more indicating high quality.

4.2.4 Data abstraction

Data on humoral factor levels were gathered and the results of each trial summarized on an intention-to-treat basis in prospectively designed 2 x 2 tables. The data were categorised by humoral factor measured and time interval at which this was done. For the purposes of meta-analysis, standard deviation of the outcome data was used if provided by the authors, or calculated
from $P$ values, confidence intervals, or data ranges if they were not. The corresponding author for each publication was contacted if information was missing or unclear to obtain as much raw data as possible.

4.2.5 Statistical analysis

Analysis of combined data was performed using Review Manager Version 5.0 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008). Results of the meta-analysis were assessed by graphical presentations of standardised mean difference with 95 per cent confidence intervals on forest plots using the random effects model for more conservative estimates; $(231) P < 0.050$ was considered statistically significant. Statistical heterogeneity was evaluated using $I^2$ statistics, with values up to 25 per cent, up to 50 per cent, and above 50 per cent indicating low, moderate, and high levels of heterogeneity. A $\chi^2$ test for heterogeneity was performed, in which $P < 0.100$ was regarded as significant. Funnel plots were used to screen for publication bias. An a priori sensitivity analysis was performed to assess the effect of serum versus plasma cytokine measurements on data heterogeneity.
4.3 RESULTS

A total of 1,616 search results were entered into a unified database, after which 111 duplicate results were removed. Application of the inclusion and exclusion criteria yielded a total of 14 randomised controlled trials for inclusion in the review.\(^{(193, 194, 267-278)}\) One of these trials was subsequently excluded\(^{(278)}\) when it was realised that a subset of the data presented had been obtained from a non-randomised trial published previously by the same authors.\(^{(279)}\) This had not been explicitly stated in the text of the manuscript. A QUORUM diagram is provided in Figure 38.

The 13 included studies were published over a nine year period between 1997 and 2006. Nine of these studies described surgery for colorectal neoplasia exclusively,\(^{(193, 194, 267-273)}\) one study described inflammatory bowel disease and familial adenomatous polyposis,\(^{(274)}\) one study rectal prolapse,\(^{(275)}\) and two studies resections for heterogeneous indications (including colonic neoplasia and benign pathology).\(^{(276, 277)}\) Three of these studies\(^{(193, 194, 273)}\) were rated as high quality according to the Jadad score, and the rest as low quality. A summary of study characteristics is presented in Table 8, and a summary of results in Table 9.
Figure 38: QUORUM Diagram.
N: Number of Papers, RCT: Randomised Controlled Trial, Mini-lap: Mini-laparotomy
<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Indication</th>
<th>N Lap</th>
<th>N Open</th>
<th>Randomisation</th>
<th>Blinded</th>
<th>Jadad Score</th>
<th>Comparable Groups?</th>
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<tbody>
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<td>39</td>
<td>58</td>
<td>Computer generated</td>
<td>N</td>
<td>2</td>
<td>Excluded conversions, more left sided cases in open group</td>
</tr>
<tr>
<td>Dunker (274)</td>
<td>IBD, FAP</td>
<td>16</td>
<td>14</td>
<td>Did not state</td>
<td>N</td>
<td>1</td>
<td>Excluded transfused patients</td>
</tr>
<tr>
<td>Hasegawa (268)</td>
<td>Colon and recto-sigmoid Ca.</td>
<td>24</td>
<td>26</td>
<td>Did not state</td>
<td>N</td>
<td>1</td>
<td>Excluded conversions</td>
</tr>
<tr>
<td>Hewitt (269)</td>
<td>Colon Ca</td>
<td>8</td>
<td>8</td>
<td>Did not state</td>
<td>N</td>
<td>1</td>
<td>Excluded conversions</td>
</tr>
<tr>
<td>Leung (270)</td>
<td>Recto-sigmoid Ca above 5cm</td>
<td>17</td>
<td>17</td>
<td>Computer generated</td>
<td>N</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>Ordemann (271)</td>
<td>Colon and recto-sigmoid Ca above 12cm</td>
<td>20</td>
<td>20</td>
<td>Did not state</td>
<td>N</td>
<td>1</td>
<td>Y</td>
</tr>
<tr>
<td>Schwenk (272)</td>
<td>Colon and rectal Ca</td>
<td>30</td>
<td>30</td>
<td>Did not state</td>
<td>N</td>
<td>1</td>
<td>Y</td>
</tr>
<tr>
<td>Solomon (275)</td>
<td>Full thickness rectal prolapse</td>
<td>20</td>
<td>19</td>
<td>Did not state</td>
<td>Single</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>Stage (273)</td>
<td>Colon Ca</td>
<td>15</td>
<td>14</td>
<td>Random numbers</td>
<td>N</td>
<td>3</td>
<td>Excluded conversions</td>
</tr>
<tr>
<td>Svendsen (276)</td>
<td>Colon Ca, adenoma, diverticular, sigmoid volvulus</td>
<td>23</td>
<td>30</td>
<td>Did not state</td>
<td>N</td>
<td>1</td>
<td>Excluded conversions</td>
</tr>
<tr>
<td>Wu (193)</td>
<td>Colon Ca</td>
<td>12</td>
<td>14</td>
<td>Computer generated</td>
<td>N</td>
<td>3</td>
<td>More advanced stage disease in lap</td>
</tr>
<tr>
<td>Wu (194)</td>
<td>Colon Ca</td>
<td>12</td>
<td>14</td>
<td>Computer generated</td>
<td>N</td>
<td>3</td>
<td>More advanced stage disease in lap</td>
</tr>
<tr>
<td>Ytting (277)</td>
<td>Colon Ca, adenoma, diverticular, sigmoid volvulus</td>
<td>26</td>
<td>34</td>
<td>Did not state</td>
<td>N</td>
<td>1</td>
<td>Y</td>
</tr>
</tbody>
</table>

**Table 8: Summary Characteristics and Quality Assessment of Included Studies.**
N: Number of Patients, Lap: Laparoscopic, Ca: Cancer, IBD: Inflammatory Bowel Disease, FAP: Familial Adenomatous Polyposis, Cm: Centimetres.
<table>
<thead>
<tr>
<th>Study (Reference)</th>
<th>Sample</th>
<th>Humoral factor</th>
<th>Summary of results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delgado (267)</td>
<td>Serum</td>
<td>IL-6</td>
<td>IL-6 sig higher in open group at 4h, 12h, 24h.</td>
</tr>
<tr>
<td>Dunker (274)</td>
<td>Plasma</td>
<td>IL-6</td>
<td>IL-6 no sig difference at d1, d7.</td>
</tr>
<tr>
<td>Hasegawa (268)</td>
<td>Plasma</td>
<td>IL-6</td>
<td>IL-6 no sig difference at d1, d7.</td>
</tr>
<tr>
<td>Hewitt (269)</td>
<td>Serum</td>
<td>IL-6</td>
<td>Serum IL-6 no sig difference at 0h, 4h, 8h, d1, d2. PDF IL-6 no sig difference at 0h.</td>
</tr>
<tr>
<td>Hewitt (269)</td>
<td>PDF</td>
<td>IL-6</td>
<td>IL-6 sig higher peak in open. IL-1β sig higher peak in open. TNF-α no sig difference at 2h, 8h, d1, d2, d3, d7, d28.</td>
</tr>
<tr>
<td>Leung (270)</td>
<td>Serum</td>
<td>IL-6, IL-1β, TNF-α</td>
<td>IL-6 sig higher peak in open. IL-1β sig higher peak in open.</td>
</tr>
<tr>
<td>Ordemann (271)</td>
<td>Plasma</td>
<td>IL-6, TNF-α</td>
<td>IL-6 sig higher in open group at 1h, 4h, d1. TNF-α sig higher in open group at 1h, 4h, d1, d2.</td>
</tr>
<tr>
<td>Schwenk (272)</td>
<td>Plasma</td>
<td>IL-6, IL-10, IL-1RA</td>
<td>IL-6 sig higher peak and AUC in open. IL-1RA no sig difference in peak or AUC. IL-10 no sig difference in peak or AUC.</td>
</tr>
<tr>
<td>Solomon (275)</td>
<td>Serum</td>
<td>IL-6</td>
<td>IL-6 no sig difference at 4h, d1, d2.</td>
</tr>
<tr>
<td>Stage (273)</td>
<td>Plasma</td>
<td>IL-6</td>
<td>IL-6 higher increase in lap group (d1, d3, d10).</td>
</tr>
<tr>
<td>Svendsen (276)</td>
<td>Plasma</td>
<td>VEGF</td>
<td>VEGF no sig difference 1h, 2h, 6h, 24h, 48h, d8, d30.</td>
</tr>
<tr>
<td>Wu (193)</td>
<td>Serum</td>
<td>IL-6, IL-8, TNF-α</td>
<td>Serum IL-6 higher in open group at 2h. Serum IL-8 higher in open group at 2h. Serum TNF-α undetectable 2h, d1, d4. PDF IL-8 higher in lap group on 24h collection d4. PDF IL-6 no sig difference on 24h collection d1, d4. PDF TNF-α undetectable on 24h collection d1, d4.</td>
</tr>
<tr>
<td>Wu (194)</td>
<td>Serum</td>
<td>VEGF</td>
<td>Serum VEGF no sig difference 2h, d1, d4. PDF VEGF higher in lap group on 24h collection d4.</td>
</tr>
<tr>
<td>Ytting (277)</td>
<td>Plasma</td>
<td>IL-6</td>
<td>Plasma IL-6 no sig difference at 1h, 2h, 6h, d1, d2, d8, d30.</td>
</tr>
</tbody>
</table>

Table 9: Summary of Study Results.
Significant differences in green, no difference in red.
4.3.1 Systemic response

All thirteen studies included a measure of the humoral response in the systemic circulation. Seven studies measured plasma levels of humoral markers, and six studies used serum levels (Table 9).

4.3.1.1 Interleukin - 6

IL-6 was the most commonly measured humoral factor. Five studies found no significant difference between systemic IL-6 levels in laparoscopic and open colorectal surgery,(268, 269, 274, 275, 277) and five studies demonstrated higher levels in the open group.(193, 267, 270-272) Only one study reported higher levels of plasma IL-6 in laparoscopic resections for colonic cancer.(273) While measures of IL-6 levels were performed at a variety of time-points, and on patients with diverse pathologies, eight studies including only colorectal resections for neoplasia were congruous in that they measured systemic levels of IL-6 on day 1.(193, 267-273) Therefore, a meta-analysis of the data from these studies and at this time point was performed, and the results of this are presented in Figure 39, with funnel plot analysis in Figure 40. Patients in the open group (n = 187) had a significantly higher level of IL-6 on day 1 compared with patients in the laparoscopic group (n = 165, p = 0.040). However, there was very significant heterogeneity in the results ($\hat{I}^2 = 80\%$; $\chi^2 = 35.11$, $p < 0.0001$).

Plasma and serum data were analysed separately as per an a priori sensitivity analysis for sources of heterogeneity. This revealed the majority of heterogeneity to be caused by studies measuring plasma levels of IL-6. As can be seen in Figure 41, serum IL-6 was significantly higher in the open group (n = 97) than in the laparoscopic group (n = 76, p = 0.0008) with no significant heterogeneity in the results (p = 0.280). Data for plasma was still heterogeneous, with no apparent difference between groups.
### Figure 39: Forest Plot of Plasma and Serum IL-6 Levels on Day 1.

SD: Standard Deviation, CI: Confidence Interval, Std: Standardised, %: Percentage.

<table>
<thead>
<tr>
<th>Study</th>
<th>Laparoscopic Mean</th>
<th>SD</th>
<th>Total</th>
<th>Open Mean</th>
<th>SD</th>
<th>Total</th>
<th>Weight</th>
<th>Std. Mean Difference Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delgado</td>
<td>96.6</td>
<td>78.8</td>
<td>39</td>
<td>166.6</td>
<td>178</td>
<td>58</td>
<td>14.6%</td>
<td>-0.47 [-0.89, -0.06]</td>
</tr>
<tr>
<td>Hasegaw</td>
<td>90</td>
<td>65</td>
<td>15</td>
<td>87.5</td>
<td>65</td>
<td>14</td>
<td>12.2%</td>
<td>0.04 [-0.69, 0.77]</td>
</tr>
<tr>
<td>Hewitt</td>
<td>40</td>
<td>35</td>
<td>8</td>
<td>75</td>
<td>45</td>
<td>8</td>
<td>9.7%</td>
<td>-0.82 [-1.85, 0.21]</td>
</tr>
<tr>
<td>Leung</td>
<td>36</td>
<td>22.04</td>
<td>17</td>
<td>64</td>
<td>22.04</td>
<td>17</td>
<td>12.1%</td>
<td>-1.24 [-1.98, -0.50]</td>
</tr>
<tr>
<td>Ordeman</td>
<td>62.5</td>
<td>35.63</td>
<td>20</td>
<td>150</td>
<td>103.8</td>
<td>20</td>
<td>12.7%</td>
<td>-1.11 [-1.78, -0.43]</td>
</tr>
<tr>
<td>Schwenk</td>
<td>62.5</td>
<td>12.25</td>
<td>30</td>
<td>87.5</td>
<td>24.38</td>
<td>30</td>
<td>13.5%</td>
<td>-1.28 [-1.84, -0.72]</td>
</tr>
<tr>
<td>Stage</td>
<td>440</td>
<td>395.1</td>
<td>24</td>
<td>140</td>
<td>395.1</td>
<td>26</td>
<td>13.4%</td>
<td>0.75 [0.17, 1.32]</td>
</tr>
<tr>
<td>Wu</td>
<td>83</td>
<td>17.79</td>
<td>12</td>
<td>105</td>
<td>83.88</td>
<td>14</td>
<td>11.8%</td>
<td>-0.34 [-1.12, 0.44]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>165</td>
<td>187</td>
<td>100.0%</td>
<td>-0.55 [-1.06, -0.03]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.42; Chi² = 35.11, df = 7 (P < 0.0001); I² = 80%
Test for overall effect: Z = 2.09 (P = 0.04)
Figure 40: Funnel Plot of Plasma and Serum IL-6 Levels on Day 1.
SE: Standard Error, SMD: Standardised Mean Difference.
Figure 41: Forest Plot of (a) Plasma IL-6 Levels on Day 1, (b) Serum IL-6 Levels on Day 1.

SD: Standard Deviation, CI: Confidence Interval, Std: Standardised, %: percentage.
4.3.1.2 Tumour Necrosis Factor – α

Three studies measured systemic levels of TNF-α. Leung et al found no significant difference between groups,(270) and Ordemann et al found significantly higher levels in the open group at every time interval where this was measured (Table 9).(271) The analysis performed by Wu et al was not sensitive enough to detect TNF-α levels.(193) Meta-analysis of this data was not performed as this would have only included two studies.

4.3.1.3 Interleukin – 1

IL-1β exhibited significantly higher peak serum levels after open surgery compared with laparoscopic surgery for recto-sigmoid carcinoma in one study.(270) This was the only trial that measured levels of this cytokine directly. However, Schwenk et al measured plasma levels of IL-1 receptor antagonist (IL-1RA) after resection for colorectal cancer.(272) They argued that since IL-1RA can block the pro-inflammatory effects of IL-1β, it is a true IL-1β antagonist. No difference was demonstrable between the two groups.

4.3.1.4 Other humoral factors

IL-8 and IL-10 levels were each only reported by one study. Serum IL-8 was significantly higher after open surgery for colonic cancer,(193) while no difference in plasma IL-10 was demonstrable after surgery for colorectal cancer.(272) Two studies measured levels of plasma and serum Vascular Endothelial Growth Factor (VEGF), with no difference demonstrable between groups in either study.(194, 276) There was no data on any of the other humoral factors defined in Table 1.

4.3.2 Local response

Only 2 randomised trials (results reported in 3 publications) measured peritoneal levels of humoral factors, and both of these were in patients with colonic resection for neoplasia.(193, 194, 269) Hewitt et al did not detect a significant difference in peritoneal IL-6 in fluid aspirated from the
peritoneal cavity at the end of surgery. (269) Wu et al performed a 24h collection of peritoneal fluid at day 1 and 4 after surgery, and while there was no difference in levels of IL-6 or TNF-α, significantly higher levels of IL-8 were detected in the laparoscopic group on day 4. (193) In a follow-up analysis on the same samples, the same authors presented results showing higher levels of peritoneal VEGF in the laparoscopic group on day 4. (194)
4.4 DISCUSSION

This systematic review included 13 randomised controlled trials comparing the local and systemic humoral response after laparoscopic versus open colorectal surgery. The identified papers were heterogeneous in surgical indication, humoral factor measured, measurement timing, and method of sample analysis. Nevertheless, meta-analysis of the data on colorectal resection for neoplasia demonstrated a statistically significant higher serum level of IL-6 on day 1 after open surgery. No other trends were identified, and there were not enough data for other humoral factors and at other time points to allow further meta-analysis.

The finding that serum IL-6 is significantly higher after open colorectal surgery for neoplasia is an important one, signifying a potentially greater systemic stress response. IL-6 is considered to be a major mediator of the acute phase protein response following injury(22) and, in comparison with other cytokines, the concentration of IL-6 is most consistently increased in the circulation of injured patients.(266) The main producers of IL-6 are endothelial cells of the peritoneal capillaries.(105) Production occurs within the first hour of surgery and significantly increases after 4 h, with a significant rise in serum detected after 6 h.(73) Post-operatively, an early exaggerated IL-6 response has been shown in one study to precede the clinical onset of major complications by 12 – 48 h.(22) Although this is likely to be effect rather than cause, in experimental animal studies systemic IL-6 administration has been shown to have a direct detrimental effect on the healing of colonic anastomoses.(106)

In this study, the difference in IL-6 levels was only demonstrable in analysis of serum data, with no significant difference in plasma data despite an equivalent combined number of patients in each data-set. Centrifugation of samples after blood is collected in plain tubes and allowed to clot yields serum, whereas spinning non-clotted samples collected in anticoagulant tubes provides plasma. It is unclear which technique more accurately reflects the in vivo state, but it has been previously reported (at least for VEGF) that plasma rather than serum assays may be more appropriate.(263)
This is because humoral factors stored in WBCs and platelets are released when blood clots, which may result in overestimation. (280)

The reason for the difference in the systemic humoral response between equivalent laparoscopic and open operations for colorectal neoplasia is unclear. One answer is seemingly obvious: a smaller access incision leads to a reduced burden of injury. This is true, but simplistic, because in colorectal surgery the intra-abdominal wound is considerably more extensive than the access incision, dwarfing its systemic impact by comparison. (21, 193, 274, 281) In the study by Dunker et al., patients were grouped into two groups based on the size of the access incision regardless of operation (>8cm and <8cm), and there was no demonstrable difference in plasma IL-6 concentrations on day 1 and day 7 postoperatively between these two groups. (274)

Differences between the two techniques are not limited to the size of the access incision, and there are other factors at play that may influence the systemic response. Laparoscopic colorectal procedures tend to be associated with a longer operating time, a variable which has been directly and positively correlated with systemic IL-6 levels post-operatively. (282) Anaesthetic and analgesia protocols are also confounding variables, with spinal afferent blockade (143) and opiate usage (269) both known to blunt the systemic humoral response. Both of these modalities are generally utilised to a greater extent in open surgery due the higher post-operative analgesia requirement. (283, 284) While the above would seem to favour a diminished humoral response after open surgery (which is counter to what the current meta-analysis results suggest), the post-operative course may have the opposite effect. Laparoscopic surgery is generally associated with faster recovery, and earlier resumption of mobilisation and gastrointestinal function. (285) How much of this is attributable to surgical technique rather than differing post-operative care, and patient / care-giver attitudes towards the size of the incision, is a matter of debate. (285-287) The only blinded study performed demonstrated almost identical functional recovery of various organ functions after equivalent laparoscopic and open colorectal procedures. (287) This randomised controlled trial was performed
in the setting of a multi-modal enhanced recovery after surgery (ERAS) program, which was credited with bridging the gap between laparoscopic and open technique as far post-operative clinical outcome.(287) Furthermore, a recent study by Wichmann et al demonstrated that ERAS techniques lead to better preserved cell mediated immune function.(288) It is notable that in the two studies included in this meta-analysis that were performed in an ERAS setting, no difference in the systemic response was demonstrable between the laparoscopic and open groups. (276, 277)

Another point worthy of discussion is that levels of local peritoneal factors, rather than systemic levels, are thought to be of greater clinical importance. (192, 259) There is a much higher concentration of humoral factors in peritoneal fluid than in plasma after colorectal surgery suggesting that cytokine production occurs in a compartmentalized fashion within the abdominal cavity and at the site of dissection. (192, 193) While some of these factors are thought to be incompletely absorbed into the portal circulation, and end up in the systemic system after being degraded by the liver and then diluted in the plasma, there is an apparent independence of the peritoneal response from the systemic one. (25) For example, the IL-6 level in the peritoneal fluid peaks later and lasts longer than the systemic level measured in plasma. (193) Other observed contrasting physiological responses to the same humoral factor after intra-abdominal stimulation serve to further illustrate this point. (25) Only 2 studies identified in this review measured levels of local peritoneal humoral factors (results reported in 3 papers). While one study did not identify a difference between open and laparoscopic surgery, it is of interest that the other found higher levels of IL-8 and VEGF after laparoscopic surgery.

Further studies to elucidate any differences in the local humoral response between open and laparoscopic colorectal surgery are required. This is particularly important because it is thought that inflation and deflation of the abdomen for laparoscopy may result in organ ischaemia-reperfusion injury. (203) Furthermore, carbon dioxide pneumoperitoneum has known local immunological effects. (264) Local acidification caused by the formation of carbonic acid in the
peritoneal fluid results in microscopically visible damage to the mesothelial ultra-structure and reduced numbers of activated neutrophils, which is more pronounced than when peritoneum is exposed to room air. (220, 289-293) This acidification is independent of systemic acidosis. (294) There is also evidence that CO$_2$ directly blunts peritoneal macrophage function in vivo. (221) Another recognised issue with pneumoperitoneum is the cold temperature (room temperature) and dryness (0% relative humidity) of the gas typically used. (205) Large volumes are often required in prolonged procedures (up to 500L) owing to the imperfect seal of the laparoscopic ports, and peritoneal carbon dioxide absorption. (205) The effect of this cold, dry gas flow on the peritoneal environment is significant, producing visible structural changes in the peritoneal mesothelial surface layer and an increased local inflammatory response. (219-222) Warming the insufflated CO$_2$ has been shown to be associated with a reduced peritoneal cytokine response after laparoscopic cholecystectomy in humans. (295)

This systematic review is limited by the quality of the included studies. Only 3 out of the 13 studies were rated as high quality based on the Jadad scale, and only one study was blinded (single blinded). While this is unlikely to have influenced measured humoral factor concentrations, true group allocation may be biased or concealed by selective patient inclusion, data collection, and reporting. Also, there is a fundamental difficulty in the interpretation of any studies on immune function. While reduced levels of humoral factors tend to be interpreted as representing lesser injury, they could potentially also signify suppression of host immunity by a more major surgical insult. (264) Unless the concentration change is correlated directly with clinical outcome, the distinction is almost impossible to make. Addressing this fundamental issue should be a priority of future studies in this area.
4.5 CONCLUSION

Open colorectal resection for neoplasia is associated with higher post-operative serum levels of IL-6 on day 1 than equivalent laparoscopic surgery. The aetiology and clinical significance of this finding is uncertain, and further studies are required to elucidate any differences in the local humoral response which may be more clinically relevant in surgery for this indication.
CHAPTER 5: CLINIAL TRIAL – THE PERITONEAL CYTOKINE RESPONSE AFTER LAPAROSCOPIC VS OPEN COLONIC SURGERY
5.1 BACKGROUND

Cytokines are polypeptides that function by means of paracrine and autocrine mechanisms. They form a subgroup of humoral mediators that are directly responsible for the inflammatory processes that define the injury response. (21, 22) There is a sequential, high concentration cytokine response after major abdominal surgery. (24, 73, 95) This contributes to the systemic hemodynamic instability and global metabolic derangement that is observed post-operatively, (154-156) and is thought to be a determinant of post-operative fatigue which can last for up to 3 months after surgery. (34)

The local peritoneal micro-environment has a specific role to play in the post-operative course. There is evidence that peritoneal cytokine up-regulation has an inhibitory effect on the musculares externae of the bowel, potentially contributing to post-operative ileus. (92, 139-141) Furthermore, after peritoneal injury, the imbalance between local growth promoting and fibrinolytic mediators results in increased interstitial collagen deposition, resulting in peritoneal adhesions. (7, 110, 111, 118, 259) It is also postulated that an exaggerated peritoneal cytokine response may compromise oncological outcomes. (19, 20, 296) During and after surgery for neoplasia, exfoliated neoplastic cells bind to the endothelium and mesothelium facilitated by interactions between ligands induced by cytokines. (19, 146, 297) Surgical peritoneal disruption dramatically increases levels of these local mediators leading to a tumour promoting effect. (19, 20)

The systemic humoral response may be reduced after laparoscopic surgery compared with equivalent open procedures. (182, 184, 260-263) The meta-analysis of randomised controlled trials in the previous chapter demonstrated significantly lower serum interleukin 6 (IL-6) levels on day 1 after laparoscopic colorectal resection. However, conclusions were limited by heterogeneity in surgical indication, humoral factor measured, measurement timing, method of sample analysis, and lack of correlation of cytokine levels with clinical outcomes. In addition, despite randomisation, small subject numbers and exclusion of laparoscopic cases converted to laparotomy resulted in
poor matching between the study and control groups in several included trials. Furthermore, the local peritoneal response may be the more important determinant of post-operative recovery.(192, 264) Only 2 studies identified in the meta-analysis above measured levels of local peritoneal humoral factors, and their results were conflicting.

The aim of this study is to compare the local and systemic cytokine response in laparoscopic and open colonic surgery and relate this to post-operative recovery parameters, using a prospectively collected patient database and case-control design.
5.2 METHODS

The following is a summary of the methods used in this study. Further details can be found in CHAPTER 3: Methods.

5.2.1 Study Group

The study group consisted of a subset of consecutive patients having laparoscopic colonic resection at any of the three public hospitals in the Auckland region (Auckland City Hospital, North Shore Hospital, and Middlemore Hospital). All elective patients undergoing laparoscopic colonic resection for any indication between April 2008 and June 2009 were screened for inclusion. Exclusion criteria were: Patients under 15 years of age, acute colonic resections, hand-assisted colonic resection, decision to perform open surgery pre-operatively (intra-operative conversions were included as intention to treat), surgery for rectal lesions defined as within 15 cm of the anal verge, stoma formation (preoperative or intra-operative decision), pre-operative steroid dependence, inability to consent due to cognitive impairment or language barrier, patients with ASA ≥ 4, and deviation from anaesthetic protocol (patients who had an epidural or spinal anaesthesia were excluded).

Study group patients were matched to patients in the control group for age, sex, Body Mass Index (BMI), ASA, Colorectal Physiological and Operative Severity Score (Cr POSSUM), side of surgery (right versus left sided resection), diagnosis, presence or absence of malignancy, and Dukes’ histological stage.

5.2.2 Control Group

The historical control group consisted of consecutive patients recruited as part of a completed double-blinded randomized controlled trial investigating the use of intravenous dexamethasone (dexamethasone sodium phosphate Injection, 4mg/ml, Hospira NZ Limited, Wellington, New Zealand) in open colonic surgery.(35) All patients undergoing elective open colonic resection within
an Enhanced Recovery After Surgery (ERAS) program at the Manukau Surgical Centre between June 2006 and March 2008 were screened for inclusion. Exclusion criteria were equivalent to the study group. Only patients in the intervention arm of the dexamethasone study were included in the comparison because dexamethasone administration results in an attenuated early peritoneal cytokine response.(35) All patients in the Study Group above had also received a single dose of intravenous dexamethasone after anaesthesia induction as part of the study protocol.

5.2.3 Sample collection and storage
Patients in both arms of the study had a size 15F Blakes drain (Blake Silicone Drain, Ethicon inc, Somerville, New Jersey 08876-0151) inserted into the peritoneal cavity at the conclusion of surgery. This was brought out through the abdominal wall, and sutured to the skin. The drain was attached to a low vacuum drain bottle (Low vacuum wound drainage system 80kPa / neg 150mmHg, Leur-lock replacement bottle, Van Straten Medinorm, 66583 Spiesen, Germany). At 20 - 24 hours post-operatively, 4 mL of fluid from the drain bottle as well as a simultaneous sample of 4 mL of venous blood were collected into two separate vacutainer tubes containing either ethylenediamine tetra-acetic acid or sodium citrate to prevent clotting (BD Vacutainer, BD Diagnostics Franklin Lakes, NJ 07417 USA). The timing of sample collection was chosen because peritoneal IL-6 levels peak at 20 – 24 hours after abdominal surgery.(24) All collected samples were sent immediately on ice to the Middlemore Hospital Laboratory, where they were centrifuged for 10 minutes at 1000 x g and the supernatant removed. Samples were then aliquoted and stored in polypropylene tubes at -80 °C for future batch analysis.

5.2.4 Cytokine analysis
At the end of recruitment of the Study Group patients, all samples from both groups were retrieved, thawed, and assayed together (analysis of Control Group samples was repeated to ensure equivalent analysis technique). Median concentrations of IL-6, IL-8, IL-10, TNFα and were compared between groups. Cytokine assays were carried out by multiplexed cytometric bead
immunoassays using the Milliplex™ human cytokine kit (#MPXHCYTO-60K 96 well plate assay, Millipore Corporation, Billerica, MA 01821, USA). All samples were assayed in duplicate, and peritoneal fluid samples were re-assayed for IL-6 in 1:5 dilution, as peritoneal fluid levels of this cytokine were frequently above the detection limit of the assay (> 10000 pg/ml). The minimum assay detection limits for TNFα, IL-6, IL-8, and IL-10 were 0.1, 0.3, 0.2, and 0.3 pg/ml, respectively. Data were acquired using Luminex 100™ laser-based fluorescent analytical test instrumentation (Luminex 100™ IS, Luminex Corporation, Austin, TX 78727, USA). The Median Fluorescent Intensity (MFI) data obtained using Luminex IS 2.3 software were saved and analysed using a weighted 5-parameter logistic curve-fit.

5.2.5 Post-operative assessment

Data on post-operative course were prospectively collected in both Study and Control groups. Day that discharge criteria were achieved and actual day of discharge were recorded. Discharge criteria were defined as return of bowel function (passage of flatus or bowel motion, resumption of full solid oral diet, and the absence of nausea and vomiting), independent mobilisation (or mobilisation back to baseline function), and adequate pain relief using oral analgesia only. Readmission was defined as return to hospital within 30 days post-discharge requiring hospital stay of 24 hours or more. Total hospital stay (day stay on index admission plus hospital stay on readmission) was derived and recorded. Functional recovery was measured using the Surgical Recovery Score (SRS), a comprehensive recovery assessment questionnaire derived from the previously validated multi-dimensional Identity-Consequence Fatigue Scale.(241, 242) The SRS questionnaire was filled out by all patients pre-operatively (baseline) and at 3, 7, 30, and 60 days post-operatively. Further details regarding the SRS can be found in Appendix E: Surgical Recovery Score.

Post-operative complications up to 30 days after surgery were recorded prospectively using pre-defined criteria. Complications were defined as per the standardised “definitions of operation and / or disease related complications” proposed by Buzby et al.(243) In addition to this, ileus was
defined as post-operative obstipation and vomiting requiring nasogastric tube insertion, but without radiological evidence of bowel obstruction. All complications were also graded as per the Clavien-Dindo classification.(244, 245)

### 5.2.6 Power calculation

Power calculation was performed using a two tailed Mann–Whitney U test for non-parametric data. An estimate of peritoneal fluid IL-6 concentration for the Control Group patients was based on the concentrations measured in the intervention arm of the Dexamethasone trial.(159) Twenty five patients had complete peritoneal fluid IL-6 concentrations measured in that study (mean 3800 pg/ml, standard deviation 2300 pg/ml). We estimated that matching 50 patients in the Study Group in 2:1 design would be able to detect a 35% reduction in peritoneal fluid IL-6 concentration between groups with an alpha of 0.05 and power of 0.8.(249)

### 5.2.7 Statistical analysis

Results were analysed using SPSS® for Windows® version 17.0 (SPSS, Chicago, Illinois, USA). Continuous variable parametricity was tested using the Shapiro-Wilk test. Groups were compared using the Fisher’s Exact or χ² test for categorical variables, the Mann–Whitney U test for non-parametric continuous variables, and the t test for parametric continuous variables. Spearman’s rho was used for correlations. Statistical significance was accepted at the 0.050 level.
5.3 RESULTS

5.3.1 Baseline and intra-operative parameters

Groups were very well matched at baseline, with the exception of operation time which was significantly longer in the Study Group (Table 10). There were two conversions to laparotomy in the Study Group, one due to extensive adhesions to the inferior aspect of the liver, and one due to inadvertent damage to a gonadal vessel which resulted in bleeding that could not be controlled laparoscopically.

<table>
<thead>
<tr>
<th></th>
<th>Study Group (n = 50)</th>
<th>Control Group (n = 25)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>73.5 (21-94)</td>
<td>71 (37-92)</td>
<td>0.218**</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (34%)</td>
<td>8 (32%)</td>
<td>1.000**</td>
</tr>
<tr>
<td>Female</td>
<td>33 (66%)</td>
<td>17 (68%)</td>
<td></td>
</tr>
<tr>
<td>Mean BMI (SD)</td>
<td>26.0 (5.6)</td>
<td>27.1 (4.0)</td>
<td>0.331†</td>
</tr>
<tr>
<td>ASA score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7 (14%)</td>
<td>8 (16%)</td>
<td>0.944*</td>
</tr>
<tr>
<td>II</td>
<td>28 (56%)</td>
<td>26 (52%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>15 (30%)</td>
<td>16 (32%)</td>
<td></td>
</tr>
<tr>
<td>Mean Cr-POSSUM (SD)</td>
<td>18 (3)</td>
<td>17(3)</td>
<td>0.206†</td>
</tr>
<tr>
<td>Operation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R sided</td>
<td>34 (68%)</td>
<td>17 (68%)</td>
<td>1.000**</td>
</tr>
<tr>
<td>L sided</td>
<td>16 (32%)</td>
<td>8 (32%)</td>
<td></td>
</tr>
<tr>
<td>Mean operating time (SD)</td>
<td>172.2 (45.1)</td>
<td>109.6 (30.3)</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>36 (72%)</td>
<td>18 (72%)</td>
<td>0.573*</td>
</tr>
<tr>
<td>Adenoma</td>
<td>7 (14%)</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>Diverticulosis</td>
<td>3 (6%)</td>
<td>4 (16%)</td>
<td></td>
</tr>
<tr>
<td>IBD</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Other Benign</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36 (72%)</td>
<td>18 (72%)</td>
<td>1.000**</td>
</tr>
<tr>
<td>No</td>
<td>14 (28%)</td>
<td>7 (28%)</td>
<td></td>
</tr>
<tr>
<td>Dukes stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3 (8.3%)</td>
<td>1 (5.6%)</td>
<td>0.815*</td>
</tr>
<tr>
<td>B</td>
<td>20 (55.6%)</td>
<td>9 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>13 (36.1%)</td>
<td>8 (44.4%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 10: Baseline Characteristics.

N: Number of Patients, SD: Standard Deviation, ASA: American Society of Anaesthesia Score, Cr-POSSUM: Colorectal Physiological and Operative Severity Score, * χ² test, ** Fisher’s Exact test, † T test, ++ Mann-Whitney U test.
5.3.2 Post-operative recovery

There were no significant differences between groups in days to meeting discharge criteria, actual discharge day, and total day stay (Table 11). Post-operative recovery, as measured by the SRS score, was not significantly different at any time point. The readmission rate, post-operative complication rate (percentage of patients with any complication), and complication severity were similar in both groups. There were no deaths in either group during the 30 day follow-up period.

5.3.3 Cytokine response

The peritoneal fluid IL-6 level was significantly lower in the study group than the open group, with no other significant differences in the peritoneal fluid or plasma (Table 11). Cytokine concentrations are depicted graphically in Figure 42 and Figure 43.

There were several significant correlations between cytokine concentrations and post-operative recovery parameters (Table 12). Plasma and peritoneal fluid TNFα correlated positively with days to meeting discharge criteria, plasma IL-6 correlated positively with actual discharge day and total day stay, and plasma IL8 correlated positively with actual discharge day and negatively with the SRS score at baseline and day 30.

Patients who developed a complication had a significantly higher concentration of IL-10 and TNFα in the peritoneal fluid than those who did not (510.92 ± 390.00 vs. 365.08 ± 372.61, \( P = 0.031 \)) pg/ml and 18.61 ± 25.40 vs. 9.61 ± 13.23 pg/ml, \( P = 0.038 \), respectively). There were no significant correlations between cytokine concentrations and complication severity.
<table>
<thead>
<tr>
<th></th>
<th>Study Group</th>
<th>Control Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Discharge (range)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day meeting discharge criteria</td>
<td>3 (1-29)</td>
<td>3 (2-12)</td>
<td>0.078**</td>
</tr>
<tr>
<td>Actual discharge day</td>
<td>5 (2-47)</td>
<td>4 (3-34)</td>
<td>0.084**</td>
</tr>
<tr>
<td>Total day stay</td>
<td>6 (2-47)</td>
<td>6 (3-34)</td>
<td>0.526**</td>
</tr>
<tr>
<td><strong>Readmission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (12%)</td>
<td>5 (20%)</td>
<td>0.490**</td>
</tr>
<tr>
<td>No</td>
<td>44 (88%)</td>
<td>20 (80%)</td>
<td></td>
</tr>
<tr>
<td><strong>Complication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25 (50%)</td>
<td>12 (48%)</td>
<td>1.000**</td>
</tr>
<tr>
<td>No</td>
<td>25 (50%)</td>
<td>13 (52%)</td>
<td></td>
</tr>
<tr>
<td><strong>Complication Grade</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 (8%)</td>
<td>0 (0%)</td>
<td>0.630*</td>
</tr>
<tr>
<td>2</td>
<td>18 (72%)</td>
<td>10 (83.3%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 (16%)</td>
<td>1 (8.3%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 (4%)</td>
<td>1 (8.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean SRS score (SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>74.3% (13.9)</td>
<td>71.0% (14.1)</td>
<td>0.348†</td>
</tr>
<tr>
<td>Day 3</td>
<td>55.3% (16.2)</td>
<td>56.5% (10.1)</td>
<td>0.704†</td>
</tr>
<tr>
<td>Day 7</td>
<td>56.7% (14.5)</td>
<td>59.0% (8.6)</td>
<td>0.410†</td>
</tr>
<tr>
<td>Day 30</td>
<td>67.7% (15.0)</td>
<td>70.8% (10.7)</td>
<td>0.339†</td>
</tr>
<tr>
<td>Day 60</td>
<td>75.2% (14.3)</td>
<td>80.3% (11.7)</td>
<td>0.141†</td>
</tr>
<tr>
<td><strong>Peritoneal Cytokine Concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>5389.2 (5516.5)</td>
<td>8149.0 (4196.7)</td>
<td>0.030**</td>
</tr>
<tr>
<td>IL-8</td>
<td>639.7 (900.4)</td>
<td>862.3 (3072.0)</td>
<td>0.591**</td>
</tr>
<tr>
<td>IL-10</td>
<td>462.4 (402.9)</td>
<td>371.3 (438.5)</td>
<td>0.322**</td>
</tr>
<tr>
<td>TNFα</td>
<td>13.6 (17.1)</td>
<td>17.2 (24.2)</td>
<td>0.322**</td>
</tr>
<tr>
<td><strong>Plasma Cytokine Concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>37.1 (54.9)</td>
<td>33.7 (44.9)</td>
<td>0.787**</td>
</tr>
<tr>
<td>IL-8</td>
<td>19.3 (30.9)</td>
<td>16.9 (9.3)</td>
<td>0.393**</td>
</tr>
<tr>
<td>IL-10</td>
<td>11.6 (10.3)</td>
<td>8.0 (10.8)</td>
<td>0.138**</td>
</tr>
<tr>
<td>TNFα</td>
<td>5.1 (4.3)</td>
<td>6.0 (3.0)</td>
<td>0.298**</td>
</tr>
</tbody>
</table>

**Table 11: Post-Operative Recovery and Cytokine Assay Results.**
Cytokine concentrations in pg/ml are expressed as Medians (Interquartile Range)
Peritoneal IL-6 sample diluted 1:5
N: Number of Patients, IL: Interleukin, TNF: Tumour Necrosis Factor, SD: Standard Deviation, * χ2 test, ** Fisher’s Exact test, † T test, ‡ Mann-Whitney U test.
Figure 42: Peritoneal Cytokine Concentrations in Study Versus Control Group. Coloured bars: medians with interquartile ranges, error bars: 95% confidence intervals. pg / mL: Picogram Per Millilitre, IL: Interleukin, TNF: Tumour Necrosis Factor
Figure 43: Plasma Cytokine Concentrations in Study Versus Control Group.
Coloured bars: medians with interquartile ranges, error bars: 95% confidence intervals.
pg / mL: Picogram Per Millilitre, IL: Interleukin, TNF: Tumour Necrosis Factor
<table>
<thead>
<tr>
<th><strong>PERITONEAL</strong></th>
<th><strong>DISCHARGE</strong></th>
<th><strong>SRS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-6</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Actual</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.064 (0.594)</td>
<td>-0.152 (0.204)</td>
</tr>
<tr>
<td><strong>IL-8</strong></td>
<td>0.129 (0.279)</td>
<td>0.020 (0.865)</td>
</tr>
<tr>
<td><strong>IL-10</strong></td>
<td>0.014 (0.905)</td>
<td>0.185 (0.120)</td>
</tr>
<tr>
<td><strong>TNFα</strong></td>
<td><strong>0.295</strong> (0.012)</td>
<td>0.145 (0.224)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>PLASMA</strong></th>
<th><strong>DISCHARGE</strong></th>
<th><strong>SRS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-6</strong></td>
<td><strong>Criteria</strong></td>
<td><strong>Actual</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.174 (0.135)</td>
<td><strong>0.278</strong> (0.016)</td>
</tr>
<tr>
<td><strong>IL-8</strong></td>
<td>0.147 (0.207)</td>
<td><strong>0.243</strong> (0.036)</td>
</tr>
<tr>
<td><strong>IL-10</strong></td>
<td>0.018 (0.877)</td>
<td>0.106 (0.367)</td>
</tr>
<tr>
<td><strong>TNFα</strong></td>
<td><strong>0.284</strong> (0.014)</td>
<td>0.158 (0.175)</td>
</tr>
</tbody>
</table>

**Table 12: Spearman’s Correlations between Cytokines and Recovery Parameters.**
Results presented as correlation co-efficients, with p values in brackets. Positive correlations are highlighted in blue, negative correlations in red and significant correlations in bold. Peritoneal IL-6 sample diluted 1:5.

IL: Interleukin, TNF: Tumour Necrosis Factor, Pre-op: Preoperative, SRS: Surgical Recovery Score.
5.4 DISCUSSION

We conducted a case-control study comparing patients undergoing laparoscopic colonic surgery with patients undergoing open colonic surgery, with the primary endpoint of peritoneal fluid and plasma cytokine concentrations. Patients were very well matched at baseline, and all the data were prospectively collected using standardised definitions. The peritoneal fluid IL-6 concentration was significantly lower at 20 – 24 hours after laparoscopic surgery, but no differences were detected in any of the other cytokines measured in peritoneal fluid or plasma. There were no other differences between the groups in any of the post-operative recovery outcomes measured. Significant positive correlations between cytokine levels and discharge criteria achievement, actual day stay, post-operative complications, and negative correlations with the SRS score were demonstrated.

This is the largest study so far undertaken evaluating the peritoneal cytokine response after colonic surgery, and the results support previously postulated relationships between levels of inflammatory cytokines and post-surgical recovery.(34) The lower level of peritoneal fluid IL-6 after laparoscopic colonic surgery is a novel finding. Hewitt et al randomised 16 patients to laparoscopic and open colonic resection for neoplasia, and sampled peritoneal fluid at the end of surgery. No difference was found in peritoneal IL-6 between groups, but the study was weakened by small numbers and exclusion of conversions to laparotomy.(269) Wu et al similarly randomised 26 patients and also did not detect a difference in peritoneal fluid IL-6 at day 1 or day 4 post-operatively. A higher concentration of IL-8 at day 4 in the laparoscopic arm was reported; however, only 16 patients (5 in the laparoscopic arm, and 11 in the open arm) actually had peritoneal fluid measurements.(193, 194) To the authors knowledge, only one other small case-control study measured IL-6 concentrations in peritoneal drain fluid at 24 hours in 13 patients undergoing laparoscopic or open colectomy and once again no difference was demonstrated.(298)
In the systemic circulation, IL-6 is considered to be a major mediator of the acute phase protein response,(22, 23) with levels appearing to be proportional to the extent of tissue injury.(94) The role of peritoneal fluid IL-6 in the post-surgical course is uncertain. An association between peritoneal IL-6 on day 1 and anastomotic leakage after mesorectal excision for rectal cancer has been demonstrated in one study, but the impact of pre-operative radiation may have been significant, and applicability to colon resection is limited.(100) It is notable that in our study we did not find a correlation between peritoneal fluid IL-6 and post-operative complications or any measures of post-operative recovery. Therefore, the clinical relevance of the higher level of peritoneal IL-6 in the study group is unclear, particularly since there were no differences between the study and control groups in terms of discharge parameters, complication rates, or the SRS score. With the exception of the lower peritoneal IL-6 response, the local cytokine response was otherwise similar after laparoscopy compared with laparotomy, with no difference in the 3 other cytokines measured. This may be because of the ischaemia-reperfusion injury caused by pneumoperitoneum,(203) or the known local immunological effects of carbon dioxide.(205, 220, 264, 289-293, 299-301)

The equivalent plasma cytokine response after laparoscopic and open surgery is consistent with the results of the meta-analysis conducted by the authors, in which a lower IL-6 on day 1 after laparoscopic colorectal resection was demonstrable only in serum (and not in plasma). Centrifugation of samples after blood is collected in plain tubes and allowed to clot yields serum, whereas centrifugation of samples collected in anticoagulant tubes provides plasma. It is thought that plasma rather than serum assays are more reflective of the in vivo state,(263) because humoral factors stored in WBCs and platelets are released when blood clots, resulting in overestimation.(280) Overall, our results suggest an equivalent systemic inflammatory response after colonic surgery regardless of the access incision. We attribute this to the fact that, in colonic surgery, the trauma caused by the intra-peritoneal dissection is considerably more extensive than that caused by the access incision, perhaps dwarfing its inflammatory impact by comparison.(21,
As can be seen in this study, plasma cytokine concentrations were much lower than peritoneal concentrations. This has been noted in several earlier publications and is thought to be due to incomplete absorption into the portal circulation, hepatic metabolism, and dilution in plasma, causing an apparent independence of the peritoneal response from the systemic one.(25, 192, 193)

This study has several limitations. Patients in the two groups were recruited from different hospitals, with their surgery and care undertaken by different clinicians.(302) Secondly, patients in the Control Group were managed within a formal enhanced recovery after surgery program, whereas patients in the Study Group were not. As a result, all patients in the Control Group were given spinal afferent blockade via a thoracic epidural peri-operatively. Epidural blockade has been shown to blunt the systemic humoral response.(143) While this means that there was some co-intervention bias in this study, it is generally accepted standard practice in our hospital to use an epidural for open colonic resections and not for laparoscopic resections, due to the differences in post-operative analgesia requirement. This co-intervention is therefore present in current clinical practice.

5.5 CONCLUSION

With the exception of a lower peritoneal IL-6 response, the systemic and peritoneal cytokine response at 20-24 hours is similar after laparoscopic versus open colonic resection, with corresponding equivalent rates of post-operative recovery. There are significant correlations between cytokine concentrations and post-operative complications, discharge parameters, and the SRS score.
CHAPTER 6: SYSTEMATIC REVIEW – OXIDATIVE STRESS ASSOCIATED WITH PNEUMOPERITONEUM
6.1 BACKGROUND

In prolonged laparoscopy, attenuation of the inflammatory humoral response appears to be minimal and not corroborated by differences in clinical outcome.\(^{(303)}\) Nevertheless, prolonged laparoscopic surgery for major abdominal surgery such as colectomy is now common place.\(^{(181, 304)}\) While generally considered safe, there have been several reports on mesenteric ischaemia and bowel infarction after routine laparoscopic procedures.\(^{(211, 212, 305-312)}\) Most have occurred in patients with evidence of pre-operative cardiovascular, hepatic or renal compromise, \(^{(211, 308, 311)}\) however, there have also been rare reports of complications in healthy young patients.\(^{(212, 305, 309, 310, 312)}\)

These findings have led to the hypothesis that abdominal insufflation and the consequent raised intra-abdominal pressure may produce significant organ ischaemia, followed by reperfusion injury upon deflation of the abdomen.\(^{(203)}\) One of the main consequences of this ischaemia – reperfusion is an imbalance between oxidants, such as reactive oxygen species (ROS), and antioxidants.\(^{(218)}\) This imbalance is defined as oxidative stress, and may result from enhanced ROS formation, or from the malfunction of the scavenging systems. Several enzymatic antioxidant defence systems have been described and characterized, including the superoxide dismutases, catalases and a number of glutathione peroxidases.\(^{(313)}\) There are also endogenous and exogenous compounds that act as anti-oxidants.\(^{(313)}\) The surplus reactive oxygen species that result in oxidative stress can oxidise macromolecular constituents of cells, such as membrane phospholipids, proteins, and DNA, causing cellular injury and organ dysfunction.\(^{(314)}\)

Given the increasing complexity and length of laparoscopic procedures, organ ischaemia-reperfusion injury and oxidative stress associated with pneumoperitoneum may become a more significant problem. The aim of this review was to answer four questions: i) what is the evidence for a reduction in splanchnic blood flow during pneumoperitoneum, ii) what is the nature of the oxidative stress that ensues, iii) what is the relationship between this oxidative stress and...
insufflation pressure and nature of the gas used, and iv) what measures have been shown to reduce the oxidative stress associated with pneumoperitoneum.
6.2 METHODS

6.2.1 Systematic literature search

Medline, Medline in-process & other non-indexed citations, Cochrane Library, Pubmed, and Embase 1947+ were searched independently by two investigators (Tarik Sammour, Anubhav Mittal) for papers on oxidative stress and pneumoperitoneum, using the search terms outlined in Table 13. Papers spanning a period of time from 1947 (earliest EMBASE records) to March 2008 were searched with no language restriction. Reference lists were then reviewed and an online search of scientific meeting abstracts performed by a third investigator (Arman Kahokehr) to identify further papers. The results were pooled into a single database, and duplicate entries removed.

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<tr>
<th>Database</th>
<th>Search terms</th>
</tr>
</thead>
<tbody>
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<td>Medline ® and Medline in-process &amp; other non-indexed citations</td>
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</tr>
<tr>
<td>Cochrane library</td>
<td>(Laparoscop* OR Endoscop* OR Coelioscop* OR Celioscop* OR Pneumoperitoneum) AND (ischaemi* OR ischemi* OR precondition* OR sequential OR oxida*)</td>
</tr>
<tr>
<td>Pubmed</td>
<td>(Laparoscop* [Title] OR Endoscop* [Title] OR Coelioscop* [Title] OR Celioscop* [Title] OR Pneumoperitoneum [Title]) AND (ischaemi* [Title] OR ischemi* [Title] OR precondition* [Title] OR sequen* [Title] OR oxida* [Title])</td>
</tr>
<tr>
<td>EMBASE</td>
<td>(Laparoscop$ OR Endoscop$ OR Coelioscop$ OR Celioscop$ OR Pneumoperitoneum) AND (ischaemi$ OR ischemi$ OR precondition$ OR sequential OR oxida$).m_titl.</td>
</tr>
</tbody>
</table>

Table 13: Search Terms Used in Different Databases.

6.2.2 Study selection

All laboratory trials, clinical trials, reviews, and comments were identified. There was no restriction on trial design, with randomised controlled trials, non-randomised controlled trials, case-control studies, case series and case reports all included. Exclusion criteria were any papers that did not
investigate pneumoperitoneum as a causative factor, or did not report outcome measures related to oxidative stress, specifically splanchnic blood flow, oxidant / anti-oxidant marker levels, gastric / intestinal pH, or histology scores. These criteria were applied by two investigators (Tarik Sammour, Anubhav Mittal) and any disagreement resolved by consultation with senior doctoral supervisors (Anthony R J Phillips, John A Windsor, Andrew G Hill).

6.2.3 Data abstraction

All results were reviewed by a single investigator (Tarik Sammour), and entered into data tables designed prior to data abstraction. Data were reported only if stated in the text, tables, graphs or figures of the articles, but critical review of the results is also presented. The data was grouped into animal and human studies and further categorised by outcome measure. No meta-analyses were possible, and study quality was not assessed due to the heterogeneity of study design and outcome measures.
6.3 RESULTS

6.3.1 Search results

Five hundred and sixty one papers were identified in the initial database search (QUORUM diagram in Figure 44). Fifty seven of these met the search criteria, and a review of their reference lists and meeting abstracts lead to the identification of a further 16 papers. Thus, a total of 73 publications were retrieved for review. Thirty six of these were animal studies, 21 were human clinical trials, 9 were case reports, 5 were review articles, and 2 were comments.

![Figure 44: QUORUM Diagram](image)

N: Number of Papers.

6.3.2 Reduction in splanchnic venous and arterial blood flow

6.3.2.1 Animal Studies

Increased intra-abdominal pressure, from any cause, leads to increased systemic vascular resistance and afterload, and a decrease in cardiac output. Early canine studies with high insufflation pressures (20 – 40mmHg) demonstrated this, with an associated marked blood flow
reduction in all viscera, with the exception of the adrenal gland. (316) In another canine model, within 30 min of establishing pneumoperitoneum at a pressure of 16 mmHg, a reduction in blood flow of up to 30 per cent was seen in the superior mesenteric artery and portal vein. (317) This effect can be partially explained by the intra-abdominal pressure exceeding the normal portal venous pressure of 7–10 mm Hg. (318) but the reduction was also noted to be gradual, progressive with time, and beyond that attributable to the decrease in cardiac output alone, suggesting that auto-regulatory vasoconstriction makes a significant contribution. (319) Splanchnic vasoconstriction (predominantly at the arteriolar level) is due to several different mechanisms. Vasopressin, produced by a central nervous system pathway in response to increased intra-abdominal pressure, constricts renal, superior mesenteric and celiac vasculature. (315, 317, 320, 321) Levels of renin and angiotensin (both potent vasoconstrictors) are increased in the renal vein after laparoscopy in a rabbit model. (322) Furthermore, splanchnic vessels possess a well-developed intrinsic myogenic mechanism for local control of vascular tone, and compression of the venous outflow triggers this causing vasoconstriction. (317)

The type of gas used can also influence the mesenteric vascular response. Blobner et al. compared abdominal insufflation in pigs with air and CO\(_2\) at sequentially increasing pressures using direct arterial and venous measurements. (323) demonstrating a relative mesenteric hyper-perfusion in the CO\(_2\) group at lower insufflation pressures. The increase in mesenteric artery and portal venous blood flow was however reversed between 8mmHg and 12mmHg, corresponding with an increase in mesenteric artery resistance, and a reduction in cardiac output and inferior vena cava flow. This suggests that hyperaemic effects of CO\(_2\) at lower pressures are more than counteracted by the vascular compression effects at 12mmHg and above.

**6.3.2.2 Human studies**

Similar haemodynamic changes have been demonstrated in human laparoscopy studies. (315, 324-326) Insufflation pressure is generally set at 12–15 mm Hg, which raises the intra-abdominal
pressure above that of the normal portal circulation pressure. (181, 318) The reduction in cardiac output and accompanying mesenteric vasoconstriction has been reported to cause a significant reduction in organ perfusion and reduced portal venous flow during routine laparoscopic procedures such as cholecystectomy. (324-326) While the raised intra-abdominal pressure is contributory, it should be noted that, in the clinical setting, cardiac output and splanchnic perfusion changes are the result of a complex interaction between anaesthesia, the surgical insult, patient position, and the nature of the gas used (see below). (181, 327)

6.3.3 Oxidative stress associated with pneumoperitoneum

Various animal (203, 218, 314, 324, 328-332) and human (207, 208, 333-341) studies have investigated the ischaemia-reperfusion injury that occurs as a result of pneumoperitoneum. These studies used a wide range of outcome measures of oxidative stress, including endogenous antioxidant levels, peroxidation markers, derived gastric intra-mucosal pH, cytokine levels, and histology. A simplified schematic representation of the rationale behind these measurements is presented in Figure 45.

It should be noted at this point that the large variation in oxidative stress measures weakens inter-study comparisons. Furthermore, there is no consensus on which peroxidation markers or methods of detection are most valid. (313) However, measurements of lipid peroxidation marker levels, specifically malondialdehyde (MDA) and F2-Isoprostanes, are generally regarded to be the most reliable and reproducible markers in the clinical setting. (313, 342, 343)
Figure 45: Simplified Diagram of Oxidants, Endogenous Anti-oxidants, and Enzymatic Defence / Repair Mechanisms.
Figure modified from Grune et al.(313) MB: Metal Binding, SOD: Superoxide Dismutase, GPX: Glutathione Peroxidase, TBARS: Thiobarbituric Acid Reactive Substances, HPLC: High Performance Liquid Chromatography, oLDL: Oxidized Low Density Lipoprotein, oLAb: Oxidized Low Density Lipoprotein Autoantibodies, AOPP: Plasma Advanced Oxidation Protein Products, 8OHdG: 8-Hydroxydeoxyguanosine, IL6: Interleukin 6, TNFα: Tumour Necrosis Factor Alpha, HETEs: Hydroxyeicosatetraenoic Acids.
6.3.3.1 Animal studies

There is clear evidence from animal studies that pneumoperitoneum directly results in end organ ischaemia and injury. A recent study by Nickkhohlg et al demonstrated that pneumoperitoneum at 12mmHg for 90 minutes induced histological (in-vivo light microscopy) and biochemical (plasma liver function enzyme levels) evidence of liver reperfusion injury in rats. (203) A significant increase in kidney markers of oxidative stress (MDA, protein carbonyls) was also observed in a rat model of laparoscopic donor nephrectomy compared to controls that underwent a sham operation and were not subjected to pneumoperitoneum. (218, 328) These markers were shown to rise in a time dependent manner, with increased protein carbonyls and decreased protein sulphhydryls and tissue superoxide dismutase activities after 240 minutes of pneumoperitoneum compared with 120 minutes. (218) Increased xanthine oxidase activity was observed in rabbit colonic tissue after only 20 minutes of laparoscopic insufflation. (330) Other studies have shown similar patterns of tissue oxidative stress in open and retro-peritoneoscopic donor nephrectomy. (314)

Extra-abdominal organs also demonstrate oxidative stress in the context of pneumoperitoneum, with significantly increased MDA levels in rat lungs at 2 and 6 hours after deflation. (331)

6.3.3.2 Human studies

Thirteen out of the 37 human studies identified in the search examined oxidative stress in patients undergoing laparoscopic surgery, and details of these are summarised in Table 14. (207, 208, 210, 324, 333-341)
<table>
<thead>
<tr>
<th>Author</th>
<th>Methods</th>
<th>Exposure (n)</th>
<th>Comparison (n)</th>
<th>Insufflation pressure</th>
<th>Marker measured</th>
<th>Timing of measure</th>
<th>Significant result summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eleftheriadis et al 1996</td>
<td>Not randomised, uncontrolled.</td>
<td>Lap chole (8)</td>
<td>Open chole (8)</td>
<td>12 mmHg</td>
<td>Gastric mucosal pH</td>
<td>Intraop, Immediately after deflation in lap</td>
<td>Lower pH in lap intraop. Returned to normal after deflation.</td>
</tr>
<tr>
<td>Windsor et al 1997</td>
<td>Not randomised, uncontrolled.</td>
<td>Lap chole (5)</td>
<td>NA</td>
<td>4 – 8 mmHg</td>
<td>Gastric mucosal pH</td>
<td>After induction, After insufflation, Postop</td>
<td>pH reduced intraop. Pressure dependent.</td>
</tr>
<tr>
<td>Gal et al 1997</td>
<td>Randomised, uncontrolled</td>
<td>Lap chole (21)</td>
<td>Open chole (21)</td>
<td>Not stated</td>
<td>Plasma: MDA, GPX, Myeloperoxidase</td>
<td>Preop, Postop up to 5 days (unclear how frequently)</td>
<td>Higher levels of all three measures in open</td>
</tr>
<tr>
<td>Seven et al 1999</td>
<td>Case-control 2 exposure arms</td>
<td>Open chole (14)</td>
<td>Healthy volunteers who didn't have surgery (15)</td>
<td>Not stated</td>
<td>Plasma: Lipid peroxidation index</td>
<td>Immediately postop</td>
<td>Open &gt; lap &gt; control</td>
</tr>
<tr>
<td>Glantzounis et al 2001</td>
<td>Not randomised, uncontrolled.</td>
<td>Lap chole (30)</td>
<td>Open chole (20)</td>
<td>13 mmHg</td>
<td>Plasma: TBARS, TAS, Uric acid</td>
<td>Preop, 5min after deflation, end of operation, 24h postop</td>
<td>Open vs baseline: TAS, uric acid higher at 24h.</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lap vs baseline: TBARS higher at 5min.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Open vs lap: TBARS higher in lap at 5min, TAS and uric acid higher in open at 24h.</td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Methods</td>
<td>Exposure (n)</td>
<td>Comparison (n)</td>
<td>Insufflation pressure</td>
<td>Marker measured</td>
<td>Timing of measure</td>
<td>Significant result summary</td>
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<tr>
<td>Zulfikaroglu et al 2002</td>
<td>Randomised, uncontrolled</td>
<td>Lap chole (25)</td>
<td>Open chole (25)</td>
<td>12 mmHg</td>
<td>Plasma: MDA, TAS, NO</td>
<td>Plasma: Intraop after 30min of insuffl, 30min post incision, 24h postop; Tissue: immediately after removed from abdomen</td>
<td>Open vs intraop: NO lower, MDA higher at 24h postop; Lap vs intraop: MDA higher at 24h postop; Open vs lap: MDA higher in open intraop, TAS higher in open at 24h, Gallbladder MDA higher in lap</td>
</tr>
<tr>
<td>Ozmen et al 2002</td>
<td>Randomised, uncontrolled</td>
<td>Lap chole (20)</td>
<td>Open chole (20)</td>
<td>12 mmHg</td>
<td>Plasma: MDA, NO</td>
<td>Plasma: preop, midop, immediately postop; Tissue: preop, after induction, intraop, after deflation, closure of abdomen, 1h and 24h postop</td>
<td>Plasma MDA higher in lap than open mid-operation only. No other differences found.</td>
</tr>
<tr>
<td>Polat et al 2003</td>
<td>Randomised, uncontrolled</td>
<td>Lap chole at 15 mmHg (12)</td>
<td>Lap chole at 10 mmHg (12)</td>
<td>15 mmHg 10 mmHg</td>
<td>Plasma: TBARS, Carbonyl, Sulphhydryl</td>
<td>Preop, Immediately postop, 6h postop, 24h postop</td>
<td>Significant increase compared to baseline for both groups. Carbonyl levels higher in 15 mmHg than 10 mmHg group immediately postop.</td>
</tr>
<tr>
<td>Author</td>
<td>Methods</td>
<td>Exposure (n)</td>
<td>Comparison (n)</td>
<td>Insufflation pressure</td>
<td>Marker measured</td>
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</tbody>
</table>
| Bentes de Souza et al 2003 | Not randomised, uncontrolled.                | Lap uterine myoma or ovarian cyst excision (14) | Open uterine myoma excision (14)                   | 15 mmHg               | Tissue: 8-isoprostane, HETEs, MDA | 1x1cm peritoneal square removed on breaching peritoneum (1st sample) | Open 1st vs 2nd sample: 15-HETE lower in 2nd sample  
Lap 1st vs 2nd sample: 8-isoprostane, 5-HETE higher in 2nd sample. Correlated with insufflation time and volume.  
Open vs lap: MDA higher in open. 8-isoprostane, HETEs higher in lap. |
| Zhang et al 2004         | Not randomised, uncontrolled.                | Lap uterine myoma enucleation (20)               | Open uterine myoma enucleation (20)                | Not stated            | Plasma: MDA, AOA, GPX            | Preop, 5min after deflation, end of operation, 24h postop  | Open vs baseline: MDA higher, GPX and AOA lower at 24h  
Lap vs baseline: MDA higher, GPX and AOA lower immediately postop. Back to normal at 24h.  
Open vs lap: Open higher MDA and lower GPX at 24h. |
<table>
<thead>
<tr>
<th>Author</th>
<th>Methods</th>
<th>Exposure (n)</th>
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<th>Marker measured</th>
<th>Timing of measure</th>
<th>Significant result summary</th>
</tr>
</thead>
</table>
| Hakan Bukan et al 2004 | Randomised, uncontrolled | Lap chole (21) | Open chole (21) | Not stated             | Plasma: MDA, Nitrite, Nitrate | 1 day preop, 45 min after incision, 1 day postop | **Open vs baseline:** MDA and Nitrite higher intraop, back to normal day 1.  
**Lap vs baseline:** MDA lower day 1 postop. Nitrite higher intraop, back to normal day 1.  
**Open vs lap:** Open higher nitrite day 1 postop. |
| Zhang et al 2005    | Randomised, uncontrolled | Lap uterine myoma enucleation (20) | Open uterine myoma enucleation (20) | Not stated | Plasma: MDA, AOPP, AOA, GPX | Preop, 5min after deflation, end of operation, 24h postop | **Open vs baseline:** AOPP and MDA higher, GPX and AOA lower at 24h  
**Lap vs baseline:** AOPP and MDA higher, GPX and AOA lower end of OT. All except AOA back to normal at 24h.  
**Open vs lap:** Open higher AOPP and MDA, lower GPX at 24h. |
<table>
<thead>
<tr>
<th>Author</th>
<th>Methods</th>
<th>Exposure (n)</th>
<th>Comparison (n)</th>
<th>Insufflation pressure</th>
<th>Marker measured</th>
<th>Timing of measure</th>
<th>Significant result summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stipancic et al 2005</td>
<td>Case-control 2 exposure arms</td>
<td>Lap chole (21)</td>
<td>Control: Healthy volunteers who didn’t have surgery (20)</td>
<td>13 mmHg</td>
<td>Plasma: POX, oLAb, neopterin, TAS, SOD</td>
<td>24h preop, 1 day postop, 2 days postop, 3 days postop</td>
<td>Open vs baseline: POX lower on day 1, higher on day 7. oLAb, neopterin higher on day 7. Lap vs baseline: POX higher on day 7. Open vs lap: POX lower in open on day 1. oLAb, neopterin higher in open on day 7.</td>
</tr>
</tbody>
</table>

**Table 14: Summary of Clinical Trials on Laparoscopic Oxidative Stress (Organised by Date).**

- *All patients subjected to a period of low pressure, then normal pressure insufflation.*
Three randomised clinical trials (208, 334, 341) and two case-control studies (207, 337) have compared plasma markers of lipid peroxidation (see Table 14) between laparoscopic and open cholecystectomy, and found these to be elevated in both groups, but significantly higher in open surgery. The difference was demonstrable up to day 7 in one of the case-control studies when plasma oxidized low density lipoprotein autoantibodies and neopterin were utilised as markers.(207) One non-randomised trial found that there was a significantly higher level of thiobarbituric acid–reactive substances (TBARS) in laparoscopic versus open cholecystectomy. This was seen only at 5 minutes post deflation, with no significant difference at 24 hours.(335)

Studies conducted in China on female patients who underwent laparoscopic and open enucleation of uterine myomas consistently demonstrated higher levels of MDA and advanced oxidation protein products (AOPP), and lower levels of glutathione peroxidise (GPX) and antioxidant activity (AOA) in laparoscopic cases immediately postoperatively.(339, 340) This picture reversed at 24h, with a deterioration in the open cases at this time point, when levels in the laparoscopic group had essentially normalised. Another gynaecologic study (which included both uterine myomas and ovarian cysts) showed a greater increase of MDA in the open group, but a higher level of isoprostanes and hydroxyeicosatetraenoic acids in the laparoscopic group. The authors hypothesised that the oxidative stress was occurring through different pathways (thromboxane synthesis in open cases, and lipid peroxidation in the laparoscopic cases).(333) However, the comparison was weakened by the significant difference in the baseline uterine pathology between groups. Interestingly, the extent of the oxidative stress in the laparoscopic group appeared to correlate significantly with the volume of gas used, as well as the duration of insufflation.(333)

Gastric mucosal pH has also been used as a measure of tissue oxygenation in human studies.(344, 345) This technique assumes equilibrium across the superficial gastric mucosa between pCO$_2$ in the gut lumen and the bicarbonate concentration in the blood. It is inherently
limited by these assumptions, but serves as an indirect measure of splanchnic perfusion.(346) In 1997, Windsor et al published a preliminary study showing that there was a measurable and continuous reduction in gastric mucosal pH during laparoscopic cholecystectomy.(338) It is not known whether this reduction is more pronounced compared to that for open cholecystectomy. An early study by Eleftheriadis et al performed a non-randomised comparison of laparoscopic and open cholecystectomy patients using a single intraoperative gastric mucosal pH measurement, and demonstrated a significantly lower pH in the laparoscopic group.(324) However, this result has been challenged by a larger randomised trial conducted by Ozmen et al. that did not show a significant difference in gastric mucosal pH between laparoscopic and open cholecystectomy patients when this was measured at multiple points intra-operatively and postoperatively (see Table 14).(336) In addition to study design, another possible reason for the different outcomes of these two studies, is that Ozmen et al. excluded all patients with significant comorbidities and therefore the study patients may have been better able to tolerate pneumoperitoneum than the general patient population. Both studies used similar insufflation pressures of approximately 12 mmHg.

The data presented suggests that pneumoperitoneum causes oxidative stress, with significant elevations in local and plasma markers in the laparoscopy groups compared to baseline in most studies, and even in relatively minor surgery. It is notable that the elevation in biochemical markers after laparoscopy is not necessarily higher than in corresponding open procedures.(182, 183, 187) The finding that laparoscopic surgery is associated with oxidative stress requires some explanation, and it is reasonable to ask the question whether it is the raised intra-abdominal pressure alone that might be blamed, or whether other factors such as the nature of the gas used for insufflation may contribute as well.
6.3.4 Impact of insufflation pressure

6.3.4.1 Animal studies

Pneumoperitoneum applied in rats with 5, 10, or 15 mmHg pressure increased the formation of free oxygen radicals in the lung and liver tissues incrementally.(347) Similar increases were seen in MDA levels in the small intestine of rabbit subjected to pneumoperitoneum at 0, 15, and 25 mmHg,(332) and in the kidneys of rats in the laparoscopic donor nephrectomy model mentioned previously.(328) In the study by Nickkholgh et al, the histological and biochemical evidence of liver reperfusion injury was demonstrable at 12mmHg but not at 8mmHg.(203) In a pig model, Windberger et al found a significantly reduced portal venous flow and hepatic tissue pH when 14 mmHg pneumoperitoneum was performed compared to 7 mmHg, at 60 min post insufflation.(348)

6.3.4.2 Human studies

Laplace’s law describes the effect of pneumoperitoneum on pressure across the abdominal wall (the higher the tension on the abdominal wall the higher the intra-abdominal pressure for a given fixed radius at maximal abdominal expansion).(349) This pressure reduces peritoneal microcirculation during laparoscopy, in addition to the systemic haemodynamic effect discussed previously.(317, 333) Schilling et al demonstrated that this reduction in organ perfusion is pressure dependent by studying the effect of different insufflation pressures in laparoscopic cholecystectomy using a laser Doppler flow probe to measure organ perfusion.(326) Increasing pressure from 10 to 15 mmHg significantly decreased the blood flow in the stomach by 40 percent, duodenum by 11 percent, jejunum by 32 percent, colon by 44 percent, liver by 39 percent, and the parietal peritoneum by 60 percent. Splanchnic blood flow also decreased significantly with operative time at a constant intra-arterial pressure.(326) It has also been shown that there is a significant reduction in gastric mucosal pH in laparoscopic cholecystectomy patients when an insufflation pressure of 12-15 mmHg is used compared to a pressure of 4-8 mmHg.(338) Polat et al. demonstrated a trend towards increased oxidative markers when laparoscopic cholecystectomy was performed in patients at 15mmHg rather than 10mmHg, but this was only statistically significant for carbonyl
levels immediately postoperatively (Table 14). It is interesting to draw an analogy with abdominal compartment syndrome, where the detrimental effects of elevated intra-abdominal pressure are frequently realised, usually in surgical intensive care units. In this setting, intra-abdominal hypertension defined as intra-abdominal pressure ≥ 12 mmHg.

6.3.5 Impact of the nature of gas used

6.3.5.1 Type of gas

An ideal gas for establishing pneumoperitoneum is neutral (so as not to support combustion), easily absorbed, and completely non-toxic to the peritoneal environment. The most widely used gas, CO₂, is not ideal, but does have the advantage of being readily available, quickly absorbed and excreted, inexpensive and nonflammable. When insufflated into the abdominal cavity, CO₂ normally diffuses across the peritoneum, and is carried by the circulation to the lung, where it is expired. However, adverse effects have been associated with the increased systemic CO₂ load manifested by recordable increases in arterial pCO₂ after laparoscopy. This increase in pCO₂ results in a reduced tissue pH causing vasoconstriction and reducing mesenteric and hepatic blood flow. Furthermore, local acidity caused by the CO₂ being converted to carbonic acid in the peritoneal fluid, results in microscopically visible histological damage to the mesothelial ultra-structure, which is more pronounced than when peritoneum is exposed to room air. There is also evidence that CO₂ directly blunts peritoneal macrophage function in vivo.

Non-reactive gases such as helium and argon have been considered as alternatives. Yilmaz et al. showed a significantly higher increase in oxidative stress markers when CO₂ was used compared with helium for a given insufflation pressure in a rat model. Helium pneumoperitoneum did not produce any significant MDA and carbonyl elevation even at 15mmHg as compared to control, but CO₂ pneumoperitoneum caused a significant increase, even at pressures as low as 10mmHg. Despite these apparent advantages it is likely that CO₂ will remain the preferred gas for
pneumoperitoneum, largely due to concerns that non-absorbable gases might be at greater risk of contributing to potentially fatal gas emboli, and pneumothoraces.\(^{(220, 353)}\)

Nitrous oxide was commonly used in the 1970's. While it may offer some advantages in terms of patient tolerance,\(^{(220)}\) its combustibility makes it unsafe for use with electrocautery. Its use as an insufflation agent has declined dramatically in parallel with the increased use of electrocautery, and its relative effects on pneumoperitoneum associated oxidative stress have not been studied.

### 6.3.5.2 Gas temperature and humidity

The gas used in laparoscopy is typically cold and dry, at \(21^\circ\text{C}\) and 0% relative humidity.\(^{(205)}\) Large volumes of gas may be required for a single case (up to 500L), owing to the imperfect seal of the laparoscopic ports, and peritoneal carbon dioxide absorption. The effect of this cold, dry gas flow on the peritoneal environment is significant,\(^{(205)}\) with the desiccating and cooling effect producing visible structural, morphological, and biochemical changes (increased cytokine response) in the peritoneal mesothelial surface layer.\(^{(219-222)}\) This includes bulging of mesothelial cells, widening of intercellular junctions, and exposure of the basement membrane underneath.\(^{(221)}\) This effect can apparently be attenuated by warming and humidification of insufflated gas.\(^{(222, 226)}\)

Despite postulation of causality, there have not been any animal or human studies investigating whether this directly contributes to oxidative stress during pneumoperitoneum,\(^{(195)}\) although warming insufflated \(\text{CO}_2\) has been shown to be associated with a reduced peritoneal cytokine response after laparoscopic cholecystectomy in humans.\(^{(295)}\)
6.3.6 Measures to reduce oxidative stress associated with pneumoperitoneum

6.3.6.1 Practical measures

Routine laparoscopy is well tolerated in fit patients, but the clinical significance of the oxidative stress caused by pneumoperitoneum in those with significant co-morbidities needs further investigation. Nevertheless, several authors have recommended simple measures that might reduce its impact in situations where physiological reserves are compromised, such as in patients with renal or cardiovascular disease. It is recommended that the lowest possible inflation pressure is used, with insufflation started at a standard pressure (12 – 15 mm Hg), and then gradually lowered as much as possible without compromising visibility ("dial down" approach). (354, 355) Some have also advocated releasing gas intermittently during the procedure. (317, 356) Gasless surgery using various abdominal wall lifting devices has also been employed, although this may not provide uniform exposure and increases operating time. (354, 357) From a haemodynamic point of view, the head-up position should be avoided, as this exacerbates venous stasis, and thus reduces venous return. (327, 358, 359) Intravascular volume expansion with intravenous fluids prior to commencement of pneumoperitoneum can enhance renal perfusion and reduce the extent of renal injury. (360) The evidence for this is limited, however, and there are no clear administration protocols or guidelines as to which subset of patients would benefit most. Indeed, in certain patients with severe cardiovascular or renal function compromise, volume expansion can be detrimental to end organ perfusion, as the extra fluid cannot be distributed or excreted adequately. This has led to the concept of individually tailored fluid replacement, where the volume given intra-operatively is goal directed (based on direct measures of cardiac output). (361, 362) These protocols have been shown to result in improved short term postoperative outcomes, although their relationship with oxidative stress has not been investigated. (125, 362)

A mechanical solution raised by Bickel involves using an intermittent sequential pneumatic compression device to improve venous return. (363) This device compresses the lower limb veins using two sleeves wrapped around the patient’s legs from ankle to groin. The pressure generated
is distributed into overlapping air compartments sequentially from distal to proximal, and to both legs simultaneously in 30-second cycle consisting of 18 seconds of compression and 12 seconds of decompression. This has been shown to reduce venous stasis and deep venous thrombosis,(247, 358, 364-366) improve haemodynamic parameters intraoperatively,(363, 367, 368) and reduce plasma levels of total lipid peroxides during the reperfusion phase in laparoscopic cholecystectomy.(369, 370) Peroneal nerve compression has been reported as a rare complication of this system of lower leg compression.(371, 372)

6.3.6.2 Ischaemic pre-conditioning

Ischemic-preconditioning is a concept that has been employed to avoid the harmful effects of ischaemia-reperfusion injury in cardiac(209), liver,(373, 374) and reconstructive surgery.(375) It consists of short periods of ischaemia-reperfusion cycles, which are performed prior to the main insult taking place. These cycles can be followed by increased resistance to cellular re-oxygenation injury.(206) This theory has been tested in rats using various protocols of short periods of inflation and deflation upon establishment of pneumoperitoneum. The results consistently demonstrated a reduction in oxidative stress as measured by various plasma and tissue markers. (206, 210, 376, 377) Furthermore, a reduction in peritoneal and plasma cytokine levels, and an improvement in small bowel, kidney and liver histological injury scores were seen in preconditioned animals compared to those that were not preconditioned.(206, 210, 376-378) Cevrioglu et al. also included a low pressure group in their study (insufflated at 10mmHg). While this improved oxidative stress compared to 15mmHg, preconditioning (with 10 min of 15mmHg followed by deflation for 10min) prior to the usual 15mmHg insufflation resulted in significantly reduced levels of oxidative stress markers compared to the low pressure group. They concluded that preconditioning “can be more effective than low-pressure pneumoperitoneum in reducing the oxidative stress and inflammatory cytokine response associated with laparoscopy”.(376) Despite this success in animals, no human studies on preconditioning in pneumoperitoneum have been conducted to the authors’ knowledge, and preconditioning has not found a definite place in clinical practice. This may be because
experimental protocols of preconditioning are conceived by some to be too lengthy for practical use. (206, 376)

**6.3.6.3 Pharmacologic agents**

Various pharmacological agents have been tested to combat oxidative stress, and some antioxidants and vasodilators have been shown to be successful in animal models. (354, 379, 380) Administration of dopamine and endothelin-1 antagonists greatly improves the portal circulation in rats subjected to CO₂ and helium insufflation, but oxidative stress markers have not been measured. (380) Pre-treatment with the calcium channel antagonist verapamil significantly reduces oxidant levels and increased antioxidant levels in a rabbit model of retroperitoneoscopy. (381) This was based on previous studies of verapamil showing a reduction in renal and hepatic ischaemia–reperfusion injury by acting on calcium influx, although a specific molecular interaction between calcium channel antagonists and reactive oxygen species is not evident. (382, 383)

Erythropoietin is a hypoxia-inducible growth factor expressed mainly in the kidney. It has multiple protective effects against oxidants and apoptosis. (377) Administration of erythropoietin in a rat model at a dose of 1,000 U/kg prior to laparoscopy significantly decreases plasma MDA levels compared to controls. (377) Similarly, melatonin (10 mg/kg) administered 5 min before insufflation and immediately before desufflation in a rat model significantly reduces mean MDA levels in liver, small intestine and kidney, and improves small bowel histological parameters. (384)

Other endogenous antioxidants (e.g. tocopherol, glutathione, superoxide dismutase) and various synthetic antioxidant drugs (e.g. xanthine oxidase inhibitors) (385) provide possible avenues to minimize tissue injury in laparoscopic surgery, and indeed open surgery as well. Clinical trials are needed before these can be accepted into general use.
6.4 CONCLUSION

This review has examined the relationship between pneumoperitoneum and oxidative stress. It is clear that pneumoperitoneum causes a reduction in splanchnic blood flow due to both systemic and local effects, in a pressure and time dependent manner, resulting in biochemical evidence of oxidative stress and histological evidence of tissue injury.

It is important to note that corresponding open abdominal procedures are associated with a similar oxidative stress response, indicating that this phenomenon is not simply due to elevated intra-abdominal pressure. There is some evidence that the use of carbon dioxide as the insufflation may be contributory, but the impact of other factors such as the temperature and humidity of the gas have not been elucidated despite evidence of histological injury caused by desiccation. It is now appropriate to investigate this, and evaluate whether warming and humidification of insufflated carbon dioxide could be beneficial in this respect.
CHAPTER 7: SYSTEMATIC REVIEW AND META-ANALYSIS – THE EFFECT OF WARM, HUMIDIFIED INSUFFLATION ON PAIN AFTER LAPAROSCOPY
7.1 BACKGROUND

Working space in laparoscopic surgery is created by using pneumoperitoneum to lift the abdominal wall. The gas used is typically cold, dry carbon dioxide (CO$_2$) at 21°C, and 0% relative humidity. The effect of this gas on the peritoneal environment has been probably underestimated. Carbon dioxide is a peritoneal irritant that, when dry, also has a desiccating effect. The result is structural, morphological and biochemical changes in the peritoneal mesothelial surface layer.(219-222, 301) Some studies describing laparoscopy in awake patients have shown that this translates into a conscious sensation of pain, although results have been conflicting.(386-388)

Warming and humidification of the insufflated carbon dioxide is relatively easily achieved by incorporating a commercially available humidifier into the tubing of the insufflation apparatus. Several randomized controlled trials have been published evaluating the use of warming and humidification in laparoscopic cholecystectomy, gastric bypass, gastric fundoplication, and gynaecologic procedures. The European Association for Endoscopic Surgery clinical practice guideline on the establishment of pneumoperitoneum suggests that data on the influence of warming and humidification on postoperative pain is contradictory. It also states that, because most studies had small sample sizes with possible type II error, no firm conclusions can be drawn.(389) There is also the question of whether the beneficial effects noted by some authors are due to prevention of hypothermia alone, as this is perhaps more easily achieved using an external warming device (such as a forced air warming blanket) and other conservative measures.(389)

While there are no clinical trials evaluating the effect of warming and humidification on the peritoneal inflammatory response, most randomised controlled trials have used postoperative pain as the main outcome measure, with this endpoint regarded as a proxy measure of peritoneal inflammation. We conducted a meta-analysis of these randomised controlled trials.
7.2 METHODS

7.2.1 Research question

The principal question is: Does humidification and warming of insufflated CO\textsubscript{2} (HWI) reduce postoperative pain compared with using standard cold and dry CO\textsubscript{2} after laparoscopic abdominal surgery in adults.

7.2.2 Systematic literature search

Medline including in-process and other non-indexed citations, Cochrane Library, Cochrane Central Register of Controlled Trials (CENTRAL/CCTR), Pubmed, and EMBASE were searched independently by two investigators (Tarik Sammour, Arman Kahokehr) using the search terms outlined in Table 15. Papers spanning a period of time from 1947 (earliest EMBASE records) to January 2008 were searched with no language restriction. Relevant scientific meeting abstracts were also manually searched by a single investigator (Arman Kahokehr) to identify further papers. All the results were pooled into a single database, and duplicate entries removed. The titles and abstracts of these studies were scrutinized, and reference lists were manually searched to identify further relevant articles.

7.2.3 Study selection

Inclusion criteria were randomised controlled clinical trials investigating humidification and warming of insufflated carbon dioxide during elective laparoscopic surgery under general anaesthesia for any indication in adults. The comparison group was patients receiving standard cold, dry insufflated carbon dioxide for the same indication. The outcome measure was post-operative pain as measured by visual analogue scale (VAS) and/or morphine equivalent usage for up to 72 hours after surgery.\textsuperscript{238, 239} Exclusion criteria were: animal studies, thoracoscopy studies, awake laparoscopy studies, studies in patients less than 15 years of age, non-randomised trials (no restriction on randomisation method), studies that compared warm versus cold gas without humidification, studies that compared humidified, cold gas versus heated gas, and studies that did
not measure post-operative pain as an outcome measure. Study selection was undertaken independently by two investigators (Tarik Sammour, Arman Kahokehr), with any disagreement resolved by consensus, and consultation with the senior doctoral supervisor (Andrew G Hill) if consensus could not be reached.

<table>
<thead>
<tr>
<th>Database</th>
<th>Search terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>CENTRAL</td>
<td>Humidif$ AND (Laparoscop$ OR endoscop$ OR coelioscop$ OR insuffl$ OR CO2 OR carbon dioxide OR hysteric$ OR gyne$ OR gynae$ OR cholecystec$ OR gallbladder OR bariatric OR bypass).</td>
</tr>
<tr>
<td>Medline ® and Medline in-process &amp; other non-indexed citations</td>
<td>Humidif$ AND (Laparoscop$ OR endoscop$ OR coelioscop$ OR insuffl$ OR CO2 OR carbon dioxide OR hysteric$ OR gyne$ OR gynae$ OR cholecystec$ OR gallbladder OR bariatric OR bypass).</td>
</tr>
<tr>
<td>Cochrane library</td>
<td>(Humidification OR humidified OR humidify OR humidifying) AND (Laparoscopy OR endoscopy OR coelioscopy OR insufflation OR CO2 OR carbon dioxide OR hysterectomy OR gynecology OR gynaecology OR cholecystectomy OR gallbladder OR bariatric OR bypass).</td>
</tr>
<tr>
<td>Pubmed</td>
<td>(humidif* [Title]) AND (laparoscop* [Title/Abstract] OR endoscop* [Title/Abstract] OR coelioscop* [Title/Abstract] OR insuffl* [Title/Abstract] OR CO2 [Title/Abstract] OR carbon dioxide [Title/Abstract] OR hysterectomy [Title/Abstract] OR gyne* [Title/Abstract] OR gynae* [Title/Abstract] OR cholecystectomy [Title/Abstract] OR gallbladder [Title/Abstract] OR bariatric [Title/Abstract] OR bypass [Title/Abstract])</td>
</tr>
<tr>
<td>Embase</td>
<td>Humidif$ AND (Laparoscop$ OR endoscop$ OR coelioscop$ OR insuffl$ OR CO2 OR carbon dioxide OR hysterectomy OR gyne$ OR gynae$ OR cholecystectomy OR gallbladder OR bariatric OR bypass)</td>
</tr>
</tbody>
</table>

Table 15: Search Terms Used in Different Databases.

7.2.4 Validity assessment

Trial assessment was performed by a single investigator (Tarik Sammour), who was blinded to the journal title, article title, and authors of the publications. Methodological quality was determined using the Jadad scale. (228) This scale consists of 3 items: random allocation, masking of patients, and dropouts and withdrawals. The scale ranges from 0 to 5 points, with 2 or less indicating low quality and 3 or more indicating high quality.
7.2.5 Data abstraction

The corresponding author for each publication was contacted to obtain as much raw data as possible. Data on pain by visual analogue scale and morphine equivalent analgesia usage were gathered and summarized on an intention-to-treat basis in 2 × 2 tables for each outcome. As different studies measured pain scores at slightly different intervals, values for visual analogue scores were collated and grouped into the following post-operative time intervals: up to 6 h, on day 1, on day 2, and on day 3. Morphine equivalent usage data were similarly grouped into usage up to 6 h, on day 1 and on day 2. When an outcome was measured more than once within a particular defined time interval, the mean value was calculated and used.

7.2.6 Statistical analysis

Analysis of combined data was performed using RevMan version 4.2 (The Nordic Cochrane Centre, Rigshospitalet, Copenhagen, Denmark). Standard deviation of the outcome data was used if provided by the authors, or calculated from $P$ values, confidence intervals (C.I.), or data ranges if they were not. Results of the meta-analysis were assessed by graphical presentations of mean difference with 95% C.I. on forest plots using the fixed-effects model, with $P < 0.050$ considered statistically significant. A Chi$^2$ test for heterogeneity was also performed, in which $P < 0.100$ was regarded as significant. A priori subset analyses based on the use of an external warming device was performed.
7.3 RESULTS

A total of 195 results were entered into a unified database and duplicate results removed. Twenty eight potentially relevant publications were identified and retrieved after title and abstract review. Of these, 9 papers met the inclusion criteria. A QUORUM diagram is provided in Figure 46. Two studies had to be excluded from the analysis at this stage: the first reported pain outcome as the presence or absence of pain, with no reported data on analogue score or analgesia use,(390) and the second had no measure of data distribution, standard deviation, or statistical significance (this could not be obtained by contacting the corresponding author).(391)

Thus, seven studies were included in the data analysis. Three described gastric bypass,(392-394) 2 laparoscopic cholecystectomy,(238, 395) 1 Nissen fundoplication,(396) and 1 gynaecological procedures.(397) Studies were performed in two countries (6 in the USA, and 1 in Australia) and were published between 1999 and 2006. A total of 327 patients were included, with 163 in the HWI group and 164 in the control group. A summary of included studies is presented in Table 16, and a summary of results in Table 17.
Figure 46: QUORUM Diagram.
N: Number of Papers, RCT: Randomised Controlled Trial, Vs.: Versus
<table>
<thead>
<tr>
<th>Study</th>
<th>Humidified (n)</th>
<th>Control (n)</th>
<th>Withdrawals (n)</th>
<th>External warming device?</th>
<th>Indication</th>
<th>Mean Age (approx)</th>
<th>OT Time (min)</th>
<th>Double blinding</th>
<th>Randomised</th>
<th>Adequate control</th>
<th>Jadad score</th>
<th>Outcome reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farley 2004</td>
<td>49</td>
<td>52</td>
<td>16 patients</td>
<td>Anaesthetist discretion</td>
<td>Lap chole</td>
<td>52 years</td>
<td>91</td>
<td>Y – method adequate</td>
<td>Y – method adequate</td>
<td>Y</td>
<td>5</td>
<td>Morph</td>
</tr>
<tr>
<td>Nguyen 2002</td>
<td>10</td>
<td>10</td>
<td>Not stated</td>
<td>External warming blanket for all patients</td>
<td>Nissen fundo</td>
<td>44 years</td>
<td>108</td>
<td>N</td>
<td>Y – not adequately described</td>
<td>Y</td>
<td>1</td>
<td>Pain VAS Morph</td>
</tr>
<tr>
<td>Savel 2005</td>
<td>15</td>
<td>15</td>
<td>Not stated</td>
<td>Anaesthetist discretion</td>
<td>Gastric bypass</td>
<td>40 years</td>
<td>89</td>
<td>Y – method inadequate</td>
<td>Y – method inadequate</td>
<td>Y</td>
<td>1</td>
<td>Pain VAS Morph</td>
</tr>
<tr>
<td>Champion 2006</td>
<td>25</td>
<td>25</td>
<td>Not stated</td>
<td>No</td>
<td>Gastric bypass</td>
<td>43 years</td>
<td>62</td>
<td>Y – method inadequate</td>
<td>Y – method inadequate</td>
<td>Y</td>
<td>0</td>
<td>Pain VAS</td>
</tr>
<tr>
<td>Ott 1998</td>
<td>25</td>
<td>25</td>
<td>Not stated</td>
<td>No</td>
<td>Gynaec</td>
<td>Not stated</td>
<td>Not stated</td>
<td>N</td>
<td>Y – not adequately described</td>
<td>Y</td>
<td>1</td>
<td>Pain VAS</td>
</tr>
<tr>
<td>Hamza 2005</td>
<td>23</td>
<td>21</td>
<td>6 patients</td>
<td>No (patients needing this excluded)</td>
<td>Gastric bypass</td>
<td>44 years</td>
<td>114</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>5</td>
<td>Pain VAS Morph</td>
</tr>
<tr>
<td>Mouton Australia 1999</td>
<td>16</td>
<td>16</td>
<td>8 patients</td>
<td>Not stated</td>
<td>Lap chole</td>
<td>Not stated</td>
<td>Not stated</td>
<td>N</td>
<td>Y – method not adequately described</td>
<td>Y</td>
<td>2</td>
<td>Pain VAS</td>
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</table>

**Table 16: Summary Characteristics and Quality Assessment of Included Studies.**
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Subset</th>
<th>No. of trials Included</th>
<th>Humidified Mean (n)</th>
<th>Control Mean (n)</th>
<th>Test for overall effect (Z)</th>
<th>P value</th>
<th>SMD</th>
<th>CI</th>
<th>Chi²</th>
<th>P value</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5</td>
<td>3.58 (104)</td>
<td>4.82 (104)</td>
<td>2.75</td>
<td>0.006 *</td>
<td>-0.39</td>
<td>-0.67 to -0.11</td>
<td>5.97</td>
<td>0.200</td>
</tr>
<tr>
<td></td>
<td>EW used</td>
<td>1</td>
<td>4.30 (15)</td>
<td>4.00 (15)</td>
<td>Only one study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EW not used</td>
<td>4</td>
<td>3.40 (89)</td>
<td>5.03 (87)</td>
<td>3.27</td>
<td>0.001 *</td>
<td>-0.50</td>
<td>-0.81 to -0.20</td>
<td>2.36</td>
<td>0.500</td>
</tr>
<tr>
<td>Pain VAS 6 hours</td>
<td>Total</td>
<td>6</td>
<td>3.50 (111)</td>
<td>4.62 (110)</td>
<td>2.47</td>
<td>0.010 *</td>
<td>-0.34</td>
<td>-0.61 to -0.07</td>
<td>14.02</td>
<td>0.02 c</td>
</tr>
<tr>
<td></td>
<td>EW used</td>
<td>2</td>
<td>3.5 (25)</td>
<td>4.60 (25)</td>
<td>2.92</td>
<td>0.003 *</td>
<td>-0.88</td>
<td>-1.48 to -0.29</td>
<td>2.20</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>EW not used</td>
<td>4</td>
<td>3.5 (86)</td>
<td>4.63 (85)</td>
<td>1.28</td>
<td>0.200</td>
<td>-0.50</td>
<td>-0.11 to 0.11</td>
<td>7.75</td>
<td>0.050 c</td>
</tr>
<tr>
<td>Pain VAS day 1</td>
<td>Total</td>
<td>5</td>
<td>2.16 (93)</td>
<td>3.30 (92)</td>
<td>0.17</td>
<td>0.870</td>
<td>-0.27</td>
<td>-0.32 to 0.32</td>
<td>13.07</td>
<td>0.010 c</td>
</tr>
<tr>
<td></td>
<td>EW used</td>
<td>1</td>
<td>2.30 (15)</td>
<td>1.60 (15)</td>
<td>Only one study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EW not used</td>
<td>4</td>
<td>3.38 (78)</td>
<td>3.73 (77)</td>
<td>1.92</td>
<td>0.060</td>
<td>-0.40</td>
<td>0.24 to 0.09</td>
<td>10.56</td>
<td>0.010 c</td>
</tr>
<tr>
<td>Pain VAS day 2</td>
<td>Total</td>
<td>3</td>
<td>1.57 (50)</td>
<td>4.2 (49)</td>
<td>4.02</td>
<td>&lt;0.0001 *</td>
<td>-1.3</td>
<td>-0.45 to 0.45</td>
<td>3.87</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>EW used</td>
<td>No studies</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EW not used</td>
<td>3</td>
<td>1.57 (50)</td>
<td>4.2 (49)</td>
<td>4.02</td>
<td>&lt;0.0001 *</td>
<td>-1.3</td>
<td>-0.45 to 0.45</td>
<td>3.87</td>
<td>0.140</td>
</tr>
<tr>
<td>Pain VAS day 3</td>
<td>Total</td>
<td>3</td>
<td>9.17 (87)</td>
<td>10.9 (88)</td>
<td>0.46</td>
<td>0.650</td>
<td>-0.37</td>
<td>0.23 to 0.37</td>
<td>4.84</td>
<td>0.090 c</td>
</tr>
<tr>
<td></td>
<td>EW used</td>
<td>2</td>
<td>11.3 (64)</td>
<td>11.4 (67)</td>
<td>0.57</td>
<td>0.570</td>
<td>-0.24</td>
<td>0.44 to 0.24</td>
<td>0.98</td>
<td>0.320</td>
</tr>
<tr>
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<td>EW not used</td>
<td>1</td>
<td>5.00 (23)</td>
<td>10.00 (21)</td>
<td>Only one study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morph 6 hours</td>
<td>Total</td>
<td>3</td>
<td>33.33 (48)</td>
<td>35.00 (46)</td>
<td>1.59</td>
<td>0.110</td>
<td>-0.33</td>
<td>-0.74 to 0.08</td>
<td>1.85</td>
<td>0.400</td>
</tr>
<tr>
<td></td>
<td>EW used</td>
<td>2</td>
<td>34.00 (25)</td>
<td>34.00 (25)</td>
<td>0.61</td>
<td>0.540</td>
<td>-0.37</td>
<td>0.39 to 0.07</td>
<td>1.19</td>
<td>0.270</td>
</tr>
<tr>
<td></td>
<td>EW not used</td>
<td>1</td>
<td>32.00 (23)</td>
<td>37.00 (21)</td>
<td>Only one study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morph day 1</td>
<td>Total</td>
<td>2</td>
<td>29.00 (38)</td>
<td>32.50 (36)</td>
<td>2.01</td>
<td>0.040 *</td>
<td>-0.48</td>
<td>-0.95 to -0.01</td>
<td>2.13</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>EW used</td>
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<td>43.00 (15)</td>
<td>44.00 (15)</td>
<td>Only one study</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>EW not used</td>
<td>1</td>
<td>15.00 (23)</td>
<td>21.00 (21)</td>
<td>Only one study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morph day 2</td>
<td>Total</td>
<td>2</td>
<td>29.00 (38)</td>
<td>32.50 (36)</td>
<td>2.01</td>
<td>0.040 *</td>
<td>-0.48</td>
<td>-0.95 to -0.01</td>
<td>2.13</td>
<td>0.140</td>
</tr>
</tbody>
</table>

Table 17: Summary of Results.
No.: Number, N: Number of patients in each arm, EW: External Warming Device, SMD: Standardised Mean Difference, CI: Confidence Interval, Chi²: Chi squared test of heterogeneity, *: P < 0.050 for overall effect, c: P < 0.100 for heterogeneity by Chi²
Yellow highlighted: reached statistical significance with no heterogeneity between studies.
7.3.1 Pain by visual analogue scale

7.3.1.1 All studies
Forest plots for pain by visual analogue scale are displayed in Figure 47 to Figure 50. There was a statistically significant reduction in VAS pain scores in the HWI group at 6 hours \((p < 0.006)\), on day 1 \((p = 0.010)\), and on day 3 \((p < 0.0001)\). There was significant heterogeneity between studies for the data on day 1 only \((p = 0.020)\).

7.3.1.2 Studies using an external warming device
Subset analysis including only the studies where an external warming device had been used demonstrated significantly lower scores on day 1 \((p = 0.003)\) in the HWI group, with no significant heterogeneity in the data (Figure 51). It was not possible to perform this analysis at other intervals as there were not enough data to combine.

7.3.1.3 Studies without an external warming device
Subset analysis of only studies without an external warming device, demonstrated a significant reduction in pain in the HWI group at 6h \((p = 0.001)\) and day 3 \((p < 0.0001)\), with no significant heterogeneity (Figure 52 and Figure 55). There was no significant difference at day 1 or day 2 (Figure 53 and Figure 54).

7.3.2 Morphine equivalent usage

7.3.2.1 All studies
Forest plots for morphine equivalent usage are displayed in Figure 56 to Figure 58. A statistically significant reduction in morphine equivalent usage was seen on day 2 \((p = 0.040)\) in the HWI group, with no significant heterogeneity in the data.
7.3.2.2 Studies using an external warming device

Subset analysis including only the studies where an external warming device had been used did not show any significant difference between the two groups at 6 hours or day 1 post-operatively (Figure 59 and Figure 60). Analysis of morphine usage on day 2 could not be performed as there were not enough data to combine.

7.3.2.3 Studies without an external warming device

Subset analysis of only studies without an external warming device could not be performed as there were not enough data to combine.
Figure 47: Forest Plot for Pain by Visual Analogue Scale up to 6 Hours Postoperatively.

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
**Figure 48: Forest Plot for Pain by Visual Analogue Scale on Day 1 Postoperatively.**

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Humidified Mean (SD)</th>
<th>N</th>
<th>Control Mean (SD)</th>
<th>SMD (fixed)</th>
<th>95% CI</th>
<th>Weight %</th>
<th>Quality</th>
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<tr>
<td>Hamza et al</td>
<td>23</td>
<td>5.00 (0.50)</td>
<td>21</td>
<td>5.00 (0.50)</td>
<td>0.00</td>
<td>[-0.59, 0.59]</td>
<td>20.97</td>
<td>A</td>
</tr>
<tr>
<td>Savel et al</td>
<td>15</td>
<td>3.50 (1.10)</td>
<td>15</td>
<td>3.00 (0.85)</td>
<td>-1.29</td>
<td>[-2.00, -0.49]</td>
<td>11.87</td>
<td>C</td>
</tr>
<tr>
<td>Champion et al</td>
<td>25</td>
<td>5.10 (1.05)</td>
<td>25</td>
<td>4.80 (0.90)</td>
<td>0.30</td>
<td>[-0.26, 0.86]</td>
<td>23.59</td>
<td>D</td>
</tr>
<tr>
<td>Mouton et al</td>
<td>13</td>
<td>2.00 (3.38)</td>
<td>14</td>
<td>5.00 (3.38)</td>
<td>-0.86</td>
<td>[-1.66, -0.07]</td>
<td>11.62</td>
<td>D</td>
</tr>
<tr>
<td>Nguyen et al</td>
<td>10</td>
<td>4.50 (2.24)</td>
<td>10</td>
<td>5.40 (2.24)</td>
<td>-0.38</td>
<td>[-1.27, 0.50]</td>
<td>9.34</td>
<td>D</td>
</tr>
<tr>
<td>Oe et al</td>
<td>25</td>
<td>1.90 (3.17)</td>
<td>25</td>
<td>3.70 (3.17)</td>
<td>-0.56</td>
<td>[-1.12, 0.01]</td>
<td>22.91</td>
<td>D</td>
</tr>
</tbody>
</table>

Total (95% CI): Favours treatment
Test for heterogeneity: Chi² = 14.02, df = 5 (P = 0.02)
Test for overall effect: Z = 2.47 (P = 0.01)
**Figure 49: Forest Plot for Pain by Visual Analogue Scale on Day 2 Postoperatively.**

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Humidified Mean (SD)</th>
<th>N</th>
<th>Control Mean (SD)</th>
<th>SMD (fixed) 95% CI</th>
<th>SMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamza et al</td>
<td>23</td>
<td>4.00 (0.25)</td>
<td>21</td>
<td>4.00 (0.50)</td>
<td>0.00 [-0.59, 0.59]</td>
<td></td>
<td>24.83</td>
<td>A</td>
</tr>
<tr>
<td>Sevel et al</td>
<td>15</td>
<td>2.90 (1.10)</td>
<td>15</td>
<td>1.60 (0.80)</td>
<td>0.57 [-0.17, 1.30]</td>
<td></td>
<td>16.22</td>
<td>C</td>
</tr>
<tr>
<td>Champion et al</td>
<td>25</td>
<td>4.50 (1.10)</td>
<td>25</td>
<td>4.00 (1.10)</td>
<td>0.54 [-0.03, 1.10]</td>
<td></td>
<td>27.21</td>
<td>D</td>
</tr>
<tr>
<td>Mouton et al</td>
<td>5</td>
<td>1.00 (2.07)</td>
<td>6</td>
<td>4.00 (2.07)</td>
<td>-1.33 [-2.70, 0.05]</td>
<td></td>
<td>4.61</td>
<td>D</td>
</tr>
<tr>
<td>Ott et al</td>
<td>25</td>
<td>0.90 (3.52)</td>
<td>25</td>
<td>2.90 (3.52)</td>
<td>-0.56 [-1.13, 0.01]</td>
<td></td>
<td>27.13</td>
<td>D</td>
</tr>
</tbody>
</table>

Total (95% CI): 53 N, 92 N

Test for heterogeneity: $\chi^2 = 13.07$, df = 4 ($p = 0.01$)

Test for overall effect: $Z = 0.17$ ($p = 0.87$)
Figure 50: Forest Plot for Pain by Visual Analogue Scale on Day 3 Postoperatively.

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
Figure 51: Forest Plot for Pain by Visual Analogue Scale on Day 1 Postoperatively (Only Studies Using an External Warming Device).

<table>
<thead>
<tr>
<th>Study</th>
<th>Humidified</th>
<th>Control</th>
<th>SMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Mean (SD)</td>
<td>N Mean (SD)</td>
<td>95% CI</td>
</tr>
<tr>
<td>Sevel et al</td>
<td>15 2.50 (1.10)</td>
<td>15 3.80 (0.85)</td>
<td>-1.29 [-2.08, -0.49]</td>
</tr>
<tr>
<td>Nguyen et al</td>
<td>10 4.50 (2.24)</td>
<td>10 5.40 (2.24)</td>
<td>-0.38 [-1.27, 0.50]</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25</td>
<td>-0.88 [-1.48, -0.29]</td>
</tr>
</tbody>
</table>

Total (95% CI)

Test for heterogeneity: $\chi^2 = 2.20$, df = 1 ($P = 0.14$)

Test for overall effect: $Z = 2.02$ ($P = 0.003$)

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
Table 52: Forest Plot for Pain by Visual Analogue Scale to 6 Hours Postoperatively (Only Studies without an External Warming Device).

<table>
<thead>
<tr>
<th>Study</th>
<th>Humidified N Mean (SD)</th>
<th>Control N Mean (SD)</th>
<th>SMD (fixed)</th>
<th>Weight %</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23 3.00 (3.30)</td>
<td>21 5.00 (3.30)</td>
<td>-0.60 [-1.20, 0.01]</td>
<td>24.83</td>
<td>A</td>
</tr>
<tr>
<td>Champion et al</td>
<td>25 5.50 (1.10)</td>
<td>25 5.70 (1.20)</td>
<td>-0.17 [-0.73, 0.38]</td>
<td>29.53</td>
<td>D</td>
</tr>
<tr>
<td>Moulin et al</td>
<td>15 4.00 (2.30)</td>
<td>15 6.00 (2.30)</td>
<td>-0.85 [-1.58, -0.12]</td>
<td>17.21</td>
<td>D</td>
</tr>
<tr>
<td>Ott et al</td>
<td>25 1.10 (4.04)</td>
<td>25 3.40 (4.04)</td>
<td>-0.56 [-1.10, 0.01]</td>
<td>20.42</td>
<td>D</td>
</tr>
</tbody>
</table>

Total (95% CI): $\chi^2 = 2.36$, of $\chi^2 (P = 0.50)$

Test for heterogeneity: $\chi^2 = 2.36$, of $\chi^2 (P = 0.50)$

Test for overall effect: $Z = 3.27$ ($P = 0.001$)

Figure 52: Forest Plot for Pain by Visual Analogue Scale to 6 Hours Postoperatively (Only Studies without an External Warming Device).

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
Figure 53: Forest Plot for Pain by Visual Analogue Scale on Day 1 Postoperatively (Only Studies without an External Warming Device).

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
Figure 54: Forest Plot for Pain by Visual Analogue Scale on Day 2 Postoperatively (Only Studies without an External Warming Device).

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
**Figure 55: Forest Plot for Pain by Visual Analogue Scale on Day 3 Postoperatively (Only Studies without an External Warming Device).**

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Control Mean (SD)</th>
<th>N</th>
<th>Control Mean (SD)</th>
<th>SMD (fixed) 95% CI</th>
<th>SMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hainza et al</td>
<td>23</td>
<td>3.00 (0.25)</td>
<td>21</td>
<td>3.50 (0.50)</td>
<td>-1.26 [-1.91, -0.61]</td>
<td>42.76 A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouton et al</td>
<td>2</td>
<td>1.00 (0.00)</td>
<td>0</td>
<td>7.00 (0.00)</td>
<td>-4.96 [-11.07, 1.95]</td>
<td>0.30 D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ott et al</td>
<td>25</td>
<td>0.70 (2.46)</td>
<td>25</td>
<td>2.10 (2.46)</td>
<td>-0.56 [-1.18, 0.01]</td>
<td>56.86 D</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td></td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: Chi² = 3.87, df = 2 (P = 0.14)
Test for overall effect: Z = 4.02 (P < 0.0001)
**Figure 56: Forest Plot for Morphine Equivalent Usage up to 6 Hours Postoperatively.**

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Humidified Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>SMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>SMD (fixed) 95% CI</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanza et al</td>
<td>22</td>
<td>32.00 (10.00)</td>
<td>31 27.00 (9.00)</td>
<td>46.00 -6.51 [-1.12, 0.09]</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Savel et al</td>
<td>15</td>
<td>36.00 (8.50)</td>
<td>15 41.00 (13.50)</td>
<td>31.95 -6.43 [-1.16, 0.29]</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nguyen et al</td>
<td>10</td>
<td>32.00 (23.50)</td>
<td>10 27.00 (23.50)</td>
<td>21.73 0.20 [-0.58, 1.08]</td>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI)
Test for heterogeneity: Chi² = 1.85, df = 2 (P = 0.40), I² = 0%
Test for overall effect: Z = 1.69 (P = 0.11)

**Figure 57: Forest Plot for Morphine Equivalent Usage on Day 1 Postoperatively.**
N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
Figure 58: Forest Plot for Morphine Equivalent Usage on Day 2 Postoperatively.

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Humidified Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>SMD (fixed) 95% CI</th>
<th>SMD (fixed) 95% CI</th>
<th>Weight %</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farley et al</td>
<td>49</td>
<td>3.50 (4.09)</td>
<td>52 2.70 (4.09)</td>
<td>0.19 [-0.20, 0.59]</td>
<td>77.12</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Sevel et al</td>
<td>15</td>
<td>19.00 (3.00)</td>
<td>15 20.00 (5.50)</td>
<td>-0.22 [-0.94, 0.50]</td>
<td>22.86</td>
<td></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>67</td>
<td></td>
<td>0.10 [-0.24, 0.44]</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI): Test for heterogeneity: Chi² = 0.98, df = 1 (P = 0.32) Favor treatment Favor control
Test for overall effect: Z = 0.57 (P = 0.57)

Figure 59: Forest Plot for Morphine Equivalent Usage up to 6 Hours Postoperatively (Only Studies Using an External Warming Device).
N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
Figure 60: Forest Plot for Morphine Equivalent Usage on Day 1 Postoperatively (Only Studies Using an External Warming Device).

N: Number, SD: Standard Deviation, %: Percent, CI: Confidence Interval, SMD: Standardised Mean Difference.
7.4 DISCUSSION

This meta-analysis demonstrates a reduction in postoperative pain with humidification and warming of insufflation gas in laparoscopic surgery. The reduction appears to be consistent at different intervals in the postoperative period; whether measured by visual VAS or morphine-equivalent dose usage, reaching statistical significance at 6h, day 1, and day 3 in the former, and at day 2 in the latter (see Table 17). There is significant heterogeneity between studies only for pain by VAS on day 1, implying a variation in effect estimates beyond chance. The rest of the results stand with no detectable heterogeneity statistically.

There are two ways in which preconditioning of insufflation gases might exert a beneficial effect. The first is by counteracting a reduction in the patient's core temperature, thereby preventing hypothermia; and the second is through a direct effect on the peritoneum in which desiccation and local temperature reduction are prevented.

Insufflation of cold, dry gas during laparoscopy causes hypothermia, and it is the humidity rather than the temperature of the gas which is largely to blame. The adverse clinical effects of hypothermia are well established, but its relationship to postoperative pain and fatigue less so. Given that prevention of hypothermia can be successfully achieved with the use warming blankets, forced-air warming devices and warmed fluids, it has been argued that the role for warming and humidification of insufflation gases is diminished. Subset analysis of only those studies that employed an external warmer showed a statistically significant reduction in pain scores on day 1 when the insufflate was warmed and humidified compared to when it was not. This is an important finding. Assuming hypothermia had been adequately prevented in the control group by external warming, this implies that the beneficial effects of warming and humidifying of the insufflation gases was above and beyond that of hypothermia prevention alone.

Damage to the peritoneum has been demonstrated microscopically when dry and cold CO₂ is used. Therefore, it may be that a reduction in peritoneal desiccation using HWI
plays an important role in reducing pain; or that local peritoneal hypothermia contributes to pain sensation independently from overall core body temperature.

The conclusions to be drawn from this meta-analysis are limited by the quality of the included studies. (404) Five of the 7 RCTs were methodologically poor as assessed by the Jadad scale which leads to the possibility that biases were combined in meta-analysis. Furthermore, variation between the studies in terms of surgical indication, the use of other external warming measures, and reported outcome variables weakens the analysis. Further high-quality trials are required, particularly in prolonged laparoscopy, such as laparoscopic colorectal surgery (research in this area is limited to a small case-matched study). (405) For this particular indication, as well as for other prolonged laparoscopies, the effect of peritoneal desiccation and hypothermia may be more pronounced. In addition, the effect of warming and humidification on the peritoneal inflammatory response and the consequent post-operative recovery has yet to be determined.

7.5 CONCLUSION

This analysis of 7 randomized controlled trials shows that humidification and warming of laparoscopic insufflation gas reduces postoperative pain after major laparoscopic surgery. This effect is also demonstrable in patients where an external warming device is used. Further high quality studies are required, particularly in procedures where the insufflation time is prolonged, to determine the effect of warming and humidification on the peritoneal inflammatory response and the consequent post-operative recovery.
CHAPTER 8: LABORATORY STUDY - INDEPENDENT TESTING OF A LAPAROSCOPIC HUMIDIFICATION SYSTEM
8.1 BACKGROUND

The effect of cold, dry gas flow on the peritoneal environment is potentially significant, with the desiccating and cooling effect producing visible structural, morphological, and biochemical changes in the peritoneal mesothelial surface layer.\(^{(219-222)}\) Warming and humidification of the insufflated carbon dioxide is relatively easily achieved by incorporating a commercially available humidifier into the tubing insufflation apparatus, and the results of the meta-analysis presented in the previous chapter suggest that this may provide some clinical benefit, although further studies are needed.\(^{(205, 303)}\)

There is very little information available in the public domain regarding the performance of laparoscopic humidifiers, with most of the data available related to humidifiers used for breathing circuits.\(^{(233, 234, 406, 407)}\) In laparoscopic surgery carbon dioxide is insufflated at very variable flow rates in contrast to breathing circuit humidifiers that tend to work with Room air / Oxygen at much more constant flow rates at any given time. Therefore, data on these humidifiers may not be applicable.

Prior to conducting further clinical studies, it is prudent to test a commercially available laparoscopic humidifier to ensure that it effectively warm and humidify carbon dioxide at clinically relevant flow rates. The aim of this study was to conduct independent testing of the Fisher & Paykel MR860 laparoscopic humidification system (Fisher & Paykel Healthcare, Auckland, New Zealand).
8.2 METHODS

The following is a summary of the methods used in this study. Further details can be found in CHAPTER 3: Methods.

A 2.5L insulated plastic chamber was constructed with the inlet port connected to a laparoscopic insufflator (Karl Storz – Endoskope, electronic endoflator, Medipak Surgical, Auckland, NZ) via the humidifier (Fisher & Paykel MR860 Laparoscopic Humidification System, Fisher & Paykel Healthcare, Auckland, New Zealand). The outlet port was open to the external environment. A flow meter and temperature sensor with computer readout was incorporated into the setup proximal to the humidifier (Red-y Smart Meter, Vogtlin Instruments AG, Switzerland), and a thermo-hygrometer was placed inside the chamber (ITW JT-07CRL Humidity & Temperature Meter, Shenzen Jingtengwei Industry Co. Ltd, Guangdong, China).

The humidifier water vessel was weighed using an electronic scale accurate to 0.001g (Mettler PE360 weight scale, Mettler instruments AG, Greifensee, Zurich) and exactly 30.0g of water poured in. After the humidifier was allowed to warm up to 37.0°C, the water vessel was re-weighed. 50.0L of CO₂ was insufflated into the chamber via the humidifier at 2.0L/min. Measurements of gas inflow and outflow temperature, and chamber temperature and humidity were taken at 30s intervals. After 50.0L of gas was insufflated the water left in the humidifier was weighed, and the mean absolute humidity (AH) of insufflated gas calculated using the formula:(233, 234)

\[ AH = \frac{(m \times 1000)}{[(50 \times T_0 / T_a) + (1.4081 \times m)]} \]

Where \( m \) = mass of evaporated water (grams), \( T_0 \) = mean temperature of outflow gas (Kelvin), \( T_a \) = mean temperature of inflow gas (Kelvin)

The absolute humidity at saturation (\( A_{H_s} \)) was derived, and the relative humidity calculated (RH). The procedure was repeated at flow rates of 4.0L/min, 6.0L/min, 8.0L/min, and 10.0L/min.
8.3 RESULTS

8.3.1 Chamber measurements (thermo-hygrometer reading)

The relative humidity recorded inside the chamber by the hygrometer reached > 98.0% after less than 30 seconds of insufflation at all flow rates. Table 18 shows the temperature recorded in the chamber at various intervals and at different flow rates. As can be seen, the temperature did not reach 37.0 °C, but was elevated to a greater degree with higher flow rates.

<table>
<thead>
<tr>
<th>Volume of insufflated CO₂ (L)</th>
<th>Chamber temperature at different flow rates (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 L/min</td>
</tr>
<tr>
<td>0</td>
<td>24.2</td>
</tr>
<tr>
<td>4.0</td>
<td>26</td>
</tr>
<tr>
<td>8.0</td>
<td>25.7</td>
</tr>
<tr>
<td>12.0</td>
<td>25.3</td>
</tr>
<tr>
<td>16.0</td>
<td>25</td>
</tr>
<tr>
<td>20.0</td>
<td>25.2</td>
</tr>
<tr>
<td>24.0</td>
<td>26.5</td>
</tr>
<tr>
<td>28.0</td>
<td>27.7</td>
</tr>
<tr>
<td>32.0</td>
<td>28.8</td>
</tr>
<tr>
<td>36.0</td>
<td>29.3</td>
</tr>
<tr>
<td>40.0</td>
<td>29.6</td>
</tr>
<tr>
<td>44.0</td>
<td>29.6</td>
</tr>
<tr>
<td>48.0</td>
<td>29.8</td>
</tr>
<tr>
<td>50.0</td>
<td>29.7</td>
</tr>
</tbody>
</table>

Table 18: Temperature in the Chamber at Different Flow Rates.
*: estimate, °C: Celsius, L: Litres, Min: Minute.
8.3.2 Absolute Humidity output (gravimetric calculation)

The mass of water evaporated after 50.0L of insufflation, and the mean temperature of the gas outflow as recorded by the humidifier are shown in Table 19. The calculated absolute humidity at every flow rate was close to or above absolute humidity at saturation, ranging from 48.1 to 56.3 mg/L. This corresponded to a relative humidity ranging from 98.7 to 110.1%.

<table>
<thead>
<tr>
<th>Flow Rate (L/min)</th>
<th>Mass of water evaporated (g)</th>
<th>Mean Inflow Temp (°C)</th>
<th>Mean Outflow Temp (°C)</th>
<th>Absolute Humidity at saturation (g/L)</th>
<th>Absolute Humidity calculated (g/L)</th>
<th>Relative Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>3.24</td>
<td>22.04</td>
<td>39.99</td>
<td>51.08</td>
<td>56.25</td>
<td>110.12</td>
</tr>
<tr>
<td>4.0</td>
<td>3.04</td>
<td>22.35</td>
<td>39.57</td>
<td>50.01</td>
<td>53.15</td>
<td>106.28</td>
</tr>
<tr>
<td>6.0</td>
<td>2.86</td>
<td>22.49</td>
<td>39.87</td>
<td>50.89</td>
<td>50.20</td>
<td>98.65</td>
</tr>
<tr>
<td>8.0</td>
<td>2.90</td>
<td>22.33</td>
<td>39.74</td>
<td>50.44</td>
<td>50.85</td>
<td>100.81</td>
</tr>
<tr>
<td>10.0</td>
<td>2.72</td>
<td>22.29</td>
<td>38.65</td>
<td>47.74</td>
<td>48.06</td>
<td>100.66</td>
</tr>
</tbody>
</table>

Table 19: Absolute Humidity Output at Different Flow Rates.
L: litres, Min: Minute, G: Grams, °C: Celsius, Kg: Kilograms, %: Percent.
8.4 DISCUSSION

These results confirm that the humidifier was able to saturate 50L of insufflated CO₂ to an absolute humidity that is close to saturation humidity at every flow rate tested.

Several studies have been conducted to test humidifiers for breathing circuits. (406-412) However only a few of these utilised the gravimetric method to calculate absolute humidity, which is the technique generally regarded as the most accurate. (413) A recent bench study by Schumann et al comparing a counter-flow humidifier and a conventional breathing circuit humidifier (of very similar design to the humidifier tested in our study) showed that the humidification capability of both humidifiers was independent of flow rate. (406) The gravimetric method was used in this study. However, to simulate the required clinical scenario, air was used and at a flow rate ranging from 30-120 L/min. This limits the applicability of their results to our humidifier which is intended for use with CO₂ and at generally lower flow rates in the surgical setting.

The gravimetric estimate in our study revealed that the gas was humidified to at least 98.7% relative humidity at the operating temperatures recorded. The gas even appeared to be “super-saturated” at the lowest flow rates tested. The reason for this is uncertain. Despite this being an air-tight closed circuit system, the possibility of an undetected gas leak leading to water evaporation that was unaccounted for cannot be ruled out. Any inaccuracies such as this would be expected to be more pronounced at a lower flow rate, as the insufflation occurred over a longer period of time. Another limitation of the study is the fact that the insufflator delivers the gas in a pulsatile fashion. We did however attempt to control for this as much as possible by incorporating an independent flow meter to compensate for any inaccuracies in the output flow rate.

It is also worth noting that while the gas output temperatures were above 37.0 °C the maximum recorded temperature inside the chamber was 33.4°C. Therefore, while the humidifier was able to humidify the gas effectively, it was unable to warm the chamber up to its operating temperature of 37.0°C. Nevertheless, this observation is largely inconsequential as in the clinical setting the baseline peritoneal cavity temperature is much higher. It is also known that the humidity of the gas
is substantially more important than the temperature in regulating the local effects on the peritoneum. (398) This is because most of the hypothermia is caused by latent heat loss in evaporation of peritoneal fluid. (398-400)

8.5 CONCLUSION

The Fisher & Paykel MR860 laparoscopic humidification system effectively humidifies insufflated CO\textsubscript{2} at a range of flow rates commonly used in the clinical setting.
9.1 BACKGROUND

In laparoscopic surgery, the abdominal wall is distended using pneumoperitoneum to provide room for instrument insertion and tissue dissection. Carbon dioxide (CO$_2$) is used almost universally as the insufflation gas of choice. (220) This is commonly delivered as defined by the United States Pharmacopeia and National Formulary, which requires impurity of less than 200 parts per million, including water vapour. (414) Medical grade CO$_2$ is supplied as a compressed liquid in cylinders with a release temperature of approximately -90°C. (221) In the absence of active conditioning, the gas is passed through the insufflator and tubing apparatus raising its temperature to that of the room (19 - 21°C) with a relative humidity approaching 0% at the point of entry into the peritoneal cavity. (391)

Despite several advances in technique, little has changed with this aspect of laparoscopic practice since it was established by Kurt Semm in the 1960’s. (415) The effect of this continuous flow of cool, dry carbon dioxide on the peritoneal micro-environment has probably been under-estimated, (183, 263, 264, 416) and there is evidence that it causes structural, morphological, and biochemical injury to the peritoneal mesothelium. (215, 219, 221, 222, 289, 303) How much of this effect relates to intrinsic properties of CO$_2$, and how much is due to the desiccative effect of the gas is debated; (299, 417) but there is some evidence that conditioning of insufflation gas by warming and humidification may be beneficial in this regard. (222, 226, 295, 418)

The clinical benefits have also been investigated. Proponents have argued that warming and humidification may reduce the incidence of intra-operative hypothermia, reduce post-operative pain and analgesia use, and improve post-operative recovery. (238, 392-397) Results have been conflicting, however. (390, 419) Two meta-analyses have been published (including the one presented in Chapter 9), and while they were able to demonstrate some significant benefits, conclusions were limited by the inclusion of mainly unblinded (or inadequately blinded) studies, and heterogeneity in surgical indication. (205, 420) In addition, most trials did not control for the use of an external patient warming device (such as a warming blanket), and were conducted for laparoscopic procedures of relatively short duration. (205) There have not been any randomised
controlled trials evaluating the use of warming and humidification in prolonged laparoscopic surgical procedures such as colonic surgery, where it might be expected the effects would be most pronounced.

We aimed to design and execute a prospective, double-blinded, randomised controlled trial to test the hypothesis that warming and humidification of insufflation gas during laparoscopic colonic surgery would lead to reduced post-operative pain and improved recovery by reducing peritoneal inflammation.

9.2 METHODS
The following is a summary of the methods used in this study. Further details can be found in CHAPTER 3: Methods.

9.2.1 Participants
The study population included all New Zealand citizens and permanent residents residing within the catchment area of the three District Health Boards serving Auckland city (Auckland District Health Board (DHB), Waitemata DHB, and Counties Manukau DHB). All patients undergoing elective laparoscopic colonic resection for any indication and at any of the three public hospitals between April 2008 and June 2009 were screened for inclusion. Exclusion criteria were: Patients under 15 years of age, acute colonic resection, hand-assisted colonic resection, decision to perform open surgery pre-operatively (intra-operative conversions were included as intention to treat), surgery for rectal lesions defined as within 15 cm of the anal verge on sigmoidoscopy / colonoscopy, stoma formation (preoperative or intra-operative decision), patients who did not have colon resected despite initial surgical plan, pre-operative steroid dependence, inability to consent or complete visual analogue scores in study questionnaires due to cognitive impairment or language barrier, patients with ASA ≥ 4, and deviation from anaesthetic protocol (as defined below). Conversion to open colectomy was at the discretion of the individual surgeon for concerns of patient safety, technical difficulties, or associated unexpected conditions requiring treatment by
laparotomy. Conversions were recorded and analyzed in the allocated group on an intention to treat basis.

9.2.2 Interventions

9.2.2.1 Surgery

All patients underwent routine laparoscopic-assisted colonic resection either by, or under the supervision of, consultant colorectal specialists employed by the three District Health Boards. Technical aspects of the surgical procedure, and post-operative care not related to analgesia protocol (see below) were left up to the discretion of the surgical team.

9.2.2.2 Anaesthesia and analgesia

All patients were administered standardised premedication and peri-operative analgesia as per a protocol designed in conjunction with the Department of Anaesthesia at Auckland City Hospital. This was agreed upon by each individual anaesthetist prior to commencement of the operation. All patients received paracetamol 1g orally as pre-medication (Panadol tablets, GlaxoSmithKline Consumer, Auckland, NZ). No sedation or non-steroidal anti-inflammatories were given. All patients received dexamethasone 8mg intravenously after induction (DBL dexamethasone sodium phosphate injection, Hospira NZ Limited, Wellington, NZ), and morphine / fentanyl intravenously as required for analgesia during the procedure (morphine sulphate injection, Biomed Ltd, Auckland, NZ; fentanyl injection, AstraZeneca Ltd, Auckland, NZ). Choice of induction agent and muscle relaxant was left to the discretion of the theatre anaesthetic team. Epidural, spinal, and intra-thecal analgesia / anaesthesia were not used. (236) Room temperature was set at 19°C before the start of the case, and all patients were covered with a forced-air-rewarming blanket (The Original Bair Hugger Forced Air Warming Temperature Management Units, Arizant Healthcare Inc, Eden Prairie, MN 55344, USA). Choice, volume, and temperature of intra-venous fluid given intra-operatively were left up to the discretion of the anaesthetic team. Prior to incision, all laparoscopic port sites were infiltrated with 0.25% bupivacaine with adrenaline (Marcain 0.25% with adrenaline 1:400,000 Injection, AstraZeneca Ltd, Auckland, NZ), and at the end of the procedure all skin wounds were infiltrated with 0.25% bupivacaine with adrenaline (up to a maximum total dose of 2
mg/kg). Post-operatively all patients received regular paracetamol 1g orally until discharge, and morphine / fentanyl intravenously via patient controlled analgesia until pain could be satisfactorily controlled using oral opiates. Oral opiates used were tramadol 100mg orally as first line (tramadol hydrochloride, AFT Pharmaceuticals Ltd, Auckland, NZ), and morphine / oxycodone orally as second line (morphine sulphate, Douglas Pharmaceuticals Ltd, Auckland, NZ; oxycodone hydrochloride, Mundipharma NZ Ltd, Auckland, NZ).

9.2.2.3 Study Group
The Study Group received warm, humidified insufflation gas. Insufflation pressure was set at 12 – 15 mmHg with a variable flow rate. The gas used was carbon dioxide (Carbon dioxide medical gas, BOC Ltd, Auckland, NZ), and this was warmed to 37°C and humidified to 98% RH using a Fisher & Paykel laparoscopic humidifier (Fisher & Paykel MR860 Laparoscopic Humidification System, Fisher & Paykel Healthcare, Auckland, New Zealand). This humidification system is specifically designed to deliver warm, humidified CO₂ to patients undergoing laparoscopic surgery, and has previously undergone independent testing by our group to confirm the effectiveness of gas conditioning.(421) The gas is passed from the insufflator through a chamber which is filled with 30 mL of sterile water and sits on a heater plate. Water evaporates from the chamber into the gas which flows over it. The temperature of the gas is maintained as it travels along a heated tube to the laparoscopic port and into the patient’s abdomen. The humidifier monitors the temperature and flow rate of the gas at the chamber outlet with a probe attachment, controlling the amount of power delivered to the heater plate to maintain the chamber set point temperature.(232)

9.2.2.4 Control Group
The Control Group received standard dry carbon dioxide for insufflation (Carbon dioxide medical gas, BOC Ltd, Auckland, NZ), delivered at room temperature (19 °C) and 0% RH. Insufflation pressure was set at 12 – 15 mmHg with a variable flow rate.
9.2.3 Objectives

The objective of this study was to test the hypothesis that warming and humidification of insufflation gas during laparoscopic colectomy would lead to reduced post-operative pain and improved recovery by reducing peritoneal inflammation.

9.2.4 Outcomes

All data were collected by a single, blinded investigator (TS) to ensure standardisation of data collection.

9.2.4.1 Baseline data

Patient data
Baseline patient data recorded included: National Health Index (NHI) number, the hospital that the patient was treated at, patient surname, age, sex, ethnicity as coded in hospital electronic records (self-identified), weight in kilograms, height in centimetres, past medical history, past surgical history, ASA score, and Cr Possum score.

Operative data
Operative data recorded included: pre-operative diagnosis, post-operative diagnosis on histology, lesion location, date of surgery, operation performed, approach (laparoscopic, laparoscopic-assisted, conversion to open), operation start time (scalpel to skin), gas insufflation start time, gas insufflation end time, operation end time (all wound dressings applied), volume of gas used for insufflation, use of intra-peritoneal fluid washout, contamination with pus or faeces, dose of morphine / fentanyl used intra-operatively, dose of any anti-emetics used intra-operatively, and type and volume of intravenous fluid used intra-operatively. At the end of the surgery the operating surgeon was asked to rate the camera fogging on a Likert scale from 1 to 10 (1 = perfect image, 10 very poor quality image).
Other data

Other relevant data recorded post-operatively were: volume of fluid in the intra-peritoneal drain at the time of sample collection, total wound size on day 2 in cm (all wounds measured with a ruler and lengths added), and specimen histology including full TNM staging for neoplastic lesions.

9.2.4.2 Primary outcome

The primary outcome was total opiate analgesia use during the index inpatient stay. This was calculated using the Mean Equivalent Daily Dose method. (238, 239) All opiates administered to the patient via patient controlled analgesia, intravenous, and oral routes were converted to an equivalent MEDD dose and collated. These data were collected in the post-operative recovery unit, day 0 (day of operation), day 1, day 2, day 3, and day 4 until discharge. Days 1 to 3 were defined as a 24 hour period from midnight to midnight. Absolute MEDD dose, and MEDD dose per kilogram of patient weight were determined.

9.2.4.3 Secondary outcomes

Pain

Pain at rest, on moving, and on coughing was assessed using three separate 10 point Likert VAS. The patient was asked to fill out the VAS questions pre-operatively (baseline), and at 2 hours, 4 hours, 8 hours, 12 hours, day 1, day 2, day 3, day 7, day 14, day 30, and day 60 post-operatively. If patients were discharged from hospital, a questionnaire with specific instructions was mailed to them with a pre-paid return envelope.

Intra-operative core temperature

Intra-operative core temperature was measured at 15 min intervals using an oesophageal probe. The change in temperature between the start and end of the procedure, as well as the minimum, maximum, and mean / median temperatures were compared between groups.
Cytokine response

Patients had a size 15F Blakes drain (Blake Silicone Drain, Ethicon inc, Somerville, NJ 08876-0151, USA) inserted into the peritoneal cavity at the conclusion of surgery. This was brought out through the abdominal wall, and sutured to the skin. The drain was attached to a low vacuum drain bottle (Low vacuum wound drainage system 80kPa / neg 150mmHg, Leur-lock replacement bottle, Van Straten Medinorm, 66583 Spiesen, Germany). At 20 hours post-operatively, 4 mL of fluid from the drain bottle as well as a simultaneous sample of 4 mL of venous blood were collected into two separate vacutainer tubes containing ethylenediamine tetra-acetic acid (K2 EDTA BD Vacutainer, BD Diagnostics Franklin Lakes, NJ 07417, USA). The timing of sample collection was chosen because peritoneal IL-6 levels peak at 20 – 24 hours after abdominal surgery.(24) All collected samples were sent immediately on ice to the Middlemore Hospital Laboratory, where they were centrifuged for 10 minutes at 1000 x g and the supernatant removed. Samples were then aliquoted and stored in polypropylene tubes at -80 °C for future batch analysis.

All samples from both groups were retrieved, thawed to room temperature, and assayed together. Median concentrations of IL-1α, IL-6, IL-8, IL-10, and TNFα were compared between groups. Cytokine assays were carried out by multiplexed cytometric bead immunoassays using the Milliplex™ human cytokine kit (#MPXHCYTO-60K 96 well plate assay, Millipore Corporation, Billerica, MA 01821, USA). All samples were assayed in duplicate, and peritoneal fluid samples were re-assayed for IL-6 in 1:5 dilution, as peritoneal fluid levels of this cytokine are frequently above the maximum detection limit of the assay (> 10000 pg/ml). The minimum assay detection limits for IL-1α, IL-6, IL-8, IL-10, and TNFα were 3.5, 0.3, 0.2, 0.3 and 0.1 pg/ml, respectively. Data were acquired using Luminex 100™ laser-based fluorescent analytical test instrumentation (Luminex 100™ IS, Luminex Corporation, Austin, TX 78727, USA). The Median Fluorescent Intensity (MFI) data obtained using Luminex IS 2.3 software were saved and analysed using a weighted 5-parameter logistic curve-fitting method. Peritoneal fluid concentrations were corrected for dilution using the urea method. Since urea readily diffuses throughout the body, the plasma and peritoneal fluid urea concentrations are the same. Consequently, when the urea concentrations in
plasma and peritoneal samples are known, the dilution of the initial volume of peritoneal fluid obtained can be calculated. (240)

**9.2.4.4 Other variables recorded**

Discharge criteria were defined as return of bowel function (passage of flatus or bowel motion, resumption of full solid oral diet, and the absence of nausea and vomiting), independent mobilisation (or mobilisation back to baseline function), and adequate pain relief using oral analgesia only. Day that discharge criteria were achieved and actual day of discharge were recorded. Readmission was defined as return to hospital within 30 days post-discharge requiring hospital stay of 24 hours or more. Total hospital stay (day stay on index admission plus hospital stay on readmission) was derived and recorded. Post-operative anti-emetic use was recorded, and collated total units used during the hospital stay were calculated (1 unit = 10mg metoclopramide, 25mg cyclizine, 4mg ondansetron, 0.625mg droperidol, or 10mg stemetil orally or intra-venously). Volume of post-operative intravenous fluid use was also recorded.

Functional recovery was measured using the SRS score, a comprehensive recovery assessment questionnaire derived from the previously validated multi-dimensional Identity-Consequence Fatigue Scale questionnaire (Appendix E: Surgical Recovery Score). (241, 242) This score assesses 5 categories of post-operative recovery: Fatigue, Vigor, Mental Function, Impact on Patient Activity and Impact on Activities of Daily Living and is expressed as a percentage of maximum possible score. The SRS questionnaire was filled out by all patients pre-operatively (baseline) and at 1, 3, 7, 30, and 60 days post-operatively.

Post-operative complications up to 30 days after surgery were recorded prospectively using pre-defined criteria. Complications were defined as per the standardised “definitions of operation and / or disease related complications” proposed by Buzby et al. (243) In addition to this, ileus was defined as post-operative obstipation and vomiting requiring nasogastric tube insertion, but without radiological evidence of bowel obstruction. All complications were recorded per patient and graded by the Clavien-Dindo classification. (244, 245)
9.2.5 Sample size

The *a priori* power calculation was based on the primary outcome of total opiate analgesia use (measured in MEDD dose) during the index inpatient stay. Data from three previously published studies that measured opiate use after laparoscopic colectomy were used to calculate MEDD dose expected. (246-248) The total MEDD dose during the hospital stay was estimated at 137 mg (SD 41 mg). For the power calculation, we used a conservative rounded figure of 150 mg (SD 50 mg) MEDD. Using a two tailed Mann-Whitney U test for difference between two independent groups, 37 patients would be required in each arm to detect a difference of 20% between groups with an alpha of 0.05 and power of 0.8. (249) No interim analyses were planned or undertaken.

9.2.6 Randomization

9.2.6.1 Sequence generation

Randomisation was conducted using random numbers obtained from an open source computer-based random number generator (www.random.org). Randomisation was stratified by hospital to ensure equal distribution of intervention and control group patients between these, and minimise bias due to differences in pre, intra and post-operative protocols between sites.

9.2.6.2 Allocation concealment

Allocations were concealed in opaque numbered envelopes. They were kept in a central location and not used until interventions were assigned on the day of surgery.

9.2.6.3 Implementation

The randomisation sequence was generated by a third party not involved in the study. Patients were recruited on the day of surgery. All patients were seen on a one to one basis pre-operatively by Tarik Sammour, and trial rationale and procedure were explained verbally. Patients were then given a participant information sheet, after which written informed consent was obtained prior to randomisation. Allocation of each individual patient into either Study or Control Group was performed intra-operatively by an unblinded research assistant (see below), after the patient was anaesthetised and before the insufflation was started.
9.2.7 Blinding

The patient, study investigators, surgeon, and medical staff responsible for patient care were all blinded to patient allocation. This was achieved by having the humidifier connected to the insufflation apparatus and power supply regardless of allocation, and covered with a specially designed plastic casing which concealed its LCD screen and water chamber. This was to ensure that none of the theatre occupants were able to tell if the humidifier was switched on or not. A research assistant not involved in patient management, study design, data collection, data analysis or results write-up was responsible for setting up the humidifier. After the patient was asleep, the assistant opened an opaque envelope with allocation instructions and set-up the humidifier away from view of the theatre staff and investigator. If the patient was in the Study Group, 30ml of sterile water was added to the chamber and the humidifier was switched on and muted so that it did not make any noise. If the patient was in the Control Group water was not added and humidifier was not switched on.

The blinding protocol was practised in simulation several times and tested in March 2008 (prior to study commencement) on a consented patient undergoing a laparoscopic colonic resection at the Counties Manukau DHB. The patient, study investigators, surgeon, and all medical staff responsible for patient care were blinded successfully.

Data analysis was also blinded. The investigator undertaking statistical analysis on study completion was only allowed access to modified data tables with the allocation concealed. These specified patients as being allocated to “Group 1” and “Group 2”.

9.2.8 Statistical methods

Results were analysed using SPSS® for Windows® version 17.0 (Lead Technologies Inc, Chicago, Illinois, USA). Continuous variable parametricity was tested using the Shapiro-Wilk test. Results are presented as Mean (Standard Deviation) for parametric data and Median (Inter-quartile Range) for non-parametric data. Groups were compared using the Fisher’s Exact or χ2 test for
categorical variables, the Mann–Whitney U test for non-parametric continuous variables, and the t test for parametric continuous variables. Statistical significance was accepted at the 0.050 level. No subgroup analyses were planned or undertaken.

9.2.9 Ethics Approval and Trial Registration

Ethics approval was granted by the Ministry of Health, Northern X Regional Ethics Committee in March 2008 (trial number NTX/08/02/010). Approval was also granted by the Clinical Boards of all three DHB’s. The trial was prospectively registered with ClinicalTrials.gov (trial identifier: NCT00642005, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894, USA).
9.3 RESULTS

9.3.1 Participant recruitment and flow

Detailed patient flow is shown in the CONSORT diagram (Figure 61).(422) Between April 2008 and June 2009, 111 patients were screened for inclusion. Of these, 82 patients were randomised equally between the Study and Control Groups. Six patients in the Study Group were excluded after randomisation: 2 patients had a rectal lesion below 15cm found intra-operatively, 2 patients did not have any colon resected despite initial plan, 1 patient had an unplanned diverting ileostomy performed due to a positive anastomotic air-leak test, and in 1 case the investigator was unblinded when a nurse inadvertently lifted the plastic cover off the humidifier during surgery. Two patients in the Control Group were excluded after randomisation: 1 patient did not have any colon resected despite initial plan, and 1 patient suffered a severe anaphylactic reaction on induction of anaesthesia, and the procedure was abandoned.

9.3.2 Number analysed

Seventy four patients were analysed, 35 in the Study Group and 39 in the Control Group. All patients had complete data collected for the primary outcome and for all variables measured during the index hospital stay. Complete follow-up data at 7, 14, 30, and 60 days were obtained for 74 (100%), 71 (96%), 67 (91%), and 60 (81%) patients, respectively. Peritoneal and plasma cytokine data were obtained for 70 (95%) and 73 (99%) patients, respectively.
Figure 61: CONSORT Diagram Documenting Patient Flow.
N: number of patients, Incl: Inclusion.
### 9.3.3 Baseline characteristics

Groups were well matched at baseline, with no significant differences in age, sex, BMI, ASA, Cr Possum, previous abdominal surgery, operation performed, diagnosis, or stage (Table 20).

<table>
<thead>
<tr>
<th></th>
<th>Study Group (n = 35)</th>
<th>Control (n = 39)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> (median in years, IQR)</td>
<td>71 (29)</td>
<td>69 (22)</td>
<td>0.959**</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (42.9%)</td>
<td>16 (41.0%)</td>
<td>1.000**</td>
</tr>
<tr>
<td>Female</td>
<td>20 (57.1%)</td>
<td>23 (59.0%)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong> (mean in kg/m², SD)</td>
<td>26.5 (4.8)</td>
<td>25.5 (5.4)</td>
<td>0.401*</td>
</tr>
<tr>
<td><strong>ASA score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6 (17.1%)</td>
<td>3 (7.7%)</td>
<td>0.355*</td>
</tr>
<tr>
<td>II</td>
<td>21 (60.0%)</td>
<td>23 (59.0%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>8 (22.9%)</td>
<td>13 (33.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>CR-POSSUM</strong> (median, IQR)</td>
<td>17 (5)</td>
<td>19 (5)</td>
<td>0.178**</td>
</tr>
<tr>
<td><strong>Previous abdominal surgery</strong></td>
<td>15 (42.9%)</td>
<td>13 (33.3%)</td>
<td>0.475**</td>
</tr>
<tr>
<td><strong>Operation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileocolic resection</td>
<td>5 (14.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Right hemicolecotmy</td>
<td>14 (40.0%)</td>
<td>11 (28.2%)</td>
<td></td>
</tr>
<tr>
<td>Extended Right hemicolecotmy</td>
<td>5 (14.3%)</td>
<td>3 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Transverse colectomy</td>
<td>1 (2.9%)</td>
<td>1 (2.6%)</td>
<td></td>
</tr>
<tr>
<td>Left hemicolecotmy</td>
<td>2 (5.7%)</td>
<td>6 (15.4%)</td>
<td></td>
</tr>
<tr>
<td>Sigmoid colectomy</td>
<td>1 (2.9%)</td>
<td>2 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>High anterior resection</td>
<td>7 (20.0%)</td>
<td>16 (41.0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>21 (60.0%)</td>
<td>27 (69.2%)</td>
<td>0.587*</td>
</tr>
<tr>
<td>Adenoma</td>
<td>6 (17.1%)</td>
<td>3 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Diverticulitis</td>
<td>3 (8.6%)</td>
<td>3 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>3 (8.6%)</td>
<td>2 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>Carcinoid</td>
<td>2 (5.7%)</td>
<td>2 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>Other benign</td>
<td>0 (0%)</td>
<td>2 (5.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>AJCC staging</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6 (26.1%)</td>
<td>5 (17.2%)</td>
<td>0.316*</td>
</tr>
<tr>
<td>II</td>
<td>11 (47.8%)</td>
<td>9 (31.0%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>5 (21.7%)</td>
<td>13 (44.8%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1 (4.3%)</td>
<td>2 (6.9%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 20: Baseline Characteristics.**

N: Number of Patients, IQR: Inter-Quartile Range, SD: Standard Deviation, R: Right, L: Left

* χ² test, ** Fisher’s Exact test, † t test, ‡ Mann-Whitney U test
9.3.4 Intra-operative parameters

The operative time, pneumoperitoneum time and volume of CO₂ used were similar between groups (Table 21). There was a higher conversion rate in the Control Group (15.4 vs 5.7%) but this was not statistically significant. Out of 6 conversions in the Control Group, 2 were due to extensive intra-operative adhesions preventing dissection, 1 due to invasive disease requiring en-bloc resection, 1 due to intra-operative bleeding from inadvertent injury to a gonadal vessel, 1 due to inability to localise the lesion tattoo, and 1 due to failure of the bean bag used to secure the patient to the operating table. The first conversion in the Study Group was due to difficulty extracting a large specimen, and the second due to invasive disease requiring partial bladder resection. The total wound size was similar in both groups despite this.

<table>
<thead>
<tr>
<th></th>
<th>Study Group (n = 35)</th>
<th>Control (n = 39)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Operating Time</strong> (mean in min, SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total operating time</td>
<td>176.3 (48.8)</td>
<td>184.7 (57.5)</td>
<td>0.504†</td>
</tr>
<tr>
<td>Pneumoperitoneum time</td>
<td>105.1 (39.0)</td>
<td>116.9 (55.0)</td>
<td>0.295*</td>
</tr>
<tr>
<td><strong>Volume CO₂ used</strong> (mean in L, SD)</td>
<td></td>
<td></td>
<td>0.057*</td>
</tr>
<tr>
<td>Converted</td>
<td>2 (5.7%)</td>
<td>6 (15.4%)</td>
<td>0.267**</td>
</tr>
<tr>
<td>Not converted</td>
<td>33 (94.3%)</td>
<td>33 (84.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>Total wound size</strong> (median in cm, IQR)</td>
<td>10.0 (5.0)</td>
<td>11.3 (6.0)</td>
<td>0.451**</td>
</tr>
<tr>
<td><strong>Core temperature</strong> (median in °C, IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start temperature</td>
<td>35.8 (0.7)</td>
<td>35.9 (0.8)</td>
<td>0.533**</td>
</tr>
<tr>
<td>Final temperature</td>
<td>36.4 (0.7)</td>
<td>36.3 (0.6)</td>
<td>0.602**</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>35.6 (0.6)</td>
<td>35.6 (0.8)</td>
<td>0.827**</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>36.4 (0.6)</td>
<td>36.4 (0.7)</td>
<td>0.844**</td>
</tr>
<tr>
<td>Temperature change</td>
<td>0.4 (0.7)</td>
<td>0.6 (0.9)</td>
<td>0.324**</td>
</tr>
<tr>
<td>Mean temperature during surgery</td>
<td>36.0 (0.6)</td>
<td>35.9 (0.6)</td>
<td>0.981**</td>
</tr>
<tr>
<td><strong>Surgeon fogging score</strong> (median, IQR)</td>
<td>4 (5)</td>
<td>2 (3)</td>
<td>0.040**</td>
</tr>
</tbody>
</table>

Table 21: Intra-operative Parameters.
N: Number of Patients, IQR: Inter-Quartile Range, SD: Standard Deviation
** Fisher’s Exact test, † t test, ‡ Mann-Whitney U test
The intra-operative core temperature increased in both groups during the surgery, with no differences in the start, final, minimum, maximum or mean temperatures. In addition, the temperature was similar in both groups at all time points that it was measured (Figure 62). Median camera fogging score as rated by the principal operating surgeon was significantly worse in the Study Group versus the Control Group (4 vs. 2, \( P = 0.040 \)).

![Figure 62: Intra-operative Core Temperature.](image)

**Figure 62: Intra-operative Core Temperature.**
Min: Minutes

### 9.3.5 Morphine equivalent usage and pain scores

There were no significant differences in MEDD usage at any time point measured (Table 22). MEDD per kilogram of patient weight was not different between groups (data not shown). Pain perception at rest and on moving as measured by VAS was higher in the Study Group on day 1. Pain on coughing was higher in the Control Group on day 7. There were no significant differences in pain by VAS at any other time point.
|                      | Study Group  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 35)</td>
<td>Control (n = 39)</td>
</tr>
<tr>
<td><strong>MEDD (median, IQR)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-operative</td>
<td>35.0 (17.5)</td>
<td>35.0 (26.0)</td>
</tr>
<tr>
<td>PACU</td>
<td>10.0 (16.0)</td>
<td>8.0 (18.0)</td>
</tr>
<tr>
<td>Day 0</td>
<td>12.0 (22.0)</td>
<td>9.0 (17.0)</td>
</tr>
<tr>
<td>Day 1</td>
<td>22.0 (28.5)</td>
<td>36.0 (41.0)</td>
</tr>
<tr>
<td>Day 2</td>
<td>16.0 (21.5)</td>
<td>20.0 (32.0)</td>
</tr>
<tr>
<td>Day 3</td>
<td>7.5 (21.0)</td>
<td>10.0 (21.0)</td>
</tr>
<tr>
<td>3 day total</td>
<td>77.0 (95.0)</td>
<td>94.4 (107.0)</td>
</tr>
<tr>
<td>Total during admission</td>
<td>86.0 (116.5)</td>
<td>121.5 (183.5)</td>
</tr>
<tr>
<td><strong>Day PCA removed (median, IQR)</strong></td>
<td>1 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td><strong>Pain at rest (median, IQR)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>2 hours</td>
<td>5 (4)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>4 hours</td>
<td>3 (4)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>8 hours</td>
<td>1 (2)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>12 hours</td>
<td>2 (3)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Day 1</td>
<td>4 (3)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Day 2</td>
<td>3 (2)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Day 3</td>
<td>3 (3)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Day 7</td>
<td>2 (1)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Day 14</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Day 30</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Day 60</td>
<td>1 (1)</td>
<td>1 (0)</td>
</tr>
<tr>
<td><strong>Pain on moving (median, IQR)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>2 hours</td>
<td>5 (4)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>4 hours</td>
<td>5 (4)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>8 hours</td>
<td>5 (4)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>12 hours</td>
<td>4 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Day 1</td>
<td>6 (3)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Day 2</td>
<td>5 (3)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Day 3</td>
<td>4 (4)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Day 7</td>
<td>2 (2)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Day 14</td>
<td>2 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Day 30</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Day 60</td>
<td>1 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><strong>Pain on coughing (median, IQR)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>2 hours</td>
<td>6 (3)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>4 hours</td>
<td>5 (4)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>8 hours</td>
<td>5 (4)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>12 hours</td>
<td>4 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Day 1</td>
<td>5 (4)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Day 2</td>
<td>6 (4)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Day 3</td>
<td>5 (4)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Day 7</td>
<td>3 (3)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Day 14</td>
<td>2 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Day 30</td>
<td>1 (1)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Day 60</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Table 22: Pain by Morphine Equivalent Daily Dose and Visual Analogue Score.
N: Number of Patients, MEDD: Morphine Equivalent Daily Dose, IQR: Inter-Quartile Range, PACU: Post-Anaesthesia Care Unit, PCA: Patient Controlled Analgesia.
All variables analysed using the Mann-Whitney U test.
9.3.6 Inflammatory response

Median peritoneal drain volume at the time of sample collection was significantly higher in the Control Group (210.0 mls (192.5) vs. 100.0 mls (157.5), \( P = 0.005 \)). As can be seen in Table 23, there were no significant differences in the median peritoneal and plasma cytokine concentrations between groups (peritoneal fluid cytokine concentrations were corrected for dilution).

Concentrations are depicted graphically on a logarithmic scale in Figure 63 and Figure 64.

<table>
<thead>
<tr>
<th>Peritoneal fluid cytokine</th>
<th>Study Group (n = 35)</th>
<th>Control Group (n = 38)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>47.7 (78.0)</td>
<td>43.5 (90.0)</td>
<td>0.613</td>
</tr>
<tr>
<td>IL-6</td>
<td>5158.6 (5578.5)</td>
<td>3018.4 (4549.0)</td>
<td>0.051</td>
</tr>
<tr>
<td>IL-8</td>
<td>560.3 (1587.2)</td>
<td>755.8 (655.9)</td>
<td>0.735</td>
</tr>
<tr>
<td>IL-10</td>
<td>552.8 (429.2)</td>
<td>431.2 (273.9)</td>
<td>0.223</td>
</tr>
<tr>
<td>TNFα</td>
<td>13.6 (15.1)</td>
<td>11.0 (12.8)</td>
<td>0.520</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma cytokine</th>
<th>Study Group (n = 35)</th>
<th>Control Group (n = 38)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>31.9 (181.0)</td>
<td>40.3 (132.3)</td>
<td>0.886</td>
</tr>
<tr>
<td>IL-6</td>
<td>42.4 (115.5)</td>
<td>48.8 (63.8)</td>
<td>0.969</td>
</tr>
<tr>
<td>IL-8</td>
<td>24.0 (29.3)</td>
<td>19.3 (37.3)</td>
<td>0.996</td>
</tr>
<tr>
<td>IL-10</td>
<td>12.0 (13.5)</td>
<td>13.2 (11.1)</td>
<td>0.703</td>
</tr>
<tr>
<td>TNFα</td>
<td>4.6 (3.6)</td>
<td>5.2 (4.6)</td>
<td>0.623</td>
</tr>
</tbody>
</table>

Table 23: Peritoneal Fluid and Plasma Cytokine Response.

N: Number of Patients, IL-1α: Interleukin 1α, IL-6: Interleukin 6, IL-8: Interleukin 8, IL-10: Interleukin 10, TNFα: Tumour Necrosis Factor α.

Cytokine concentrations in pg/ml are expressed as Medians (Inter-quartile Range). Groups were compared using the Mann-Whitney U test. All peritoneal fluid samples were corrected for dilution using the Urea method, and peritoneal fluid IL6 samples were diluted 1:5.
Figure 63: Peritoneal Fluid Cytokine Concentrations in Study Versus Control Group.
pg / mL: Picogram Per Millilitre, IL-1α: Interleukin 1α, IL-6: Interleukin 6, IL-8: Interleukin 8, IL-10: Interleukin 10, TNFα: Tumour Necrosis Factor α.
Coloured bars: medians with inter-quartile ranges, error bars: 95% confidence intervals.
Figure 64: Plasma Cytokine Concentrations in Study Versus Control Group.
pg / mL: Picogram Per Millilitre, IL-1α: Interleukin 1α, IL-6: Interleukin 6, IL-8: Interleukin 8, IL-10: Interleukin 10, TNFα: Tumour Necrosis Factor α.
Coloured bars: medians with inter-quartile ranges, error bars: 95% confidence intervals.
9.3.7 Post-operative recovery

There were no significant differences in any recovery parameters measured (Table 24). Return of bowel function as measured by passing flatus, passing bowel motion, eating a full meal, as well as post-operative intravenous fluid and anti-emetic use were similar in both groups. Median days to meeting discharge criteria and actual discharge day were the same. Although patients in the Study Group had a higher re-admission rate (22.9% vs. 7.7%), and thus a prolonged total day stay (7 vs. 5 days), neither of these differences were statistically significant.

Complication rates and grades were equivalent. Functional recovery as measured by the SRS and patients’ self-reported time to return to work / normal activity was also similar in both groups.

9.3.8 Ancillary analyses and adverse events

No unplanned subgroup or adjusted analyses were performed, and no other adverse events specific to the intervention or control are reported.
<table>
<thead>
<tr>
<th></th>
<th>Study Group (n = 35)</th>
<th>Control Group (n = 39)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Return of bowel function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of flatus passage</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>0.834**</td>
</tr>
<tr>
<td>Day of bowel motion passage</td>
<td>5 (2)</td>
<td>5 (3)</td>
<td>0.297**</td>
</tr>
<tr>
<td>Day of full solid meal</td>
<td>2 (3)</td>
<td>2 (2)</td>
<td>0.772**</td>
</tr>
<tr>
<td>Antiemetic units used</td>
<td>4 (9.0)</td>
<td>3.2 (12.0)</td>
<td>0.907**</td>
</tr>
<tr>
<td><strong>Intravenous Fluids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replacement fluids</td>
<td>2.6 (2.3)</td>
<td>2.0 (3.6)</td>
<td>0.968**</td>
</tr>
<tr>
<td>Maintenance fluids</td>
<td>0.5 (2.7)</td>
<td>0 (1.5)</td>
<td>0.730**</td>
</tr>
<tr>
<td>Total fluids</td>
<td>3.0 (4.2)</td>
<td>2.75 (3.8)</td>
<td>0.708**</td>
</tr>
<tr>
<td><strong>Discharge</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day meeting discharge criteria</td>
<td>3 (3)</td>
<td>3 (2)</td>
<td>0.755**</td>
</tr>
<tr>
<td>Actual discharge day</td>
<td>5 (5)</td>
<td>5 (6)</td>
<td>0.750**</td>
</tr>
<tr>
<td>Total day stay</td>
<td>7 (7)</td>
<td>5 (7)</td>
<td>0.873**</td>
</tr>
<tr>
<td><strong>Readmission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (22.9%)</td>
<td>3 (7.7%)</td>
<td>0.102**</td>
</tr>
<tr>
<td>No</td>
<td>27 (77.1%)</td>
<td>36 (92.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Complication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19 (54.3%)</td>
<td>19 (48.7%)</td>
<td>0.650**</td>
</tr>
<tr>
<td>No</td>
<td>16 (45.7%)</td>
<td>20 (51.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Complication Grade</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1 (5.3%)</td>
<td>1 (5.3%)</td>
<td>0.543*</td>
</tr>
<tr>
<td>II</td>
<td>15 (78.9%)</td>
<td>13 (68.4%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3 (15.8%)</td>
<td>3 (15.8%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0 (0%)</td>
<td>2 (10.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>SRS score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>72.9 (13.7)</td>
<td>72.9 (14.4)</td>
<td>0.977†</td>
</tr>
<tr>
<td>Day 1</td>
<td>52.2 (21.0)</td>
<td>52.3 (16.1)</td>
<td>0.986†</td>
</tr>
<tr>
<td>Day 3</td>
<td>54.3 (14.3)</td>
<td>50.6 (18.5)</td>
<td>0.381†</td>
</tr>
<tr>
<td>Day 7</td>
<td>58.8 (14.5)</td>
<td>52.2 (15.1)</td>
<td>0.077†</td>
</tr>
<tr>
<td>Day 30</td>
<td>67.4 (15.6)</td>
<td>69.5 (14.6)</td>
<td>0.598†</td>
</tr>
<tr>
<td>Day 60</td>
<td>74.7 (13.5)</td>
<td>72.4 (16.4)</td>
<td>0.580†</td>
</tr>
<tr>
<td><strong>Days to return to work / normal function</strong></td>
<td>30 (21)</td>
<td>31 (47)</td>
<td>0.969**</td>
</tr>
</tbody>
</table>

Table 24: Post-operative Recovery Parameters.
N: Number of Patients, IQR: Inter-Quartile Range, SRS: Surgical Recovery Score, SD: Standard Deviation, * χ² test, ** Fisher’s Exact test, † t test, ‡ Mann-Whitney U test
9.4 DISCUSSION

We conducted a multicenter, double-blinded, randomised controlled trial investigating warming and humidification of insufflation carbon dioxide in laparoscopic colonic surgery. There was no statistically significant difference in post-operative opiate analgesia usage between groups. Subjective pain perception at rest and on moving was higher in the study arm on day 1, and pain on coughing was higher in the control arm on day 7. There were no other significant differences in pain by VAS at any time point. In addition, there were no differences in the intra-operative core temperature, the peritoneal or plasma cytokine response at 20 hours, or in any of the post-operative recovery parameters measured. Camera fogging as reported by the primary surgeon was significantly worse in the study arm.

There are no other published randomised trials comparing warming and humidification of insufflation gases in laparoscopic colonic surgery. A small case-control study conducted by Yeh et al did not demonstrate a clinical benefit. (405) Eleven randomised controlled trials have been published for other indications, with conflicting results. (238, 390-397, 419, 423) While 2 recent meta-analyses demonstrated a reduction in the incidence of intra-operative hypothermia, post-operative pain, and opiate analgesia use, these conclusions were significantly limited by the quality of included studies. (205, 420) In fact, the literature is limited to only two studies which were adequately blinded. (238, 393) Hamza et al. randomised 44 patients undergoing gastric bypass, with the intervention group receiving warm, humidified CO₂ using the Insuflow device (Lexion Medical, St. Paul, MN, USA). They found that the study group experienced reduced intra-operative heat loss, decreased pain and reduced morphine usage in the post-anaesthetic recovery unit, and improved functional recovery on day 2. External warming measures were not used in this study except as “rescue” treatment, and it is unclear if the benefits observed were due to hypothermia prevention alone. (205, 402, 403) Farley et al. randomised 101 patients undergoing laparoscopic cholecystectomy, again using the Insuflow device. A marginal but statistically significant benefit was found in intra-operative core temperature maintenance, with the study group also experiencing reduced shoulder pain in the post-anaesthetic recovery unit, and reduced abdominal pain and MEDD use at 2 weeks post-operatively. (238) One weakness of this study is that cases converted
to open cholecystectomy (of which there were 11) were excluded from further analysis, as were two patients in which the Insuflow device was disconnected for technical reasons. It is unclear from which groups these patients were excluded, and this may have caused significant bias in the final results. Nevertheless, the authors concluded that although some outcome measures reached statistical significance, no major clinically relevant benefits were observed.\(^{(238)}\)

There were marginal differences in pain perception in the current study, with higher early pain scores in the Study Group on day 1, and higher late pain scores in the Control Group on day 7. While this may be of significance, indicating a possible delayed benefit from warming and humidification, these findings need to be interpreted in the context of multiple measures. In total, 33 pain scores were measured for each patient (3 pain scores at 11 post-operative time-points) and only 3 of those were significantly different between groups. Since there were no significant differences in total opiate usage or any measured recovery parameters, it is the authors’ opinion is that overall, warming and humidification of the insufflation gas did not seem to confer any clinical benefit.

In addition, the inflammatory response as measured by local and systemic cytokine concentrations was equivalent in both groups. Although warming insufflated CO\(_2\) (without humidification) has been shown to reduce the relatively minor peritoneal humoral response after laparoscopic cholecystectomy in humans, no clinical studies with the exception of the current one have investigated immunological consequences of warming and humidification.\(^{(295, 418)}\) The equivalent cytokine response in our study maybe due to the fact that the inflammatory response after laparoscopic colonic surgery is so extensive, that it over-shadows any immunological effects caused by the insufflation gas.\(^{(21, 263, 303, 416)}\) Another possible explanation is that warming and humidification does not actually reduce the inflammatory response. Evidence from animal studies is inconclusive, and it is possible that the acidic nature of carbon dioxide, rather than its desiccative effect is to blame for the observed inflammatory reaction.\(^{(119, 299-301, 417, 424, 425)}\) The importance of the local peritoneal inflammatory response in this setting relates largely to its use a surrogate marker for post-operative peritoneal adhesion formation.\(^{(230, 259)}\) While this
endpoint is difficult to assess in humans, exaggerated animal models have demonstrated that pneumoperitoneum conditioning does reduce peritoneal adhesion formation. (224-226) However, there appears to be a counterbalancing effect between prevention of desiccation and cooling (which also reduces adhesion formation), and it may be that cold humidification should be further investigated. (217, 224)

Optical visibility during laparoscopic surgery is another endpoint of relevance in laparoscopic surgery. (420) Camera fogging as rated by the primary surgeon was significantly worse in the warmed, humidified group. This is a novel finding. The two other double-blinded studies discussed above both similarly assessed fogging at the end of the procedure, but no difference between the groups was detected. (238, 393) It is possible that the longer duration of surgery and pneumoperitoneum in the current study may have had an effect, with a greater degree of condensation in the laparoscopic ports. This is speculative however, and it is difficult to draw any further conclusions with such a subjective outcome measured at a single time point. It is worth noting that any difference in visibility did not lead to any detectable adverse consequences, as there were no differences in operation times or conversion rates. Although the current study was not powered to detect these differences, results tended to favour the Study Group for both these outcomes.

We note some weaknesses of this study. There was a trend towards more right sided cases in the Study Group, and more left sided cases and conversions in the Control Group. While this is likely to be due to sampling error, these differences were not statistically significant, and any resulting differences in the operating time, pneumoperitoneum time and volume of gas used were also not statistically significant. Also, had these seemingly random differences been important, they would all have favoured the Study Group. Despite this, there was still no detectable difference in any of the post-operative outcomes measured. Larger numbers would have probably reduced the risk of a type II error in this study. However, we note that the small reduction in MEDD usage in first 3 days was not corroborated by the pain VAS which was marginally (but not significantly) higher in the Study Group.
Therefore, in the absence of any demonstrable clinical benefit the use of warming and humidification of insufflation gases in laparoscopic colonic surgery cannot be recommended. As well as guiding clinical practice, the findings of this study hold important implications for future trials investigating alternative gases for laparoscopy, and the recently proposed theory of wound humidification in open surgery.\(426, 427\)

**9.5 CONCLUSION**

Warming and humidification of insufflation carbon dioxide does not attenuate the early inflammatory cytokine response, and confers no clinically significant benefit in laparoscopic colonic surgery.
CHAPTER 10: ANIMAL STUDY – THE EFFECT OF WARMING AND HUMIDIFICATION ON PNEUMOPERITONEUM ASSOCIATED OXIDATIVE STRESS
10.1 BACKGROUND

There is evidence that pneumoperitoneum during laparoscopy induces systemic and local tissue oxidative stress as measured by markers of lipid and protein peroxidation. This imbalance between reactive oxygen species production and anti-oxidant capacity is physiologically deleterious, potentially contributing to the post-operative stress and adhesiogenic response.

Data from animal studies suggest that the possible etiology of pneumoperitoneum associated oxidative stress is a reduction in splanchnic blood flow in a pressure and time dependent manner, with a subsequent reperfusion injury on deflation of the abdomen. It has also been reported that inherent reactive properties of the carbon dioxide gas used for insufflation may be contributory. However, findings from randomised clinical trials have demonstrated similarly high levels of oxidative stress markers after both laparoscopic and equivalent open surgery, indicating that the oxidative stress is not specific to being caused by elevated intra-abdominal pressure or the acidic nature of carbon dioxide pneumoperitoneum.

It has recently been proposed that the surgical oxidative stress response maybe related to the exposure of the peritoneal cavity to a non-physiological environment; room air in open surgery, and dry, cold carbon dioxide insufflation in laparoscopic surgery. In the case of laparoscopic surgery, carbon dioxide gas used in laparoscopic insufflation is typically delivered at room temperature and at 0% relative humidity (significantly dryer than the usual 40-60% humidity of room air). The resulting cooling and desiccation produces visible structural changes in the peritoneal mesothelial surface layer a response which may be attenuated by warming and humidification of insufflation gas.

To date the effect of using warm, humidified gas on the oxidative stress response has not been directly investigated. While the results of the trial presented in last chapter appear contradictory, with no difference in the level of inflammatory cytokines response if the insufflation was warmed.
and humidified,(251) oxidative stress markers may provide a more sensitive measure of inflammation, and measurement of these under controlled circumstances may provide valuable additional information. Thus the aim of the current study is to investigate the effect of warming and humidification on the oxidative stress response in a physiologically appropriate rat model of pneumoperitoneum.
10.2 METHODS

10.2.1 Animals

Twenty adult inbred male Wistar rats (mean weight 510 ± 85 g) were alternately assigned to the Warm Humidified group (WH group, n = 10) and Control group (n = 10). These rats had been subjected to a 12 hour day / night cycle in a controlled room environment (25 ± 2 °C and relative humidity of 30 – 70%). They were ad libitum fed standard rodent chow (Teklad 2018, Madison, WI, USA) and had free access to tap water. Food was withdrawn 6 hours prior to surgery.

10.2.2 Anaesthesia

Rats were anaesthetised in a standard Perspex induction chamber using isoflurane 5% + 5 L / min oxygen (isoflurane, Luman Better Pharmaceutical Co. Ltd, Shandong 276006, China), and maintained initially by nose cone ventilation using Isoflurane 3% + 2 L / min inspired 40% oxygen. A single subcutaneous dose of buprenorphine (Temgesic) 0.05 mg / kg in 0.2 ml solution was administered for analgesia. All animals had a tracheostomy performed via a 2 cm midline neck incision and were then ventilated using a pressure controlled ventilator (Kent Scientific Corporation, Torrington, CT, USA). The fraction of inspired oxygen/air was kept at 40%; the respiratory rate was maintained at 40 – 60 breaths per minute; and the peak inspiratory pressure at 15 – 25 cm H₂O. This maintained the expired CO₂ at 35 – 55 mL / L as measured by an in-line respiratory profile monitor and capnograph (CO₂SMO+, Novametrix Medical Systems Inc, Wallingford, CT, USA). Once required ventilation parameters were achieved, the animals were administered a muscle relaxant via an intravenous cannula inserted in the right femoral vein (pancuronium 0.2 mL of 4mg / 2 mL solution). Body temperature was maintained by means of a warming plate placed beneath the animal. A double layer of aluminium foil was also placed on top of the animal once insufflation was started, simulating a warming blanket.

10.2.3 Monitoring

Continuous HR and MAP were measured using a right femoral artery intra-arterial pressure transducer (Millar Mikro-Tip catheter, Millar Instruments Incorporated, Houston, Texas, USA). The MAP was maintained at > 70 mmHg. Rectal temperature was measured using a calibrated digital
rectal thermometer. A second calibrated thermometer was inserted through a 1 cm midline incision in the abdominal wall and secured with an air tight purse string suture. All data were collected using the PowerLab data acquisition system and recorded using LabChart 5 (ADInstruments, Dunedin, New Zealand).

10.2.4 Intravenous fluids
A continuous intravenous fluid infusion of 0.9% NaCl was administered using a syringe driver (Genie™, Kent Scientific Corporation, Torrington, CT, USA). The flow rate was calculated individually and corrected for the weight of each rat based on data from 74 human laparoscopic colonic resections performed in 3 hospitals in Auckland, New Zealand from May 2008 to June 2009.(303) In this human trial, the median volume of intravenous fluid given to these patients intra-operatively was 2000 mL, the mean duration of surgery was 180 min, and the mean patient weight was 72.4 kg. Thus the fluid infusion rate was calculated to be 9.17 mL / kg / hour.

10.2.5 Pneumoperitoneum
A 16G cannula was inserted in the midline of the abdomen in the supra-pubic region and directed cranially. This served as the gas inflow port, and was connected to a gas insufflator (CO₂-OP-Pneu insufflator, Wisap, Munich, Germany) set at 5 mmHg and 1 L / min.(432) Carbon dioxide gas was used for insufflation (Medical grade, BOC Gases Ltd, Auckland, NZ).

A humidifier (MR860 Laparoscopic Humidification System, Fisher & Paykel Healthcare, Auckland, New Zealand) was connected in all cases, but only activated in the WH group. Thus the WH group received warmed (37 °C) and humidified (98% RH) carbon dioxide and the Control Group received standard carbon dioxide at room temperature (19 °C) and 0% RH.

Insufflation was started, and once the abdomen distended sufficiently (5 mmHg; calibrated electronic insufflator), 3 x 24G outflow cannulas were inserted through the abdominal wall in the right iliac fossa via the incision previously made for femoral access. This mimicked the laparoscopic surgery working ports. Gas escape was adjusted by inserting or removing cannulas to
maintain the intra-abdominal pressure of 5 mmHg at the desired flow rate. The flow rate was precisely controlled by means of an adjustable mechanical flow limiter (Precision Flow Control Valve, GRPO-10-PK-3, Esslingen, Germany) located downstream from a reservoir which served to blunt the pulsed flow from the insufflator. A flow meter (TSI model 4140, TSI Incorporated, MN, USA) was incorporated into the apparatus. This allowed real-time monitoring and continuous recording of the insufflation rate during the experiment (Figure 32).

The insufflation flow rate was determined individually and corrected for the peritoneal surface area of each rat based on data from 74 human laparoscopic colonic resections (details of the calculation are provided in section 3.4.3 Pneumoperitoneum).(251-253) The insufflation was continued for a total of 110 minutes (based on the median pneumoperitoneum time from the same clinical trial data).(251)

10.2.6 Specimen collection

At the end of 110 min of continuous pneumoperitoneum, a laparotomy was performed. Blood was collected by a euthanizing trans-diaphragmatic cardiac puncture and immediately distributed between microtainers containing Ethylenediamine Tetra-acetic Acid (EDTA) and Lithium Heparin (BD Diagnostics, Franklin Lakes, NJ 07417, USA). These were then centrifuged for 15 min at 3000 rpm and 4° C (Eppendorf centrifuge, Hamburg, Germany). The supernatant was aliquoted and stored at -80° C until analysis.

The left lobe of the liver, both kidneys, whole pancreas, 5 cm of mid-jejenum, and both lungs were excised. Approximately 1 cm³ of the tissue specimens were snap frozen in liquid nitrogen, and stored at -80° C until analysis. The remaining specimens were fixed in 10% neutral buffered formalin for histology.
10.2.7 Outcomes

10.2.7.1 Baseline data
Baseline data were recorded for age (wks), weight (g), nose to base of tail length (cm), total operating time (min from induction of anaesthesia to termination of pneumoperitoneum), and mean gas flow rate (L / min). The mean HR, MAP, RR, PIP and EtCO₂ served as a baseline comparison of haemodynamic and respiratory stability.

10.2.7.2 Primary outcome
The primary outcome was oxidative stress as measured by malondialdehyde (MDA) and protein carbonyl (PC) levels at the conclusion of pneumoperitoneum.

MDA levels in plasma and tissue homogenates were measured using a published Thiobarbituric Acid Reactive Substances (TBARS) method,(255) based on the Animal Models of Diabetic Complications Consortium Protocol.(256) Results were corrected for protein concentrations determined using a Biuret assay and expressed as nM / g of protein.

PC levels in plasma and tissue homogenates were measured using a commercial ELISA test kit (BioCell PC Test kit, BioCell Corporation Ltd, New Zealand) using published methodology and according to the recommendations of the manufacturer.(257) Results were corrected for protein concentrations determined using a Biuret assay and expressed as nM / g of protein.

10.2.7.3 Secondary outcomes
Organ histology
Light microscopy was performed by a blinded consultant histopathologist (B.D.) on 5 μm thick longitudinal paraffin sections using haematoxylin and eosin staining. A previously published standardised scoring system for organ inflammation was used.(254)
Temperature parameters
Continuous recording of intra-abdominal and rectal temperature was undertaken (mean values, as well the change from baseline were calculated).

10.2.8 Statistical analysis
Results were analysed using SPSS® for Windows® version 17.0 (Lead Technologies Inc, Chicago, Illinois, USA). Continuous variables were tested using the Shapiro-Wilk test for normality. Groups were compared using the Mann–Whitney U test for non-parametric continuous variables, and the t test / ANOVA for parametric continuous variables (ANOVA post-hoc analysis was performed using t tests with Bonferroni correction). Statistical significance was accepted at the 0.05 level. Results are presented as Mean (Standard Deviation) for parametric data and Median (Inter-Quartile Range) for non-parametric data.

10.2.9 Ethical approval
This study was approved by the Animal Ethics Committee of the University of Auckland (Protocol approval number R533).
10.3 RESULTS

10.3.1 Baseline data

All animals survived the experimental protocol. There were no significant differences in any baseline parameters recorded (Table 25).

<table>
<thead>
<tr>
<th></th>
<th>WH group (n = 10)</th>
<th>Control Group (n = 10)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
<td>26 (14)</td>
<td>26 (14)</td>
<td>0.912</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>501 (90)</td>
<td>519 (84)</td>
<td>0.654</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>26 (2)</td>
<td>26 (2)</td>
<td>0.570</td>
</tr>
<tr>
<td>Operating time (min)</td>
<td>172 (8)</td>
<td>173 (8)</td>
<td>0.767</td>
</tr>
<tr>
<td>Insufflation flow rate (L / min)</td>
<td>0.070 (0.010)</td>
<td>0.073 (0.013)</td>
<td>0.623</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>391 (24)</td>
<td>390 (20)</td>
<td>0.908</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mm Hg)</td>
<td>97 (11)</td>
<td>93 (10)</td>
<td>0.365</td>
</tr>
<tr>
<td>Respiratory Rate (bpm)</td>
<td>54 (9)</td>
<td>56 (11)</td>
<td>0.756</td>
</tr>
<tr>
<td>Peak Inspiratory Pressure (mm H₂O)</td>
<td>20 (3)</td>
<td>21 (2)</td>
<td>0.471</td>
</tr>
<tr>
<td>End Tidal CO₂ (mL / L)</td>
<td>47 (8)</td>
<td>48 (8)</td>
<td>0.805</td>
</tr>
</tbody>
</table>

Table 25: Baseline Comparison between WH (Humidified) Versus Control (Dry) Groups. Results expressed as Mean (Standard Deviation). T test used for comparison.

n: Number of Rats, g: Grams, cm: Centimetres, Min: Minutes, BPM: Beats Per Minute, mm: Millimetres, Hg: Mercury, H₂O: Water, mL: Millilitres, L: Litres.

10.3.2 Oxidative stress markers

There were no significant differences between groups for MDA or PC levels in plasma, or in any of the organ tissues (Table 26). Data for pancreatic tissue MDA was excluded from final analysis as protein concentrations were inconsistent due to variable fat content on homogenization (concentrations could not be corrected for dilution).
Table 26: Oxidative Stress Marker Levels in WH (Humidified) Versus Control (Dry) Groups.

Results in nM / g of protein are expressed as Mean (Standard Deviation). T test used for comparison, n: Number of Rats, nM: Nanomoles, g: Grams

* assay failure for MDA in this tissue.

10.3.3 Histology

There were no significant differences in histological score between groups for any organs on light microscopy (Table 27).

Table 27: Histological Grading of Inflammation on Light Microscopy in WH (Humidified) Versus Control (Dry) Groups.

Results expressed as Median (Inter-Quartile Range). Mann-Whitney U test used for comparison. n: Number of Rats
10.3.4 Temperature

In both groups, the rectal and intra-abdominal temperatures increased during the 110min of pneumoperitoneum (Figure 65). Rats in the WH group had a modest but significantly greater increase compared to controls in rectal temperature from baseline (2.2 ± 0.8 °C vs 1.3 ± 0.9 °C, respectively, P = 0.023) and in intra-abdominal temperature from baseline (2.2 ± 0.6 °C vs 0.4 ± 1.0 °C, respectively, P < 0.0001). There were however no significant differences in mean rectal temperature (37.6 ± 0.9 vs 37.9 ± 0.8, P = 0.495) or mean intra-abdominal temperature (35.6 ± 0.5 vs 35.0 ± 0.9, P = 0.136) in the WH versus the Control group.
Figure 65: Graph of Rectal and Intra-abdominal Temperature During Pneumoperitoneum.
Median (Inter-Quartile Range), °C: Celsius, Min: Minutes
10.4 DISCUSSION

We have conducted a study investigating the impact of warming and humidification of insufflation carbon dioxide on pneumoperitoneum-associated oxidative stress markers in a clinically relevant animal model. Animals that received warmed; humidified insufflation (WH) had a modest increase in rectal and intra-abdominal temperature from baseline during the period of pneumoperitoneum. This was expected given the additional heating for these animals from the insufflation gas. WH caused no significant difference to levels of the oxidative stress markers or to histology scores compared to non-WH controls.

The current study is the first to specifically investigate the impact of warming and humidification on pneumoperitoneum and associated oxidative stress markers. Under our study conditions there was no impact of WH on the underlying oxidative stress markers or any other variables compared to controls. This was surprising as previous electron microscopy studies using rat models have reported attenuation of histological peritoneal damage by warming the insufflation gas. (222, 226) There are two possible reasons for this disparity. The first is that electron microscopy is more sensitive at detecting superficial peritoneal histological damage, the attenuation of which may not necessarily translate to a reduction in whole tissue or plasma oxidative stress. A second possible reason for the disparity is that there is a significant difference in insufflation protocols between our study and the previous electron microscopy studies. (222, 226) In the first of these other studies, pneumoperitoneum was maintained for 2 hours at 10 mmHg, and the abdomen desufflated and re-insufflated every 15 min, raising the possibility of repeated reperfusion injury during the surgery. (222) We did not undertake these cyclical pressure changes as we did not see the clinical relevance of such an insufflation pattern model, and further we were interested in humidification effects only. In a more recent and larger electron microscopy study by Peng et al, pneumoperitoneum was maintained for 3 – 5 hours at 9 mmHg at a flow rate of 0.3 L/min. (226) Based on the scaling calculations performed in the our study, we calculate that the pneumoperitoneum time was approximately twice as long, and the flow rate 10 times as high.
(given a mean rat weight of 275 g) as that of a typical prolonged laparoscopic procedure in the human clinical setting (section 3.4.3 Pneumoperitoneum).(251-253) Thus, the effects of hypothermia and desiccation were probably exaggerated, as evidenced by the significant drop in core temperature despite use of a warming blanket in the control group of that study.

Furthermore, it became clear after scaling calculations were performed for the current study that almost all the previously published rat studies investigating oxidative stress associated with pneumoperitoneum had employed exaggerated models, using gas flow rates only loosely adapted from the clinical setting without corrections for animal size or physiology.(206, 210, 218, 331, 347, 377, 433-435) Insufflation pressures have also tended to be over-estimated. It has recently been described that the optimal insufflation pressure that should be used in a rat model to simulate human laparoscopy approximates 5 mmHg, which is significantly lower than that used by the majority of the studies investigating oxidative stress (10 – 15 mmHg).(432) These inaccuracies in the scaling of insufflation parameters limit the applicability of these rat models to the clinical setting.

Both MDA and PC are considered validated and reliable markers of oxidative stress with MDA measuring lipid peroxidation and PC measuring protein oxidation.(255, 428, 436) Table 28 presents a summary of MDA levels from previously published rat studies investigating pneumoperitoneum-associated oxidative stress.(206, 210, 218, 331, 347, 377, 433-435) It is notable that MDA levels measured in our appropriately scaled model of pneumoperitoneum were much lower than most of the values presented in these studies. This was despite our relatively longer period of pneumoperitoneum. While we could not find any pneumoperitoneum studies that measured PC levels, our measurements of tissue PC levels are equivalent to those reported in control groups of several studies investigating other interventions in rats.(437-439).
<table>
<thead>
<tr>
<th>Study</th>
<th>Flow Rate (L/min)</th>
<th>Duration (min)</th>
<th>Pressure (mmHg)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
<th>Small Bowel</th>
<th>Lung</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pross (331)</td>
<td>0.4</td>
<td>20</td>
<td>Control 6</td>
<td>40 53</td>
<td>80 108</td>
<td>60 104</td>
<td>-</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Sare (347)</td>
<td>NS</td>
<td>30</td>
<td>Control 5</td>
<td>123 174 150 128</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>130</td>
<td>206 206</td>
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<tr>
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<td>60</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1900 3420 4350</td>
</tr>
<tr>
<td>Yilmaz (210)</td>
<td>NS</td>
<td>60</td>
<td>Control 15</td>
<td>37670 57200</td>
<td>61070 75230</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yilmaz (218)</td>
<td>NS</td>
<td>60</td>
<td>Control 15</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>500 500 900</td>
</tr>
<tr>
<td>Yilmaz (210)</td>
<td>NS</td>
<td>60</td>
<td>Control 15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>150 500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oksuz (433)</td>
<td>NS</td>
<td>60</td>
<td>Control 15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unsal (434)</td>
<td>0.5</td>
<td>60</td>
<td>Control 15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>76 145</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Xu (435)</td>
<td>NS</td>
<td>60</td>
<td>Control 15</td>
<td>4690 9830</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3980 7640</td>
</tr>
<tr>
<td>Ates (377)</td>
<td>NS</td>
<td>60</td>
<td>Control 15</td>
<td>25000 40000</td>
<td>60000 90000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 28: Summary of MDA levels in rat studies investigating pneumoperitoneum associated oxidative stress.

All studies used the Thiobarbuturic Acid Reactive Substances (TBARS) method to measure MDA levels. Units have been converted to nM / g protein if not reported as such in the original paper. **Some intermediate groups omitted, NS: Not Stated, MDA: Malondialdehyde, Min: Minutes, mm: millimetres, Hg: Mercury, L: Litres, nM: Nanomoles, g: grams

An unexpected limitation of our study that only became apparent when we discovered the lack of peritoneal damage from cold, dry gas, was that the light microscopy performed may not have been sensitive enough to visualise differences in organ inflammation. Also we did not include a sham control group that underwent anaesthesia and / or laparotomy alone. This was because we were specifically interested in the impact of insufflation warming and humidification, rather than whether pneumoperitoneum causes oxidative stress per se, as this had been borne out by previous studies.
as discussed above (albeit in exaggerated models). (206, 210, 218, 331, 347, 377, 433-435)
Importantly, however, our findings have now led us to question the basic assumption from these other experiments, namely that pneumoperitoneum induces oxidative stress at all. We now suggest that future studies need to re-assess the occurrence and aetiology of oxidative stress in more carefully controlled and clinically relevant models of pneumoperitoneum.

10.5 CONCLUSION
Warming and humidification of insufflation gas has no effect on measures of oxidative stress compared to un-warmed and non-humidified controls when physiologically relevant insufflation parameters are used. The apparent lack of marked damage compared to other more exaggerated protocols reported in the literature raise an important issue of the validity of the current existing models and their oxidative marker findings.
SUMMARY OF RESULTS
This thesis aims to answer the following three questions: Is the local inflammatory reaction significantly reduced after prolonged laparoscopic surgery compared with equivalent open surgery? Does the pneumoperitoneum itself contribute to the local peritoneal inflammatory response? If so, can this response be attenuated by conditioning the insufflation gas to improve post-operative clinical outcomes after prolonged laparoscopic surgery?

In a review of current concepts (presented in the introduction) the structure and function of the peritoneum was outlined, with a focus on the inflammatory response after major abdominal surgery. The peritoneum is described as a metabolically active organ, responding to insult through a complex array of immunologic cascades which are amplified with the duration and extent of injury. The consequences of this response are paramount in the post-operative clinical course, contributing to post-operative adhesions, gastro-intestinal ileus, tumour progression, metabolic derangement, pain perception, and fatigue. The conclusion from this review was that interventions that reduce the peritoneal inflammatory response should be investigated with the aim of enhancing recovery after abdominal surgery. Minimally invasive laparoscopic surgery is one such intervention that has been touted to reduce peritoneal injury. However, it is also evident from this review that the association between laparoscopic surgery and a reduced peritoneal reaction has not been convincingly demonstrated, particularly in prolonged laparoscopic procedures such as laparoscopic colonic surgery, where the peritoneal damage is greatest, and the clinical consequences more pronounced.

The first study conducted was a systematic review of the literature investigating the humoral inflammatory response after laparoscopic and equivalent open colorectal surgery, with this indication discussed as a model of prolonged laparoscopy. A highly-sensitive search using Cochrane methodology was undertaken independently by two investigators with no language restriction. Studies were identified from the Cochrane Central Register of Controlled Trials (CENTRAL/CCTR), Cochrane Library, Medline (January 1966 to January 2009), PubMed (1950 to
January 2009) and Embase (1947 to January 2009). Relevant meeting abstracts and reference lists were manually searched. Thirteen randomised controlled trials were systematically reviewed and 8 of these included in meta-analysis which demonstrated a significantly higher serum IL-6 on day 1 after open colorectal resection for neoplasia (n = 97) compared with laparoscopic resection (n = 76, p = 0.0008) without significant heterogeneity. Data for plasma IL-6 were heterogeneous, with no apparent difference between groups. No other significant differences were identified, and there were not enough data on local peritoneal humoral factors to allow meta-analysis. In addition, it was apparent that data on the local peritoneal humoral response which may be more clinically relevant (as discussed above), was relatively sparse.

The second study conducted compared the local (and systemic) cytokine response in laparoscopic and open colonic surgery and correlated this with post-operative recovery parameters. Using a prospectively collected patient database, a Study Group (n = 50) of patients undergoing elective laparoscopic colonic resection was compared with a Control Group (n = 25) of patients undergoing equivalent open colonic surgery within an ERAS program. Patients were matched for age, sex, BMI, ASA, Cr Possum, side of resection, diagnosis, and histological stage. Plasma and peritoneal fluid concentrations of IL-6, IL-8, IL-10, and TNFα were measured at 20 – 24 hours after surgery. The SRS score, a validated score for measuring recovery after surgery was determined pre-operatively and at 3, 7, 30, and 60 days post-operatively. All data were prospectively collected, and a priori definitions were used for discharge parameters, complications, and complication severity.

With the exception of a marginally lower peritoneal IL-6 level (5389.2 ± 5516.5 pg/ml versus 8149.0 ± 4196.7 pg/ml, P 0.030), the systemic and peritoneal cytokine response at 20-24 hours was found to be similar after laparoscopic versus open colonic resection, with corresponding equivalent rates of post-operative recovery. Significant correlations were found between other cytokine levels (which were similar in the two groups) and discharge criteria achievement, day stay, post-operative complications, and the Surgical Recovery Score.
Thus in prolonged laparoscopy, attenuation of the inflammatory humoral response appears to be minimal and not corroborated by differences in clinical outcome. The next study undertaken was a systematic review of the literature on the concept of pneumoperitoneum-associated oxidative stress which is put forth as a possible explanation for the apparently significant inflammatory response after laparoscopy. A highly-sensitive search was performed independently by two investigators with no language restriction or restriction on trial design. Medline, Medline in-process, Cochrane Library, Pubmed, and Embase were searched from 1947 to March 2008. Papers that did not investigate pneumoperitoneum as a causative factor, or did not report outcome measures related to oxidative stress were excluded. Seventy-three relevant papers were identified, including 36 animal studies, 21 human clinical trials, 9 case reports, 5 review articles, and 2 comments. The published data indicate that pneumoperitoneum causes a reduction in splanchnic blood flow resulting in biochemical evidence of oxidative stress in a pressure and time dependent manner. It is also clear that corresponding open abdominal procedures are associated with a similar oxidative stress response, indicating that this phenomenon is not simply due to elevated intra-abdominal pressure. There was some evidence that the use of carbon dioxide as the insufflation may be contributory, but the impact of other factors such as the temperature and humidity of the gas have not been elucidated despite evidence of histological injury caused by desiccation. It was now appropriate to investigate this, and evaluate whether warming and humidification of insufflated carbon dioxide could be beneficial in this respect.

Several randomised controlled trials have investigated warming and humidification using postoperative pain as the main outcome measure, with this endpoint regarded as a proxy measure of peritoneal inflammation. Results have been inconclusive due to small sample sizes. A meta-analysis of published randomised controlled trials on the effect of warming and humidification on post-operative pain as determined by visual analogue score or morphine usage was performed. A highly-sensitive search using Cochrane methodology was conducted independently by two investigators with no language restriction. Studies were identified from the Cochrane Central
Register of Controlled Trials (CENTRAL/CCTR), Cochrane Library, Medline, PubMed and Embase spanning a period of time from 1947 (earliest EMBASE records) to January 2008. Relevant meeting abstracts and reference lists were also manually searched. Randomised controlled trials on adults undergoing elective laparoscopic abdominal surgery under general anaesthetic, where the exposure group received warmed, humidified insufflation, and the control group received standard cold and dry insufflation were included (if they reported pain by visual analogue score, or morphine equivalent usage as an outcome measure). Seven RCTs were included. In the warmed, humidified group there was a significant reduction in pain visual analogue scores at 6 hours (p < 0.006), day 1 (p = 0.010), and day 3 (p < 0.0001); and in morphine equivalent usage on day 2 (p = 0.040). The conclusion was that warming and humidification of insufflation gas may reduce postoperative pain after laparoscopy; however these conclusions were limited by the quality of the included studies. Five of the 7 RCTs were methodologically poor as assessed by the Jadad scale which leads to the possibility that biases were combined in meta-analysis. Furthermore, variation between the studies in terms of surgical indication, the use of other external warming measures, and reported outcome variables weakened the analysis. Further high-quality trials were required, particularly in prolonged laparoscopy where the effect of peritoneal desiccation and hypothermia may be more pronounced. In addition, the effect of warming and humidification on the peritoneal inflammatory response and the consequent post-operative recovery had yet to be determined.

Prior to conducting further clinical studies, an independent test of a commercially available laparoscopic humidifier (Fisher & Paykel MR860 laparoscopic humidification system, Fisher & Paykel Healthcare, Auckland, New Zealand) was conducted to ensure effective warming and humidification of carbon dioxide gas at clinically relevant flow rates. A 2.5L insulated chamber was constructed and a digital thermo-hygrometer placed inside it. The humidifier water vessel was weighed and exactly 30.0g of water poured in. 50.0L of CO₂ was insufflated into the chamber via the humidifier at 2.0L/min, 4.0L/min, 6.0L/min, 8.0L/min, and 10L/min using a laparoscopic insufflator. Measurements of temperature and humidity in the chamber were taken at 30 second
intervals. After 50.0L of gas was insufflated the water left in the humidifier was weighed, and this used to calculate the mean absolute humidity of the insufflated gas by the gravimetric method. At every flow rate tested, > 100.0% relative humidity was achieved in the chamber after less than 30 seconds of insufflation. Using the gravimetric estimate, the humidifier was able to saturate 50.0L of carbon dioxide to close to saturation humidity at every flow rate tested. The conclusion from this study was that the device tested was able to effectively humidify insufflated carbon dioxide at a range of flow rates commonly used in the laparoscopic setting.

After confirmation of the effectiveness of the device, a multi-centre, double blinded, randomised controlled trial investigating warming and humidification conducted in laparoscopic colonic surgery was undertaken. The hypothesis tested was that warming and humidification of insufflation gas during laparoscopic colonic surgery would lead to reduced post-operative pain and improved recovery by reducing peritoneal inflammation. The Study Group received warmed, humidified insufflation carbon dioxide (37°C, 98%RH) delivered using the Fisher & Paykel MR860 laparoscopic humidification system (Fisher & Paykel Healthcare, Auckland, New Zealand). The Control Group received standard carbon dioxide gas (19°C, 0%RH). Randomisation was conducted using computer-generated random numbers stratified by hospital, and allocation was concealed in opaque numbered envelopes until the day of surgery. The patient, study investigators, surgeon, and medical staff responsible for patient care were all blinded to patient allocation. Data analysis was also blinded. Anaesthesia and analgesia protocols were standardised. The primary outcome was morphine equivalent use determined using Morphine Equivalent Daily Dose (MEDD). Secondary outcomes included pain at rest / moving / coughing measured by visual analogue scores, intra-operative oesophageal temperature measured at 15min intervals, camera fogging as rated by the primary surgeon on a Likert scale, peritoneal and plasma cytokine concentrations at 20h post-operatively, and patient recovery using defined discharge and complication criteria, and the Surgical Recovery Score. Eighty two patients were randomised, with 41 in each arm. Groups were well matched at baseline. Intra-operative core temperature was
similar in both groups. Median camera fogging score was significantly worse in the Study Group (4 vs. 2, \( P = 0.040 \)). There were marginal differences in pain scores, but no significant differences were detected in MEDD usage, cytokine concentrations or any recovery parameters measured.

The results of this trial suggested that warming and humidification of insufflation carbon dioxide did not attenuate the inflammatory cytokine response after prolonged laparoscopy. These results were inconsistent with previously published animal model studies demonstrating a significant reduction in histological peritoneal damage if insufflation gas is conditioned. Using data from the previously conducted clinical trial, a final study was conducted to re-assess the effect of warming and humidification in an accurate animal model of pneumoperitoneum using measurement of oxidative stress markers as a sensitive measure of inflammation. Ten male Wistar rats were included in each arm, with the Study Group receiving warmed, humidified insufflation carbon dioxide (37°C, 98%RH) delivered using the Fisher & Paykel MR860 laparoscopic humidification system (Fisher & Paykel Healthcare, Auckland, New Zealand), and the Control Group receiving standard carbon dioxide gas (19 °C, 0%RH). Pneumoperitoneum was maintained for 110 minutes by insufflating the abdomen through a 16G cannula inserted in the midline. Intra-abdominal pressure was kept at 5 mmHg and the insufflation flow rate was determined individually and corrected for the peritoneal surface area of each rat based on data from the clinical trial. Continuous invasive heart rate, and mean arterial pressure measurements were made using an arterial line pressure transducer inserted into the right femoral artery. Respiratory rate, peak inspiratory pressure and end tidal CO\(_2\) were also measured every 10min for the duration of pneumoperitoneum. Rectal temperature was measured using a calibrated digital rectal thermometer. A second calibrated thermometer was inserted through a 1 cm midline incision in the abdominal wall and used to measure intra-abdominal temperature. A continuous intravenous fluid infusion of 0.9% Sodium Chloride was administered at a flow rate determined individually and corrected for the weight of each rat based on data from the clinical trial. At the end of the period of pneumoperitoneum, a laparotomy was performed to collect blood and harvest samples of liver, kidney, jejunum, pancreas and lung. The
primary outcome was the levels of Malondialdehyde (MDA) and Protein Carbonyls (PC) in plasma and tissue. Secondary outcomes measured included histology by light microscopy performed by a blinded histopathologist, and rectal / intra-abdominal temperature change. Animals who received warmed, humidified insufflation had a significantly more pronounced increase in rectal and intra-abdominal temperature during the surgery. Despite this, there were no detectable differences in levels of oxidative stress markers, or histology on light microscopy between the two groups. The conclusion from this study was that dessication caused by cold, dry gas insufflation does not contribute significantly to the oxidative stress response associated with pneumoperitoneum.
CONCLUSION
Several conclusions can be made based on the research presented in this thesis.

The first is that the peritoneal inflammatory humoral response is clinically relevant, and that its magnitude can be directly correlated with post-operative patient recovery and complications.

The second conclusion is that this inflammatory response does not appear to be significantly attenuated by performing major abdominal surgery laparoscopically. This may either be due to the effects of pneumoperitoneum, or to the fact that the peritoneal destruction caused by the surgical dissection (which is generally similar regardless of the access incision) over-shadows any other differences in technique.

The third conclusion is that, should the pneumoperitoneum be independently responsible for an inflammatory response, the theory that dessication caused by cold, dry insufflation gas is the etiological cause remains largely unsubstantiated.
FUTURE RESEARCH
Interventions that reduce the peritoneal inflammatory response should be investigated with the aim of enhancing recovery after abdominal surgery. The research outlined in this thesis, and the resulting conclusions reveal several interesting areas that require further inquiry.

First and foremost, the basic tenet that pneumoperitoneum is associated with a significant oxidative stress response independent of surgical dissection requires validation within an accurate animal model prolonged pneumoperitoneum. Perhaps one of the most important findings in this thesis, is the extremely exaggerated nature of most published animal studies on which many of the theories are based. In addition, the clinical significance of the oxidative stress response requires investigation. Without correlation with clinical outcome within the setting of a randomised controlled trial, the absolute value of the measurements cannot be ascertained.

Should pneumoperitoneum be found to be associated with an independent inflammatory response that is clinically relevant, the logical next step is to further investigate the aetiology. Further studies evaluating warming and humidification are required to confirm the negative findings in this thesis.

Other areas that warrant investigation include the inherent nature of carbon dioxide gas, specifically its acidity in solution and known immunological effects. It may be that benefits previously observed using a different gas (such as helium) still hold true in a non-exaggerated model. Modification of the contents of the insufflated gas using gas mixtures (including oxygen supplementation) may confer additional benefits. The concept of cold humidification is also on the horizon, with the suggestion that heat may promote inflammation, opposing any benefits gained by humidification. Advances in the nebulisation technology used to achieve cold humidification hold great promise, potentially allowing the concurrent administration anti-inflammatory, anti-adhesiogenic, local anaesthetic and antibiotic medications directly into the site of peritoneal injury.
APPENDIX A: MATERIALS
Anaesthetic machine
Gem 9100, CIG Health, Datex Ohmeda, GE Healthcare, Auckland, New Zealand

Arterial line pressure transducer
Millar Mikro-Tip catheter, Millar Instruments Incorporated, Houston, Texas, USA

Bair hugger
The Original Bair Hugger Forced Air Warming Temperature Management Units, Arizant Healthcare Inc, Eden Prairie, MN 55344, USA

Blood collection tubes
K2 EDTA BD Vacutainer, BD Diagnostics, Franklin Lakes, NJ 07417, USA
K2 EDTA BD Microtainer, BD Diagnostics, Franklin Lakes, NJ 07417, USA
Lithium Heparin Microtainer, BD Diagnostics, Franklin Lakes, NJ 07417, USA
Serum Separator Microtainer, BD Diagnostics, Franklin Lakes, NJ 07417, USA

Bupivacaine with Adrenaline
Marcain 0.25% with Adrenaline 1:400,000 Injection, AstraZeneca Ltd, Auckland, New Zealand

Buprenorphine
Temgesic Injection 300mcg/ml, Reckitt Benckiser Ltd, Auckland, New Zealand

Cannulas
14G BD Insyte intravenous catheter, BD, Franklin Lakes, NJ 07417, USA.
16G BD Insyte intravenous catheter, BD, Franklin Lakes, NJ 07417, USA.
24G BD Insyte intravenous catheter, BD, Franklin Lakes, NJ 07417, USA.

Capnograph
CO₂SMO+ respiratory profile monitor, Novametrix Medical Systems Inc, Wallingford, CT, USA

Carbon dioxide gas
Carbon dioxide medical gas, BOC Ltd, Auckland, New Zealand

Centrifuge
Eppendorf centrifuge, Hamburg, Germany

COBAS MIRA analyser
Roche, Basel, Switzerland

COBAS MIRA analyser reagent sets
Pointe Scientific, Michigan, USA

Cytokine ELISA kit
Milliplex™ Human cytokine kit, #MPXHCYTO-60K 96 well plate assay, Millipore Corporation, Billerica, MA 01821, USA

Dexamethasone (intravenous)
DBL Dexamethasone Sodium Phosphate Injection, Hospira NZ Limited, Te Aro, Wellington, NZ

Drain
Blake Silicone Drain, 15 FR round, hubless with 3/6” trocar, Ethicon inc, Somerville, New Jersey 08876-0151, USA.
Drain bottle
Low vacuum wound drainage system 80kPa (neg 150mmHg), Leur-lock replacement bottle, Van Straten Medinorm, 66583 Spiesen, Germany

Eppendorf tubes
1.5ml Eppendorf graduated microcentrifuge tubes, Raylab NZ Ltd, Auckland, New Zealand

Fentanyl (intravenous)
Fentanyl injection, AstraZeneca Ltd, Auckland, NZ

Flow Limiter
Precision Flow Control Valve, GRPO-10-PK-3, Esslingen, Germany

Flow meters (electronic mass flow meters)
Red-y Smart Meter, Vogtlin Instruments AG, Switzerland
TSI model 4140, TSI Incorporated, MN, USA

Humidifier

Insufflators
Karl Storz – Endoskope, electronic endoflator, Medipak Surgical, Auckland, NZ
Wisap CO2-OP-Pneu insufflator, Wisap, Munich, Germany

Isoflurane
Isoflurane, Luman Better Pharmaceutical Co. Ltd, Shandong 276006, China

Morphine (intravenous)
Morphine sulphate injection, Biomed Ltd, Auckland, NZ

Morphine (oral)
Morphine sulphate, Douglas Pharmaceuticals Ltd, Auckland, NZ

Needles
21G BD Precision Glide, BD, Franklin Lakes, NJ 07417, USA

Operating microscope
Leica M500 microscope, Leica Microsystems Pty. Ltd., Gladesville, NSW, Australia)

Oxycodone (oral)
Oxycodone Hydrochloride, Mundipharma NZ Ltd, Auckland, NZ

Paracetamol
Panadol tablets, GlaxoSmithKline Consumer, Auckland, NZ

Plate reader
Spectramax 340PC384, MDS Analytical Technologies, CA, USA

PowerLab data acquisition system and PowerLab LabChart 5 software
ADInstruments, Dunedin, New Zealand

Protease inhibitor
Roche complete mini tablet, Roche Applied Science, Mannheim 68298, Germany
Protein Carbonyl ELISA kit
BioCell PC Test kit, BioCell Corporation Ltd, Manukau, New Zealand

Rodent chow
Standard rodent chow, Teklad 2018, Madison, WI, USA

Scavenger
VetTech vacuum scavenger, Independent Vaccum Services, Auckland, NZ

Solutions
Liquid paraffin
10% neutral buffered formalin (NBF)
50% ethanol
70% ethanol
Chloride free phosphate buffer (20 mM KH$_2$PO$_4$ and 20 mM Na$_2$HPO$_4$)
RIPA buffer (25mM Tris stock, 150mM NaCl, 1% Na Deoxycholate, 1% Triton-x 100, 0.1% SDS)

Stains
Haematoxylin
Eosin

Sutures and ligatures
Ethicon Perma-Hand 3/0 surgical silk braided, Ethicon Inc, Somerville, NJ, USA.

Synchroon CX5 analyser
Beckman Coulter, CA, USA

Syringe driver
Genie precision programmable syringe driver, Kent Scientific Corporation, Torrington, CT, USA.

Thermometer and hygrometer
Humidity & Temperature Meter ITW JT-07CRL, Shenzen Jingtengwei Industry Co. Ltd, Guangdong, China

Tissue glue
Histocryl glue 0.5 mL, Aesculap AG & Co., Tuttingen 78532, Germany

Tissue lyser
TissueLyser II, Qiagen GmbH, Hilden, Germany

Tramadol (oral)
Tramadol Hydrochloride, AFT Pharmaceuticals Ltd, Auckland, NZ

Ventilator
Pressure controlled ventilator, Kent Scientific Corporation, Torrington, CT, USA

Weight scale
Mettler PE360 electronic weight scale, Mettler instruments AG, Greifensee, Zurich
APPENDIX B: CLINICAL TRIAL DATA COLLECTION FORM
Laparoscopic Humidification Study

Day of operation

<table>
<thead>
<tr>
<th>Preop booklet filled?</th>
<th>Consent filled?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tell anaesthetist and give anaesthetic front sheet</td>
<td></td>
</tr>
<tr>
<td>Set room thermostat to 19 degrees</td>
<td></td>
</tr>
<tr>
<td>Give unblinded nurse envelope with instructions and describe these</td>
<td></td>
</tr>
<tr>
<td>OT start time (knife to skin)</td>
<td></td>
</tr>
<tr>
<td>Humidification start time</td>
<td></td>
</tr>
<tr>
<td>Name of operation</td>
<td></td>
</tr>
<tr>
<td>Indication for operation</td>
<td></td>
</tr>
<tr>
<td>Did they have an epidural?</td>
<td></td>
</tr>
<tr>
<td>What sort of epidural</td>
<td></td>
</tr>
<tr>
<td>Did they have a bair-hugger?</td>
<td></td>
</tr>
<tr>
<td>ASA score of patient</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
</tr>
<tr>
<td>Background medical history?</td>
<td></td>
</tr>
<tr>
<td>Pre anaesthetic Heart Rate</td>
<td></td>
</tr>
<tr>
<td>Pre anaesthetic BP</td>
<td></td>
</tr>
<tr>
<td>Premed analgesia used and dosage</td>
<td></td>
</tr>
<tr>
<td>Premed Dexamethasone used? What was the dosage</td>
<td></td>
</tr>
<tr>
<td>Any intraop soiling with pus or faeces (name which one)</td>
<td></td>
</tr>
<tr>
<td>Did the surgeon wash out the abdomen?</td>
<td></td>
</tr>
<tr>
<td>Tell surgeon to put a drain in at end of OT for cytokines tomorrow</td>
<td></td>
</tr>
<tr>
<td>Humidification end time</td>
<td></td>
</tr>
<tr>
<td>OT end time</td>
<td></td>
</tr>
<tr>
<td>CO2 volume used</td>
<td></td>
</tr>
<tr>
<td>How much morphine used in total during OT</td>
<td></td>
</tr>
<tr>
<td>How much fentanyl used in total during OT</td>
<td></td>
</tr>
<tr>
<td>Record intraop antiemetics used and dosage</td>
<td></td>
</tr>
<tr>
<td>Volume of crystalloids used intraop (N saline, plasmalyte)?</td>
<td></td>
</tr>
<tr>
<td>Volume of colloids used intraop (gelofusin)?</td>
<td></td>
</tr>
<tr>
<td>Volume of maintenance fluid used intraop (dex/saline)?</td>
<td></td>
</tr>
<tr>
<td>Volume of blood used intraop?</td>
<td></td>
</tr>
<tr>
<td>Ask surgeon to rate fogging from 1 to 10 (10 being the worst)</td>
<td></td>
</tr>
</tbody>
</table>

Patient temperature intraoperatively

<table>
<thead>
<tr>
<th>0min</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>105</th>
<th>120</th>
<th>135</th>
<th>150</th>
<th>165</th>
<th>180</th>
<th>195</th>
</tr>
</thead>
</table>

Make sure patient fills out workbook for pain scores at 2, 4, 8, and 12h postoperatively (fill these times in the workbook).
### Day 1

<table>
<thead>
<tr>
<th>Task</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ask patient to compete Day 1 in workbook</td>
<td></td>
</tr>
<tr>
<td>Note volume of fluid in drain</td>
<td></td>
</tr>
<tr>
<td>Shake drain and take sample from this and put in purple top on ice</td>
<td></td>
</tr>
<tr>
<td>Take blood sample and put in a purple top on ice</td>
<td></td>
</tr>
<tr>
<td>Document all analgesia and antiemetics used in PACU with doses</td>
<td></td>
</tr>
<tr>
<td>Document all analgesia and antiemetics used between PACU and midnight last night with doses (don’t forget to include morphine PCA)</td>
<td></td>
</tr>
<tr>
<td>Document IV fluids with volumes between the operation and midnight last night.</td>
<td></td>
</tr>
</tbody>
</table>

### Day 2

<table>
<thead>
<tr>
<th>Task</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ask patient to compete Day 2 in workbook</td>
<td></td>
</tr>
<tr>
<td>Measure total wound length in cm</td>
<td></td>
</tr>
<tr>
<td>Analgesia used until midnight last night with doses (Day 1: 12am -12am)</td>
<td></td>
</tr>
<tr>
<td>Antiemetics used until midnight last night with doses (Day 1: 12am -12am)</td>
<td></td>
</tr>
<tr>
<td>IV fluids with volumes given until midnight last night (Day 1: 12am -12am)</td>
<td></td>
</tr>
</tbody>
</table>

### Day 3

<table>
<thead>
<tr>
<th>Task</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ask patient to compete Day 3 in workbook</td>
<td></td>
</tr>
<tr>
<td>Analgesia used until midnight last night with doses (Day 2: 12am -12am)</td>
<td></td>
</tr>
<tr>
<td>Antiemetics used until midnight last night with doses (Day 2: 12am -12am)</td>
<td></td>
</tr>
<tr>
<td>IV fluids with volumes given until midnight last night (Day 2: 12am -12am)</td>
<td></td>
</tr>
</tbody>
</table>
### Day of Discharge

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the dc date?</td>
<td></td>
</tr>
<tr>
<td>What day was IDC was removed?</td>
<td></td>
</tr>
<tr>
<td>If epidural, what day was epidural was removed?</td>
<td></td>
</tr>
<tr>
<td>What day was PCA discontinued?</td>
<td></td>
</tr>
<tr>
<td>What day was the drain removed?</td>
<td></td>
</tr>
<tr>
<td>What day did they first pass wind?</td>
<td></td>
</tr>
<tr>
<td>What day did they first pass a bowel motion?</td>
<td></td>
</tr>
<tr>
<td>What day did they first eat a full solid meal?</td>
<td></td>
</tr>
<tr>
<td>What day did they first start mobilising independently?</td>
<td></td>
</tr>
</tbody>
</table>

**Analgesia used on day 3 (12am to 12am) with doses**

**Antiemetics used on day 3 (12am to 12am) with doses**

**IV fluids with volumes given on day 3 (12am to 12am)**

**Analgesia used between day 3 (above) and discharge with doses**

**Antiemetics used between day 3 (above) and discharge with doses**

**IV fluids with volumes used between day 3 (above) and discharge**

Give patient fatigue and physiology questionnaire with a self addressed envelope. They are to fill this out on day 7 and return it by mail.
APPENDIX C: PATIENT BOOKLET
Humidification in laparoscopic colonic surgery
Patient booklet

Please ask patient to fill out as below:

- Before the operation....................... Page 4 – 7
- Day of operation.......................... Page 9 – 10
  (after you’ve had it)
- Day 1 in the morning...................... Page 12 – 15
- Day 2 in the morning...................... Page 17 – 18
- Day 3 in the morning...................... Page 20 – 23
Before the operation
Investigating feelings of tiredness

Some things to be aware of while you complete this questionnaire:

- There are no right or wrong answers to the questions.

- It is best not to spend long thinking about any one answer; normally the first response is best.

- Some questions may seem very similar, but for measurement purposes it is often important to ask a question in slightly different ways. We would appreciate your patience and willingness to answer all of the questions.

- Please remember your answers to this questionnaire are completely confidential.

Thank you for taking the time to fill out this questionnaire
**Part 1**

Please think about the **last two days** and tick the box that best describes how you have been feeling.

<table>
<thead>
<tr>
<th>During the last two days ...</th>
<th>Not at all ▼</th>
<th>Almost Never ▼</th>
<th>Some of the time ▼</th>
<th>Fairly Often ▼</th>
<th>Very Often ▼</th>
<th>All of the time ▼</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I have been feeling drained</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. I start things without difficulty then get tired</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. I have been feeling energetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. I have had trouble paying attention</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. I have been feeling worn out</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. I have been feeling refreshed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. My body has been feeling heavy all over</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. I have been feeling vigorous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. I have been forgetful</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. It has been hard for me to get motivated to do my regular activities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>During the last two days ...</th>
<th>Not at all ▼</th>
<th>Almost Never ▼</th>
<th>Some of the time ▼</th>
<th>Fairly Often ▼</th>
<th>Very Often ▼</th>
<th>All of the time ▼</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. I do very little in a day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. I have been able to concentrate on things</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. My thoughts have wandered easily</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. I lack the energy to do things I normally do</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. I have been feeling fatigued</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. I have had the energy to do lots of things</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. Physically, I have felt tired</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. I have made more mistakes than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. I have had to restrict how much I try and do in a day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. I have been feeling lively</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Humidification Patient Booklet v 2 / Feb 08*
Part 2

The following questions ask how much fatigue interferes with the things you can do.

For activities you aren’t doing, for reasons other than fatigue, tick the box labelled “N/A” (not applicable).

Examples of why you might tick the “N/A” box include:
- You are still in hospital and are not required to do things like run errands.
- You are not the person who usually cooks in your household.
- Or, you have a wound that is vacuum-sealed and you are not able to do household chores because of this.

During the last two days, I have had enough energy to...

<table>
<thead>
<tr>
<th>Activities</th>
<th>Not at all</th>
<th>Only occasionally</th>
<th>Sometimes, but less than usual</th>
<th>Nearly as often as usual</th>
<th>As often as usual</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>21. Read a newspaper/book or watch TV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. Bath/Wash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. Dress</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. Do household chores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25. Cook</td>
<td></td>
<td></td>
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<td>26. Work</td>
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<td>27. Visit or socialize with family and friends</td>
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<tr>
<td>28. Engage in leisure or recreational activities</td>
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<tr>
<td>29. Shop or do errands</td>
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<tr>
<td>30. Walk</td>
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<tr>
<td>31. Exercise other than walk</td>
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</tbody>
</table>

How would you describe your overall energy levels at the present time on a scale from 1 to 10?

<table>
<thead>
<tr>
<th>Energy Level</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fit</td>
<td>1</td>
</tr>
<tr>
<td>Slightly Tired</td>
<td>2-3</td>
</tr>
<tr>
<td>Tired</td>
<td>4-5</td>
</tr>
<tr>
<td>Fatigued</td>
<td>6-10</td>
</tr>
</tbody>
</table>
Preop: Please fill this out before your operation
Circle the appropriate answer or write in the space provided.

1. How would you describe your pain level at the present time, *while in bed*?  
\[
\begin{array}{ccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\
\text{No Pain} & \text{Moderate Pain} & \text{Severe Pain}
\end{array}
\]

2. How would you describe your pain level at the present time, *when you move*?  
\[
\begin{array}{ccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\
\text{No Pain} & \text{Moderate Pain} & \text{Severe Pain}
\end{array}
\]

3. How would you describe your pain level at the present time, *when you cough*?  
\[
\begin{array}{ccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\
\text{No Pain} & \text{Moderate Pain} & \text{Severe Pain}
\end{array}
\]

4. How would you describe your energy levels at the present time?  
\[
\begin{array}{ccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\
\text{Fit} & \text{Slightly Tired} & \text{Tired} & \text{Fatigued}
\end{array}
\]

5. How would you describe your level of nausea at present time?  
0. No nausea at all  
1. Mild, tolerable nausea  
2. Moderate nausea, requiring medication  
3. Severe nausea  
\[
\begin{array}{ccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\
\text{None} & \text{Mild} & \text{Moderate} & \text{Severe}
\end{array}
\]

6. Have you vomited in the past 6 hrs?  
0. No  
1. Yes, only once  
2. Yes 2-3 times  
3. Yes more than 3 times
7. At the present time, do you feel...

Hungry?
[Scale from 1 to 10]

Not Hungry
At all

Moderately
Hungry

Very
Hungry

Thirsty?
[Scale from 1 to 10]

Not Thirsty
At All

Moderately
Thirsty

Very
Thirsty

8. How would you describe your anxiety level at present time?
[Scale from 1 to 10]

Very
Relaxed

Moderately
Anxious

Very
Anxious

9. How would you rate your Sleep quality over the past 24 Hrs?
[Scale from 1 to 10]

No
Sleep

Moderate

Excellent

Sleep

10. How would you rate the quality of the care you have received thus far?
[Scale from 1 to 10]

Poor

Moderate

Excellent

11. Did you pass flatus today? ____ Did you pass flatus yesterday? ____

12. Did you pass a bowel motion today? ____ Did you pass a bowel motion yesterday? ____

13. Did you have a full solid meal today? ____ Did you have a full solid meal yesterday? ____
After the operation
Pain 2 hours after your surgery (Please fill this out at _____)

How would you describe your pain level at the present time, while resting in bed?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
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<td>Pain</td>
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</tbody>
</table>

How would you describe your pain level at the present time, when you move?

<table>
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<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
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</tbody>
</table>

How would you describe your pain level at the present time, when you cough?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
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</tbody>
</table>

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Pain 4 hours after your surgery (Please fill this out at _____)

How would you describe your pain level at the present time, while resting in bed?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
<td></td>
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</tbody>
</table>

How would you describe your pain level at the present time, when you move?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
<th>7</th>
<th>8</th>
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</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
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</tbody>
</table>

How would you describe your pain level at the present time, when you cough?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
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</table>
### Pain 8 hours after your surgery (Please fill this out at _____)

How would you describe your pain level at the present time, *while resting in bed?*

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How would you describe your pain level at the present time, *when you move?*

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</table>

How would you describe your pain level at the present time, *when you cough?*

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### Pain 12 hours after your surgery (Please fill this out at _____)

How would you describe your pain level at the present time, *while resting in bed?*

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<tr>
<td>No</td>
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<td>Severe</td>
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How would you describe your pain level at the present time, *when you move?*

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</table>

How would you describe your pain level at the present time, *when you cough?*

<p>| | | | | | | | | | | |</p>
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<td>10</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
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</tbody>
</table>
On the morning of:

Day 1
Part 1
Please think about the last two days and tick the box that best describes how you have been feeling.

**During the last two days ...**

1. I have been feeling drained
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

2. I start things without difficulty then get tired
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

3. I have been feeling energetic
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

4. I have had trouble paying attention
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

5. I have been feeling worn out
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

6. I have been feeling refreshed
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

7. My body has been feeling heavy all over
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

8. I have been feeling vigorous
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

9. I have been forgetful
   - Not at all
   - Almost Never
   - Some of the time
   - Fairly Often
   - Very Often
   - All of the time

10. It has been hard for me to get motivated to do my regular activities
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

**During the last two days ...**

11. I do very little in a day
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

12. I have been able to concentrate on things
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

13. My thoughts have wandered easily
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

14. I lack the energy to do things I normally do
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

15. I have been feeling fatigued
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

16. I have had the energy to do lots of things
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

17. Physically, I have felt tired
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

18. I have made more mistakes than usual
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

19. I have had to restrict how much I try and do in a day
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

20. I have been feeling lively
    - Not at all
    - Almost Never
    - Some of the time
    - Fairly Often
    - Very Often
    - All of the time

Humidification Patient Booklet v 2 / Feb 08

12
**Part 2**

The following questions ask how much fatigue interferes with the things you can do.

For activities you aren’t doing, for reasons other than fatigue, tick the box labelled “N/A” (not applicable).

Examples of why you might tick the “N/A” box include:
- You are still in hospital and are not required to do things like run errands.
- You are not the person who usually cooks in your household.
- Or, you have a wound that is vacuum-sealed and you are not able to do household chores because of this.

### During the last two days, I have had enough energy to...

<table>
<thead>
<tr>
<th>Activity</th>
<th>Not at all</th>
<th>Occasionally</th>
<th>Sometimes, but less than usual</th>
<th>Nearly as often as usual</th>
<th>As often as usual</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>21. Read a newspaper/book or watch TV</td>
<td></td>
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</tr>
<tr>
<td>22. Bath/wash</td>
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<tr>
<td>23. Dress</td>
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<td></td>
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<tr>
<td>24. Do household chores</td>
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<tr>
<td>25. Cook</td>
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<tr>
<td>26. Work</td>
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<td>29. Shop or do errands</td>
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<tr>
<td>30. Walk</td>
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<tr>
<td>31. Exercise other than walk</td>
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</tr>
</tbody>
</table>

**How would you describe your overall energy levels at the present time on a scale from 1 to 10?**

<table>
<thead>
<tr>
<th>Fit</th>
<th>Slightly Tired</th>
<th>Tired</th>
<th>Fatigued</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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<tr>
<td>2</td>
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<td>10</td>
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</tbody>
</table>
Day 1: Please fill this out on the morning after the day of the operation
Circle the appropriate answer or write in the space provided.

1. How would you describe your pain level at the present time, while in bed?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
<td></td>
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<td>Pain</td>
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</tr>
</tbody>
</table>

2. How would you describe your pain level at the present time, when you move?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
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</tr>
</tbody>
</table>

3. How would you describe your pain level at the present time, when you cough?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
<td></td>
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<tr>
<td>Pain</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. How would you describe your energy levels at the present time?

   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
   | Fit | Slightly Tired | Tired | Fatigued |

5. How would you describe your level of nausea at present time?
   0. No nausea at all
   1. Mild, tolerable nausea
   2. Moderate nausea, requiring medication
   3. Severe nausea

   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
   | Nil | Mild | Moderate | Severe |

6. Have you vomited in the past 6 hrs?
   0. No
   1. Yes, only once
   2. Yes 2-3 times
   3. Yes more than 3 times
7. At the present time, do you feel...

Hungry?

1  2  3  4  5  6  7  8  9  10
Not Hungry
At all  Moderately  Very
Hungry

Thirsty?

1  2  3  4  5  6  7  8  9  10
Not Thirsty
At All  Moderately  Very
Thirsty

8. How would you describe your anxiety level at present time?

1  2  3  4  5  6  7  8  9  10
Very
Relaxed  Moderately  Very
Anxious
Anxious

9. How would you rate your Sleep quality over the past 24 Hrs?

1  2  3  4  5  6  7  8  9  10
No
Sleep  Moderate  Excellent
Sleep

10. How would you rate the quality of the care you have received thus far?

1  2  3  4  5  6  7  8  9  10
Poor  Moderate  Excellent

11. Did you pass flatus today?    Did you pass flatus after your op yesterday?    

12. Did you pass a bowel motion today?    Did you pass one after your op yesterday?    

13. Did you have a full solid meal today?    Did you have one after your op yesterday?    

14. Did you walk independently today?    Did you walk independently after your op yesterday?    

Humidification Patient Booklet v 2 / Feb 08
On the morning of:

Day 2
Day 2: Please fill this out on the morning of day 2 after the operation
Circle the appropriate answer or write in the space provided.

1. How would you describe your pain level at the present time, while in bed?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
<td></td>
<td></td>
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<td>Pain</td>
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<td></td>
</tr>
</tbody>
</table>

2. How would you describe your pain level at the present time, when you move?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

3. How would you describe your pain level at the present time, when you cough?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Moderate</td>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pain</td>
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<td></td>
</tr>
</tbody>
</table>

4. How would you describe your energy levels at the present time?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fit</td>
<td>Slightly</td>
<td>Tired</td>
<td>Fatigued</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Tired</td>
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</tr>
</tbody>
</table>

5. How would you describe your level of nausea at present time?

0. No nausea at all
1. Mild, tolerable nausea
2. Moderate nausea, requiring medication
3. Severe nausea

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

6. Have you vomited in the past 6 hrs?

0. No
1. Yes, only once
2. Yes 2-3 times
3. Yes more than 3 times
7. At the present time, do you feel…

Hungry?

1 2 3 4 5 6 7 8 9 10

Not Hungry
At all

Moderately Hungry

Very Hungry

Thirsty?

1 2 3 4 5 6 7 8 9 10

Not Thirsty
At All

Moderately Thirsty

Very Thirsty

8. How would you describe your anxiety level at present time?

1 2 3 4 5 6 7 8 9 10

Very Relaxed

Moderately Anxious

Very Anxious

9. How would you rate your Sleep quality over the past 24 Hrs?

1 2 3 4 5 6 7 8 9 10

No Sleep

Moderate

Excellent Sleep

10. How would you rate the quality of the care you have received thus far?

1 2 3 4 5 6 7 8 9 10

Poor

Moderate

Excellent

11. Did you pass flatus today? _____ Did you pass flatus yesterday? _____

12. Did you pass a bowel motion today? _____ Did you pass a bowel motion yesterday? _____

13. Did you have a full solid meal today? _____ Did you have a full solid meal yesterday? _____

14. Did you walk independently today? _____ Did you walk independently yesterday? _____

Humidification Patient Booklet v 2 / Feb 08
On the morning of:
Day 3
Part 1
Please think about the last two days and tick the box that best describes how you have been feeling.

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>Almost Never</th>
<th>Some of the time</th>
<th>Fairly Often</th>
<th>Very Often</th>
<th>All of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I have been feeling drained</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>2. I start things without difficulty then get tired</td>
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<tr>
<td>3. I have been feeling energetic</td>
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<tr>
<td>4. I have had trouble paying attention</td>
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<tr>
<td>5. I have been feeling worn out</td>
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<tr>
<td>6. I have been feeling refreshed</td>
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<tr>
<td>7. My body has been feeling heavy all over</td>
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<tr>
<td>8. I have been feeling vigorous</td>
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<td>9. I have been forgetful</td>
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<tr>
<td>10. It has been hard for me to get motivated to do my regular activities</td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>Almost Never</th>
<th>Some of the time</th>
<th>Fairly Often</th>
<th>Very Often</th>
<th>All of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. I do very little in a day</td>
<td></td>
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<tr>
<td>12. I have been able to concentrate on things</td>
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<tr>
<td>13. My thoughts have wandered easily</td>
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<tr>
<td>14. I lack the energy to do things I normally do</td>
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<tr>
<td>15. I have been feeling fatigued</td>
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<tr>
<td>16. I have had the energy to do lots of things</td>
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</tr>
<tr>
<td>17. Physically, I have felt tired</td>
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<tr>
<td>18. I have made more mistakes than usual</td>
<td></td>
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</tr>
<tr>
<td>19. I have had to restrict how much I try and do in a day</td>
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</tr>
<tr>
<td>20. I have been feeling lively</td>
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</tr>
</tbody>
</table>

Humidification Patient Booklet v 2 / Feb 08

20
Part 2

The following questions ask how much fatigue interferes with the things you can do.

For activities you aren’t doing, for reasons other than fatigue, tick the box labelled “N/A” (not applicable).

Examples of why you might tick the “N/A” box include:
- You are still in hospital and are not required to do things like run errands.
- You are not the person who usually cooks in your household.
- Or, you have a wound that is vacuum-sealed and you are not able to do household chores because of this.

**During the last two days, I have had enough energy to...**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Not at all</th>
<th>Occasionally</th>
<th>Sometimes, but less than usual</th>
<th>Nearly as often as usual</th>
<th>As often as usual</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>21. Read a newspaper/book or watch TV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>22. Bath/wash</td>
<td></td>
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</tr>
<tr>
<td>23. Dress</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>24. Do household chores</td>
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<tr>
<td>25. Cook</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>26. Work</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>27. Visit or socialize with family and friends</td>
<td></td>
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</tr>
<tr>
<td>28. Engage in leisure or recreational activities</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>29. Shop or do errands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30. Walk</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31. Exercise other than walk</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

How would you describe your overall energy levels at the present time on a scale from 1 to 10?

1  2  3  4  5  6  7  8  9  10

Fit  Slightly Tired  Tired  Fatigued

Humidification Patient Booklet v 2 / Feb 08
Day 3: Please fill this out on the morning of day 3 after the operation
Circle the appropriate answer or write in the space provided.

1. How would you describe your pain level at the present time, while in bed?
   
   [Scale 1-10: No Pain, Moderate Pain, Severe Pain]

2. How would you describe your pain level at the present time, when you move?
   
   [Scale 1-10: No Pain, Moderate Pain, Severe Pain]

3. How would you describe your pain level at the present time, when you cough?
   
   [Scale 1-10: No Pain, Moderate Pain, Severe Pain]

4. How would you describe your energy levels at the present time?
   
   [Scale 1-10: Fit, Slightly Tired, Tired, Fatigued]

5. How would you describe your level of nausea at present time?
   
   0. No nausea at all
   1. Mild, tolerable nausea
   2. Moderate nausea, requiring medication
   3. Severe nausea

6. Have you vomited in the past 6 hrs?
   
   0. No
   1. Yes, only once
   2. Yes 2-3 times
   3. Yes more than 3 times

Humidification Patient Booklet v 2 / Feb 08

22
7. At the present time, do you feel...

Hungry?

Not Hungry

At all

Moderately

Hungry

Very

Hungry

Thirsty?

Not Thirsty

At All

Moderately

Thirsty

Very

Thirsty

8. How would you describe your anxiety level at present time?

Very

Relaxed

Moderately

Anxious

Very

Anxious

9. How would you rate your Sleep quality over the past 24 Hrs?

No

Sleep

Moderate

Excellent

Sleep

10. How would you rate the quality of the care you have received thus far?

Poor

Moderate

Excellent

11. Did you pass flatus today? ___ Did you pass flatus yesterday? ___

12. Did you pass a bowel motion today? ___ Did you pass a bowel motion yesterday? ___

13. Did you have a full solid meal today? ___ Did you have a full solid meal yesterday? ___

14. Did you walk independently today? ___ Did you walk independently yesterday? ___
Consent Form

Humidification in Laparoscopic Colonic Surgery: A randomised controlled trial.

Principal Investigator: Dr Tarik Sammour, Research Fellow, Department of Surgery, Middlemore Hospital, Phone 021 317417

Patient Name: ___________________________ Date of Birth: _______________________

Request for Interpreter

<table>
<thead>
<tr>
<th>Language</th>
<th>Interpreter Request</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>I wish to have an interpreter</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Maori</td>
<td>E hiahia ana ahau l tetahi hei korero Maori ki ahau</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Samoan</td>
<td>Ouie mana' o o iai se fa'amatalu upu</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Tongan</td>
<td>Oka fiema'a ha fakatoulele</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cook Island</td>
<td>Ka inangiro na l tetali tangata uri reo</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Niuean</td>
<td>Fia manako au ke fakaangata e tagata fakahoko hoko va gahau</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

I have read and I understand the information sheet dated Feb 2008 for volunteers taking part in this study. I have had the opportunity to discuss the study. I am satisfied with the answers I have been given. I have had this project explained to me by ____________________________.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and that this will in no way affect my continuing health care.

I understand that my participation in this study is confidential and that no material that could identify me will be used in any reports on this study.

I understand that to participate in the study I am required to disclose my medical history.

I agree to details of my admission to hospital being entered onto a data sheet that will be confidential.

I understand the compensation provisions for this study.

I have had time to consider whether to take part.

I know whom to contact if I have concerns or questions about the study.

I consent to the researchers using a specimen of my blood and surgical drain fluid for analysis of inflammatory chemicals. Yes / No

I have had an opportunity to discuss this with my family / iwi. Yes / No

I would like to receive a copy of the results when available. Yes / No

I consent to my GP (Family Doctor) being informed of my condition if necessary. Yes / No

Humidification Patient Booklet v 2 / Feb 98
I am clinically responsible for this patient’s care at Hospital and I have no objection to his/her participation in this project.

Signed (attending physician): __________________________ Date __________

I_________________________________________ (full name) hereby consent to take part.

Signed (subject): __________________________ Date __________

In my opinion consent was given freely and with understanding

Signed (witness): __________________________ Date __________

Consent obtained by __________________________ Date __________

If you have any concerns about the study, you may contact:
Dr Tarik Sammour, Department of Surgery: Tel 021 317417
Participant Information Sheet

Humidification in laparoscopic colonic surgery

Principal Investigator: Dr Tarik Sammour, Research Fellow, Department of Surgery, Middlemore Hospital
Phone 276 0000 ext 2100, or mobile 021317417

Introduction
You are invited to take part in a clinical research study. Your participation is entirely voluntary (your choice). You
do not have to take part in this study, and if you choose not to take part this will not affect any future care or treatment.
The information sheet and consent form gives you detailed information about the research study which your doctor will
discuss with you. Once you are happy that you understand the study you will be asked to sign the form if you wish to
participate.

About the Study
Recovery from surgery is dependent on many factors that are controlled by our bodily processes. There are many
ways that we, as doctors, can control or manipulate these processes; examples include the use of antibiotics or pain
relief. But as our knowledge about the human body improves we have realised that there are many other processes in
the body which affect the way that we feel or recover after surgery.

The usual complete recovery period following large bowel surgery is between two and three months. We are
aiming to investigate the effect of new treatments in reducing the length of this recovery period by reducing fatigue,
pain, nausea and vomiting.

About Humidification
Carbon dioxide gas is used to inflate the abdomen for ‘key-hole’ surgery. Traditionally this gas has been dry and
cold, which contributes to a drop in body temperature during the operation. There is recent evidence to suggest that
humidifying and warming the gas to body temperature may be beneficial with improved recovery times and reduced
pain.

We are inviting you to participate in a new study which is aiming to investigate improvements in the energy levels
and speed of recovery after operation with the use of humidified gas rather than dry gas for laparoscopy (‘key-hole’
surgery).

We are hoping to invite 60 patients who are going to have a laparoscopic major bowel operation to take part in this
study. If you agree to participate, you will randomly be assigned to receive either humidified or dry gas during the
operation. You will not know which one you have received and nor will your surgeon, (this is so the study can be
objective). Following that, you will have the same routine, standardised care plan as all the other patients and your
participation in this trial will not affect your standard of care in any way.

Humidification Patient Booklet v 2 / Feb 08
At the end of the operation your surgeon will insert a surgical drain to monitor for bleeding after the operation. This is common for bowel surgery. This drain will be removed the day after the operation.

During the post operative period we will ask you to complete a questionnaire about your pain and energy levels with the assistance of the researchers.

We will also collect a small sample of your surgical drainage fluid for testing of levels of some chemicals that cause swelling and pain. We will ask for your permission prior to doing so. This is painless and does not involve any genetic testing. The samples will be destroyed after the testing.

Participants must note that some will disagree with storage of tissue or blood samples citing whakapapa, and advice their people to consult prior to participation in research where this occurs. However, it is acknowledged that individuals have the right to choose to participate. You will not be subjected to any extra tests or procedures for the study and any test, such as blood tests or x-rays, will be done as is seen fit by your treating surgeon.

---

Risks

There are no known risks associated with participating in the study, as no adverse events have been reported with humidification. There will be no costs or payments to you.

---

What will happen to my sample?

The sample of drain fluid will be collected and frozen. Once we have enough samples collected form enrolled patients we will test these for markers of inflammation. The sample will then be destroyed.

---

Participation

Your participation is entirely voluntary (your choice). You do not have to take part in this study, and if you choose not to take part this will not affect any future care or treatment. If you do agree to take part you are free to withdraw from the study at any time, without having to give a reason and this will in no way affect your continuing health care. No material which could personally identify you will be used in any reports on this study.

---

General

Further information can be obtained from Dr Tarik Sammour, Department of Surgery (Tel 021 317417). An interpreter will be provided if you would like one.

If you have any queries or concerns regarding your rights as a participant in this research study, you can contact an independent Health and Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act.

Telephone (NZ wide): 0800 555 050
Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)
Email: advocacy@hdc.org.nz
Confidentiality

Your hospital records are confidential. Your name or any other personally identifying information will not be used in reports or publications resulting from this study. The information about your medical history and medications required to interpret the research results will be identified using a code to ensure your confidentiality.

Compensation

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator.

Results

The final results of the research will not be known until December 2009. You will be provided with study results at the completion of the study should you indicate that you want them on the consent form.

Statement of Approval:

This study has received ethical approval from the Northern X Regional Ethics Committee.
Please fill this form out as soon as possible.

You can return this form by mail in the prepaid envelope provided.

Thank you very much for all your help with this, and I wish you all the best.

Investigating tiredness

Some things to be aware of while you complete this questionnaire:

- There are no right or wrong answers to the questions.

- It is best not to spend long thinking about any one answer; normally the first response is best.

- Some questions may seem very similar, but for measurement purposes it is often important to ask a question in slightly different ways. We would appreciate your patience and willingness to answer all of the questions.

- Please remember your answers to this questionnaire are completely confidential.

Thank you for taking the time to fill out this questionnaire
Part 1
Please think about the last two days and tick the box that best describes how you have been feeling.

<table>
<thead>
<tr>
<th></th>
<th>Not at all ▼</th>
<th>Almost Never ▼</th>
<th>Some of the time ▼</th>
<th>Fairly Often ▼</th>
<th>Very Often ▼</th>
<th>All of the time ▼</th>
</tr>
</thead>
<tbody>
<tr>
<td>During the last two days ...</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1. I have been feeling drained</td>
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<tr>
<td>2. I start things without difficulty then get tired</td>
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<tr>
<td>3. I have been feeling energetic</td>
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<td>4. I have had trouble paying attention</td>
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<td>5. I have been feeling worn out</td>
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<td>6. I have been feeling refreshed</td>
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<td>7. My body has been feeling heavy all over</td>
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<td>8. I have been feeling vigorous</td>
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<td>9. I have been forgetful</td>
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<tr>
<td>10. It has been hard for me to get motivated to do my regular activities</td>
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<tr>
<td>During the last two days ...</td>
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<tr>
<td>11. I do very little in a day</td>
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<tr>
<td>12. I have been able to concentrate on things</td>
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<tr>
<td>13. My thoughts have wandered easily</td>
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<tr>
<td>14. I lack the energy to do things I normally do</td>
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<tr>
<td>15. I have been feeling fatigued</td>
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<tr>
<td>16. I have had the energy to do lots of things</td>
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<tr>
<td>17. Physically, I have felt tired</td>
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<tr>
<td>18. I have made more mistakes than usual</td>
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<tr>
<td>19. I have had to restrict how much I try and do in a day</td>
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<tr>
<td>20. I have been feeling lively</td>
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</tr>
</tbody>
</table>

Humidification Patient Booklet v 2 / Feb 08

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Part 2

The following questions ask how much fatigue interferes with the things you can do.

For activities you aren’t doing, for reasons other than fatigue, tick the box labelled “N/A” (not applicable).

Examples of why you might tick the “N/A” box include:
You are still in hospital and are not required to do things like run errands.
You are not the person who usually cooks in your household.
Or, you have a wound that is vacuum-sealed and you are not able to do household chores because of this.

### During the last two days, I have had enough energy to...

<table>
<thead>
<tr>
<th>Activity</th>
<th>Not at all</th>
<th>Only occasionally</th>
<th>Sometimes, but less than usual</th>
<th>Nearly as often as usual</th>
<th>As often as usual</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>21. Read a newspaper/book or watch TV</td>
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<tr>
<td>22. Bath/wash</td>
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<td></td>
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<tr>
<td>23. Dress</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>24. Do household chores</td>
<td></td>
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<tr>
<td>25. Cook</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>26. Work</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>27. Visit or socialize with family and friends</td>
<td></td>
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<tr>
<td>28. Engage in leisure or recreational activities</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>29. Shop or do errands</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30. Walk</td>
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<tr>
<td>31. Exercise other than walk</td>
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</tr>
</tbody>
</table>

How would you describe your overall energy levels at the present time on a scale from 1 to 10?

1  2  3  4  5  6  7  8  9  10
Fit  Slightly Tired  Tired  Fatigued

Humidification Patient Booklet v 2 / Feb 08
Preop: Please fill this out before your operation
Circle the appropriate answer or write in the space provided.

1. How would you describe your pain level at the present time, *while in bed*?

   1 2 3 4 5 6 7 8 9 10
   No     Moderate  Severe
   Pain   Pain      Pain

2. How would you describe your pain level at the present time, *when you move*?

   1 2 3 4 5 6 7 8 9 10
   No     Moderate  Severe
   Pain   Pain      Pain

3. How would you describe your pain level at the present time, *when you cough*?

   1 2 3 4 5 6 7 8 9 10
   No     Moderate  Severe
   Pain   Pain      Pain

4. How would you describe your energy levels at the present time?

   1 2 3 4 5 6 7 8 9 10
   Fit     Slightly Tired Fatigued
   Tired

5. How would you describe your level of *nausea* at present time?

   0. No nausea at all
   1. Mild, tolerable nausea
   2. Moderate nausea, requiring medication
   3. Severe nausea

   1 2 3 4 5 6 7 8 9 10
   Nil     Mild     Moderate Severe

6. Have you vomited in the past 6 hrs?

   0. No
   1. Yes, only once
   2. Yes 2-3 times
   3. Yes more than 3 times

Humidification Patient Booklet v 2 / Feb 08
7. At the present time, do you feel...

Hungry?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Hungry At all</td>
<td>Moderately Hungry</td>
<td>Very Hungry</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

Thirsty?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Thirsty At All</td>
<td>Moderately Thirsty</td>
<td>Very Thirsty</td>
<td></td>
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</tr>
</tbody>
</table>

8. How would you describe your anxiety level at present time?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Relaxed</td>
<td>Moderately Anxious</td>
<td>Very Anxious</td>
<td></td>
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</tr>
</tbody>
</table>

9. How would you rate your Sleep quality over the past 24 Hrs?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Sleep</td>
<td>Moderate</td>
<td>Excellent Sleep</td>
<td></td>
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</tr>
</tbody>
</table>

10. How would you rate the quality of the care you have received thus far?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>Moderate</td>
<td>Excellent</td>
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</tr>
</tbody>
</table>

11. How many days after your operation did you return to work / normal activity?

__________
APPENDIX E: SURGICAL RECOVERY SCORE
The Surgical Recovery Score (SRS) is an evaluative score that reliably measures functional patient recovery after surgery, and provides an outcome to assess interventions used to enhance recovery. The score measures physical capacity and performance, as well as psychological and social function. It can be used for English speaking patients of any age, sex, or ethnicity undergoing open or laparoscopic major abdominal surgery.

**STRUCTURE**

The SRS questionnaire included in the Patient Booklet and Patient Questionnaire consists of 31 items. These encompass five different recovery sub-scores: Fatigue (5 questions), Vigor (4 questions), Mental Function (5 questions), Impact on patient activity (6 questions) and Impact on ADLs (11 questions). Questions are staggered and some are reverse-scored to minimize repetition bias.

**SCORING**

The scoring for items 1 to 20 is as follows:

- Not at all 1
- Almost never 2
- Some of the time 3
- Fairly often 4
- Very often 5
- All of the time 6

For some of the items, the scoring is reversed because of the nature of the question (1 changed to 6, 2 changed to 5, and so on). If an item is not answered by the patient (or it is answered twice) then that question item is excluded from the subscale. Each subscale score is based on the percentage of the maximum possible score in that particular subscale. For example if only 4 questions were answered in a subscale with 5 questions, the maximum score for that subscale is 24 (rather than 30).
The scoring for items 21 to 31 is as follows:

- Not at all: 1
- Only occasionally: 2
- Sometimes, but less than usual: 3
- Nearly as often as usual: 4
- As often as usual: 5
- NA: excluded

If an item is marked as not applicable (N/A) or if it is not answered by the patient (or answered twice) then that item is excluded from the subscale. Each subscale score is based on the percentage of the maximum possible score in that particular subscale. For example if only 9 questions were answered in a subscale with 11 questions, the maximum score for that subscale is 45 (rather than 55).

**SUBSCALES**

**Fatigue**

This subscale is measured based on 5 items, assessed in questions number 1, 5, 7, 15 and 17. All of these are reverse scored and the maximum achievable score is 30.

**Vigor**

This subscale is measured based on 4 items, assessed in questions number 3, 6, 8 and 20. None of these are reverse scored, and the maximum achievable score is 24.

**Mental Function**

This subscale is measured based on 5 items, assessed in questions number 4, 9, 12, 13 and 18. Items number 4, 9, and 13 are reverse scored, and the maximum achievable score is 30.
Impact on Patient Activity

This subscale is measured based on 6 items, assessed in questions number 2, 10, 11, 14, 16 and 19. Items number 2, 10, 11, 14, and 19 are reversed scored, and the maximum achievable score is 36.

Impact on Activities of Daily Living

This subscale corresponds to items 21-31. None of these are reverse scored, and the maximum achievable score is 55.

The final SRS score is obtained by calculating the mean of the percentage of maximum score achieved in the above 5 subscales.

Further information on formal validation of this score, and a downloadable Excel file (Microsoft Corporation, Redmond, WA, USA) with formulas for automated scoring can be found online here:

## Animal Study Pro Forma

<table>
<thead>
<tr>
<th>Rat code:</th>
<th>Date:</th>
<th>Start time:</th>
<th>Weight (g):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry / Humid</td>
<td>Age (wks):</td>
<td>End time:</td>
<td>Length (cm):</td>
</tr>
</tbody>
</table>

### Time

<table>
<thead>
<tr>
<th>Time</th>
<th>Intervention</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temgesic</td>
<td></td>
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<tr>
<td></td>
<td>Tracheostomy</td>
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<td>Fem line</td>
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<td>Art line</td>
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<td>Pancuronium</td>
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<td></td>
<td>Insufflation</td>
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</table>

### Min 0-150

<table>
<thead>
<tr>
<th>Min</th>
<th>Temp rectal</th>
<th>Temp abdo</th>
<th>HR</th>
<th>MAP</th>
<th>RR</th>
<th>EICO2</th>
<th>PIP</th>
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<tr>
<td>0</td>
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### Sample

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- Vol gas used: __________________________
- Vol fluid used: ________________________
- Average Flow: _________________________
- Median Flow: _________________________
- ABG results: _________________________
LIST OF REFERENCES
331


Molinas CR, Koninckx PR. Hypoxemia induced by CO2 or helium pneumoperitoneum is a co-factor in adhesion formation in rabbits. Hum Reprod. 2000;15:1758-63.


316. Ivankovich AD, Miletich DJ, Albrecht RF, Heyman HJ, Bonnet RF. Cardiovascular effects of intraperitoneal insufflation with carbon dioxide and nitrous oxide in the dog. Anesthesiology. 1975;42(3):281-7.


