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THE METABOLIC AND INFLAMMATORY RESPONSE TO LAPAROSCOPIC SURGERY IN INFANTS AND CHILDREN

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ABSTRACT

The metabolic response to laparoscopy in children is not well established. The work done in this thesis investigates some of the metabolic responses and their relationship to the inflammatory responses in children. The aim was to compare the responses between open and laparoscopic surgery. The hypothesis was that laparoscopic surgery is associated with a blunting of the metabolic response to surgery compared to open surgery. Firstly the intraoperative response to pneumoperitoneum and CO_2 absorption was investigated in children undergoing laparoscopy compared to open surgery. There was a continuous increase in CO_2 elimination throughout the period of pneumoperitoneum. CO_2 absorption was greater in smaller and younger patients. There was intraoperative hyperthermia associated with increased oxygen consumption in children undergoing laparoscopy, which was also more marked in smaller and younger children. The effects on postoperative metabolism were explored in a randomised controlled trial comparing open and laparoscopic surgery. Whole body energy metabolism (indirect calorimetry) and protein metabolism (stable isotope infusion) was measured in the preoperative and early postoperative period. There was a significant decrease in resting energy expenditure after 24 hours in children undergoing open surgery but no significant change in the laparoscopic group. Concomitantly, there was a decrease in whole body protein catabolism 4 hours postoperatively in the open group, which was blunted by laparoscopy. Laparoscopy also dissociated the influence of insulin on protein metabolism and the hormonal control of energy metabolism. Laparoscopy therefore altered postoperative energy and protein metabolism by altering intraoperative thermoregulation.

The altered metabolic response may be responsible for some of the differences in the postoperative immune response, as protein catabolism may be an important source of

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glutamine for cells of the immune system. Monocyte class II MHC surface expression decreased in both groups, but tended to be better preserved by laparoscopy. This may have important implication for the risk of postoperative infections.

These studies demonstrated that laparoscopy alters intraoperative thermoregulation in children. This altered intraoperative thermoregulation translates into a preservation of early postoperative energy and protein metabolism compared to open surgery.

ACKNOWLEDGEMENTS

DECLARATION

The work presented in this thesis I performed in the Departments of Paediatric Surgery and Immunobiology at the Institute of Child Health. The analyses and laboratory work were done by me except for the following: measurement of nitrates and nitrites was performed in the biochemistry laboratories by Giorgio Stefanutti and Clare Booth; hormonal levels were measured by the laboratory of the Great Ormond Street Hospital; plasma KIC enrichment was performed by Virgillio Carnielli. The work presented in Chapters 3 and 4 were performed jointly with Lucia Corizia. The meta-analysis presented in Chapter 8 was performed along with Moti Chowdhury.

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ABBREVIATIONS

ΔCT	Change in core temperature
$\Delta \dot{V}O_2$	Change in O_2 consumption
Adr	Adrenaline
AA	Arachidonic acid
APE	Atom percent excess
APR	Acute phase response
ARDS	Adult respiratory distress syndrome
BMR	Basal metabolic rate
CD	Cluster designation
CIITA	MHC class II transactivator
C_{prot}	Whole body protein catabolism
CRP	C reactive protein
Cy5	Phycoerytherin Cy5
DIT	Diet induced thermogenesis
EDTA	Ethylenediaminetetraacetic acid
EE	Energy expenditure
ELISA	Enzyme-linked immunosorbent assay
eWMD	Estimated mean difference
FFA	Free fatty acids

FITC	Fluorescein isothiocyanate
FSC	Forward scatter
H_2O_2	Hydrogen peroxide
HLA	Human Lymphocyte Antigen
HOCl	Hypochlorous acid
HPLC	High pressure liquid chromatography
HRP	Horseradish Peroxidase
ICAM	Intercellular cell adhesion molecule
IgG	Immunoglobulin G
Ii	Invariant chain proteins
iNOS	Inducible nitric oxide synthase
IL	Interleukins
IFN-γ	Interferon-gamma
KIC	α-ketoisocaproate
LNF	Laparoscopic Nissen fundoplication
LPS	Lipopolysaccharide
MFI	Median fluorescence intensity
MHC	Major histocompatibility complex
MIIC	MHC class II compartments
MDA	Malondiadehyde
MODS	Multi-organ dysfunction syndrome

NAdr	Noradrenaline
NCA	Nurse controlled analgesia
NO	Nitric oxide
NOS	Nitric oxide synthase
NOx	Nitrates plus nitrites in plasma
NFκB	Nuclear factor kappa B
NSAID	Non-steroidal anti-inflammatory drugs
O_2^-	Superoxide anion
OD	Optical density
OH^-	Hydroxyl radical
O_{leu}	Leucine oxidation
ONF	Open Nissen fundoplication
OSS	Operative stress score
$PaCO_2$	Arterial CO_2 pressure
PBS	Phosphate buffered saline
PCA	Patient controlled infusion analgesia
PG	Prostaglandins
Q_{leu}	Leucine flux
REE	Resting energy expenditure
R-PE	R-Phycoerytherin
R-PE Cy5	R-Phycoerytherin Cy5

RQ	Respiratory quotient
RR	Relative risk
SIRS	Systemic inflammatory response syndrome
SSC	Side scatter
TBA	Thiobarbituric acid
TEE	Total energy expenditure
TCR	T-cell receptor
TG	Triglyceride
TMB	Tetramethylbenzidine
TNF-α	Tumor necrosis factor-alpha
VCAM	Vascular cell adhesion molecule
$\dot{V}CO_2$	CO_2 production
$\dot{V}ECO_2$	CO_2 elimination
$\dot{V}O_2$	Oxygen consumption

SUMMARY OF THE OVERALL AIMS OF THIS THESIS

Laparoscopy has only relatively recently (within the last 10 years) been popularised in paediatric surgery. This technique could offer an important advantage by substantially decreasing postoperative pain, stress and morbidity. Some paediatric surgeons perform laparoscopic surgery with the commonly held belief that minimally invasive surgery is associated with a dampened stress response, more rapid postoperative recovery and early discharge from hospital. There are however no studies comparing the clinical outcome, the metabolic, endocrine and inflammatory response to laparoscopic surgery in children.

Potential benefits

Laparoscopic surgery is associated with minimisation of the degree of tissue trauma (as the incisions into the abdominal wall are smaller than the comparative open operation) and is of benefit in reducing some of the adverse effects and postoperative complications. Blunting of this metabolic and stress response is a possible advantage to laparoscopy. Clinical evidence shows that, in adults, laparoscopic surgery reduces postoperative stay, respiratory complications and postoperative pain when compared to open surgery.

One of the new dimensions introduced by laparoscopy is the creation of a pneumoperitoneum. The insufflation of CO_2 or other gases into the abdominal cavity may stimulate metabolic, endocrine and inflammatory responses on its own accord.

The work done in this thesis investigates some of these responses in an attempt to understand some of the postoperative responses in children. The specific aim was to

compare the responses between open and laparoscopic surgery. The investigations performed, the outcomes collected and analysed are outlined below.

Specific investigations

i. CO_2 absorption and elimination

The absorption of CO_2 from the abdominal cavity in children has not been specifically investigated. The excretion of CO_2 via the lungs during CO_2 insufflation was investigated to understand the way in which children handle an intra-abdominal CO_2 load.

ii.   Energy Metabolism

The effects of laparoscopy on whole body metabolism were investigated by measuring whole body O_2 consumption both intraoperatively and postoperatively using indirect calorimetry. Resting energy expenditure was assessed in the postoperative period to investigate the metabolic response to open and laparoscopic surgery.

iii.   Protein metabolism

Surgical trauma affects protein metabolism in adults. Whole body protein metabolism is a significant predictor of outcome after surgery. The effects of laparoscopy on whole body protein kinetics were investigated using a stable isotopic model of protein turnover.

iv.   Endocrine response

There is a well established endocrine response to operative stress in children. Plasma insulin, glucose, cortisol and catecholamine responses were compared between groups.

v.   Inflammatory and cytokine response

Cytokines are markers and moderators of the inflammatory response. Pro- and anti-inflammatory cytokines were measured to assess the magnitude of the response. The free radical induced inflammatory response was measured by plasma malondialdehyde and nitrate plus nitrite. The effect on the immune system was investigated using monocyte class II MHC expression as a marker of postoperative immune suppression.

vi.   Clinical outcome

The postoperative clinical outcome was documented to reveal any potential advantages to laparoscopy over open surgery. The early and medium postoperative outcomes were assessed.

1.1   General Introduction

The body has developed a system of responses to deal with various noxious stimuli that threaten survival. In some respects these responses are stereotypical, and lead to the so called 'stress response'. Stress can be defined as 'factors that cause disequilibrium to an organism and therefore threaten homeostasis' (Wilmore 2002). Initiators of the stress response include infection, accidental trauma and surgery. The stress response that follows is initiated and coordinated by several messengers and affects whole body systems.

The insult of operative trauma can be considered a form of 'controlled' injury. Operative trauma initiates a constellation of inflammatory pathways which regulate a whole body response, which is similar to that seen after injury. The responses can be initiated and regulated by both chemical/hormonal signals and by neural signals. Some of the chemical signals responsible for the responses originate in the operative wound in response to local cellular injury. Cytokines, secreted in response to tissue injury, are amongst such signals. They are considered to be central regulators which act locally to bring about the alterations required to mount an inflammatory and immune response and to subsequently start the healing process. Release into the blood stream can bring about systemic features of the stress response.

Other responses may be initiated by peripheral and central nervous system stimulation. Peripheral efferent from pain receptors, for instance, can feed back to the central nervous system and produce some of the clinical signs of inflammation and the responses seen after operative stress. Indeed, blockade of this afferent stimulus is associated with dampening of the stress response (Anand et al 1987). It is one of the

clinical mechanisms (e.g. by wound infiltration of local anaesthetics, regional and spinal blocks) used to reduce the inflammatory response to surgery.

As with the signals responsible, the changes that result can also be localised or generalised. There is a whole body response that alters energy metabolism. There are changes in intermediate metabolic pathways to accompany the inflammatory response. Protein, lipid and glucose metabolism are all altered in the postoperative period.

The postoperative changes also affect the immune system. There is a period of immune stimulation that is often followed by period of immuneparesis. There is a pro-inflammatory response that is balanced by an anti-inflammatory response. The balance often determines and predicts the development of complications and outcome in terms of morbidity and mortality.

Whatever the mechanism, there are alterations in metabolic, inflammatory, endocrine and immune systems in response to operative trauma. These responses have evolved to enhance survival to trauma and infection in the absence of iatrogenic intervention. They limit patient activity in the area of injury to prevent secondary damage, and start the healing process through the inflammatory signals produced. Changes in metabolism increase the availability of substrates required by essential organs and regenerating and healing tissue. The immune stimulation allows for the swift eradication of any causal or secondary opportunistic microbial invasion, while the subsequent immuneparesis may allow for a dampening of this immune stimulation to allow for healing to ensue.

Figure 1.1 *Initiation of the inflammatory response to operative stress and the components involved.*

Various developments in the fields of surgery and anaesthesia have been aimed at reducing the stress response to surgery. It can be asked "Why dampen the stress response to surgery?" The response to trauma and operative stress can be the setting for deleterious effects leading to postoperative complications. Excessive responses can lead to systemic inflammatory response syndrome (SIRS) (Kilger et al 2003, Talmor et al 1999), and prolonged catabolism of body stores (Anand 1986), in particular proteins. Postoperative and post injury, the period of immuneparesis can predispose to secondary infection (Cheadle et al 1996, Mokart et al 2002, Muehlstedt et al 2002).

Operative insult

Vascular/cellular injury   Neuro-endocrine stimulation

Systemic release

Local inflammatory signals ⟶ Systemic mediators of inflammation

Local inflammatory response   Systemic inflammatory response

Metabolic response   Endocrine response   Immune response

Figure 1.1 *Initiation of the inflammatory response to operative stress and the components involved.*

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studies have shown that there is also evidence of dampening of some of the inflammatory and immune responses (Gupta & Watson 2001, Kehlet 1999). There are conflicting reports with respect to the metabolic response. Glerup *et al* (Glerup et al 1995) found that laparoscopic cholecystectomy largely abolishes the postoperative catabolic stress response. Conversely, Essen *et al* (Essen et al 1995) found that laparoscopic cholecystectomy improved insulin sensitivity but failed to prevent the postoperative decline in muscle protein synthesis. Overall, there seems to be minimal effect on the classical metabolic response in the postoperative period (Kehlet 1999). However, this body of evidence has been established mostly in patients undergoing cholecystectomy, an operation associated with a transient and minimal inflammatory response. Few studies have been performed in operations of a greater magnitude. The data on the inflammatory and metabolic responses after laparoscopic operations of greater magnitude are inconclusive (Fukushima et al 1996, Hildebrandt et al 2003, Nguyen et al 2002, Wu et al 2003, Yuen et al 1998).

One of the new dimensions introduced by laparoscopy is the creation of a pneumoperitoneum. The insufflation of CO_2, or any other gas, into the abdominal cavity may stimulate a metabolic response on its own accord. It is important to characterise the metabolic changes that accompany the CO_2 pneumoperitoneum. CO_2 insufflation can cause both local and systemic responses that affect the metabolic response to surgery. Local acidosis may modify the local metabolic and immune response, while systemic absorption may lead to respiratory changes and metabolic alterations that are far distant from the effect of the operation itself. The absorption of CO_2 from the abdominal cavity in children may also differ form that of adults.

The metabolic and stress response to operative stress in children often differs to that of adults (Jones et al 1994, Pierro 1999, Ward Platt et al 1990). Therefore, the scientific and clinical evidence in adults does not necessarily extrapolate into paediatric surgery, a speciality in its own right. The scientific evidence on the effects of laparoscopy on operative stress response in children is minimal. Most data have been gained from adult practice. However, children are not 'small adults'. The responses may be age related.

Intraoperative thermoregulation may be on of the main determinants of the metabolic response to surgery in children. Thermoregulation is altered intraoperatively due to the effects of anaesthetic drugs, open body cavities, with cooling due to the exposure to the environmental temperature without many of the normal regulatory control mechanisms.

Laparoscopy has relatively recently been introduced in paediatric surgery. One of the most common laparoscopic operations in children is the Nissen fundoplication. This technique could offer an important advantage by substantially decreasing postoperative pain, stress and morbidity. Nissen fundoplication has been proposed as a suitable model for studying the operative stress of an operation as it gives rise to a substantial response in adults (Zieren et al 2000). Blunting of this metabolic and stress response is a possible advantage to laparoscopy. Some paediatric surgeons perform laparoscopic Nissen fundoplication, with the commonly held belief that minimally invasive surgery is associated with a dampened stress response, more rapid postoperative recovery and early discharge from hospital. There are however no randomised studies comparing the clinical outcome, the metabolic, endocrine and inflammatory response to laparoscopic surgery in children.

The work in this thesis addresses some of the metabolic, immunological and inflammatory responses to laparoscopy in infants and children, and develops a framework for further research into this interesting and growing field of surgery in children.

The rest of this chapter discusses the metabolic response to operative stress in the following order:

i.   The chemical initiators and moderators of the inflammatory response

ii. Expansion of the role of cytokines

iii. Energy metabolism and the postoperative response

iv. Protein metabolism and changes in the postoperative period

v.   The hormonal response

vi. The immune responses with special reference to neutrophils and monocytes

vii. The effects of laparoscopy on these responses

viii. The influence of carbon dioxide insufflation on metabolism

1.2   Chemical mediators of the inflammatory stress response

After injury, there is an inflammatory response that originates in the wound or at the site of trauma. Cellular, and in particular, vascular injury leads to stimulation of inflammatory cascades that begin the clinical signs of inflammation. The local signals responsible for this process after a surgical operation can originate in the operative wound or from the viscera at the site of operation. Indeed, the levels of these mediators (e.g. cytokines) in the operative wounds and at sites of injury are often 100 times higher than circulating levels (Wiik et al 2001a, Wu et al 2003). Thereafter follows a well-documented cytokine cascade that is described later on.

CHAPTER 1

Introduction and Literature Review

The acute inflammatory response following local injury spreads outwards into adjacent uninjured areas following the release of these mediators from injured tissues. These mediators cause vasodilatation, increased vascular permeability, emigration and stimulation of white blood cells. Some of these mediators are described below.

Histamine: This is a well-known chemical mediator in acute inflammation (Robbins & Kumar 1987). It causes vascular dilatation and the immediate transient phase of increased vascular permeability. It is stored in mast cells, basophil and eosinophil leukocytes, and platelets. Histamine release is stimulated by complement components C3a and C5a, and by lysosomal proteins released from neutrophils. Despite its important role in the immediate inflammatory response, it has little role in the long term response. This is left to other chemical mediators.

5-hydroxytryptamine (serotonin): Serotonin is related to histamine both in structure and functional effects. It is present in high concentration in mast cells and platelets. It is a potent vasoconstrictor.

Lysosomal compounds: These polypeptides are released from activated neutrophils, monocytes and macrophages. Neutrophils release powerful enzymes by reverse endocytosis. They can also be released after neutrophil apoptosis. They include cationic proteins, which increase vascular permeability, and neutral proteases, which cause local increased vascular permeability and may activate complement. Collagenase, elastase and kallikrein are among components of lysosomes.

Lymphokines: This family of chemical messengers are released by lymphocytes. Apart from their major role in the inflammatory cascade, lymphokines may also have vasoactive or chemotactic properties. Cytokines are among the family of lymphokines.

Plasma factors: The plasma contains four enzymatic cascade systems that participate in the inflammatory process. They are the complement system, the kinins, the coagulation and the fibrinolytic systems. They are all inter-related. Each can interact with and simulate others, producing various mediators of inflammation and the clinical signs associated with trauma and injury.

The complement system: This is a cascade system of enzymatic proteins. It can be activated during the acute inflammatory reaction in various ways. After tissue injury, enzymes capable of activating complement are released from dying cells. During infection, the formation of antigen-antibody complexes can activate complement via the classical pathway; while the endotoxins of Gram-negative bacteria and tissue factors can activate complement via the alternative pathway. Products of the kinin system, coagulation and fibrinolytic systems can also activate complement.

The products of complement activation most important in acute inflammation, and their principal action include:

C5a: chemotactic for neutrophils; increases vascular permeability; releases histamine from mast cells

C3a: similar properties to those of C5a, but less active

C567: chemotactic for neutrophils

C56789: cytolytic activity

C4b, 2a, 3b: opsonisation of bacteria.

Kinin system. The kinins are peptides of 9-11 amino acids. The kinin system is activated by coagulation factor XII. One of the most important vascular permeability factor, bradykinin, is produced by this system. Bradykinin is also a chemical mediator of the pain which is a cardinal feature of acute inflammation and injury.

Although there are many more mediators of the inflammatory response these are some of the more common and well studied examples. The following section describes in some detail, some of the key mediators of the inflammatory response to surgery or those discussed in this thesis.

1.2.1   Arachidonic acid metabolites

Arachidonic acid (AA) is a polyunsaturated fatty acid released from membrane phospholipids by cellular phospholipases. During the inflammatory process neutrophils produce lysosomal phospholipases which release AA from cellular membranes (Robbins & Kumar 1987). The C5a product of the complement cascade can also activate lipases. AA metabolism then proceeds along 2 major distinct pathways governed by rate limiting enzymes 5-lipooxygenase and cyclooxygenase (Figure 1.2).

Products of the cyclooxygenase pathway include prostaglandins (PG) and prostacyclins that are involved in the inflammatory response. The major inflammatory mediators that are produced by the cyclooxygenase pathway include prostacyclin I_2 (PGI$_2$) which is a potent vasodilator, PGD$_2$, PGE$_2$ and PGF$_2$ which cause tissue oedema. PGE$_2$ produces pain at the sites of inflammation. Products of the 5-lipooxygenase pathway include the leukotrienes. Leukotriene B$_4$ (LTB$_4$) is involved in neutrophil and monocyte chemotaxis to site of inflammation. LTC$_4$ and LTD$_4$ cause vasoconstriction and increase

permeability of venules and are 1000 times more potent than histamine in that respect (Robbins & Kumar 1987).

Altogether AA metabolites induce many aspects of the acute inflammatory response. The pathway is prominent enough in the inflammatory response to be the target of anti-inflammatory drugs in clinical use. Steroids, by inhibiting phospholipase A_2, exhibit potent anti-inflammatory properties. Aspirin and other non-steroidal anti-inflammatory drugs (NSAID) inhibit cyclooxygenase by irreversible acetylation, thereby decrease prostaglandin synthesis.

Membrane Phospholipids

Phospholipase A_2

Arachidonic acid

Cyclooxygenase

5-Lipoxygenase

O_2 O_2

Prostaglandin H_2

Prostacyclin synthesase

Thromboxane synthesase

5-HPETE → **5-HETE**

H_2O

Prostaglandin I_2

Thromboxane A_2

Leukotriene A_4 → **Leukotriene B_4**

Leukotriene C_4

Prostaglandin D_2, E_2, F_2

Leukotriene D_4

HETE; hydroxyeicosatetrenoic acid HPETE; 5-hydroperoxyeicosatetrenoic

Figure 1.2 *Production of arachidonic acid metabolites from membrane phospholipids after initiation of the inflammatory response.*

1.2.2 Cytokines

One of the key chemical messenger systems in the control and the coordination of the inflammatory and immune response to injury is cytokines. Cytokines are a group of low molecular weight polypeptides or glycoproteins which act to regulate the local and systemic immune function and modulate the inflammatory response. They are active at very low concentrations, found usually at the picogram/ml level, and their production is usually transient. Cytokines bring about their action by altering gene expression in target cells. They act in a paracrine and autocrine manner at concentrations in the picomolar to nanomolar range, but can have systemic effects if there is release into the circulation.

Cytokines generally have a wide range of actions in the body. Cytokines do not have individual cell-specific targets, and they shares target cells. Upon stimulation with a particular cytokine different cells exhibit different actions. Their actions are generally also not specific to any one cytokine, and share some properties between them. Thus there is a moderate amount of redundancy built into the cytokine system.

Cytokines are not usually stored intracellularly and must therefore be synthesised *de novo* and released into the tissues upon appropriate stimulation and gene transcription. One of the crucial controllers of cytokine gene regulation is nuclear factor kappa B (NFκB) (Blackwell et al 1997, Blackwell & Christman 1997). NFκB is a protein transcription factor that enhances the transaction of a variety of cytokine genes. Although NFκB is not itself a stimulus for cytokine secretion, it is required for maximal transcription of many of the cytokines that it regulates. It is required for the transcription of amongst others interleukins-1 (IL-1), IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α).

One other important property of many cytokines is that stimulation of cells with one cytokine can stimulate secretion of that same cytokine, thereby creating a positive feedback loop. The secretion of other cytokines can also be stimulated. This pattern often leads to recognisable cytokine cascades in response to appropriate stimuli.

Lymphocytes are activated at the site of injury. The first cells to be recruited to the site of inflammation are monocytes and neutrophils, where they are producers of cytokines in the first few hours after the onset of a surgical or traumatic wound (Kondo et al 2002). These cytokines are chemoattractant to other white cells.

Cytokines are divided into pro- and anti-inflammatory cytokines on the basis of whether they stimulate the immune system or whether they decrease or dampen the immune response. Although most cytokines have a clear pro- or anti-inflammatory response, a few have dual properties and are classified by their major action. Some cytokines may exhibit a pro-inflammatory action in a particular cell or conditions, but an anti-inflammatory response in a different cell or under different conditions (Tilg et al 1994a, Yasukawa et al 2003). Anti-inflammatory cytokines are important in abating the immune response. The presence of naturally occurring inhibitors helps abate the otherwise catastrophic positive feedback loop that could lead to widespread tissue destruction from excessive inflammation. The following paragraphs describe the major actions of the cytokines investigated in this thesis.

Firstly the pro-inflammatory cytokines IL-1, IL-6 and TNF-α, followed by the anti-inflammatory cytokines IL-1 receptor antagonist (IL-1ra) and IL-10.

1.2.2.1   IL-1

The interleukin 1 family of proteins include IL-1α, IL-1β and IL-1ra. The anti-inflammatory properties of IL-1ra are discussed later. IL-1α and IL-1β are potent pro-inflammatory cytokines. They are produced by most cells of the immune system. IL-1α and IL-1β are 17 kDa proteins with 25% amino acid homology. They are found as pro-proteins (which show some biological activity), primarily in the cytoplasm of the cell. Cell death can lead to release of proIL-1α with eventual cleavage to active cytokine in the extracellular tissues. IL-1 is seldom detected in blood except in very severe disease, often from dying cells (Thomson 1998). However this does not preclude them from being present locally in active concentrations, where they can act in an autocrine or paracrine fashion. Also, proIL-1α remains intracellularly where it can exert direct effects in the nucleus of the cell (Mosley et al 1987a, Mosley et al 1987b). Both IL-1α and β act as a stimulant for production of their own and the other mRNA and protein secretion, thus setting up a powerful positive feed back cycle. More importantly, they confer more stability and increased translation of mRNA to protein, thereby further increasing protein levels (Thomson 1998).

IL1-α and β produce fever and chills, which are inhibited by the NSAID indomethacin (Iizumi et al 1991). The hypotension associated with septic shock is mimicked by intravenous injection of IL-1 in healthy humans (Crown et al 1993, Smith et al 1992). It produces general malaise, nausea and vomiting. IL-1 induces the production of IL-6 and TNF-α, as well as IL-1ra (Tilg et al 1994b). IL-1 induces haematopoiesis of, in particular, the monocyte and neutrophil fractions (Thomson 1998). The hypothalamus-pituitary adrenal axis is also stimulated by IL-1. This stimulation of cortisol secretion may act as a point of negative feedback control, as cortisol inhibits inflammation and cytokine mRNA production.

1.2.2.2 IL-6

IL-6 is a 21-kDa glycoprotein with pro-inflammatory activity. It is produced by lymphocytes, monocytes and fibroblasts (Thomson 1998). Adipose tissue and muscle also produce IL-6 (Mohamed-Ali et al 2001). IL-6 is increased in inflammatory conditions. It has effects on a variety of immune and non-immune cells. IL-6 is required for antigen-specific antibody production by murine B cells (Hilbert et al 1989), and stimulates B cell mRNA for secretory immunoglobulin (Kikutani et al 1985). It is involved in T cell activation, growth and differentiation (Houssiau & Van Snick 1992). It is a powerful inducer of acute phase protein production in the liver (Alonzi et al 1998); increasing, amongst others, hepatic production of C reactive protein (CRP) (Ohzato et al 1992), fibrinogen and α_1-antichymotrypsin. IL-6 also stimulates adrenocorticotrophic hormone (Naitoh et al 1988), and a variety of anterior pituitary hormones (Spangelo et al 1989).

IL-6 may play an important role in whole body energy metabolism. IL-6 knockout mice have abnormal fat and carbohydrate metabolism (Wallenius et al 2002b); injection of IL-6 intra-cerebrally decreased the obesity that resulted from IL-6 deficiency without any effect on acute phase reactants or insulin production (Wallenius et al 2002a). Furthermore, this effect seems to be primarily centrally driven, as centrally administered IL-6 acutely increased oxygen consumption in male Wistar rats, without a significant effect on food intake (Wallenius et al 2002b). These findings in rats correlate with the findings that IL-6 levels in cerebrospinal fluid in humans were inversely correlated with severe obesity (Wallenius et al 2003). It has been suggested that in the rat, the pyrogenic effect of central IL-1β is exhibited through IL-6 (Rothwell et al 1991). Peripherally, IL-6 is produced by adipose cells after sympathetic stimulation (Mohamed-Ali et al 2000) and has been shown to induce uncoupling protein 1 in brown adipose tissue through stimulation of the sympathetic system (Li et al 2002). Kotani *et al* found that

postoperative resting energy expenditure (REE) was significantly related to IL-6 levels in adults (Kotani et al 1996). The relationship between IL-6 and energy metabolism is therefore well established. Although some of the mechanisms remain obscure, it is clear that IL-6 plays an important role in the acute and chronic control of metabolism and temperature control.

IL-6 is an essential mediator of the systemic inflammatory response (acute phase response; APR) and inflammation to localised skin trauma (Fattori et al 1994). IL-6 knockout mice were unable to mount a normal inflammatory response to local injury and trauma. Locally produced IL-6 plays an important part in the wound healing process. Wound healing in IL-6 knockout mice was reduced compared to controls (Lin et al 2003). In addition, the administration of anti-IL-6 significantly delayed wild type murine wound healing, possibly due to its inhibiting effect on expression of endothelial growth factors.

1.2.2.3   TNF-α

TNF-α is a 17-kDa, 157 amino acid polypeptide. It is produced by numerous cells including cells of the monocyte/macrophage lineage, lymphocytes, neutrophils and endothelial cells (Thomson 1998). It was initially described as a potent inducer of tumor cell lysis in culture (Carswell et al 1975), but is now known to have various biological activities. It is a powerful mediator of the immune activation to stress and infection (Hill 2000, Tracey 2002, Tracey & Cerami 1989). Pro-TNF-α is a trans-membrane protein that may have potent effects on the TNF receptor (Grell et al 1995). TNF-α activates neutrophils, up regulates endothelial adhesion molecules and increase capillary permeability. In the central nervous system it induces fever. It also has significant catabolic effects by stimulating the release of triglycerides from adipose tissue, promoting amino

acid release from proteins and catabolism of skeletal proteins (Tracey et al 1988). It is a potent inducer of other cytokines, notably IL-1, IL-6 and IL-10. TNF-α is associated with laboratory and clinical evidence of adult respiratory distress syndrome (ARDS), SIRS and multi-organ dysfunction syndrome (MODS) (Horvath et al 1988, Tracey et al 1988, Zheng et al 1990).

1.2.2.4   IL-1ra

Interleukin-1 receptor antagonist (IL-1ra) is a competitive inhibitor of the pro-inflammatory cytokine IL-1. IL-1 acts on two distinct IL-1 receptors expressed on various cells. Binding to the IL-1 receptor (IL-1R) type 1 leads to intracellular signalling and gene transcription. IL-1R type 2 is a 67 kDa protein found on B cells, granulocytes and cells of the monocyte lineage. However IL-1R type 2, which lacks a cytosolic tail, seems to be unable to induce intracellular transduction. IL-1β binds more avidly than IL-1α to this receptor (Thomson 1998).

The naturally occurring IL-1 inhibitor was first described in the urine of febrile patients in the late 1980s (Balavoine et al 1986, Seckinger et al 1987). Subsequent work characterised IL-1ra (Arend et al 1989, Eisenberg et al 1990, Hannum et al 1990), and showed that it competitively inhibits the pro-inflammatory response to secreted IL-1 at the receptor level (Arend 2002, Granowitz et al 1992a).

IL-1ra is a 17 kDa protein secreted by monocyte macrophages and other cells. IL-1ra binds to the IL-1R at an affinity equal to that of IL-1α and β (Dripps et al 1991, Granowitz et al 1992a). However binding of IL-1ra fails to trigger internalisation and produces no agonist activity (Dripps et al 1991). Intracellular protein kinase activity, one of the early downstream events after IL-1R stimulation, is not triggered by IL-1ra in

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fibroblasts (Dripps et al 1991). Injection of IL-1ra into healthy humans is without biological effects (Granowitz et al 1992b) even at concentrations 1,000,000 times that which produces an effect after IL-1 injection (Crown et al 1993, Smith et al 1992).

The interaction of IL-1ra with type 2 receptor occurs with lower affinity than it does to IL-1R type 1 (Granowitz et al 1991). However, it also competitively inhibits the binding of IL-1 to this receptor.

Small amounts of IL-1ra are produced constitutively. IL-1ra release often parallels IL-1 response to stimuli, but circulating IL-1ra is often detected without any increase in circulating IL-1 levels (Fischer et al 1992). IL-1ra is detected at concentrations of up to 1,000 fold to that of IL-1 (Arend 2002, Fischer et al 1992). Such high levels are thought to protect against excessive pro-inflammatory response to IL-1. In cell culture, 10-times molar excess of IL-1ra over IL-1β greatly inhibited cytokine release following PBMC stimulation with IL-1 itself (Granowitz et al 1992a) and LPS (Granowitz et al 1992c); the inhibition was dose dependent. The 1000 fold excess of IL-1ra seen in serum can significantly dampen the pro-inflammatory cytokine cascade.

Although most of the effects of IL-1ra are due to competitive inhibition of secreted IL-1, there may be some direct anti-inflammatory effect of intracellular IL-1ra (Arend 2002). Constitutive expression of intracellular IL-1ra is thought block the binding of IL-1α to cellular DNA in epithelial cells (Arend 1993, Haskill et al 1991).

1.2.2.5   IL-10

IL-10 is a 17.5-kDa, 160 amino acid homodimer, produced mainly by monocyte/macrophages, but also by lymphocytes and dendritic cells (Thomson 1998). It

plays a crucial role in the down regulation of the immune response and has powerful immune-suppressive properties. It inhibits CD4+ T-cells while stimulating CD8+ T-cells. It also inhibits T-cell pro-inflammatory cytokine production, proliferation (Taga & Tosato 1992), and responsiveness (Groux et al 1996). IL-10 inhibits granulocyte cytokine (Cassatella et al 1993, Kasama et al 1994) and superoxide production (Chaves et al 1996). However, although predominantly inhibitory in function it does have some stimulatory capacity. It enhances B-cell function and antibody production (Itoh & Hirohata 1995, Levy & Brouet 1994).

IL-10 inhibits production of pro-inflammatory cytokines (IL-1, 6,8,12 and TNF-α) and inhibits release of IL-10 itself (autoregulation) (de Waal et al 1991a, Thomson 1998). The IL-10 enhanced production of IL-1ra (de Waal et al 1991a, Jenkins et al 1994) and soluble TNF receptor (Hart et al 1996, Joyce & Steer 1996) indicates that it induces a shift from pro- to anti-inflammatory response. By upregulating expression of complement receptors it increases phagocytosis of opsonised particles (Capsoni et al 1995, Spittler et al 1995a) but reduces subsequent killing by superoxide and nitric oxide production (Cunha et al 1992, Kuga et al 1996, Roilides et al 1997).

One key role of IL-10 is its powerful anti-inflammatory effects on cells of the monocyte-macrophage lineage. It downregulates monocyte/macrophage major histo-compatibility complex class II (MHC II) expression and thereby decreases antigen presentation to T-cells (de Waal et al 1991b, Fiorentino et al 1991). Increased circulating IL-10 is thought to be a major cause of the fall in monocyte MHC II expression after sepsis, trauma and operative stress (Fumeaux & Pugin 2002, Klava et al 1997b, Muehlstedt et al 2002). The IL-10 induced endocytosis of MHC molecules from the cell surface (Fumeaux & Pugin 2002) thereby induces a state of immuneparesis.

1.2.2.6   Cytokine cascade after operative stress

Both pro- and anti-inflammatory cytokines are produced in response to operative stress. The actual cytokine cascade is heterogeneous, and is determined by various factors including the type and magnitude of the operation. However, there is a generalised response that can be formulated. The postoperative cytokine cascade response in adults has been well characterised. There have been limited studies in children. The following overview is derived mainly from the response in adults with some reference to the literature on children.

Circulating IL-1α or β are not detected after most operations (Bellon et al 1997, O'Nuallain et al 1993, Wiik et al 2001a). However, IL-1β is probably the earliest induced cytokine in the intraoperative period. Extensive intraoperative sampling demonstrated a short lived circulating IL-1β response in adults undergoing major abdominal surgery (Baigrie et al 1992), which preceded the increase in IL-6 (Baigrie et al 1991). However, this IL-1β response has not been demonstrated in children (O'Nuallain et al 1993). There was also no detectable circulating IL-1β in children after cardiac bypass (Duval et al 1999). The limited data seems to indicate a lack of response with respect to IL-1α. In spite of the lack of systemic levels, there is a significant local IL-1β response in the postoperative period (Tsukada et al 1993, Wiik et al 2001a).

In contrast to the limited response in circulating pro-inflammatory members of the IL-1 family, there is a substantial IL-1ra response in both adults (Ertel et al 1995, Mokart et al 2002) and children (Duval et al 1999, O'Nuallain et al 1993, O'Nuallain et al 1995). IL-1ra is on of the earliest detected circulating cytokine in the postoperative period. Children undergoing cardiac bypass had elevation in IL-1ra levels before an increase in

IL-6 (Duval et al 1999). Levels tend to peak early, usually between the 2nd and 12th hours and decreases to preoperative levels by 24 to 48 hours.

Minor operations in adults are not associated with a significant rise in circulating TNF-α (Baigrie et al 1992, Parry-Billings et al 1992). However, operations of greater magnitude can be associated with a TNF-α response, although this is not a consistent finding. After abdominal aortic aneurysm repair in adults, there was only a tendency towards a TNF-α response (Galle et al 2000). Circulating TNF-α is seldom detected in the uncomplicated paediatric surgical patient (Hansen et al 1998). Studies have shown a wide variability in circulating levels after major operative stress in infants, with increased levels associated with severe operations and death (Chwals et al 1993). Overall, the postoperative rise in circulating TNF-α is not as frequent as other cytokines (e.g. IL-6). Some investigators believe that TNF-α is one of the initial cytokines that initiate the postoperative cytokine cascade (Hall et al 1997). The lack of increased circulating levels does not necessarily conflict with this theory. TNF-α is produced locally at the site of operation even in the absence of circulating levels (Tokunaga et al 1993, Wiik et al 2001b) and can stimulate the release of other cytokines. Locally produced TNF-α has been shown to have a secondary increase with the development of complications (van Berge Henegouwen et al 1998).

Interleukin-6 is one of the most consistently elevated circulating cytokine in the postoperative period in both adults (Akhtar et al 1998, Cruickshank et al 1990, Shenkin et al 1989, Zieren et al 2000) and children (Cruickshank et al 1990, Jones et al 1994). However, elevated levels are not detected in all operations and the levels seem to correlate with either the operation length, (Shenkin et al 1989), severity (Cruickshank et al 1990, Jones et al 1994) or both (Cruickshank et al 1990). Minor operations are

associated with a lack of, or a diminished, IL-6 response. The response may also be age dependent as the response differs in neonates and infants. Bolke *et al* (Bolke et al 2002) found that there was no response in IL-6 in neonates undergoing uncomplicated abdominal surgery (excluding gastroschisis repair) compared to a significant response in the older infant (undergoing relatively more minor procedures). They partially explained these differences to the presence or absence of endotoxaemia. Interestingly, other studies have demonstrated an association between endotoxin immunity and the morbidity after surgery (Bennett-Guerrero et al 2001). The link between surgery, endotoxaemia and morbidity is interesting to the pathophysiology of the postoperative response. One of the major determinants of the postoperative cytokine response and outcome may be the degree of endotoxin levels (Berger et al 1997).

The increase in circulating IL-6 is seen as early as the 4th postoperative hour. Peak levels tend to occur around the 6th to 24th postoperative hour. IL-6 levels return to baseline around the 2nd postoperative day. The time course of IL-6 response can be significantly lengthened by the development of complications or infections (Baigrie et al 1992, Mokart et al 2002, Tsang & Tam 1994), although a causal effect can not be assumed.

Elevated plasma IL-10 is observed after moderate to severe operative stress (Klava et al 1997b, Zieren et al 2000). After abdominal surgery levels are detected in the early postoperative period (Kato et al 1998, Slotwinski et al 2002); peaking between the early hours postoperatively and the first postoperative day. In relatively long and stressful procedures increased circulating levels can be seen late in the intraoperative course. IL-10 is particularly elevated in paediatric cardiac patients, where circulating levels are

seen just after the initiation of bypass (Seghaye et al 1996). Levels usually return to preoperative values 2 to 3 days postoperatively.

An overview of the time scale of the circulating cytokine response in the postoperative period is demonstrated graphically in Figure 1.3. Factors that can modify this general response include age, magnitude of operation and the occurrence of postoperative complications. In particular, the time course of circulating IL-10 and IL-6 seems to be sensitive to the influence of postoperative complications.
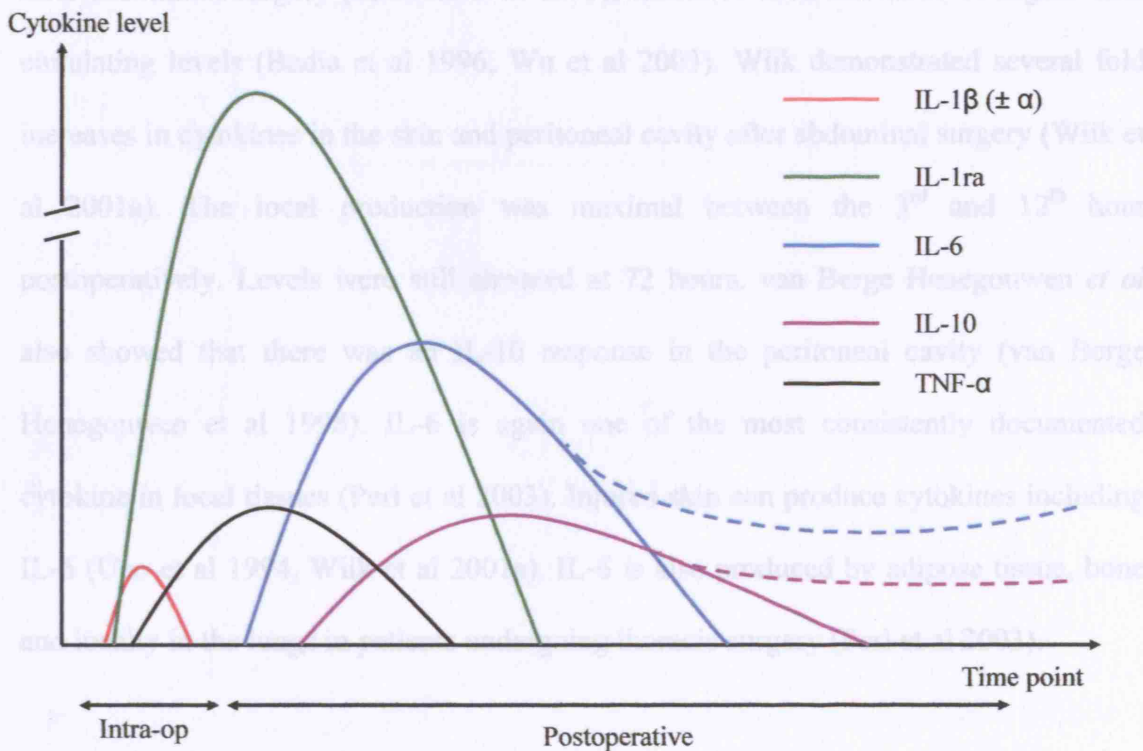
Figure 1.3 *Cytokine cascade to operative stress.*
The dashed lines indicates possible extension of the response with postoperative complications

Local cytokine response

Systemic cytokine levels are thought to originate in local tissues in the trauma/surgical patient (Perl et al 2003). However, the local cytokine response does not always correlate with the plasma levels (Badia et al 1996, Krohn et al 1999, Tsukada et al 1993). There are few studies that have failed to detect a significant increase in circulating cytokines in the postoperative period (Hansen et al 1998). There can be a significant response in local tissues in the absence of elevated plasma levels (Badia et al 1996, Muehlstedt et al 2002). After thoraco-abdominal surgery local concentration of cytokines were several-fold that of plasma (Sato et al 2001). Cytokine levels are elevated in the peritoneal fluid after abdominal surgery (Tsukada et al 1993), and have been shown to be higher than circulating levels (Badia et al 1996, Wu et al 2003). Wiik demonstrated several fold increases in cytokines in the skin and peritoneal cavity after abdominal surgery (Wiik et al 2001a). The local production was maximal between the 3rd and 12th hour postoperatively. Levels were still elevated at 72 hours. van Berge Henegouwen *et al* also showed that there was an IL-10 response in the peritoneal cavity (van Berge Henegouwen et al 1998). IL-6 is again one of the most consistently documented cytokine in local tissues (Perl et al 2003). Injured skin can produce cytokines including IL-6 (Ueo et al 1994, Wiik et al 2001a). IL-6 is also produced by adipose tissue, bone and locally in the lungs in patients undergoing thoracic surgery (Perl et al 2003).

Cellular studies have confirmed a peritoneal and visceral cytokine response to operative stress. There was an increase in IL-1 and IL-6 staining of the mesothelium after abdominal operations (Sendt et al 1999). The increase in IL-6 staining seemed to correlate in a stepwise manner with the length of operation. Kalff *et al* demonstrated increased mRNA levels for IL-1β, IL-6 and TNF-α in the muscularis externa of patients undergoing laparotomy (Kalff et al 2003). Again mRNA levels correlated with the

length of the operation. These locally produced cytokines have important immune modulating properties locally.

The possible interactions of the cytokines induced in the postoperative period are illustrated in Figure 1.4. The overall interactions are complex, and this illustration is simplified to allow an illustration of the possible initiation cascade and stimulatory interactions of the cytokines on each other.
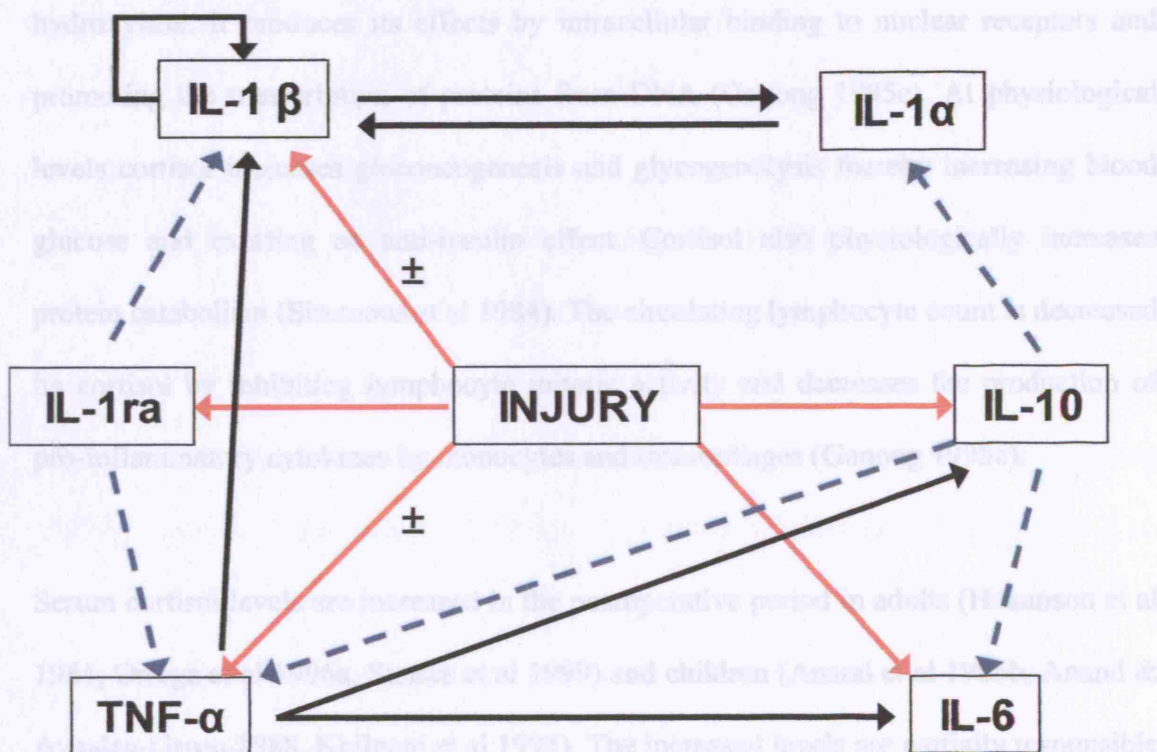
Figure 1.4 *The proposed interactions between the various cytokines in the postoperative cascade.*

The red lines indicate direct stimulation by surgical injury. ± indicates postulated or weak 'primary' stimulation of cytokines directly by injury. The black lines indicate stimulatory interactions between cytokines; blue dashed lines represent inhibitory interactions.

1.2.2.7   Adrenal hormones

The adrenal gland consists of two physiologically separate tissues that secrete hormones in response to injury and stress. The adrenal cortex produces lipid secretions that are products of cholesterol metabolism called adrenal steroids. Some of these steroids, namely the glucocorticoids and mineralocorticoids, have important function in the metabolic changes seen in the stress response. The adrenal medulla produces the amino acid derived stress hormones called catecholamines.

1.2.2.7.1   Cortisol

The glucocorticoid cortisol is produced from cholesterol via 17-hydroxylase and 11β-hydroxylase. It produces its effects by intracellular binding to nuclear receptors and promoting the transcription of proteins from DNA (Ganong 1995c). At physiological levels cortisol increases gluconeogenesis and glycogenolysis thereby increasing blood glucose and exerting an anti-insulin effect. Cortisol also physiologically increases protein catabolism (Simmons et al 1984). The circulating lymphocyte count is decreased by cortisol by inhibiting lymphocyte mitotic activity and decreases the production of pro-inflammatory cytokines by monocytes and macrophages (Ganong 1995c).

Serum cortisol levels are increased in the postoperative period in adults (Hakanson et al 1984, Ortega et al 1996a, Sietses et al 1999) and children (Anand et al 1985b, Anand & Aynsley-Green 1988, Khilnani et al 1993). The increased levels are partially responsible for the catabolic response postoperatively. It may also contribute to the hyperglycaemia and insulin insensitivity. The postoperative response usually last between 24 to 48 hours in children.

1.2.2.7.2 Catecholamines

The adrenal medulla, effectively a sympathetic ganglion, produces the catecholamines adrenaline (Adr) and noradrenaline (NAdr). Adr and NAdr are stored in granules in medullar cells associated with ATP (Ganong 1995c). Catecholamine secretion is therefore associated with concomitant ATP secretion.

Catecholamines affects many intermediary metabolic pathways in cells (Ganong 1995b, Ganong 1995c). They increase glycogenolysis in liver and muscle and increase plasma free fatty acids (FFA) (Fellander et al 1994). Catecholamine increase in the postoperative period contributes to the catabolism, hyperglycaemia and insulin resistance seen.

Catecholamines also increase the metabolic rate, partially due to vasoconstriction-induced rise in body temperature and partially due to lactate metabolism (Ganong 1995c). Heart rate and force of contraction are increased by catecholamines; this also contributes to the increase in metabolic rate seen. Adrenaline has an effect on the cardiac rate at blood levels of 0.3 nmol/L, twice the normal resting value (Ganong 1995c). Lipolysis occurs at about 0.45 nmol/L, and hyperlactaemia at 1 nmol/L.

Catecholamines also have complex interactions with the immune system. They have been shown to down-regulate the pro-inflammatory response in monocytes and lymphocytes, while stimulating the secretion of IL-10 from monocytes (Platzer et al 2000). They also inhibit TNF-α release (Severn et al 1992) while stimulating IL-10 release (van der Poll et al 1996). Catecholamines may therefore favour a switch from pro- to anti-inflammatory balance.

1.2.3 Other mediators

The list of mediators mentioned above is not exhaustive. There are a host of other chemical signals that are involved in the inflammatory response to operative stress. However, the mediators discussed above are among the most commonly studied and also the mediators investigated in this thesis.

All of the mediators coordinate the overall metabolic, inflammatory and immune response to operative stress. The stress responses in the postoperative period are now discussed, with reference to those investigated in this thesis.

1.3 Differences in paediatric anatomy and physiology relevant to the stress response to surgery

There are several anatomical and physiological differences between infants and children that impact on the response to stress and operative insult. These differences may impact on the stress response to both open and laparoscopic surgery.

Infants and children have relatively larger body surface area to mass ratio compared to adults. The relative surface areas of different body compartments are also different. The relatively larger head may provide an important area for heat loss in the intraoperative period. This may have implications for intraoperative thermoregulation (see below). The relatively larger body surface area compared to mass can also be of additional importance in laparoscopic surgery; the peritoneal cavity may absorb relatively more CO_2 compared to adults. The surface area of the intestine may also allow for either dissipation of, or absorption of, heat during surgery and laparoscopy. The open abdominal cavity allows significant heat loss (evaporation and radiation) in already thermoregulatory challenged children.

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Physiologically infants and children are able to mount a stress response. They are differences in the whole body metabolic response in neonates compared to adults in respect to energy metabolism. Both the hormonal (Anand et al 1985b) and whole body energy expenditure (Jones et al 1994) responses have unique patterns in children and infants. Also, children have less fuel stores compared to adults. It has been shown that the postoperative metabolic response is deleteriously affected by the preoperative fasting (Ljungqvist et al 1994, Nygren et al 1998). The limited fuel store may have implications in young children, who tolerate fasting poorly; this may alter the metabolic response to surgery.

1.4   Intraoperative metabolic response to surgery

1.4.1   Factors altering the intraoperative response to surgery

There are several factors that influence the metabolic response to surgery that arise in the intraoperative period. They include the effect of preoperative fasting and intraoperative fluid administration, anaesthetic drugs, temperature regulation (and in particular the exposure to cold) and the actual operative insult itself. These factors are interrelated and influence each other. For example, the observed hypothermic effect of anaesthesia has been shown to be reduced by intraoperative amino acid administration (Sellden & Lindahl 1999).

Many of these factors have different effects in infants and children compared to adults. For instance, smaller children have less body stores and tolerate fasting poorly compared to adults. Prolonged preoperative fasting is avoided in children; fasting has been shown to exacerbate the postoperative metabolic response. Intraoperative glucose (Ljungqvist et al 1994, Nygren et al 1998) and amino acid (Sellden 2002) administration

alter the metabolic response. Alterations in substrate availability may have far reaching effects in children undergoing surgery.

One factor that has a large influence on the postoperative response, and which differs substantially between children and adults, is intraoperative thermoregulation.

1.4.2 Relationship between thermoregulation and the metabolic response to surgery in children

Infants and children undergoing operations under general anaesthesia are exposed to a variety of factors which may have detrimental effects on heat production (energy expenditure) and thermoregulation, and therefore core temperature. Mild hypothermia is a common postoperative finding (Lyons et al 1996) and may increase the risk of postoperative complications by inducing peripheral vasoconstriction and anaerobic metabolism. Neonates and infants are more susceptible to hypothermia when exposed to a cool environment. Anatomically their body proportions are different from that of adults: the surface-area to body-weight ratio and larger body surface area on the head already lend to easier dissipation of body heat. Also, there is less subcutaneous fat resulting in less body insulation.

In particular, there is less brown-fat. Infants produce heat by either shivering or non-shivering thermogenesis. Anaesthesia reduces the capacity to generate heat by both shivering and non-shivering thermogenesis. Shivering causes an increase in muscular activity and thus energy expenditure and heat production. Shivering is inhibited by anaesthetic agents and in particular muscle relaxation.

Non-shivering thermogenesis is though to involve the proton leak pathway of fuel metabolism (Zamparelli et al 1999) and requires brown-fat as the fuel source. Smaller children and infants have relatively less brown-fat stores and are therefore more susceptible to the effects of cooling intraoperatively due to limited stores of brown fat for thermogenesis. Moreover, anaesthetic agents inhibit the non-shivering thermogenesis in response to hypothermia (Dicker et al 1995, Plattner et al 1997), with important effects on thermoregulation and intraoperative metabolism. The cause of the reduced heat production has been studied on the molecular level and found to be partially due to reduced sensitivity to catecholamine induced thermogenesis (Dicker et al 1995). Ohlson *et al* identified two possible sites of the inhibition of catecholamine induces thermogenesis; either inhibition of uncoupling protein-1 expression or diminished fatty acid oxidation independent of uncoupling protein-1. Zamparelli *et al* demonstrated a direct inhibition of the respiratory chain and oxygen consumption in hepatocytes incubated with fentanyl (Zamparelli et al 1999).

It has become standard practice during general anaesthesia in infancy to minimise the heat loss by wrapping the exposed areas of the body and by maintaining a high ambient temperature in the operating theatre. Warmed mattresses, water blankets, and overhead radiant heaters are also used to warm the infant and to minimise hypothermia. However, even with heat dissipation maintained at pre-anaesthetic levels, a fall in heat production has been observed during anaesthesia (Lyons et al 1996, Plattner et al 1997).

Studies on the thermogenic response to surgery have been performed mostly on experimental animals, with few studies involving preoperative, intraoperative and postoperative measurements in infants. Plattner et al demonstrated that anaesthetised infants who became cool were unable to increase their non-shivering thermogenesis

(Plattner et al 1997), with a gradual decrease in oxygen consumption as they became cooler.

Alterations in thermoregulation can play an important role in determining the postoperative metabolic response. Infants and children are more susceptible to changes in their thermoregulatory systems and environmental temperature than adults. Thermoregulatory changes in children can significantly alter the postoperative metabolic response. Anatomical and physiological differences in thermoregulation may be partially responsible for different patterns of the metabolic response between neonates, children and adults. The effects of laparoscopy on the preservation of intraoperative temperature, thermoregulation and metabolism have not been studied in children.

1.5 Changes in postoperative energy metabolism in response to operative stress

Cellular metabolism increases oxygen consumption ($\dot{V}O_2$) as oxygen is used in energy producing reactions, including ATP generation. Unless there is uncoupling of O_2 from oxidative phosphorylation, there is also an associated increase in heat and energy production. Operative stress brings about changes in whole body metabolism. This metabolic response can be a considerable challenge to the patient's homeostasis and recovery (Forsberg et al 1991). Changes in $\dot{V}O_2$ and energy expenditure (EE) provide one means of characterising and evaluating the whole body response to operative stress. There is a wealth of knowledge on this response in adults and a few reports on the response in infants, however little is know about the response in children.

Whole body energy expenditure is partitioned between various compartments (Figure 1.5). Basal metabolic rate (BMR) represents the energy required to maintain body

temperature (thermogenesis), the work of cardiac activity and respiration, the maintenance of ionic integrity of the cells, and minimum energy requirements of tissues at rest. BMR is measured at rest in a thermoneutral environment, and after a 12 hour fast. It is the major component (65 to 75%) of total energy expenditure (TEE) (Bodamer et al 1997). REE is defined as energy expenditure measured at rest in a thermoneutral environment, after a 4 hour fast. In addition to the energy expenditure measured at BMR, REE includes some diet induced thermogenesis (DIT; 5 to 10% above BMR) (Schutz et al 1999).

TEE includes the energy requirement for growth and physical activity. The contribution of activity to TEE varies, and has been estimated to be between 10 and 30% (Bodamer et al 1997, Heim 1985). This is directly proportional to the amount of physical activity and thus is subject to some individualised variation. Therefore assessment of REE is more reliable than TEE as a measure of the effect of surgical stress on whole body metabolism.
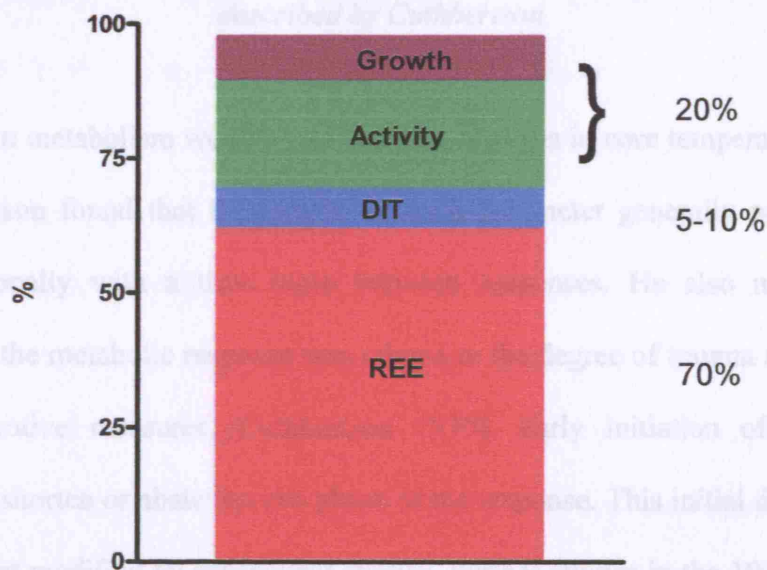
Figure 1.5 *Components of total energy expenditure in children*

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1.5.1 Postoperative whole body energy metabolism in adults

Surgical trauma significantly affects energy metabolism in adults (Kehlet 2000). In the 1930s Cuthbertson first described a brief 'ebb' period of depressed metabolic rate immediately after injury and surgery (Figure 1.6), which was followed by a 'flow phase' characterised by an increase in resting energy expenditure (REE) which can last up to several days (Cuthbertson 1970, Cuthbertson 1932, Cuthbertson 1942). Cuthbertson documented an increased metabolic rate of around 20 to 25% after injury.
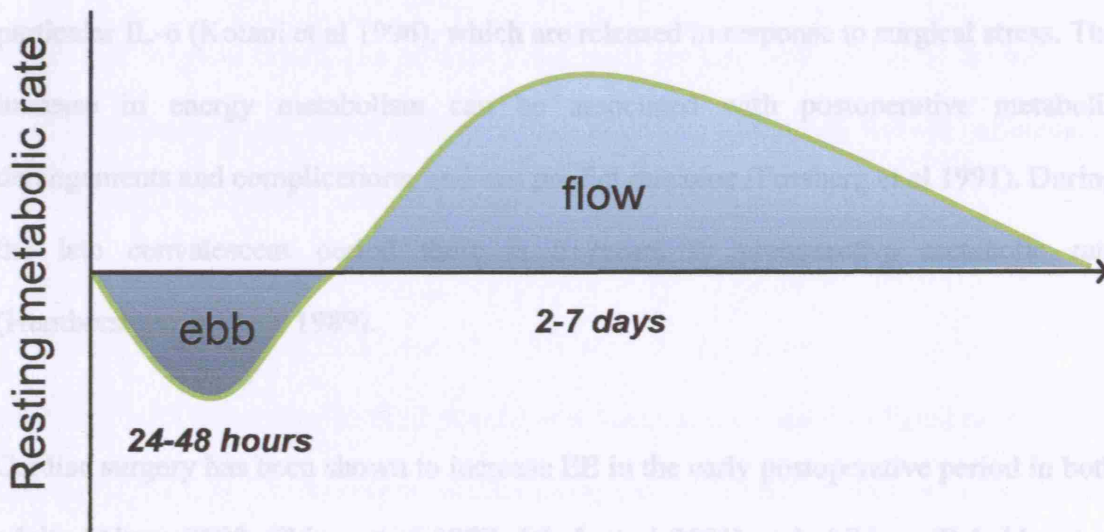
Figure 1.6 *The ebb and flow phases of the postoperative metabolic response as initially described by Cuthbertson.*

The changes in metabolism were associated with changes in core temperature and heart rate. Cuthbertson found that the curves for each parameter generally paralleled each other, occasionally with a time lapse between responses. He also noted that the magnitude of the metabolic response was related to the degree of trauma and the nature of any restorative measures (Cuthbertson 1970). Early initiation of resuscitative measures can shorten or abate the ebb phase of the response. This initial description has been somewhat modified by subsequent studies. Further studies in the 1960s confirmed some of these changes (Kinney et al 1968, Kinney & Dudrick 1970, Long et al 1969).

More recent studies have confirmed the increase in energy metabolism in the postoperative period (Brandi et al 1988, Brandi et al 1996, Forsberg et al 1991, Hersio et al 1993, Sato et al 1997, Watters et al 1993); with a hypermetabolism of between 15 and 30% in the postoperative period. The postoperative increase in REE can last up to seven days (Sato et al 1997). The increase in REE has been attributed to increased energy requirements of injured tissue and increased cycling of metabolic substrates, and is partially mediated by catecholamines (see above), glucagon, and cytokines, in particular IL-6 (Kotani et al 1996), which are released in response to surgical stress. The increase in energy metabolism can be associated with postoperative metabolic derangements and complications, and can predict outcome (Forsberg et al 1991). During the late convalescent period there is a return to preoperative metabolic rate (Humberstone & Shaw 1989).

Cardiac surgery has been shown to increase EE in the early postoperative period in both adults (Akasu 2000, Chiara et al 1987, Jakob et al 2001) and children (Puhakka et al 1994). The response is both age and stress related. Few studies have failed to show an elevation in EE in the postoperative cardiac patient (Gebara et al 1992). Mitchell *et al* measured TEE in children using doubly-labelled water technique one week before cardiac surgery and 6 hours postoperatively. They found a decrease in TEE in the postoperative period (Mitchell et al 1994). However, the preoperative study was performed 1 week prior to surgery and therefore relies on the assumption that EE did not vary in the week before surgery. In addition, they assessed TEE as apposed to REE. Differences in the degree of preoperative mobilisation and diet can account for a decrease in TEE in the face of an increased basal metabolic response. REE more accurately reflects changes in basal metabolism than TEE.

1.5.2 Energy metabolism after general surgery in infants and children

The metabolic response in infants undergoing general surgical procedures has been shown to differ to that described in adults. One early study on energy metabolism in neonates (Ito et al 1976) concluded there was a period of depressed metabolic rate (expressed as 'minimal' $\dot{V}O_2$) that was evident 2 weeks after major surgery. This finding can more accurately described as 'retarded metabolic growth' as it was compared to the normal increase in 'minimal' $\dot{V}O_2$ in the early postnatal infant with no or minimal surgical insult. The results of that study are also confounded by the inclusion of children undergoing minor surgery in the definition of normal, and the influence of various states of nutrition in these infants.

Jones et al found that REE increased by 15% after abdominal surgery in neonates (Jones et al 1993). This increase in REE peaked at 4 hours and returned to baseline within 12 hours (Figure 1.7). There was no further change in REE up to 5 days postoperatively. This transient hypermetabolism is associated with elevation of heart rate, body temperature, respiratory rate and circulating IL-6 (Jones et al 1994, Jones et al 1995).
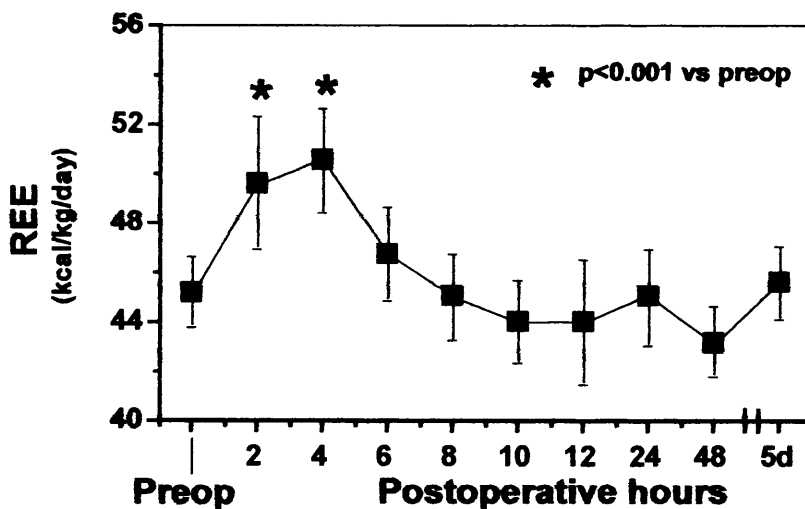
Figure 1.7 *Metabolic response to operative stress in neonates*

(From Jones et al 1994).

Contrary to the adult findings, hypermetabolism is not seen in neonates in the late postoperative period (Jones et al 1993, Shanbhogue et al 1991, Shanbhogue & Lloyd 1992). Altogether these studies have collectively shown that there is a short-lived hypermetabolic response in neonates, which usually abates by 24 hours. The different pattern in the REE response between infants and adults points to differences in the physiology of the metabolic response at different ages. Interestingly, Jones *et al* (Jones et al 1993) showed that the short-lived increase in REE in neonates was absent in infants who were less than 48 hours old.

There is limited data on energy metabolism after operative trauma in older infants or children. Groner *et al* (Groner et al 1989) measured REE in 8 to 19 year old children following major surgery and found no significant change up to 5 days postoperatively. They did not look at the early postoperative period, which has been shown to be the time of increased metabolic rate in neonates (Jones et al 1993, Jones et al 1995). Powis *et al* studied a group of children undergoing major abdominal surgery and found no significant change in REE 3 to 6 hours postoperatively (Powis et al 1998). Their patients were a mixture of neonates and infants, and may not therefore have had a homogeneous response. Small numbers or age related differences may influence the interpretation of their finding.

The factors contributing to the magnitude of the metabolic response have been alluded to. Age is an important factor that modifies the metabolic response to surgery. As already noted there is a difference between the response in neonates and adults. Therefore findings in the adult population do not necessarily transfer directly into the paediatric age group. Overall, compared to the response in adults, the postoperative changes in energy metabolism are more marked but of shorter duration in infants.

Within the paediatric age group there may be differences in the response between neonates, infants and older children (Ward Platt et al 1989).

1.5.3 Operative stress modulates the metabolic response

Cuthbertson alluded to the link between the magnitude of the insult or injury and the metabolic response (Cuthbertson et al 1972b). One of the major determinants of the metabolic response to surgery is the magnitude of the operative stress (Anand & Aynsley-Green 1988, Chwals et al 1995). Operations of greater magnitude are associated with a greater derangement in the metabolic response. This is important as the degree of postoperative metabolic derangement is a direct influence on postoperative outcome (Forsberg et al 1991). Minimising the metabolic derangements in the postoperative period may reduce postoperative complications.

Interestingly, Jones *et al* (Jones et al 1993)found the increase in REE in infants linearly correlated with the degree of operative trauma as defined by the Operative Stress Score devised by Anand *et al* (Anand & Aynsley-Green 1988). This correlation was present in infants more than 48 hours old but not those less than 48 hours old. Chwals *et al* showed that postoperative EE was also related to the operative stress as stratified by the CRP response (Chwals et al 1995).

Therefore it can be postulated that laparoscopic surgery, by decreasing the trauma associated with surgical access, may alter the metabolic response. This could then be associated with improved clinical outcome and fewer postoperative complications.

1.5.4 The relationship between thermoregulation and the metabolic response to stress

In his early experiments, Cuthbertson also made the important association between alteration in environmental temperature and the metabolic response. He noted that in both the traumatised rat and in humans, the alterations in protein and energy metabolism were depressed and in some cases abolished by alterations in environmental temperature. He showed that transferring to, or maintaining a $30^{\circ}C$ environmental temperature blunted the metabolic response to trauma (Campbell & Cuthbertson 1967, Cuthbertson et al 1968). Morbidity and mortality were also influenced by environmental temperature. As children are more susceptible to changes in thermoregulation, this may be a crucial determinant of the metabolic response to surgery in children. This may be due to factors already alluded to in Section 1.3.2.

1.5.5 Postoperative energy metabolism after laparoscopy

Various studies have been cited performed to describe intraoperative gas exchange and energy metabolism during laparoscopy compared to open surgery. Studies investigating $\dot{V}O_2$ during laparoscopic surgery in adults have yielded conflicting results. Mullet et al (Mullett et al 1993) and Kazama et al (Kazama et al 1996) used mass spectrometry and found that $\dot{V}O_2$ was stable in adults undergoing various laparoscopic procedures. Using the Deltatrac® II indirect calorimeter, Lind found a more significant rise in $\dot{V}O_2$ after skin incision in gynaecological laparotomy compared to laparoscopy (Lind 1994), while Luiz et al (Luiz et al 1992) found $\dot{V}O_2$ was stable throughout both laparoscopic and open cholecystectomy.

Although there have been many studies on various aspects of the metabolic response to laparoscopy, only one study has characterised postoperative energy metabolism (REE) in patients after laparoscopic surgery. Lou *et al* performed a trial in adults randomised to open or laparoscopic cholecystectomy (Luo et al 2003). REE, as measured by indirect calorimetry, was elevated on postoperative day 1 in both groups. The rise in REE was significantly higher in the open compared to laparoscopic group. To my knowledge, there have been no other studies that have characterised REE after laparoscopy in the literature.

1.5.6 Changes in intermediary metabolism in response to operative stress

Surgery not only affects whole body energy metabolism but also alters the intermediary pathways that are involved in tissue metabolism. In particular, there are changes in substrate utilisation and protein metabolism. These changes and some of the possible mechanisms for these changes are summarised below.

1.5.6.1 Changes in substrate utilisation

There is a shift in the main fuel used to provide energy in the postoperative period. Preoperatively, cells utilise a mixture of fat and glucose for metabolism. In children, the postoperative period is usually marked by a shift towards fat oxidation (Gebara et al 1992, Powis et al 1998). The increase in fatty acid metabolism postoperatively is thought to be due partially to change in hormonal milieu in response to stress. Postoperatively, catecholamine secretion increases triglyceride (TG) breakdown and increases FFA flux (Fellander et al 1994).

Along with the documented increase in fat oxidation there is a period of hyperglycaemia (Anand et al 1985b), indicating that the shift to fat oxidation is not due to a lack of

glucose for substrate. There is also increased lactate and total gluconeogenic substances in the blood in the postoperative period. The hyperglycaemia is due to a combination of increased gluconeogenesis and glycogenolysis (by the liver), decreased glucose utilisation by tissues, and subsequent availability of substrate in excess of requirements (Hill 2000). The decrease in glucose utilisation in the postoperative period is also due, in part, to relative insulin resistance (Brandi et al 1993, Schricker et al 2001a, Thorell et al 1994). Catecholamines have been shown to have a suppressive effect on glucose metabolism (Anand et al 1985a) while stimulating glucose output by the liver and increase glycogen breakdown in muscles. The hyperglycaemia seen in the postoperative period is important from a clinical standpoint, as hyperglycaemia is related to outcome and infective complications (Zerr et al 1997).

Several studies have documented that the increase in blood glucose in the postoperative period correlates with the magnitude of the operative stress score (Anand & Aynsley-Green 1988, Ward Platt et al 1990). The pattern of this hyperglycaemic response may also be related to age, being more long-lasting in older children (Ward Platt et al 1990).

1.5.6.2   Changes in protein metabolism

In his original descriptions on the metabolic responses to injury, Cuthbertson described an increase in protein catabolism as reflected by increase nitrogen excretion (Cuthbertson 1932). He again described a pattern of an initial decrease in metabolism followed by an increase in the 'flow' phase. The increase in protein metabolism again paralleled the changes in oxygen consumption and heart rate.

Stable isotope studies have increased our understanding of the changes in protein metabolism after operative stress. In adults, operative stress causes marked changes in

whole body protein metabolism characterised by enhanced protein turnover (Carli & Halliday 1996, Clague et al 1983), an increase in protein (in particular muscle protein) catabolism (Essen et al 1995), and a negative nitrogen balance (Carli et al 1991b). There is increased utilisation of amino acids for alternate purposes such as gluconeogenesis (Schricker et al 2001b), synthesis of acute phase proteins by the liver (Chwals et al 1993), synthesis of components of the healing process in injured tissues and immunological defence (Exner et al 2002). The protein catabolism results in marked skeletal muscle wasting as substrate is mobilised to other viscera for the above mentioned purposes. Changes in protein synthesis may depend on the severity of the surgical trauma.

Overall, during this acute phase, there is a redistribution of protein synthesis and breakdown to suit the needs of individual tissues (Wilmore 2000). These changes may be paramount to the individual's survival as vital organs (heart, respiratory system, brain and liver) benefit at the expense of less essential organs (skeletal muscle, gut). There is a shift in hepatic protein synthesis from visceral proteins to those of the acute phase proteins. This is reflected by the inverse relationship between serum prealbumin and CRP levels in the postoperative period (Chwals et al 1993), with a significant correlation between the recovery in prealbumin levels and mortality, confirming the importance of protein homeostasis to outcome.

The cytokines IL-1, IL-6 and TNF-α can partially drive the changes in protein catabolism seen after trauma (Hill 2000). Indeed, administration of IL-1ra has been shown to attenuate protein catabolism and other intermediary metabolic responses (Wilmore 2000). Complications such as sepsis can prolong the catabolic state and exacerbate protein/muscle wasting despite adequate nutrition (Plank et al 1998),

probably partially driven by cytokines. In the convalescent phase there is a reversal of the muscle wasting of the acute phase, with return to protein anabolism (Wilmore 2000).

Few studies have been performed to characterise protein metabolism in surgical children. Powis *et al* characterised the changes in protein kinetics in infants and young children who had undergone a major operation, using stable isotopic tracer techniques (Powis et al 1998). The changes in whole body protein flux, protein synthesis, amino acid oxidation and protein degradation between preoperative and 6 hours postoperative were variable, with older children exhibiting an increase in protein turnover. Overall there was no significant change. However, that study involved a heterogeneous group of children (neonates and children), with varying degrees of operative trauma. It is possible that the variability in postoperative protein turnover depends on the degree of tissue trauma.

1.6   Hormone response to operative stress in children

Various studies have characterised the endocrine response to surgery in children (Anand et al 1985b, Anand & Aynsley-Green 1988, Gruber et al 2001). These studies have revealed a significant response that last between 24 and 48 hours postoperatively. The response differs in some respects to that of adults, which usually lasts longer (Bellon et al 1998, Thorell et al 1994). Generally, the endocrine response abates with the other metabolic responses.

There is hyperglycaemia in the postoperative period, lasting between 24 and 48 hours. The increase in blood glucose can be more prolonged in operations associated with

severe surgical stress. Postoperative blood glucose can be a predictor of morbidity and mortality.

Compared to values seen after an overnight fast, there is an increase in insulin levels in the early postoperative period (Anand et al 1985a, Anand et al 1990). However, this is not universal (Anand et al 1985b, Anand & Aynsley-Green 1988). The increase in insulin levels is not proportional to the increase in glucose. There is a change in the insulin/glucose ratio postoperatively (Anand et al 1985b, Ward Platt et al 1990), which lasts more than 24 hours. Anand *et al* (Anand et al 1985b) showed neonates exhibited an initial decrease in the insulin/glucose ratio in the immediate postoperative period that was restored by 6 hours. Ward-Platt *et al* (Ward Platt et al 1990) found an early and continuous rise in the ratio in older infants and children.

Cortisol is significantly elevated postoperatively, remains elevated for the first 24 hours and is accompanied by a rise in catecholamines (Hakanson et al 1984, Rutberg et al 1984). Both these hormones have anti-insulin effects. The rise in cortisol and catecholamines partially drives the postoperative hyperglycaemic response, and may be responsible for the relative insulin insensitivity in the postoperative period. Anand *et al* found a very significant correlation between glucose and adrenaline levels in neonates at the end of abdominal surgery (Anand et al 1985b).

There is an increase in lactate levels in the postoperative period (Anand et al 1990, Anand & Hickey 1992, Fujioka et al 2000). The increase in lactate in the postoperative period is related to the alteration in glucose metabolism (Anand et al 1985b), and the presence of tissue hypo-perfusion related to surgery (Fujioka et al 2000). The increase in lactate may represent a means of discriminating the magnitude of operative stress.

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Altogether, the changes in endocrine parameters are related to the magnitude of the operative stress. However, despite the findings of quicker postoperative recovery, these responses have not been conclusively shown to be lessened by laparoscopic surgery in adults (Kehlet 1999). There are no controlled studies in children.

1.7 Innate immune response to operative stress

The cytokine and hormonal responses seen in the postoperative period bring about changes in the immune system. These are mainly coordinated by cytokines; found either locally or circulating in the bloodstream. Both the innate and the adaptive arms of the immune system are affected by operative stress. For example, there is a decrease in total leukocyte count and T-cell specific immunity in the postoperative period (Decker et al 1996). The delayed-type hypersensitivity skin reaction has been used to investigate the specific immune response in both animals and humans. There is a significant decrease in the magnitude of the response postoperatively (Little et al 1993), which may be related to the degree of the operative stress (Allendorf et al 1997).

Many of the postoperative inflammatory coordinators are intimately involved in the innate immune response. Cytokines are central regulators in this response. Activated complement is involved in opsonisation and bacterial lysis. Cytokines and complement are chemotactic.

Many cells of the immune system are involved in the innate immune response in the postoperative period. Central to the response to injury are circulating neutrophils and monocytes, which are among the first cells to be recruited to the site of injury/inflammation. Both are phagocytes. Whereas the duty of the neutrophil is only that of microbial killing, the monocyte has a part to play in linking the innate and the adaptive

arms of the immune system. The role of these two cells in the postoperative response is considered further.

1.7.1 Neutrophils role in the initiation and propagation of the inflammatory response in the postoperative period

Neutrophils are activated by mediators secreted during the inflammatory response (e.g. C5a and cytokines). Chemokines (a shortening for chemoattractant cytokines) are involved in attraction of neutrophils to the site of inflammation, while cytokines are involved in their activation and increase the surface expression of adhesion molecules on neutrophils, thereby activating rolling, adherence and subsequent migration of neutrophils (Robbins & Kumar 1987).

The circulating neutrophil pool is made up of a mixture of activated and quiescent neutrophils. Cell surface markers that indicate activation of neutrophils are up-regulated in inflammatory states. CD11a (Toft et al 1998), intercellular cell adhesion molecule (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and P-selectin are among surface markers which increase following trauma and surgically induced inflammation (Kalff et al 1999, Tarnok et al 2001).

These activated neutrophils are themselves involved in production of potent inflammatory cytokines. They phagocytose foreign bacterial and altered endogenous particles. During endocytosis of foreign material there is fusion of the endocytotic vesicle with lysosomes within the neutrophils. Within these lysosomes are powerful cytotoxic chemicals. These powerful antibacterial compounds are crucial to the defence against infection by neutrophils.

Both oxygen dependent and non-oxygen dependent killing methods are within the arsenal of the neutrophils. Neutrophils produce antimicrobial proteases within lysosomes, which can leak into the extracellular space during phagocytosis. These proteases include elastases, collagenases and matrix metalloproteinases (Robbins & Kumar 1987). These can be released into the surrounding tissue and cause significant tissue damage and a marked inflammatory response. Their actions are usually balanced by the presence of protease inhibitors in the extracellular space; this can however be overwhelmed by severe responses. Oxygen dependent mechanisms for neutrophil defence are discussed below.

1.7.2 Neutrophil oxygen free radical production

One group of cytotoxic chemicals produced by neutrophils (and cells of monocyte lineage) in response to inflammation and phagocytosis is the reactive oxygen species. The production of these oxygen derived species is associated with an oxidative burst in the neutrophils (McCloskey & Salo 2000), as well as monocytes. Included in this group are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and hypochlorous acid (HOCl) (Ganong 1995a, Robbins & Kumar 1987). These highly reactive species not only destroy invading bacteria, but can also react with cellular components on cells causing damage and even cell death.

The generation of these oxygen free radicals causes an increase in oxygen uptake and stimulation of NADPH oxidase in neutrophils (Henderson & Chappel 1996). This enzyme complex consists of five subunits, and is expressed by neutrophils and monocytes. Inhibition of superoxide formation is associated with downregulation of NADPH oxidase mRNA expression for various subunits (Kuga et al 1996). The

increase in neutrophil oxygen uptake may contribute to the increase in $\dot{V}O_2$ in the

postoperative period. The generation of oxygen free radicals is shown in Figure 1.8.
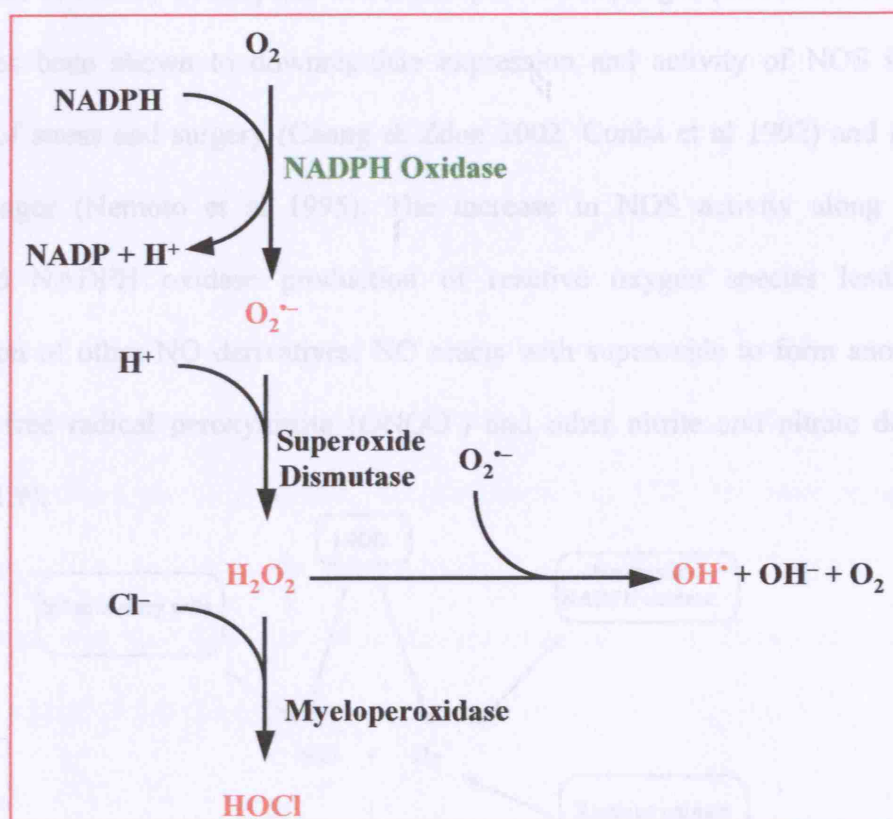
Figure 1.8 *Generation of oxygen free radicals during the respiratory burst*
in phagocytotic cells.
Free radicals are shown in red while important rate limiting enzymes are in green. See
text for names of free radical.

1.7.3 Oxygen derived free radical induced injury

1.7.3.1 Nitric oxide derivatives

Nitric oxide (NO) is an endogenous product formed from the metabolism of L-arginine

and oxygen. NO production is catalysed by the enzyme nitric oxide synthase (NOS)

(Xia & Zweier 1997a). This enzyme is constitutively expressed and may play a role in

vascular tone. A second distinct enzyme, inducible nitric oxide synthase (iNOS) is not

constitutively expressed, but requires stimulation by, for instance, endogenous or

bacterial signals. Stimulated murine macrophages are able to produce $O_2^{\cdot-}$ (as well as

NO) via iNOS in L-arginine depleted conditions, with amplified bacterial killing (Xia &

Zweier 1997b). Pro-inflammatory cytokines (TNF-α and IFN-γ), secreted during the inflammatory response, increase the expression of iNOS, thereby increasing the production of NO by neutrophils and monocytes/macrophages (Xia & Zweier 1997b). IL-10 has been shown to downregulate expression and activity of NOS in animal models of stress and surgery (Chang & Zdon 2002, Cunha et al 1992) and in human macrophages (Nemoto et al 1995). The increase in NOS activity along with the increased NADPH oxidase production of reactive oxygen species leads to the production of other NO derivatives. NO reacts with superoxide to form another very reactive free radical peroxynitrite (ONOO⁻) and other nitrite and nitrate derivatives (Figure 1.9).
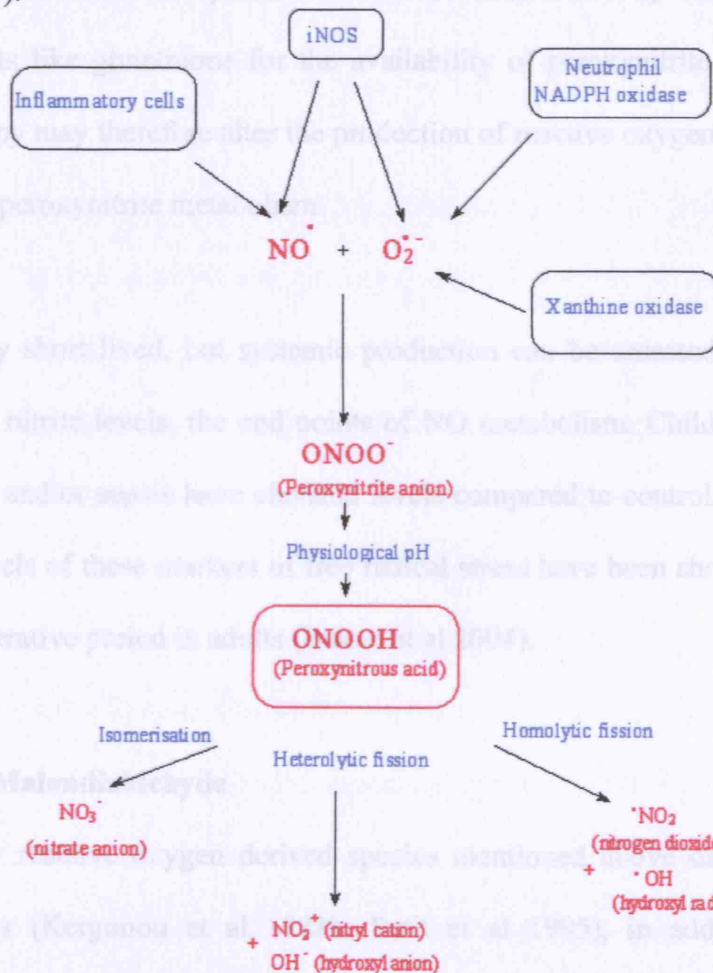
Figure 1.9 *Formation of nitrates and nitrites derivatives from NO.*
(Modified from Dr Whiteman www.med.nus.edu.sg/bioweb/faculty/metthew/matt)

Peroxynitrite can cause lipid peroxidation injury, protein degradation (Haddad et al 1993, Haddad et al 1994, Radi et al 1991) and even DNA damage (Yermilov et al 1996). The production of peroxynitrite (and superoxide) by iNOS, in the absence of L-arginine, allows the generation of these very powerful reactive oxygen species by a single enzyme, with powerful anti-microbial effects (Xia & Zweier 1997b).

Recent findings have shown that the reactivity of peroxynitrite is modified by CO_2 (Uppu et al 1996, Uppu & Pryor 1996, Yermilov et al 1996), possibly through the formation of nitrosoperoxycarbonate ($O=N-OOCO_2^-$). It was noted that the nitration of fat was increased in the presence of bicarbonate ion. CO_2 can also compete with antioxidants like glutathione for the availability of peroxynitrite (Zhang et al 1997). Laparoscopy may therefore alter the production of reactive oxygen species by the effect of CO_2 on peroxynitrite metabolism.

NO is very short lived, but systemic production can be assessed by measurement of nitrite and nitrite levels, the end points of NO metabolism. Children in intensive care with SIRS and/or sepsis have elevated levels compared to controls (Spack et al 1997). Plasma levels of these markers of free radical stress have been shown to be elevated in the postoperative period in adults (Bukan et al 2004).

1.7.3.2   Malondialdehyde

The highly reactive oxygen derived species mentioned above damage many cellular components (Kergonou et al 1988b, Petit et al 1995), in addition to doing their evolutionary duty of bacterial killing. Oxygen free radicals are very reactive and thus very short lived in the blood stream. Polyunsaturated fatty acids undergo *in vivo* oxidative damage called lipid peroxidation. This process proceeds via free radical

species with a primary stable product hydroperoxide. Because free radical products have a short half-life, detection of lipid hydroperoxides in plasma is used to investigate lipid peroxidation and free radical injury; malondiadehyde (MDA) is one such product.

MDA is the dialdehyde derivative of malonic acid and is generated during arachidonic acid catabolism, and during non-enzymatic lipid peroxidation of unsaturated fatty acid. MDA can react in *vivo* and *in vitro* with free amino groups of proteins, phospholipids and nucleic acids, leading to structural modifications of biological molecules. MDA is reactive *in vivo* and produces structural damage to proteins, which can be recognised immunologically (Kergonou et al 1988a).MDA is relatively stable and is excreted in urine. It can be considered a footprint of lipid peroxidation.

MDA levels are known to increase after ischemia/reperfusion (Soong et al 1993, Soong et al 1996, Valer et al 1994) and operative stress (Bentes de Souza et al 2003, Gal et al 1997). The response may be proportional to the operative stress, and may be less, for example, after laparoscopic surgery compared to open surgery (Gal et al 1997). No studies have been performed in children undergoing laparoscopy.

1.7.4   The role of monocytes in the inflammatory postoperative response

Monocytes play a central role in the immune response. Monocytes are producers of many cytokines involved in the inflammatory response and the constellation of cellular signalling that lead to an immune response. Monocytes express CD14 (a receptor for lipopolysaccharide (LPS) present on the surface of gram negative bacteria) on their surface. Binding stimulates cytokine release via various protein kinases. Bacterial interaction with CD14 activates monocyte and initiates cytokine release and phagocytosis (Janeway et al 2001). One of the crucial functions of monocytes in the

immune response is antigen presentation to T-cells (Figure 1.10), thereby linking the

innate and adaptive arms of the immune system.
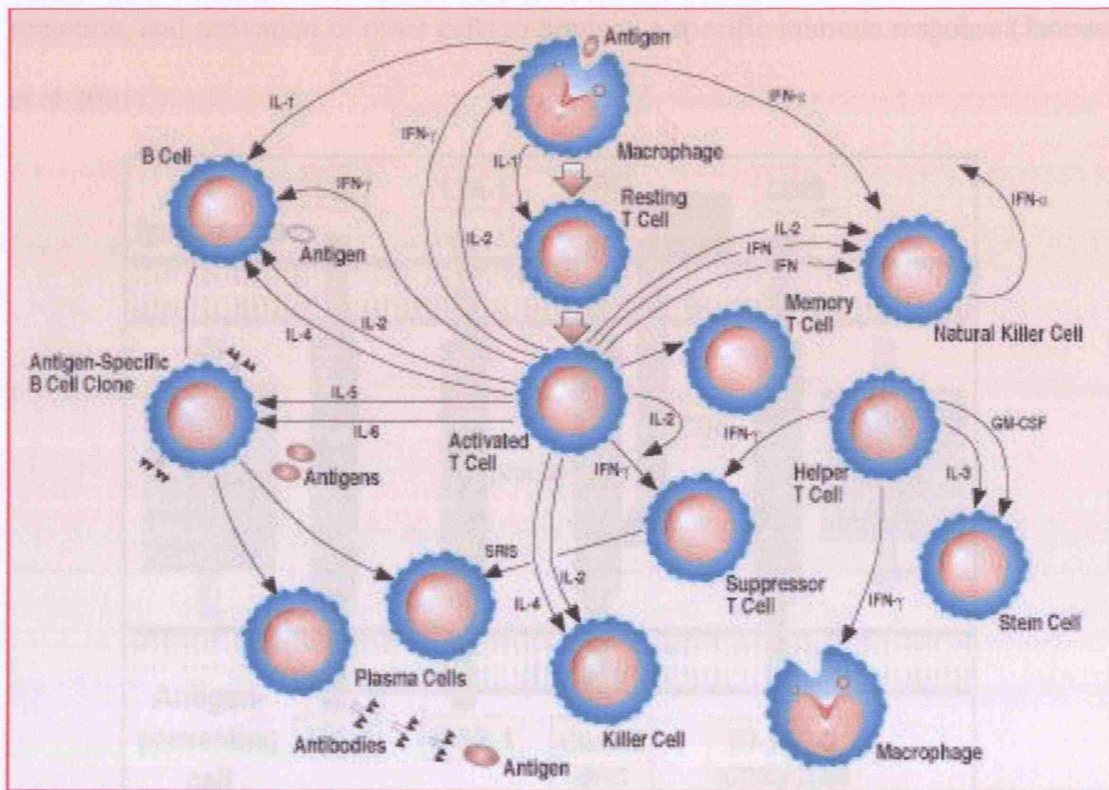
Figure 1.10 *Monocytes/macrophage play a central role in the initiation and stimulation of the specific immune response by interaction with T-cells and secretion of cytokines.*

From www. immuno-sci-lab.com

The antigen presentation capacity of monocytes is dependent on expression of class II

major histocompatibility complex (MHC) (de Waal et al 1991b, Rhodes et al 1986).

Within lysosomes, foreign antigens are digested and peptide fragments are coupled to

MHC class II vesicles to form MHC class II compartments (MIIC) (Janeway et al

2001). Interactions within the MIIC induce cleavage of a chaperone blocking protein

and allow binding of the bacterial derived peptides to MHC class II molecules. The

MIIC is recycled to the cell surface and presented to T-cells, which recognise the

foreign antigens in association with the class II MHC antigen. The receptors for the

class II MHC and the antigen binding site are CD4 and the T-cell receptor (TCR)

respectively (Figure 1.11). The T-cell receptor consists of α and β chains associated

with CD3. CD80 (a co-stimulatory molecule on the surface of the monocyte) stimulates CD28 on the surface of the T-cell. This causes CD4 +ve T-cell activation and cytokine secretion, and activation of other cells to produce a specific immune response (Janeway et al 2001).
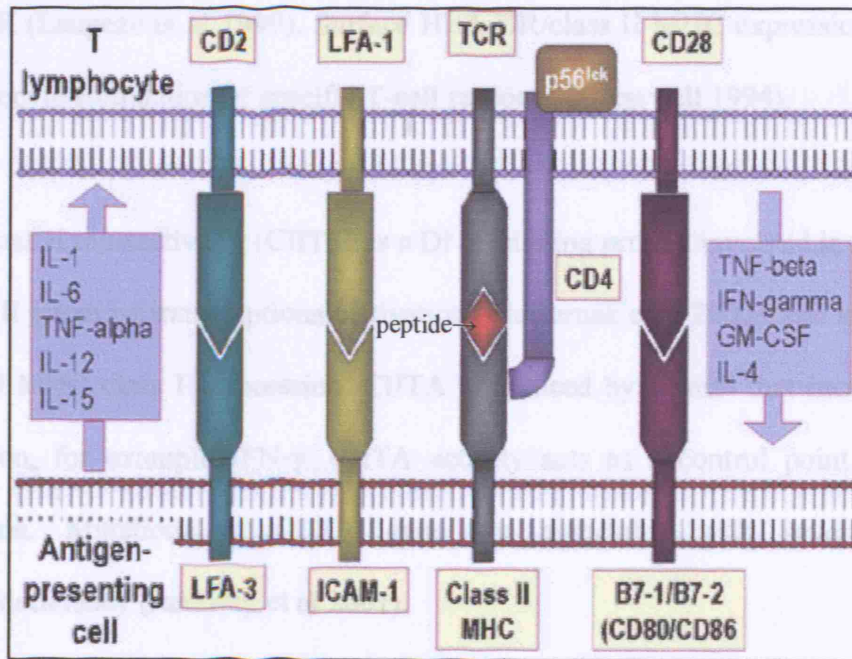
Figure 1.11 *Receptors involved in the interaction between T cells and antigen-presenting cells.*

Some cytokines produced are shown. Courtesy of www.med.sc.edu:85/ bowers/mhc.htm

Interaction between monocytes and T-cells further increase expression of stimulatory molecules on both cell types. Concomitant binding of the MHC II to CD4 and MHCII-peptide complex to TCR is required for maximal stimulation of the T-cell.

The MHC group of molecules is a group of glycoproteins coded for by Human Lymphocyte Antigen (HLA) genes, located on chromosome 6. Included at this locus are many genes involved in the immune response, including MHC class I and class II molecules. MHC class I molecules, expressed by all nucleated cells, are involved in recognition of self and non-self. MHC class II molecules, expressed only by cells of the

immune system, are involved in the interaction of antigen presentation cells and T-cells. MHC class II molecules consists of dimers alpha and beta glycoproteins non-covalently bound, which transverse the cell membrane. MHC class II molecules include HLA-DR, -DP and -DQ. The MHC class II antigen most abundantly expressed on monocytes is HLA-DR (Laupeze et al 1999). Surface HLA-DR/class II MHC expression is required for monocyte stimulation of specific T-cell response (Cresswell 1994).

MHC class II transactivator (CIITA) is a DNA binding protein involved in the induction of class II genes by transcriptional activation (Masternak et al 2000), and is required for maximal MHC class II expression. CIITA is induced by stimuli that increase class II expression, for example IFN-γ. CIITA activity acts as a control point for class II expression. Mutations in CIITA gene are associated with severe combined immunodeficiency (Janeway et al 2001).

1.7.5 Monocyte function in the postoperative period

As outline above, monocytes form the first line of the immune response, and play an important role in the stimulation of specific immunity by antigen presentation to helper T cells, thereby linking the innate and specific immune response. Foreign microbes in the blood stream encounter monocytes, and their interaction leads to killing, inflammatory response and stimulation of T cells. As mentioned above monocyte class II MHC expression is crucial to its antigen presentation and its T-cell stimulatory function.

Monocyte class II MHC/HLA-DR surface expression is decreased after sepsis (Hallwirth et al 2002b, Kanakoudi-Tsakalidou et al 2001, Schinkel et al 1998), trauma (Hershman et al 1990, Livingston et al 1988) and major surgery (Klava et al 1997b,

Kono et al 1995, Smith et al 2000, Spittler et al 2001, Zieren et al 2000). The decrease in monocyte HLA-DR expression is associated with defective antigen presentation and stimulation of T-cells (de Waal et al 1991b, Rhodes et al 1986). Decreased monocyte HLA-DR expression is associated with (Cheadle et al 1991, Hershman et al 1990, Kawasaki et al 2001, Schinkel et al 1998) and indeed predictive of post-operative infection (Allen et al 2002, Hallwirth et al 2002b). Studies have shown that the degree of monocyte HLA-DR expression is also related to clinical outcome (Gardiner et al 1994, Hershman et al 1990), and survival (Hershman et al 1988). Children who require prolonged postoperative periods of intensive care following cardiac surgery have significantly reduced levels of monocyte HLA-DR expression (Allen et al 2002). The reduced monocyte MHC class II expression is indicative of a state of immuneparesis in which the 'normal' protective inflammatory response to infection is impaired (Griscelli et al 1989, Polyak et al 1997).

The reasons for the decrease in monocyte class II expression are not fully understood. Monocyte HLA-DR expression is influenced by a number of cytokines including IL-10 and interferon-γ (Fumeaux & Pugin 2002, Klava et al 1997b, Livingston et al 1994). IL-10 inhibits pro-inflammatory cytokine release (de Waal et al 1991a) and has marked affects on specialised antigen presenting cells, including monocytes (de Waal et al 1991b). Increased levels of IL-10 can significantly decrease monocyte class II expression (Fumeaux & Pugin 2002), possibly by increased endocytosis of surface molecules. There is a correlation between postoperative levels of IL-10 and class II expression (Klava et al 1997b, Smith et al 2000). Although interferon-γ has been shown to up-regulate the expression of monocyte HLA-DR expression (Livingston et al 1994), the postoperative decrease in HLA-DR expression may be resistant to immunomodulation with interferon-γ (Klava et al 1997a).

Other investigators have focused on the role of glutamine levels on postoperative monocyte HLA-DR expression. Glutamine is an important nutrient for cells on the immune system (Exner et al 2002). *In vitro* monocytes cultured with low levels of glutamine had reduced HLA-DR expression and impaired stimulation of T cells (Spittler et al 1995b). Infusion of glutamine in the postoperative period is associated with a partial recovery in monocyte class II expression (Spittler et al 2001). The restoration was not complete, however, so other factors may also be involved. There may be a link between amino acid metabolism and monocyte HLA-DR expression in the postoperative period.

In parallel to the decrease in monocyte class II expression there are other immune suppressive effects on monocyte function in the postoperative period. There is a decrease in monocyte responsiveness to bacterial challenge *in vitro* (Haupt et al 1998, Kawasaki et al 2001, Klava et al 1997a). The reduction in the cytokine response to LPS in the postoperative period parallels the changes in class II expression (Kawasaki et al 2001). This hypo-responsiveness is thought to be due to the influence of IL-10 (Klava et al 1997b), or altered cell signalling through protein kinase C (Keel et al 1996). This combination of effects on monocytes in the postoperative period may be crucial to the well recognised increased risk of infection in the postoperative period. Recovery of monocyte responsiveness is associated with improved outcome (Hershman et al 1990). Patients succumbing to sepsis or SIRS often have a persistent and marked fall in monocyte responsiveness and class II expression (Allen et al 2002, Hershman et al 1990). Monocyte class II MHC expression may be an appropriate tool to assess the overall immune response to operative stress.

1.8   Laparoscopy and the stress response: possible effects of CO_2 metabolism

It has been assumed that laparoscopy reduces the metabolic and inflammatory response to operative stress in children. However, laparoscopy itself introduces new stresses that can alter the postoperative inflammatory response. The insufflation of CO_2 used to create a working space during laparoscopy can be associated with both mechanical and metabolic effects that alter the inflammatory response.

Gaseous insufflation of the abdomen to create a pneumoperitoneum is essential to perform laparoscopy both in children and adults. Although there have been studies investigating the role of abdominal wall lifting as a means of obtaining a working space for laparoscopy (Ishizuka et al 2000, Luks et al 1995), this has not proven to be a useful clinical tool at present. Different gases have been investigated, taking into account factors such as inertness, the tendency to cause gas embolism, combustibility and cost. Air has been shown to cause an exaggerated cytokine response compared to CO_2 (Tung & Smith 1999). CO_2 is the preferred gas for pneumoperitoneum because it is safe, non-combustible, inexpensive, and is least likely to cause embolism.

The absorption of CO_2 can lead to hypercapnia and acidosis (Bozkurt et al 1999, Ho et al 1995), while the increase in intra-abdominal pressure can lead to a decrease in lung compliance and reduced respiratory reserve (Manner et al 1998). Together, these changes can lead to deleterious side effects due to CO_2 retention. Understanding CO_2 metabolism is therefore crucial to evaluating the response to laparoscopic surgery.

1.8.1   Metabolic response to laparoscopy

There are a few studies on the metabolic response to laparoscopic surgery in adults. Only one study has characterised the change on resting energy expenditure in

postoperative period. In that study of adults undergoing cholecystectomy (Luo et al 2003), there was an increase in REE at 24 hours in both groups; the increase was significantly higher in the open group compared to the laparoscopic group. There are no studies on the metabolic response to laparoscopic surgery in children.

1.8.2 Systemic inflammatory and immune response to laparoscopy

The results of studies investigating the influence of laparoscopy on the immune response to surgery are mixed. Laparoscopy in adults has been shown to have an influence on some, but not all, of the immune responses to surgery (Carter & Whelan 2001, Gupta & Watson 2001). There seems to be less of a systemic IL-6 response after laparoscopy compared to open surgery (Wu et al 2003). CRP levels were not significantly different between the two approaches in one study (Wu et al 2003) but significantly lower after laparoscopy in others (Fornara et al 2000). The blunting of the other cytokines is not a consistent findings (Schwenk et al 2000, Zieren et al 2000).

1.8.3 Effect of laparoscopy on monocyte function

Although the interpretation of these findings are sometimes clouded by the fact that they were often non-randomised patients and the operations performed were not uniform, several studies have shown that laparoscopy preserved monocyte HLA-DR expression (Carlei et al 1999, Ordemann et al 2001, Schietroma et al 1998). In one randomised study in adults undergoing colorectal surgery there was a significant decrease in HLA-DR after both laparoscopic and open surgery, but this normalised earlier compared to open surgery (Wu et al 2003). However, there are a few contradicting studies. Klava *et al* found no significant difference in the postoperative suppression of monocyte expression, nor the level of their LPS responsiveness, between open and laparoscopic surgery (Klava et al 1997a). In a study of randomised Nissen fundoplication in adults,

Zieren found that both approaches were as suppressive on monocyte function (Zieren et al 2000). The overall verdict is still be awaited. There are no studies on the effect of laparoscopy on postoperative monocyte function in children.

1.8.4 Mechanical effects of CO_2 insufflation

Insufflation of CO_2 used during laparoscopy increases intra-abdominal pressure. The optimal intra-abdominal pressure for laparoscopy in children has been established to be between 8 and 12 mmHg (Sakka et al 2000). The increase in intra-abdominal pressure causes a rise in intra-thoracic pressure, which alters respiratory dynamics. There is an increase in airway pressures (Manner et al 1998) and a decrease in tidal volume. During laparoscopy in self-ventilating patients this translates into an increase in end-tidal CO_2 and arterial CO_2 tensions (Cheng et al 1999) that can lead to acidosis.

In children undergoing controlled ventilation there is generally a good correlation between end-tidal CO_2 and arterial CO_2 pressures ($PaCO_2$) (Cheng et al 1999, Laffon et al 1998). If ventilation parameters are maintained at pre-insufflation values, there is an increase in both end tidal CO_2 and $PaCO_2$ as intra-abdominal pressure increases. Occasionally there is an increase in $PaCO_2$ that is out of step with the increase in end-tidal CO_2 (Gehring et al 1998).

In addition to the respiratory effects of increased intra-abdominal pressure there are effects on the cardiovascular system. The cardiovascular effects of CO_2 insufflation have been characterised in animal experiments. Some of the effects are complex as there are different and interacting changes in the various cardiovascular compartments. Increased intra-abdominal pressure causes an increase in inferior vena cava pressure (Eisenhauer et al 1994, Giebler et al 1997, Ho et al 1992, Ortega et al 1996b), with

development of lower limb venous stasis. The decreased in inferior vena cava flow that results decreases cardiac preload and therefore cardiac output. Concomitantly, there is an increase in afterload on the heart as systemic blood pressure increases in response to the increased intra-abdominal pressure (Giebler et al 1997). This further decreases cardiac output. There is a compensatory tachycardia in an attempt to maintain cardiac output (Ho et al 1992). These findings in animals have been substantiated in paediatric studies (Gentili et al 2000).

There are also some effects of the CO_2 absorption in its own right, which may counteract some of the mechanical effects on the increase intra-abdominal pressure. Hypercapnia can lead to a generalized enhanced inotropic state mediated via increased sympathetic outflow (Ortega et al 1996b). CO_2 is also a potent vasodilator and may maintain constant arterial pressures by counteracting the increased vascular resistance secondary to the increased abdominal pressure.

1.8.5 CO_2 absorption from the abdominal cavity

CO_2 absorbed from the abdominal cavity during laparoscopy causes an increase in CO_2 elimination via the lungs. In adults there is usually a brief period of increase in CO_2 elimination after which a plateau is reached; usually after 10 to 30 minutes of insufflation (Mullett et al 1993). In a study by Blobner *et al* (Blobner et al 1993) there was a plateau in CO_2 that was approximately 35 ml/kg/min above preinsufflation. The length of CO_2 insufflation did not seem to influence the pattern of absorption.

There are, however, very different absorption profiles and mechanical effects between intra-peritoneal and retro-peritoneal insufflation of CO_2. Retro-peritoneal CO_2 insufflation is associated with a more rapid, greater and constant rise in CO_2 absorption

(Glascock et al 1996, Mullett et al 1993). This difference has been attributed to an increase in the absorption area during extra-peritoneal insufflation as CO_2 dissects through subcutaneous tissues. There have been no studies to directly characterise the change in CO_2 elimination in children.

The peritoneal reaction to insufflated CO_2 may also influence the postoperative response. Local acidosis at the peritoneal level may be responsible for shoulder tip pain commonly described postoperatively. This local acidosis may stimulate the inflammatory response and lead to alteration in the postoperative metabolic response.

CHAPTER 2

Materials and Methods

2.1   Materials

2.1.1   Chemicals

Chemical	Source
[1-^{13}C] L-Leucine (99.5%)	Cambridge Isotope Laboratories, Massachusetts, USA
[^{13}C] NaHCO$_3$ (99.5%)	Cambridge Isotope Laboratories, Massachusetts, USA
Bovine Serum Albumin (Fraction V)	Sigma, Poole, UK
Butylated Hydroxytoluene	Sigma, Poole, UK
Cell Fix (1% formaldehyde, 0.1% sodium azide)	BD Bioscience, Oxford, UK
Cell Lysis	BD Bioscience, Oxford, UK
Citric acid	Sigma, Poole, UK
Ethanol	Sigma, Poole, UK
Heparin 5000U/ml (preservative free)	CP Pharmaceuticals, Wrexam, UK
Hydrogen Peroxide (H$_2$O$_2$)	Sigma, Poole, UK
KH$_2$PO$_4$	BDH Merck, Poole, UK
KHCO$_3$	BDH Merck, Poole, UK
KCl	BDH Merck, Poole , UK
KOH	Sigma, Poole, UK
Methanol	VWR Scientific, Poole, UK
Methanol (HPLC grade)	Fisher Scientific
Malondialdehyde Tetrabutylammonium	Fluka Chemicals, Switzerland
Sodium Chloride (NaCl)	BDH Merck, Poole , UK
Sulphuric Acid	BDH Merck, Poole , UK
Sodium hydrogen phosphate (Na$_2$HPO$_4$.H$_2$O)	Sigma, Poole, UK
Phosphate buffered saline (PBS) tablets	Oxoid, Basingstoke, UK

Chemical	Source
Phosphoric acid (85%)	VWR Scientific, Poole, UK
Tetramethylbenzidine (TMB) tablets	Sigma, Poole, UK
Thiobarbituric acid (TBA)	Sigma, Poole, UK
Tween 20 (Polyoxethylene sorbitan monolaurate)	Sigma, Poole, UK

2.1.2   ELISA Kits

All antibodies and reagents for cytokine ELISA Cytosets™ were purchased in a kit form, from Biosource® Europe. These included the respective capture and detection antibodies, recombinant human cytokines for standards and Streptavidin-Horseradish Peroxidase.

2.1.3   Solutions and buffers for ELISA

All solutions were made up to 1000ml in Milli-Q water unless otherwise indicated.

Buffer or Solution	Composition	Concentration	pH
Blocking Solution	Coating Buffer (as below)		7.4
	Bovine Serum Albumin	0.5% (5g/litre)	
Citrate Buffer	Citric Acid	0.1 M (21g/litre)	5.0
Coating Buffer (Phosphate buffered saline)	Sodium Chloride	140 mM (8g/litre)	7.4
	Potassium Chloride	2.7 mM (0.2g/litre)	
	di-Sodium hydrogen phosphate	8.0 mM (1.42g/litre)	
	Potassium di-hydrogen phosphate	1.5mM (0.2g/litre) 1	
Diluent	Blocking Solution (as above)		7.4
	Tween 20	0.01% (v/v)	
Phosphate Buffer	di-Sodium hydrogen phosphate	0.2 M (28.5g/litre)	5.2
Phosphate Buffered saline (PBS) 1%	PBS tablet	1 per 100 MilliQ	

Phosphate Citrate buffer	Citrate	0.05M	5
	Phosphate	0.025M	
Stop Solution	H_2SO_4	1.8 N	
TMB substrate buffer	Phosphate/Citrate buffer	10ml	
	TMB	1mg	
	30% hydrogen peroxide	2µl	
Wash Buffer	Sodium Chloride	0.15M	7.4
	Tween 20	0.01% (v/v)	

2.1.4 Antibodies for flow cytometry

Antibody/fluorochrome (clone)	Manufacturer	Isotype
Anti-Human CD14/PE (TUK4)	DAKO	Mouse IgG 1
Anti-Human HLA-DP/DQ/DR FITC (CR3/43)	DAKO	Mouse IgG 1
Anti-Human CD64/PE-Cy5	DAKO	Mouse IgG 1
Mouse IgG 1 control/FITC	DAKO	

2.1.5 General equipment and consumables

Equipment	Supplier
Bijou (5ml)	SLS, Wilford, Nottingham, UK
Biohit repeating dispenser	Alpha Laboratories, UK
Dynatech MRX ELISA plate reader	Dynex technologies, UK
FACScalibur Flow Cytometer	Becton Dickinson (BD Bioscience) Oxford, UK
FACScan Research and Lysis II Software	Becton Dickinson (BD Bioscience) Oxford, UK
FACs tubes	Becton Dickinson (BD Bioscience) Oxford, UK
Falcon tubes (5ml)	BD Falcon, BD Bioscience, Oxford, UK
Falcon tubes (15ml)	TPP, Trasadingden, Switzerland

Equipment	Supplier
Falcon tubes (50ml)	TPP, Trasadingden, Switzerland
Finnipipette multichannel dispenser	Life Sciences International
Maxisorb ELISA plates (96 well)	Nalgene NUNC, Rochester, NY, USA
Mikrotek ELISA Software	Dynatech Laboratories, Chantilly, VA, USA

2.2 Methods

The methods in this chapter describe procedures performed by myself and are central to the main theme in the thesis, or those used throughout the thesis. Methods specific to other particular measurements are given in relevant chapters. The first part of this chapter describes the protocol and the design of a randomised trial used throughout Chapters 5 to 8.

2.2.1 Protocol for randomised trial of laparoscopy vs. laparotomy for Nissen fundoplication

A randomised control trial was designed to test the hypothesis that laparoscopic surgery is associated with dampening of the metabolic, endocrine and inflammatory response to surgery. Nissen fundoplication is an operation of moderate severity that is commonly performed by both laparoscopic and open methods. It was therefore chosen as the operation to study to test this hypothesis.

2.2.1.1 Patients and allocation into groups

Parents of children undergoing Nissen fundoplication for gastro-oesophageal reflux were approached for inclusion in this trial; those included were randomly allocated to laparoscopic or open procedure. Patients with sepsis, multi-organ dysfunction syndrome, cardiac, renal, immunological or metabolic abnormalities were excluded. Children requiring O_2 therapy were also excluded.

Patients were randomised to either laparotomy or laparoscopy by minimisation, using the computer program Minim® (Department of Clinical Epidemiology, London Hospital Medical School). Minimisation criteria were:

1. Age

- 1 month to 3 years

- 3 year to 6 years

- > 6 years

2. Neurological status

  - Normal

  - Impaired

3. Operating surgeon

  - EMK

  - DPD/JC

  - AP

4. Presence of major congenital gastrointestinal abnormities.

Minimisation is a method of randomisation, described as the platinum standard for randomised controlled trials (Treasure & MacRae 1998). The primary objective of minimisation is to ensure that all other factors that might influence the outcome will be equally represented in the two groups, leaving the treatment under test as the only dissimilarity. Briefly, the randomisation is biased so the minimisation criteria are fairly evenly distributed between the groups. All the minimisation criteria in this trial were equally weighted. Patients who were converted from laparoscopy to laparotomy after allocation were excluded from the analysis.

2.2.1.2   Power calculation

The primary outcome measure was REE between patients undergoing laparoscopic Nissen and those undergoing open procedure. REE data obtained from previous studies children was used in the power calculation (Jones et al 1994). Detection of a difference of 1 standard deviation in the peak REE level between groups, using a significance level

of 5%, required 16 and 21 per group for 80 and 90% power respectively. We therefore aimed to recruit 40 patients.

2.2.1.3 Preoperative management

Preoperatively, patients were fasted for at least 6 hours according to standard clinical practice. Last clear fluid intake was allowed up to 4 hours preoperatively. Thereafter a period of 'nil by mouth' was instituted; during which patients received a continuous infusion of 5% glucose and 0.18% saline. This infusion was given at normal maintenance rate, and was continued until operation.

2.2.1.4 Intraoperative management/Anaesthesia

The surgical techniques of laparoscopic and open Nissen fundoplication were standardised as were the techniques of general anaesthesia and postoperative analgesia. General anaesthesia was by inhalation of isofluorane/O_2 mixture or intravenous (propofol and fentanyl) induction. The trachea was intubated following muscle relaxation with atracurium. Intraoperatively a balanced anaesthetic technique using fentanyl (1-5 µg/kg) and isoflurane was used. A Servo 900C ventilator (Servo, Siemens, Sweden) was used in all children. General anaesthesia was maintained with a mixture of air, O_2 and 0.5 to 2% isoflurane inhalation. Muscle relaxation was maintained during the procedure with supplemental atracurium.

2.2.1.5 Blinding

Postoperatively parents, ward nurses, acute pain team nurses and nurses involved in feeding were blinded to patient allocation. An occlusive dressing was used to hide the operative site in the postoperative period.

2.2.1.6 Pain management

Post operative pain management was standardised. The total amount of analgesia given during the study period was recorded and hourly pain assessments were made using standardised pain assessment tools validated in children. In infants less than 6 years of age (i.e. groups 1 and 2) and in neurologically impaired children the pain scoring tool used was the FLACC Pain Assessment Tool (Table 2.1) which incorporates five categories of pain behaviors: facial expression; leg movement; activity; cry; and consolability (Merkel et al 2002, Merkel et al 1997).

Face	Legs	Activity	Cry	Consolability	Score
Smile	Normal position, relaxed	Quiet, moves easily	No cry	Content and relaxed	0
Occasional grimace or frown, disinterested	Uneasy, restless, tense	Squirming shifting, tense	Moans or whispers, occasional complaint	Reassured by touching or being talked to, distractible	1
Frequent quivering chin, clenched jaw	Kicking, legs drawn up	Arched, rigid or jerking	Steady cry, screams or sobs, frequent complaints	Difficult to console or comfort	2

Table 2.1 *Components of the FLACC scoring system*

In children older than 6 (group 3) a Linear Visual Analogue Scoring was used (Wong & Baker 1988).

Postoperatively, a standardised infusion of morphine was commenced, and supplementary morphine was given according to pain assessments. In groups 1 and 2 the morphine infusion was given as a nurse controlled analgesia (NCA) infusion; 10 $\mu g/kg/hr$ continuous infusion, 20 $\mu g/kg$ bolus and 20 minute lockout periods. In group 3 patient controlled analgesia (PCA) infusion was used; 4 $\mu g/kg/hr$ continuous infusion: 20 $\mu g/kg$ bolus with 5 minute lockout period. Paracetamol 15mg/kg/6hr and diclofenac 1mg/kg/8hr were given regularly to all patients for 48 hours then continued as needed. All neurologically impaired children were managed with NCA. All nurses and acute pain team members involved in postoperative pain relief management were blinded to patient allocation.

2.2.1.7   Postoperative fluid management and feeding

Postoperatively, intravenous fluids were standardised to 75% maintenance; providing a glucose intake of between 2 and 3 g/kg/day for the first 24 hours in all patients.

Nurses blinded to patient allocation were responsible for postoperative feeding. A postoperative feeding regimen was used in all patients. Any complications that did not allow for use of the regimen (e.g. ileus, intestinal obstruction) dictated individualised management as clinically indicated. Patients were considered to be fully fed when they were taking their full dietary requirements enterally without complications. Time to full enteral feeds was recorded.

Patients were discharged when they were fully fed and medically stable. The operative complications (i.e. bleeding, intestinal perforation, visceral injury) and postoperative complications (wound infection, bronchopneumonia, dysphagia, gas bloating, retching, vomiting) were recorded. The duration of hospital stay was recorded.

2.2.1.8 Measurement protocol

Resting energy expenditure was measured by computerised indirect calorimetry (Section 2.3) in the preoperative period, at 4 hours and at 24 hours postoperatively.

The components of whole-body protein turnover (flux, oxidation and breakdown) were measured by giving a continuous infusion of $[1\text{-}^{13}C]$ leucine from 4 hours before the operation and at 4 hours postoperatively (Section 2.4).

The following indices were measured preoperatively, immediately postoperatively, and postoperatively at 4 hours, 24 hours and 48 hours: insulin, cortisol, catecholamines, glucose and lactate for endocrine/metabolic markers and pro- and anti-inflammatory cytokines (IL-1ra, IL-6, IL-10 and TNF-α; Section 2.5), monocyte class II MHC expression (Section 2.6), and free-radical production (malondialdehyde, nitrate and nitrite; Chapter 7) as inflammatory markers. The overall study protocol is shown in Figure 2.1.

PREOPERATIVE

POSTOPERATIVE

Figure 2.1 *Protocol for patient investigations during randomised control trial*

2.3   Principles of indirect calorimetry measurements

Indirect calorimetry allows measurement of whole body CO_2 production ($\dot{V}CO_2$) and O_2 consumption ($\dot{V}O_2$) from respiratory gas exchange, and calculation of whole body energy expenditure. The essential assumption is that, under steady state conditions, respiratory gas exchange is in equilibrium with gas exchange at the cellular level (Figure 2.2). It is also assumed that all the O_2 is used to oxidize fuel and that all CO_2 produced is recovered. There is virtually no O_2 reserve in the body, so changes in whole body O_2 metabolism are followed very quickly by changes in measured $\dot{V}O_2$. Under normal conditions of relatively steady CO_2 production, the same is true for $\dot{V}CO_2$. Therefore, under steady state conditions, respiratory gas exchange is a measure of energy production (Ferrannini 1988). Energy production equals energy expenditure at steady state.
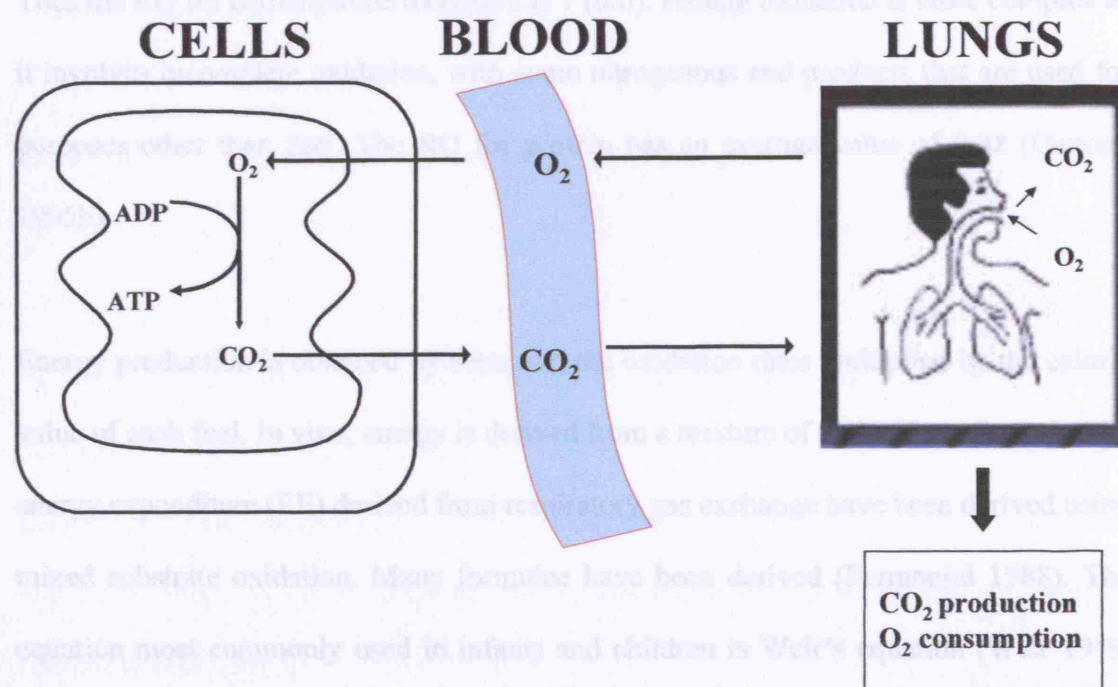
Figure 2.2 *Outline of the principles of indirect calorimetry*

Measured $\dot{V}CO_2$ and $\dot{V}O_2$ are also used to calculate the respiratory quotient (RQ), defined as $\dot{V}CO_2/\dot{V}O_2$. RQ is a measure of substrate utilisation. Substrates are oxidised to water and CO_2, utilising O_2 in proportions that depend on the fuel used. When fat is used as the substrate, and assuming complete oxidation of both fatty acid and glycerol, the oxidative reaction is:

$$2 \, C_{51}H_{99}O_6 + 145 \, O_2 \rightarrow 102 \, CO_2 + 98 \, H_2O$$
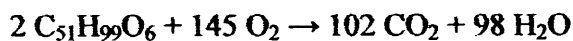
Thus, the RQ for this reaction is 0.7 (102/145). If carbohydrate is used as the sole fuel the equation for the oxidative reaction becomes:

$$C_6H_{12}O_6 + 6 \, O_2 \rightarrow 6 \, CO_2 + 6 \, H_2O$$
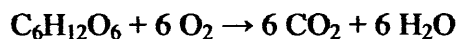
Thus the RQ for carbohydrate oxidation is 1 (6/6). Protein oxidation is more complex as it involves incomplete oxidation, with some nitrogenous end products that are used for purposes other than fuel. The RQ for protein has an average value of 0.82 (Ganong 1995b).

Energy production is obtained by summing the oxidation rates multiplied by the caloric value of each fuel. In vivo, energy is derived from a mixture of fuels. Thus, formulae for energy expenditure (EE) derived from respiratory gas exchange have been derived using mixed substrate oxidation. Many formulae have been derived (Ferrannini 1988). The equation most commonly used in infants and children is Weir's equation (Weir 1949, Wells 1998), which states:

$$EE = 3.941 \ \dot{V}O_2 + 1.106 \ \dot{V}CO_2 - 2.17 \ U_N$$

where U_N is the urinary nitrogen excretion.

Calculating EE from $\dot{V}O_2$ and $\dot{V}CO_2$ only, without U_N measurement, introduces an error of less than 4% (Ferrannini 1988). In infants and children nitrogen excretion is very low (Winthrop et al 1989) and varies little. Energy production can also be estimated from $\dot{V}O_2$ only, as $\dot{V}O_2$ is the main determinant of EE. The approximate energy liberated from one litre of O_2 is 4.82 kcal (Ganong 1995b, Heim 1985).

Under unusual physiological conditions such as gluconeogenesis, protein oxidation (specifically alanine), lipogenesis and ketone body metabolism, there are corrections that need to be introduced in the calculation of substrate utilisation. However, the effect on ignoring these corrections in calculating EE is minimal (Ferrannini 1988), and such conditions are unlikely to have prevailed during this study.

2.3.1   Deltatrac II indirect calorimetry

Respiratory gas exchange was analysed using the computerised indirect calorimeter Deltatrac® II (Datex-Ohmeda, Finland). The Deltatrac® calorimeter has the advantage of being portable, and results are reproducible and accurate once basic conditions are adhered to. Indirect calorimetry has been compared to both doubly labelled H_2O infusion and mass spectrometry as means of measuring energy expenditure. Calorimetry has been found to be as accurate and reproducible if conditions are standardised (Bodamer et al 1997).

The Deltatrac® II is an open system metabolic monitor that measures respiratory gas exchange and calculates EE and RQ. The Deltatrac® II indirect calorimeter measures CO_2 production and O_2 consumption at 1 minute intervals. An inspiratory sampling line samples inspired air. Expiratory gases are diluted into a known constant flow created by a flow generator. A paramagnetic O_2 analyser and an infrared CO_2 analyser compare the inspired and expired air to measure O_2 and CO_2 concentrations in the down-stream gas. Both these sensors are well validated and tested. The fast, differential paramagnetic O_2 sensor has a response time of 150 ms, and requires little maintenance. The calorimeter is accurate with inspired O_2 concentrations of up to 70% in the ventilator mode and 50% in the canopy mode.

The calorimeter is allowed to warm up for at least 30 minutes before any measurement or calibration is performed, according to the manufacturer recommendation. The indirect calorimeter was validated and the flow rate calibrated by burning bench alcohol of a known volume (see below).

According to manufacturer specification the flow generator was adjusted for patients' weight in the following fashion:

Weight(Kg)	Range	Deltatrac II Flow
< 3	Baby	3 L/min
3-20	Child	12 L/min
20-120	Adult	40 L/min

Ventilator Measurements

In the ventilator mode, expired air is first led to a 4 litre mixing chamber to form the mixed expired O_2 and CO_2 concentrations. An inspiratory sampling line is connected to the inspiratory arm of the ventilator. The mixing chamber of the calorimeter is connected by a 22 mm tube to the outlet of the ventilator to collect and analyse the patient's expired air. This expired air leaving the mixing chamber is mixed with room air so that the total flow is a known constant. A continuous measurement of CO_2 concentration yields $\dot{V}CO_2$ from the equation:

$$\dot{V}CO_2 = Q \times F^*CO_2$$

Where;

Q = flow;

$F^*CO_2 = CO_2$ concentration in the constant flow

RQ is calculated from the gas fractions by the following equation:

RQ = [1- FiO$_2$] / [((FiO$_2$ - FeO$_2$)/ FeCO$_2$) - FiO$_2$]

where:

FeO$_2$ = mixed expired O_2 concentration

FeCO$_2$ = mixed expired CO_2 concentration

FiO$_2$ = inspired O_2 concentration

After $\dot{V}CO_2$ and RQ have been calculated $\dot{V}O_2$ is obtained from the following equation:

$$\dot{V}O_2 = \dot{V}CO_2 / RQ$$

Canopy Measurements

Spontaneously breathing patients were studied in the canopy mode. The head and upper torso of the patient is covered with a transparent plastic canopy. The outlet of the canopy is connected to the flow generator of the calorimeter via a 22 mm tube. All expired air is collected into the constant flow. The inspiratory-expiratory O_2 difference is measured continuously with a differential O_2 sensor.

$\dot{V}CO_2$ and $\dot{V}O_2$ in mls/min and EE in kcal/24hr are calculated using the following equations:

$$\dot{V}CO_2 = Q \times FDCO_2$$

$$\dot{V}O_2 = [Q/(1- FiO_2)] \times [FDO_2 - (FiO_2 \times FDCO_2)]$$

$$RQ = \dot{V}CO_2 / \dot{V}O_2$$

$$EE = 5.50 \, \dot{V}O_2 + 1.76 \, \dot{V}CO_2 - 1.99 \, U_N$$

Where;

Q = flow;

$FDCO_2$ is the mean value of the difference between the CO_2 concentration in the constant flow and inspired CO_2 concentration each minute;

FiO_2 = inspired O_2 concentration;

FDO_2 is the mean value of the difference between FiO_2 and mixed expired O_2 concentration each minute;

U_N = Urinary nitrogen excretion in grams/24 hrs

Digital output from the calorimeter was collected via the serial output to a laptop computer. The data was stored using Collect® program (Datex-Engstrom, Instrumentarium Corp., Helsinki, Finland) as a Lotus 1-2-3 file, and data analysed using Microsoft Excel® program.

During experiments in this thesis, EE was calculated individually for each patient using Weir's equation, rather than the automatic output from the calorimeter. Urinary nitrogen excretion was not measured. However it has been shown that the error from neglecting the contribution from urinary nitrogen in children is small, and shows little variation between individuals (Weir 1949, Wells 1998). EE was therefore estimated from $\dot{V}CO_2$ and $\dot{V}O_2$ alone using the formula:

$$EE = 3.941 \ \dot{V}O_2 + 1.106 \ \dot{V}CO_2.$$

2.3.2   Calibration of the indirect calorimeter

The following maintenance calibrations were performed regularly on the Deltatrac® II calorimeter, at the periods recommended by the manufacturer.

2.3.2.1   Gas Calibration

The gas analysis system of the indirect calorimeter was calibrated before every measurement using recommended gas mixture supplied by the manufacturer. After the initial warming period, a standard calibration gas mixture of 95% O_2 and 5% CO_2 (Datex-Ohmeda, Finland) was fed through the sampling line at standard temperature and pressure. The calorimeter was allowed to reach steady state, and measured O_2 and CO_2 concentrations were then adjusted for actual concentrations in the calibration gas.

If variations between measured and actual concentrations were more than 5%, the calibration was repeated.

2.3.2.2 Flow Calibration

Flow calibration was performed monthly using an alcohol burning kit, in accordance with manufacturer recommendation. A known volume of absolute ethanol (99.7%) was burnt to complete oxidation beneath the canopy of the burning kit.

The total amount of CO_2 produced was calculated from the amount of ethanol combusted. The amount of CO_2 produced from a known volume of ethanol can be calculated from the following equation:

$$C_2H_5OH + 3\ O_2 \rightarrow 2\ CO_2 + 3\ H_2O$$
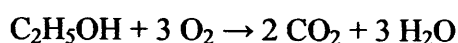
The total amount of CO_2 measured by the calorimeter can be calibrated against this calculated value. The measured CO_2 is a product of the flow and the fractional CO_2 difference over the study period. Thus the correct measurement of CO_2 is directly proportional to the flow generated.

Flow calibration was performed with the flow rate set at 40 L/min. The calorimeter was allowed to warm up and gas calibration was performed. Five mls of 99.7% ethanol was combusted in the alcohol burning set, which was connected to the flow generator of the calorimeter by a 50 mm corrugated tube. The alcohol was burnt to completion (until the measured $\dot{V}CO_2$ was zero). The total amount of CO_2 measured by the calorimeter is a total of the minute-by-minute $\dot{V}CO_2$ measurements.

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An example of the readings from a calibration experiment is shown in Figure 2.3. At standard temperature and pressure, the volume of CO_2 derived from 5 mls of 99.75% ethanol is 3808 mls. In this calibration, the total amount of $\dot{V}CO_2$ measured by the calorimeter was 3757 mls. The error in measurement of the flow rate was 1%. This was within the manufacturer recommendation of ± 5%; therefore no change in flow rate was made. Throughout calibration experiments done in this work, flow calibrations were within the recommended ± 5% of the set flow rate.

2.3.2.3 RQ calibration

The calorimeter was also calibrated for RQ as recommended by the manufacturer, using the alcohol burning set up described for flow calibration above. For the reaction with ethanol the RQ is 0.67. Measured RQ for calibration experiments were performed by burning 5 mls of ethanol. The measured RQ for the last five minutes of recordings was within 0.64 to 0.69 in accordance with manufacturer's specifications.

Figure 2.3 *Calorimeter reading during an example of flow calibration.*

2.4   Measurement of protein turnover

Protein turnover was measured by a primed stable isotopic infusion of ^{13}C leucine, using a simplified two pool model of protein dynamics (Wolfe 1992), which assumes that the plasma free amino acid pool is in continuous and instantaneous equilibrium with the intracellular pool, which together compose a single homogenous (metabolic) pool exchanging with body protein (Figure 2.4).
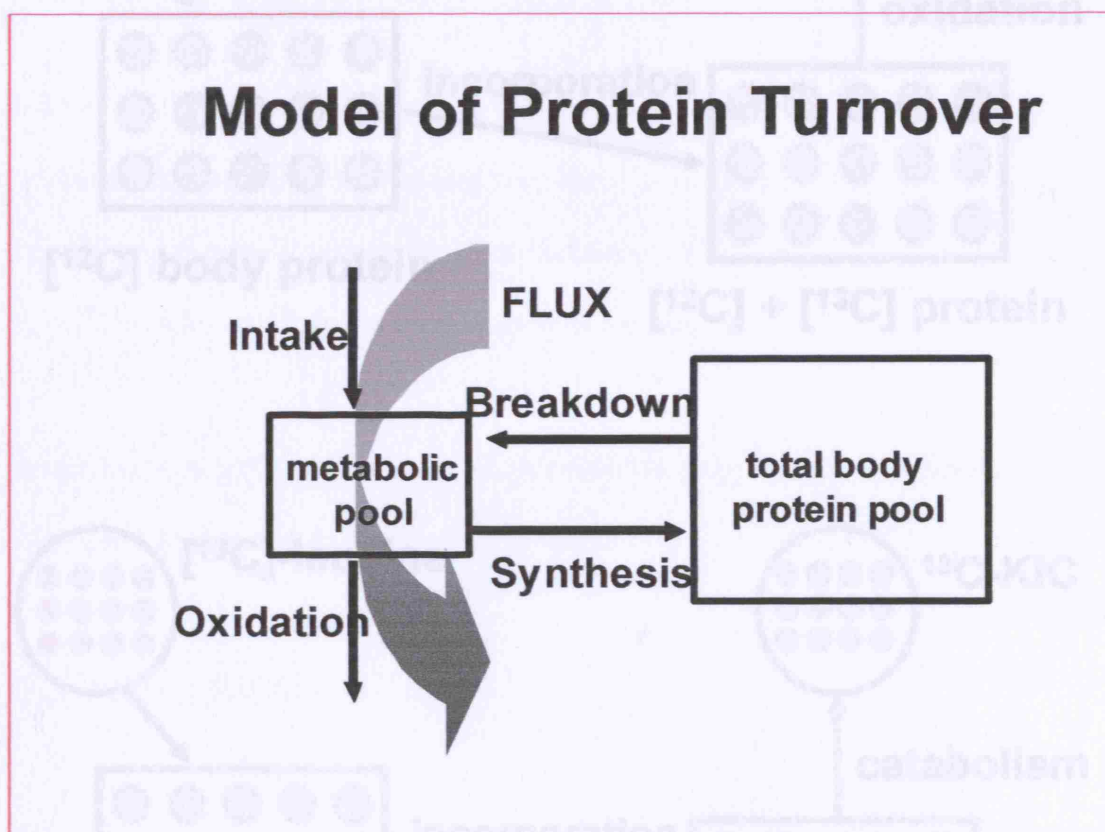
Figure 2.4 *Two pool model of protein turnover used in this study.*

Leucine enters the free amino acid pool from the diet or other exogenous source (I_{leu}) and from the catabolism of body protein (C_{leu}), and leaves by protein synthesis (S_{leu}) and oxidation (O_{leu}; Figure 2.5). At steady state:

Leucine Flux (Q_{leu}) = I_{leu} + C_{leu} = S_{leu} + O_{leu}
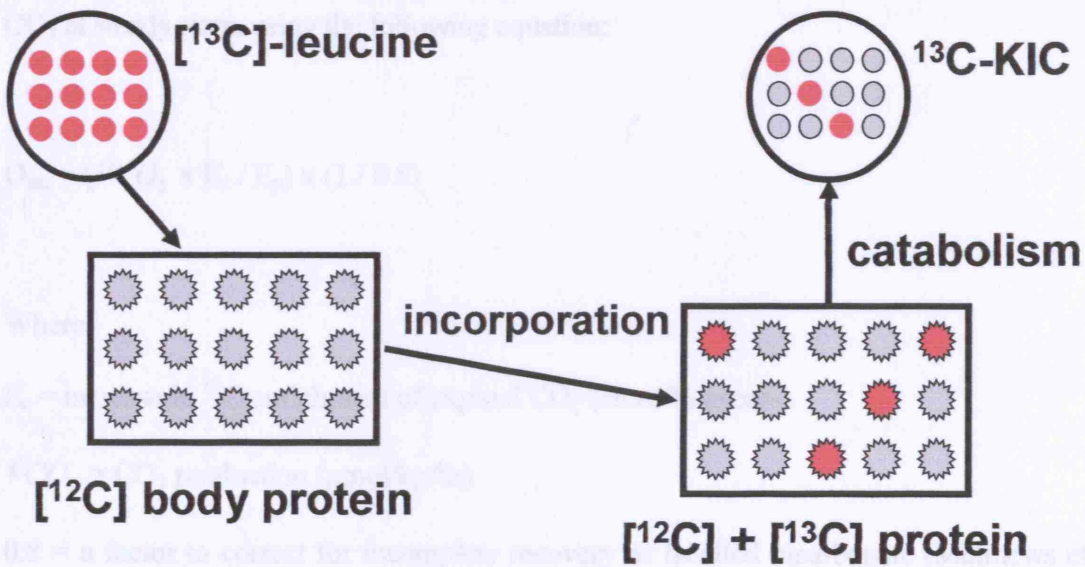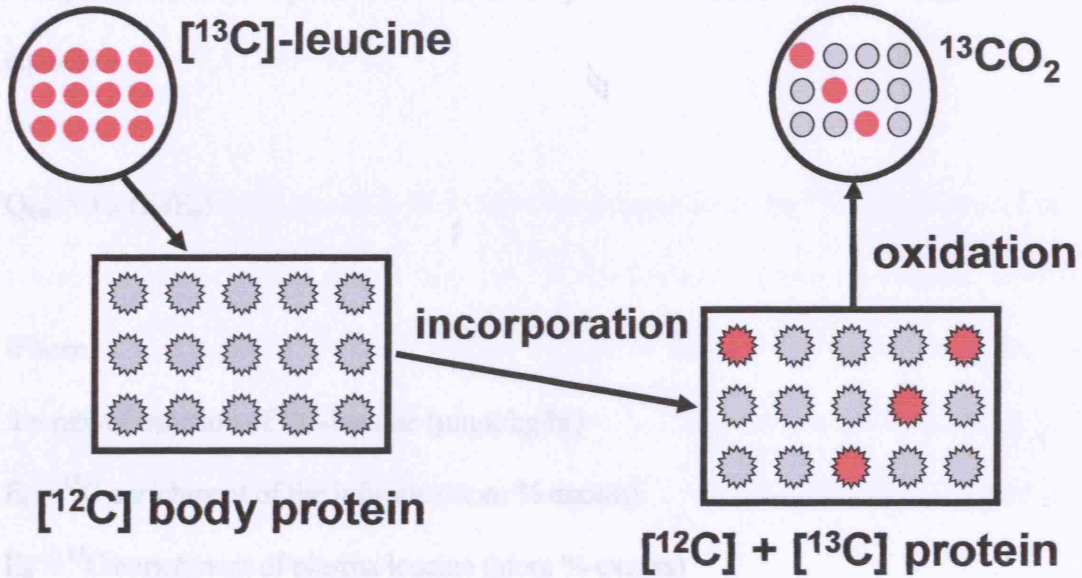
Figure 2.5 *Components of whole body protein oxidation and whole body protein catabolism after* ^{13}*C-leucine infusion.*

In the fasted state, with a constant infusion of leucine, I_{leu} is equal to rate of infusion as there is no other exogenous source of leucine. Q_{leu} ($\mu mol/kg/hr$) can be calculated from the isotopic enrichment of plasma leucine once a plateau has been reached, using the following equation:

$$Q_{leu} = i \times (E_i/E_p)$$

Where

i = rate of infusion of ^{13}C-leucine ($\mu mol/kg/hr$)

E_i = ^{13}C enrichment of the infusate (atom % excess)

E_p = ^{13}C enrichment of plasma leucine (atom % excess)

Leucine oxidation (O_{leu}) ($\mu mol/kg/hr$) is calculated from the isotopic enrichment of expired CO_2 at steady state, using the following equation:

$$O_{leu} = (\dot{V}CO_2 \times E_c / E_p) \times (1 / 0.8)$$

Where

E_c = increase in ^{13}C enrichment of expired CO_2 (atom % excess)

$\dot{V}CO_2$ = CO_2 production ($\mu mol/kg/hr$)

0.8 = a factor to correct for incomplete recovery of labelled bicarbonate (Matthews et al 1980, Van Aerde et al 1985).

Thus, since I_{leu}, i, O_{leu} and Q_{leu} are known, S_{leu} and C_{leu} can be calculated. Leucine is assumed to constitute 8% of the total protein pool; therefore oxidation and catabolism can be calculated for total body protein (Beaufrere et al 1990, de Benoist et al 1984, Mitton et

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al 1991). Whole body protein catabolism (C_{prot}; g/kg/hr) was therefore calculated from C_{leu} using the formula:

$$C_{prot} = C_{leu} \times 131.17/\ 10^6 \times 0.08.$$

Protein dynamics are calculated in an identical manner from the ^{13}C enrichment of plasma α-ketoisocaproate (KIC) rather than the ^{13}C enrichment of plasma leucine. KIC is an intracellular metabolite of leucine (Figure 2.6), is not derived from any other source, and is in equilibrium with plasma KIC (Wolfe et al 1982). Thus enrichment of KIC is thought to provide a more accurate representation of intracellular leucine metabolism (Matthews et al 1982, Schwenk et al 1985, Vazquez et al 1986).
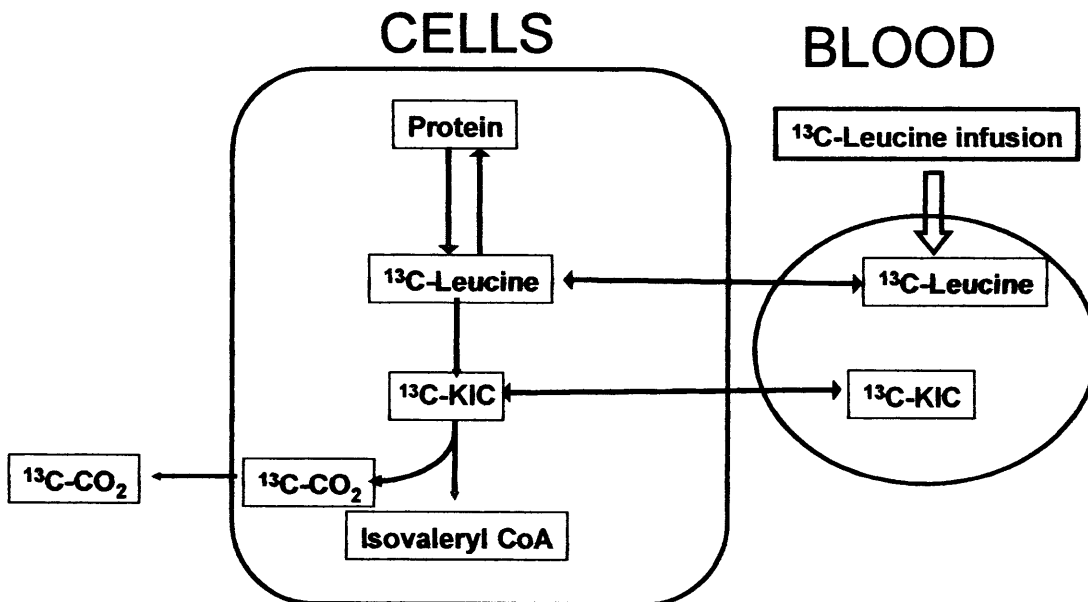
Figure 2.6 *Intracellular metabolism of leucine and KIC.*
This shows their interchangeable nature. KIC is however more indicative of intracellular metabolism.

Stable isotopic infusions were made from the powders supplied by Northwick Park Hospital, Pharmacy Department. Solutions were tested to ensure they were pyrogen free, and stability testing was done routinely to measure enrichment. After baseline blood and breath samples were taken, a loading dose of 99.5 % enriched ^{13}C-NaHCO$_3$ (6.9 μmol/kg) was given at the beginning of each phase of the study. This is given to saturate the ^{13}CO$_2$ pool in the body and facilitate measurement of enriched breath ^{13}CO$_2$. 99.5% enriched leucine was infused at a rate of 7.57 μmol/kg/hr. After a 3 hour infusion, steady state was confirmed with triplicate blood and breath samples fifteen minutes apart. Indirect calorimetry was performed during the period of sampling to determine $\dot{V}CO_2$, and enable calculation of protein oxidation.

Blood samples were collected into EDTA bottles and immediately centrifuged at 1200 rpm for 10 minutes and the supernatant frozen at −70°C until analysis. Breath samples were collected by a face mask and placed into a sealed glass container until analysis.

Protein turnover was assessed in the immediate preoperative period and in the immediate (4 hours) postoperative period (Figure 2.7). In contrast to most studies that use a continuous infusion across the intraoperative period, a time lapse of six hours was allowed between the pre- and postoperative infusions to reduce the effect of isotope recycling. A repeat baseline sample was taken to assess enrichment.
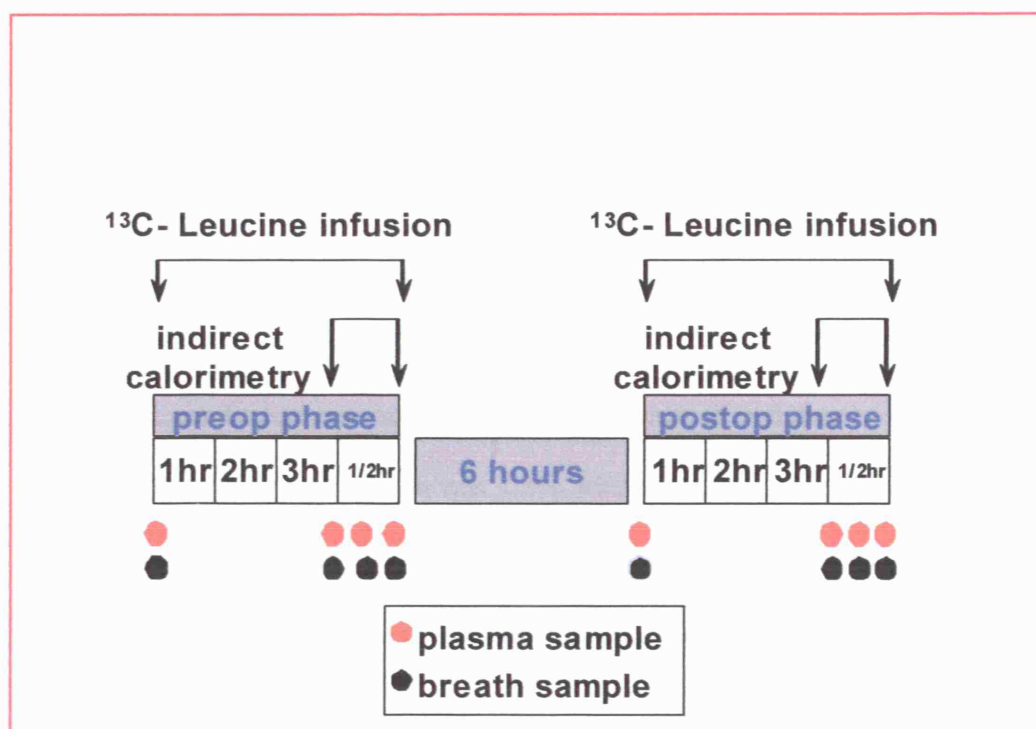
Figure 2.7 *Study design for protein turnover studies.*

2.4.1   Measurement of KIC enrichment

Enrichment of plasma ^{13}C-KIC was measured by gas chromatography mass spectroscopy (GC/MS) Virgillio Carnielli, Padova, Italy. Plasma ^{13}C enrichment of α-KIC was measured in triplicate by GC/MS: KIC was obtained from 50 μL of plasma, deproteinized by adding sulfosalycilic acid (6% w/v), after the addition of ketocaproic acid as internal standard (KCA, Sigma, Milan, Italy). KIC plasma concentration and enrichment were measured by GC-MS quadrupole (MD800, Thermoquest) operating in electron impact. Selective ion monitoring was carried out at m/z 259, 260, and 261 for natural abundance and 262 for the isotopic enrichment (Cogo et al 2002). The intra-assay variability between samples was less than 1%. Therefore, at each time point, atom percent excess compared to baseline was calculated using the formula (Wolfe 1992):

$$APE = M_1 - M_0 / (1 + (M_1 - M_0) \times 100.$$

where

APE = atom percent excess of sample

M_1 = mass percent excess of sample at steady state

M_0 = mass percent excess of baseline.

Steady state conditions were accepted if the coefficient of variation of the three time points during the fourth hour of infusion was $\leq 5\%$. Mean atom percent excess at steady state was then used to calculate leucine flux. The enrichment of ^{13}C-leucine infusion used was 99.5% and was given at a rate of 7.57 μmol/kg/hr. Therefore the equation for leucine flux becomes:

$$Q_{leu} = 7.57 \times (99.5 / E_p)$$

2.4.2 Measurement of CO_2 enrichment

Enrichment of breath $^{13}CO_2$ was analysed using gas chromatography isotope ratio mass spectroscopy. Samples were assessed in triplicate. 40μl of air from the breath sample tubes was injected with a 10:1 split into split-splitless injection of a GC with a PLOT column run isothermally at 70°C. Elution time of CO_2 was 205 seconds. Before analysis of each sample, 3 pulses of reference gas (99.95% CO_2, BOC gases, UK) were introduced into the mass spectrometer. A typical example of a mass spectroscopy trace is shown in Figure 2.8. The peaks for the $^{12}CO_2$ and $^{13}CO_2$ of each sample were then analysed using Isodat® software. The expired gas was analysed for the ratio of $^{13}CO_2/^{12}CO_2$ (molecular weight 45/44) in parts per million (ppm) with reference to standard $^{12}CO_2$ reference gas. The coefficient of variation between measurements of the repeated samples was < 5%.

APE of the expired $^{13}CO_2$ was then calculated form the formula:

$$APE = (0.0013 \times {}^{13}CO_2 \text{ ppm}) + 0.016526$$

where 0.016526 is an empirically derived factor to convert for the ${}^{13}CO_2/{}^{12}CO_2$ enrichment in the reference gas.

Mean ${}^{13}CO_2$ enrichment was then calculated from the 3 time points. $\dot{V}CO_2$ was measured by indirect calorimetry as previously described.
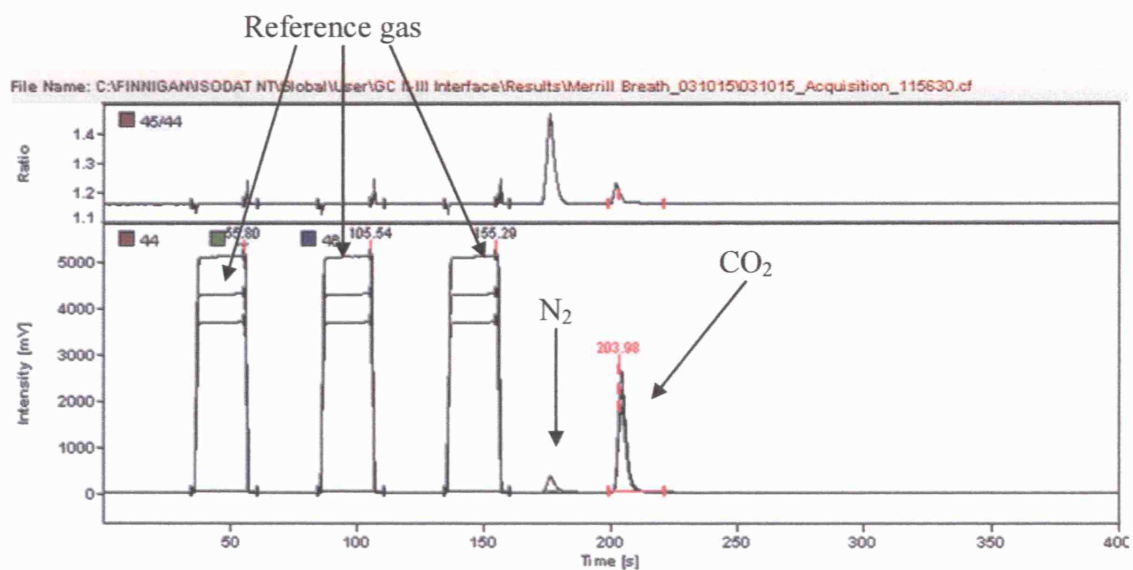
Figure 2.8 *Mass spectroscopy recording of expired ${}^{13}CO_2$ measurement*

${}^{13}CO_2$ enrichment was also analysed independently by Dr V. Carnielli laboratories using isotope ratio mass spectroscopy. Values obtained using the two laboratories were significantly correlated ($r^2 = 0.88$, $p < 0.0001$; Figure 2.9). Significant ${}^{13}C$ enrichment was not detected in expired CO_2 from the study patients, despite the enrichment of KIC achieved in plasma. Therefore protein oxidation could not be calculated.
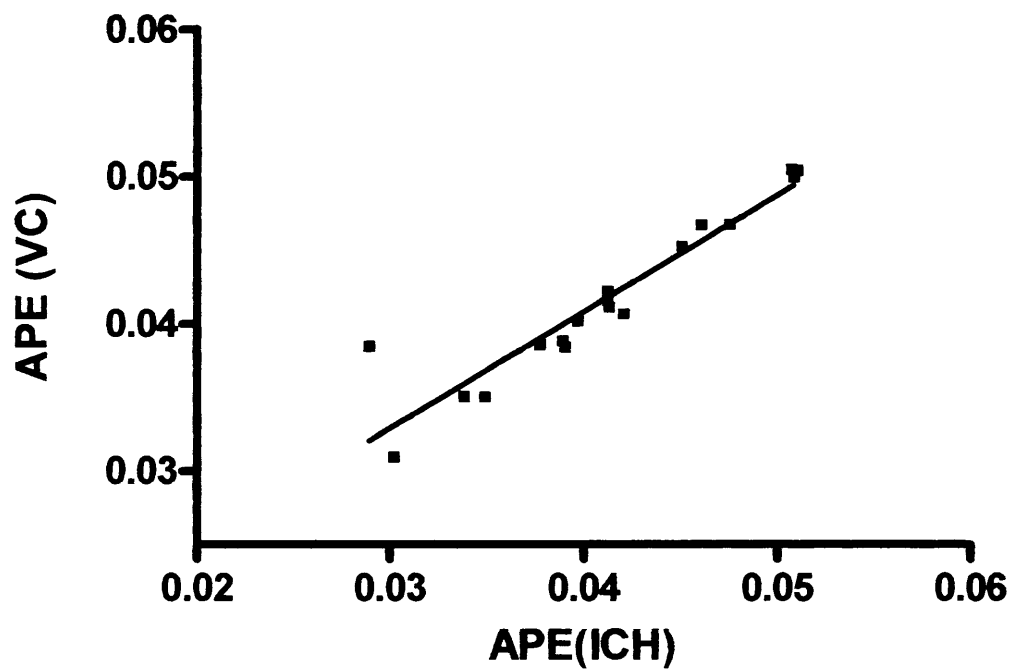
Figure 2.9 $^{13}CO_2$ enrichment as measured separately in two different labs

2.5 Measurement of plasma cytokine levels.

The cytokines IL-1ra, IL-6, IL-10 and TNF-α plasma levels were measured by highly sensitive enzyme-linked immunosorbent assays (ELISA).

2.5.1 Blood sampling and handling

Blood for cytokine analysis was taken from a peripheral vein into heparin containers (final concentration 10 IU/ml of blood) and immediately centrifuged at 3000 rpm for 5 minutes and the supernatant stored at -80°C until analysis. All cytokines were measured from each sample at the same visit to avoid freeze-thaw effects on cytokine levels. Also all samples for the same patient were analysed on the same occasion.

2.5.2 ELISA protocol

Cytokine levels were analysed using Biosource Cytosets® ELISA Kits, using a sandwich technique.

The Protocol used was as follows:

Day 1 High binding 96 well Maxisorb ELISA plates were filled with 100 μL/well of the respective capture antibody, diluted in Buffer A, at a concentration of 1 μg/ml (except TNF-α which was diluted to 5μg/ml). This was left to incubate overnight at 4°C and covered to prevent drying.

Day 2 Plates were washed twice using wash buffer and blot dried. 300 μL of Block solution was added to each well and incubated at room temperature for 2 hours on a rocker. Plates were then washed (four times), and to each well was added 100 μL of standard and samples in respective wells and left overnight in the dark at 4°C. This

incubation period was longer than the recommended 2 hours. Longer incubation time was used to maximise the discrimination of samples in the lower range. A substrate blank (in duplicate) was included by adding 100 μL of diluent only (without standards or sample). Samples were diluted 1:10 in standard diluent for IL-1ra and used 1:1 for the other cytokines. Standard concentrations were serially diluted 1:2 in diluent. Starting concentrations for standards were as follows;

IL-1ra   5,000 pg/ml

IL-6   10,000 pg/ml

IL-10   4,000 pg/ml

TNF-α   10,000 pg/ml.

Day 3 Plates were washed four times then blot dried. 100 μL of the relevant biotinylated detection antibody (0.4μg/ml) was added to each well, and allowed to incubate for 2 hours on a rocking platform. Plates were again washed four times. To each well was added 100 μL of Streptavidin-Horseradish Peroxidase 1/2500 (Streptavidin-HRP) and left to incubate for 45 minutes in the dark at room temperature, on a rocker. Plates were again washed four times and 50 μL of tetramethylbenzidine (TMB) was added and left to incubate for 30 to 60 minutes in the dark at room temperature until optimal optical density was obtained for individual plates. 50 μL of Stop solution was then added to each well, and plates were read within 15 minutes.

Plates were read using Dynatech MRX ELISA plate reader (Dynex technologies, UK) and analysed with Revelation® software. Plates were read at a wavelength of 450 nm with a reference at 650 nm. A standard curve was plotted using the mean of the optical densities (OD) for each standard vs. the standard concentrations, using a sigmoid curve

with data extrapolation. Mean optical density for each duplicate sample was then read from the standard curve to calculate the plasma concentration.

The lower limits of detection for the respective assays are as follows:

IL-1ra   50 pg/ml

IL-6   20 pg/ml

IL-10   20 pg/ml

TNF-α 10 pg/ml

Representative standard curves for cytokine ELISAs (optical density OD *vs.* concentration) are shown below (Figures 2.10 to 2.13).
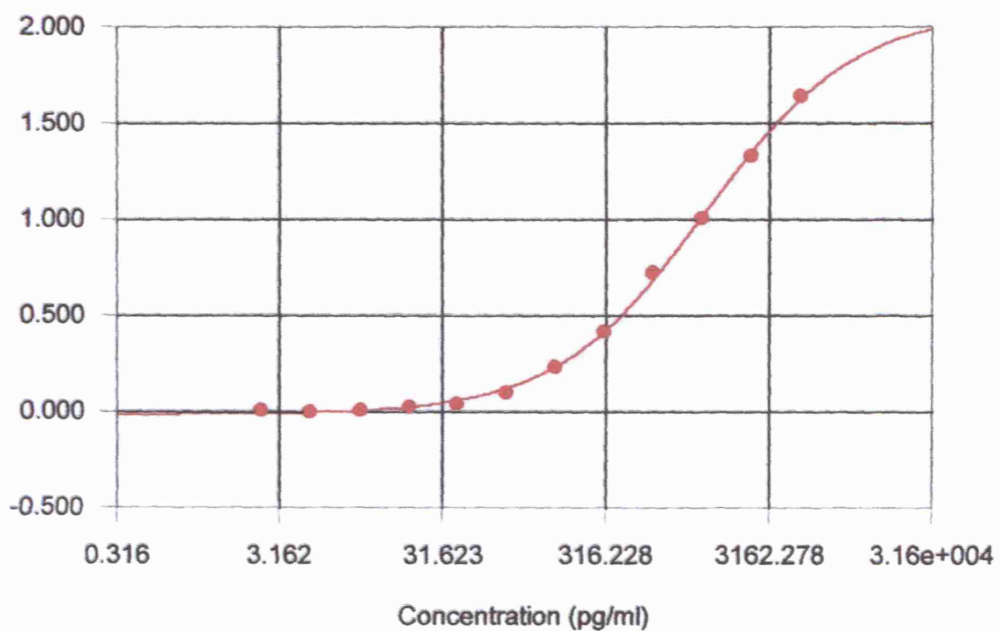
Figure 2.10 *Standard curve for IL-1ra ELISA*
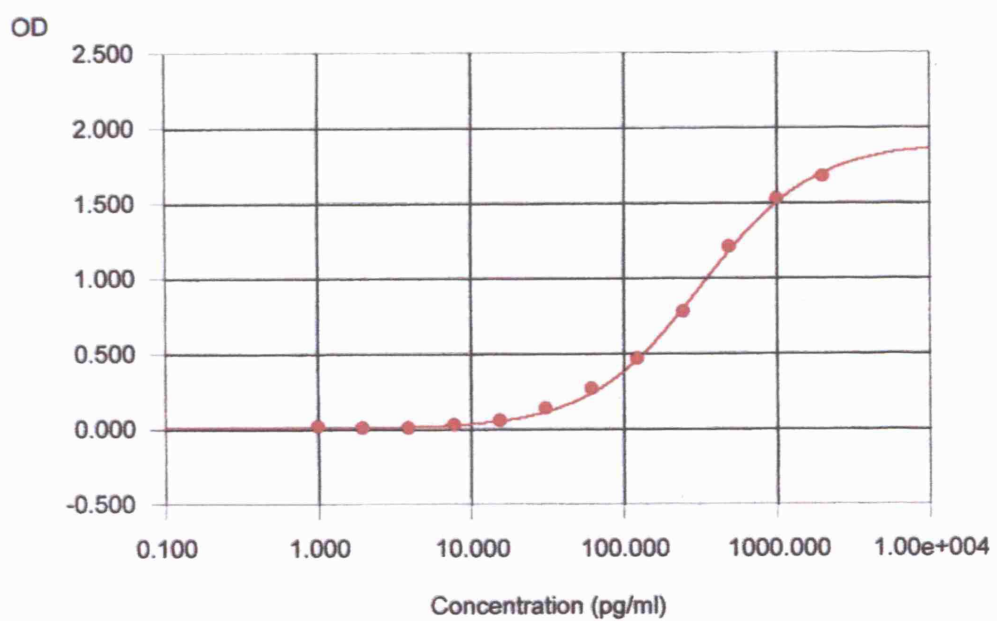
Figure 2.11 *Standard curve for IL-10 ELISA*

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OD
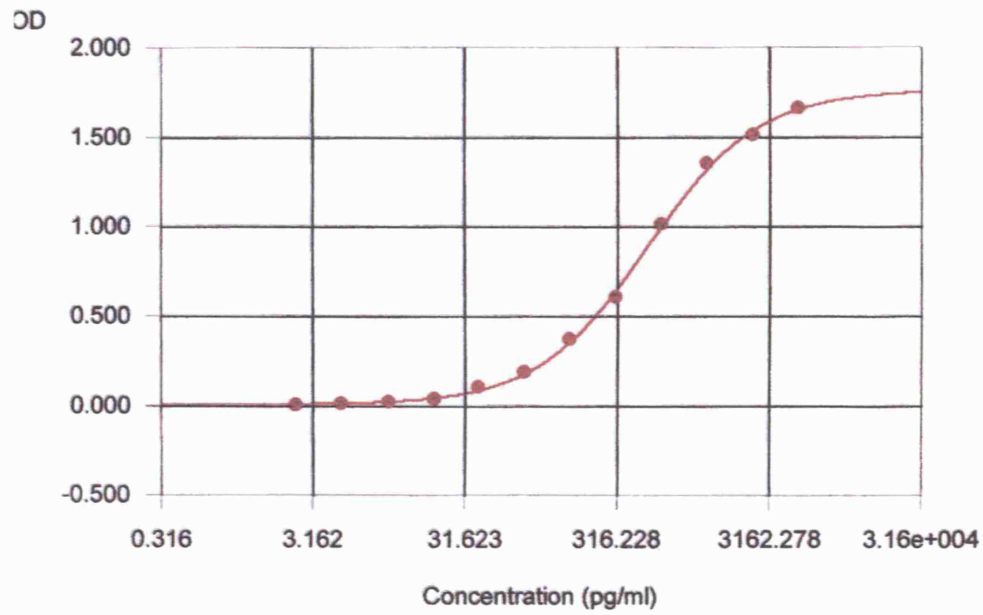
Figure 2.12 *Standard curve for IL-6 ELISA*
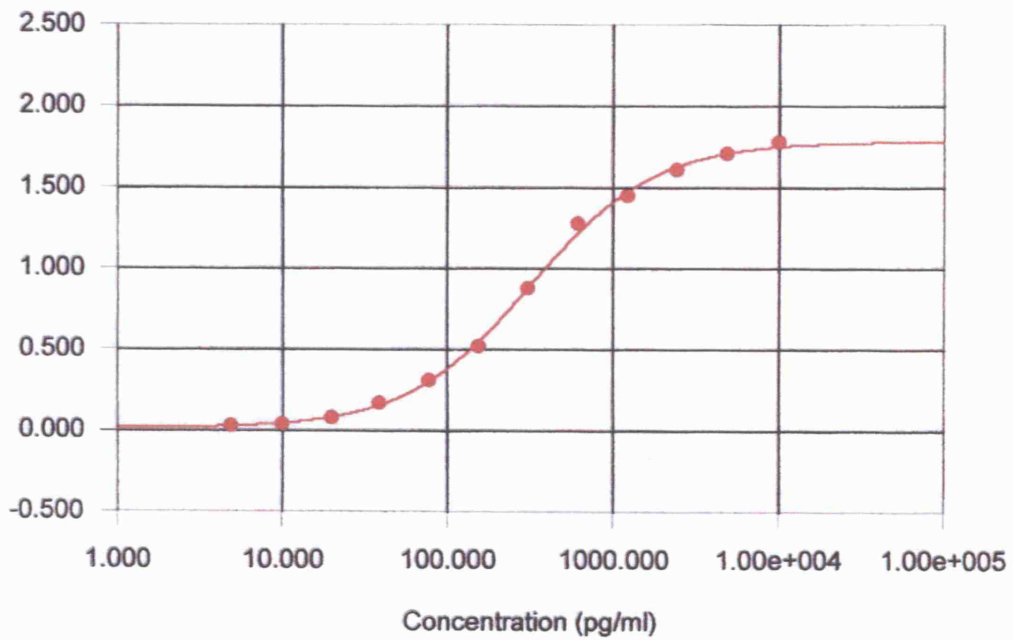
Figure 2.13 *Standard curve for TNF-α ELISA*

2.6   Flow cytometric analysis of monocyte class II MHC expression

2.6.1   Flow cytometry and fluorescence labelling

Flow cytometry allows simultaneous rapid measurement of multiple physical characteristics of single cells in suspension. Addition of fluorescent dyes coupled to antibodies against epitopes on the cell allows measurement of relative concentration of that epitope or visualisation by microscopy. The use of fluorescence in flow cytometry is described here.

The fluorescent dyes used during these experiments were:

- Fluorescein Isothiocyanate (FITC) excites at 488nm and emits at 525 nm (green). This is detected in FL1 on the cytometer.

- R-Phycoerytherin (R-PE) excites at 488 nm and emits at 575 nm (orange). This is detected in FL2 in the cytometer.

- Phycoerytherin Cy5 (Cy5) excites at 488 nm and emits at 650 nm and is detected in FL3 in the cytometer.

Fluorescent dyes used for flow cytometry were directly conjugated to specific monoclonal antibodies.

Figure 2.14 below shows a dot plot flow cytometry recording of peripheral white blood cells. Each dot represents the forward scatter (FSC) and side scatter (SSC) of a single cell on a logarithmic scale. SSC is a measure of cell granularity while FSC is a measure of cell size. The largest, most granular, cells represent granulocytes. The population of smallest and least granular cells represent lymphocytes. The middle population of cells are monocytes.
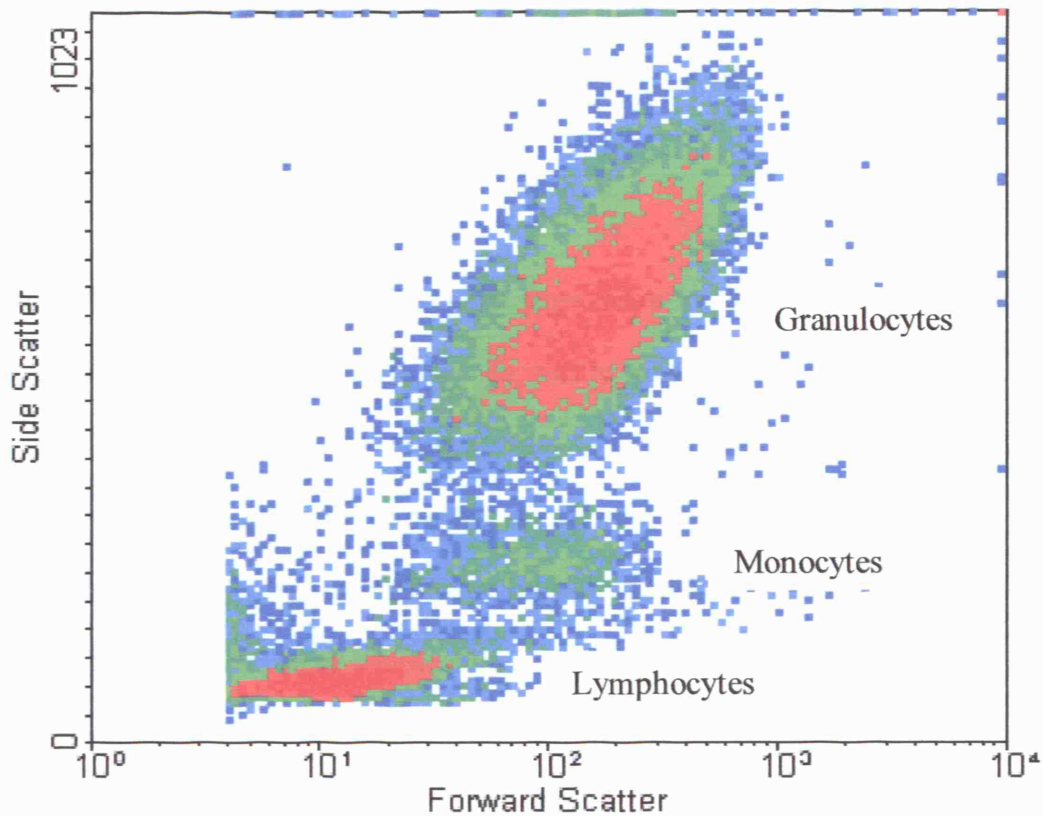
Figure 2.14 *Flow cytometric recording of peripheral white blood cells*

Electronic compensation on the cytometer was set to reduce spectral overlap between fluorophores using control negative and positive signals to generate appropriate regions on a dot plot. FITC appears primarily in FL1, but some of its fluorescence overlaps into FL2. Adjusting the FL2 – FL1% subtracts this overlap. The converse also holds for PE emissions. By adjusting the gain appropriately, the amount of spectral overlap between detectors is reduced.

Flow cytometry was performed using Becton Dickinson FACSCalibur® analyser and data acquired and analysed using Cell Quest® software. The three detectors in the flow cytometer (FL1, FL2 and FL3) optimally detect wavelengths of 530μm (green), 585μm

(orange) and 650μm (red) respectively. The FACSCalibur® was calibrated weekly using standardised fluorescent beads. This was undertaken with CaliBrite3 Beads, (Becton Dickinson, UK). These were analysed with the automated calibration programme, FACSComp.

2.6.2 Monocyte class II MHC surface expression by flow cytometry.

Heparinised whole blood, collected in sterile containers (see Section 2.6.1), was prepared within 15 minutes to minimise artefacts from monocyte adherence and artificial stimulation of cells. Experiments performed showed that there was an increase in staining for MHC surface expression if unstimulated whole blood was left for 1 hour on the bench top, at room temperature. MHC expression increased as times elapsed, with significantly increased expression at 4 to 5 hours.

A dual staining technique with negative gating was used to determine monocyte MHC expression. 50μl of whole blood was incubated with 5μl of R-PE conjugated antibody to CD14 and FITC conjugated antibody to HLA-DR/DP/DQ for 10 minutes at room temperature in the dark. Red cells were then lysed by incubating with 1ml FACS Lysing solution in the dark for 10 minutes. The samples were then washed and centrifuged at 3000 rpm, and the cell pellet fixed in Cell Fix. Non-specific staining (control for MHC class II expression) was determined by staining 50μl of whole blood with mouse FITC conjugated IgG1 monoclonal antibody. This negative control isotype antibody is raised against *Aspergillus niger* glucose oxidase, and allows measurement on non-specific (Fc portion) binding of antibody for gating purposes.

Cells were analyzed on the flow cytometer within 24 hours of staining, as it was shown that there was a significant reduction in MHC class II molecules if samples were left for

more than 24 hours post-staining. During set up experiments whole blood was collected from 3 healthy volunteers and stained with primary conjugated monoclonal antibodies to CD14 and MHC class II. Following lysis and fixation, each sample was analysed immediately and then daily for 7 days on the flow cytometer. Both the percentage positive fluorescence and the median fluorescence intensity of MHC class II surface expression were noted. Class II expression was stable in the first 24 hours post staining, but then showed a decrease thereafter (Figure 2.15).
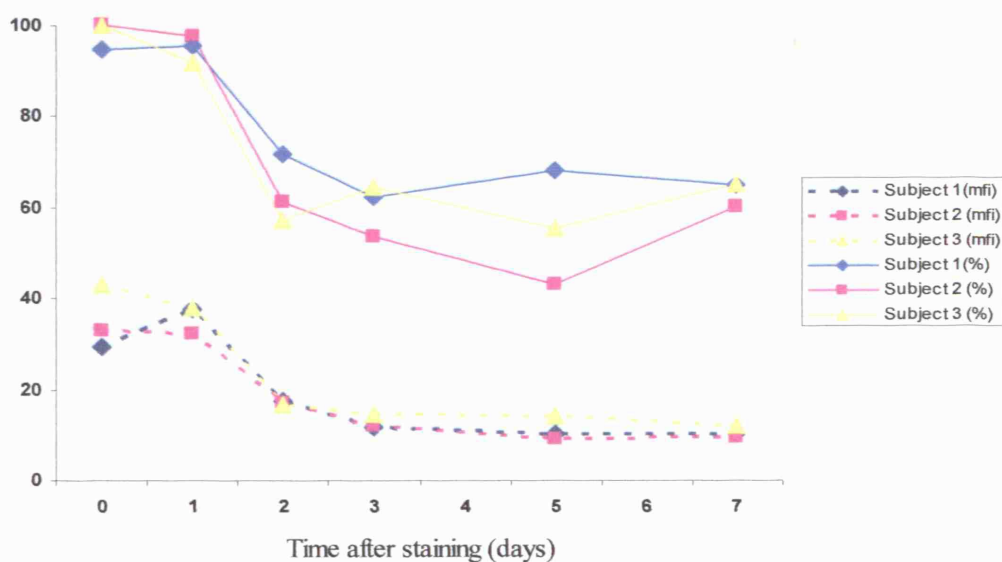
Figure 2.15 *Time course of surface expression of MHC Class II on whole blood after staining.*

In addition to the SSC *vs.* FSC dot plot shown in Figure 2.14, monocyte identification was also confirmed using CD14 fluorescence *vs.* SSC plot. Figure 2.16 shows a dot plot of CD14 expression against SSC. The region marked R1 represents CD14 positive cells and represents the cells identified as monocytes. These CD14 monocytes were then analysed for MHC expression. A minimum of 2500 monocyte events were analysed.
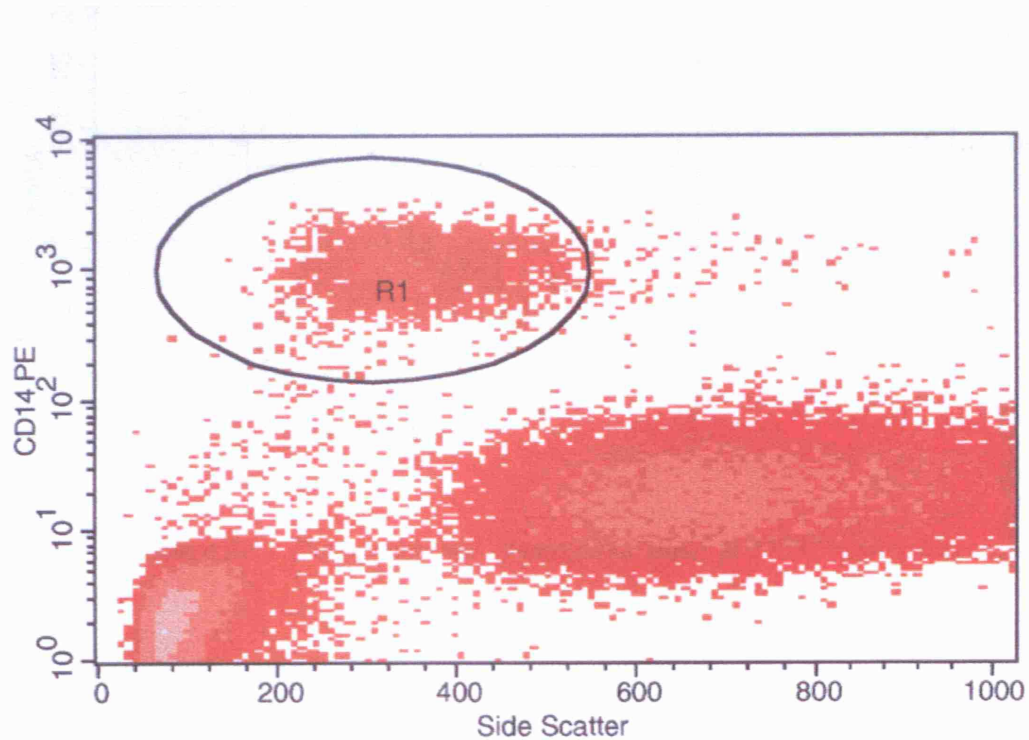
Figure 2.16 *Dot plot of CD14 expression against SSC after fluorescent staining.*

The histogram in Figure 2.17 shows monocyte MHC expression in the same patient at 2 time points. The region marked M1 contained cell that were MHC positive as defined by the control. MHC was expressed as median fluorescence intensity (MFI) of all monocytes or as the percentage of monocytes with positive fluorescence. As can be seen in this example, there was a decrease in monocyte MHC expression between preoperative and 24 hours postoperatively.
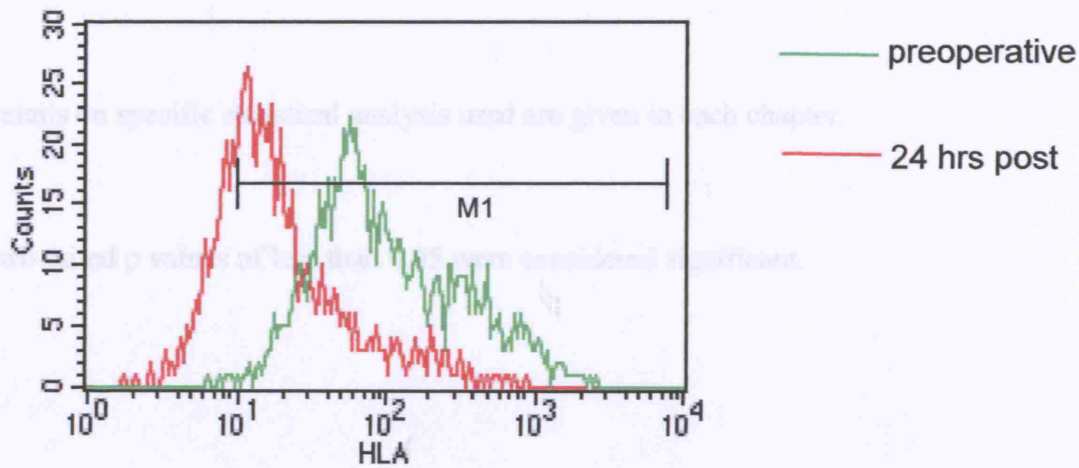
Figure 2.17 *Histogram plot of monocyte class II MHC expression*

2.7 Statistical analysis

Data was stored and analysed using SPSS for Windows (SPSS Inc., Chicago, Illinois) or GraphPad Prism software (GraphPad Prism software Inc, San Diego, CA) programs. Estimates are given with 95% confidence intervals where appropriate. Data between time points were analysed using repeated measures ANOVA with post hoc tests.

Time series data for curves were also analysed using a series of multilevel models (MLwiN®; Centre for Multilevel Modelling, Institute of Education, University of London). For these models skewed data were normalised appropriately before analysis. Outcomes were compared over time between treatment groups with correction for age, weight, operation length, and operation group (see appendix for details). These models investigated the differences in trajectories between patients allocated to open and laparoscopy.

Correlations were analysed using linear regression. Differences in correlations between groups were tested using linear regression using laparoscopic and open interactions built into the model.

Details on specific statistical analysis used are given in each chapter.

Two-tailed p values of less than 0.05 were considered significant.

CHAPTER 3

Carbon Dioxide Elimination During Laparoscopy in

Children.

3.1   Introduction

Gaseous insufflation of the abdomen to create a pneumoperitoneum is essential to perform laparoscopy both in children and adults. Although there have been studies investigating the role of abdominal wall lifting as a means of obtaining a working space for laparoscopy (Ishizuka et al 2000, Luks et al 1995), this has not proven to be a useful clinical tool at present. Gaseous insufflation increases intra-abdominal pressure, and may lead to impaired respiratory function (Pross et al 2000), including reduced functional residual capacity, increased airway pressure and decreased lung compliance (Safran & Orlando 1994). The optimal intra-abdominal pressure in children, in respect to cardio-respiratory effects, has been found to be between 8 and 12 mmHg (Gentili et al 2000, Hsing et al 1995, Laffon et al 1998, Manner et al 1998).

Different gases have been investigated for use during laparoscopy, taking into account factors such as inertness, the tendency to cause gas embolism, combustibility and cost. Carbon dioxide (CO_2) is the preferred gas for pneumoperitoneum because it is safe, non-combustible, inexpensive, and is least likely to cause embolism. Air has been shown to cause an exaggerated cytokine response compared to CO_2 (Tung & Smith 1999).

Carbon dioxide is, however, metabolically active. The absorption of CO_2 may lead to an increased CO_2 load that can contribute to respiratory impairment and hypercapnia, sometimes reported during CO_2 pneumoperitoneum (Bozkurt et al 1999, Ho et al 1995). Indirect calorimetry offers a tool to characterise the effects of CO_2 insufflation on respiratory gas exchange in children.

3.1.1 Aim

The aim of this chapter was to characterise the pattern of CO_2 elimination during laparoscopic surgery in infants and children.

3.2 Methods

This study consisted of 20 children undergoing laparoscopy for elective, major intra-abdominal surgery, and 19 patients undergoing elective laparotomy. Patients scheduled for elective surgery were approached to be included in this study. Ethical Committee approval was obtained for this study. All patients were status 1 to 3 according to the American Society of Anaesthesia. Patients with metabolic, renal, and cardiac disease, and premature babies were excluded.

3.2.1 Anaesthesia

Both groups underwent similar intraoperative care. Anaesthesia was standardised as follows: induction was either by inhalation (isoflurane) or intravenous (propofol and fentanyl) technique. Tracheal intubation was facilitated with atracurium. A cuffed endotracheal tube or a throat pack was used to minimise air leak. A Servo 300 or 900 ventilator (Servo, USA) was used in all children. General anaesthesia was maintained with a mixture of air, O_2 and isoflurane inhalation. Muscle relaxation was maintained during the procedures with atracurium, and supplemental fentanyl was given throughout general anaesthesia. End-tidal CO_2 (kPa) was measured on a continuous basis using a positive sampling system (Hewlett Packard; Boeblingen, Germany). End-tidal CO_2 levels were recorded every 10 minutes. Respiratory adjustments were made as deemed necessary by the anaesthetist to keep end-tidal CO_2 within physiological levels.

3.2.2 Laparoscopy

An open technique with insertion of a Hasson cannula was used to gain intra-abdominal access. Unheated (room temperature) CO_2 was used for peritoneal insufflation, maintaining intra-abdominal pressure between 8-12 mmHg. Maximum flow rate for CO_2 insufflation was 1 L/min. The total volume of CO_2 insufflated during the procedure was recorded. Laparoscopic procedures were performed using standard techniques.

3.2.3 Respiratory gas exchange

CO_2 elimination, expressed in ml/kg/min, was continuously measured intraoperatively at 1 minute intervals, using computerised Deltatrac® II indirect calorimeter. The details of indirect calorimetry have already been described in Section 2.3. Patients were studied in the ventilator mode. The indirect calorimeter was validated and the flow rate calibrated by burning bench alcohol of a known volume as described in Section 2.3.

$\dot{V}CO_2$ was calculated according to the principles of indirect calorimetry. During laparoscopy, measured $\dot{V}CO_2$ represents CO_2 elimination ($\dot{V}ECO_2$), the sum of CO_2 produced metabolically and CO_2 absorbed from the abdominal cavity.

Measured $\dot{V}ECO_2$ was assessed at the following times: (1) after induction of anaesthesia and before CO_2 insufflation, (2) during pneumoperitoneum and (3) after desufflation of the peritoneal cavity, until the end of anaesthesia. $\dot{V}ECO_2$ was analysed in ten minute intervals, using the mean from five minutes before to five minutes after each time point. Maximum increase in $\dot{V}ECO_2$ was measured in each patient by subtracting the baseline $\dot{V}ECO_2$ (time 1) from the highest measured $\dot{V}ECO_2$ at any time point. To characterise the immediate response to peritoneal desufflation, $\dot{V}ECO_2$ was analysed minute-by-

minute during the 10 minutes preceding desufflation and following desufflation until the end of recordings.

3.2.4 Statistical analysis

Age, weight, duration of operation and operative stress score are expressed as mean, standard deviation and range, and differences between study groups in these variables are presented with 95% confidence intervals (C.I.). $\dot{V}ECO_2$ and end-tidal CO_2 were normally distributed and are expressed as mean ± SEM. $\dot{V}ECO_2$ and end-tidal CO_2 were compared at each time-point with pre-insufflation values using paired t-test, as progressively missing values prevented repeated measures ANOVA. Correlation between $\dot{V}ECO_2$, age and weight was performed using linear regression analysis. Changes in $\dot{V}ECO_2$ and end-tidal CO_2 within patients were compared between the two groups, whilst accounting for differences in weight, age and operation length as necessary, using a series of multilevel models (MLwiN®; Centre for Multilevel Modelling, Institute of Education, University of London).

3.3 Results

There were no significant differences between the two study groups with respect to patients' age and weight (Table 3.1). The duration of operation was longer in the laparoscopic group. The operative procedures performed are listed in Table 3.2. The most commonly performed operation in both groups was a Nissen fundoplication.

3.3.1 Carbon dioxide elimination during pneumoperitoneum

In patients undergoing open surgery, $\dot{V}ECO_2$ did not change significantly during the entire study period (Figure 3.1). In patients undergoing laparoscopy, $\dot{V}ECO_2$ was 4.6 ±

0.3 ml/kg/min before pneumoperitoneum and increased after 15 minutes of pneumoperitoneum to 5.2 ± 0.3 (p<0.001 *versus* pre-insufflation). There was a steady increase in $\dot{V}ECO_2$ throughout the period of pneumoperitoneum (Figure 3.2), with maximum value of 6.7 ± 0.4 ml/kg/min. Post desufflation, $\dot{V}ECO_2$ decreased, but was still higher than pre-insufflation level by the end of operation (5.8 ± 0.3; p<0.001 *versus* pre-insufflation).

Multilevel model analysis indicated that the older, heavier children tended to have lower $\dot{V}ECO_2$ values. The effects of age and weight were not independent. After accounting for differences in age and weight, $\dot{V}ECO_2$ rose significantly in the laparoscopic group, increasing on average 0.015 ml/kg/min more per minute above those undergoing open surgery (95% C.I. 0.005, 0.02, p<0.05).

3.3.2 End tidal carbon dioxide tension increased with pneumoperitoneum

During open surgery, end-tidal CO_2 levels were not significantly different to baseline (Figure 3.3). In patients undergoing laparoscopy, end-tidal CO_2 was 4.7 ± 0.2 kPa pre-insufflation, peaked at 1 hour (5.3 ± 0.2, p<0.001 *vs.* pre-insufflation) and subsequently decreased in response to ventilatory adjustments (Figure 3.4). Post-desufflation end-tidal CO_2 (4.8 ± 0.2 kPa) was not significantly different from that measured pre-insufflation (p=0.6).

3.3.3 Carbon dioxide elimination immediately post desufflation

Seven patients (3 nephrectomy; 1 pyeloplasty; 1 cholecystectomy; 1 fundoplication; 1 Duhamel pull through) responded to desufflation with a sharp transient increase in CO_2 elimination (1.6 ± 0.4 relative to pre-desufflation). This increase in $\dot{V}ECO_2$ peaked at

5.9 ± 1.8 minutes post-desufflation, lasted for 17.0 ± 2.0 minutes and did not appear to be related to patient age, length of pneumoperitoneum, abdominal pressure or type of operation. This minute-by-minute increase in $\dot{V}ECO_2$ in 4 patients is shown graphically in Figure 3.5.

3.3.4 Correlation between carbon dioxide insufflation and elimination with patient demographics

The total amount of CO_2 insufflated positively correlated with patient age ($y = 0.2x - 58.1$; $r^2=0.27$; $p<0.01$). CO_2 absorption was age related, as indicated by the negative correlations between maximum increase in $\dot{V}ECO_2$ and both age ($y= - 0.01x + 3.0$; $r^2=0.27$; $p<0.01$; Figure 3.6) and weight ($y= - 0.05x + 3.0$; $r^2=0.29$; $p<0.01$: Figure 3.7). These regression analyses indicate that the younger or the smaller the child, the larger the relative increase in CO_2 elimination. Maximum increase in $\dot{V}ECO_2$ was not related to the length of the procedure ($r^2=0.00$; $p=0.95$), or total volume of CO_2 insufflated ($r^2=0.01$; $p=0.64$).

3.4 Discussion

One of the attributes of laparoscopic surgery is that it is less invasive than open surgery. Laparoscopic surgery, however, introduces new parameters that may modify the response to surgery. The metabolic, physiological and respiratory responses to CO_2 pneumoperitoneum are among such factors.

3.4.1 Carbon dioxide metabolism

The CO_2 used for insufflation is absorbed from the abdominal cavity, into the bloodstream. In the blood (and intracellularly) (Geers & Gros 2000, Parsons 1982), CO_2

is converted to H^+ and HCO_3^- via the carbonic anhydrase system which acts to buffer CO_2. The H^+ so produced can lead to a fall in blood pH. In the lungs CO_2 is expired, and the carbonic anhydrase reaction is reversed so the excess H^+ is 'removed'. Studies in adults and animals have characterised the effect of CO_2 absorption from the abdominal cavity. This absorption leads to an increased CO_2 load to the lungs, with an increase in CO_2 elimination (Ho et al 1995, Lind 1994, Mullett et al 1993). In otherwise healthy patients undergoing laparoscopic surgery, this CO_2 load does not produce a clinically significant respiratory or metabolic challenge. A 20 to 30% increase in minute ventilation is usually sufficient to compensate for the increased $\dot{V}ECO_2$ (Giebler et al 1997, Glascock et al 1996, Luiz et al 1992), thus avoiding an increase in end-tidal CO_2 or acidosis.

3.4.2   Pathophysiological effect of pneumoperitoneum

Increasing intra-abdominal pressure changes the dynamics of respiratory function, and may in itself alter CO_2 metabolism. Lung compliance decreases immediately following peritoneal insufflation, with an increase in airway pressure (Manner et al 1998); there may be increased pulmonary vasculature resistance (Ortega et al 1996b). These changes adversely affect respiration. Atelectasis may contribute to the pathophysiological dead space and increase in arterial-alveolar CO_2 gradient during laparoscopy (Cheng et al 1999).

These changes in pulmonary function are compounded by changes in cardiac function. Though very pertinent, they are not the subject of this work, and are not discussed fully. However they include decreased cardiac output, increased peripheral vascular resistance and decreased cardiac preload (Gentili et al 2000, Safran & Orlando 1994). The changes

in arterial pressure can be complex, depending on the interactions of the before mentioned parameters.

All of theses changes in themselves can alter CO_2 metabolism in the absence of any intra-abdominal CO_2. Lactic acidosis can contribute to the acid load. Impaired cardiac function can prejudice CO_2 excretion via the lungs. As well, the absorbed CO_2 itself has been proposed to contribute to impaired cardio-respiratory function in the absence of a pressure effect (Ho et al 1995), as insufflation of helium did not give rise to these adverse effects in pigs. It is therefore important to understand the way in which the body handles the increased CO_2 load as it may have far reaching consequences.

3.4.3 Carbon dioxide metabolism in children during laparoscopy

Children undergoing laparoscopic surgery have been shown to handle a CO_2 load without becoming acidotic or developing significant arterial-alveolar CO_2 gradient (Laffon et al 1998). Bozkurt *et al* studied 27 infants undergoing laparoscopic procedures and showed that, although there was a statistically significant rise in arterial CO_2 tension and a significant fall in blood pH, these' were within physiological levels (Bozkurt et al 1999). Generally, the respiratory burden of the increased CO_2 load is well tolerated in children without untoward clinical effect. Results in this chapter showed that end-tidal CO_2 increased from a pre-insufflation level of 4.6 kPa, to reach a plateau of 5.3 kPa between 45 and 90 minutes (Figure 3.3). Subsequently it decreased in response to increased minute ventilation. This rise in end-tidal CO_2 levels is in keeping with published figures for children undergoing laparoscopy (Manner et al 1998). In most patients undergoing controlled ventilation end-tidal CO_2 is a good reflection of arterial CO_2 tension (Cheng et al 1999). Arterial CO_2 and pH were not measured in this study, but one can assume that, due to the minor changes in end-tidal CO_2, the arterial CO_2 and

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pH did not change significantly. Alternatively, a marked decrease in cardiac output or increase in pulmonary vascular resistance could have limited end-tidal CO_2 and $\dot{V}ECO_2$, in the face of increased arterial CO_2 and acidosis. However this is unlikely. Clinically there were no observed cardiovascular or respiratory side effects noted.

3.4.4 Effect of maintaining constant ventilation settings on gas exchange

Studies in animals and healthy adults undergoing controlled ventilation have characterised the rise in CO_2 elimination and end-tidal CO_2 while maintaining constant minute ventilation. In an animal model there was a rise in end-tidal CO_2 and blood CO_2 tensions that contributed to acidosis and adverse haemodynamic effects (Ho et al 1992, Ho et al 1995). In pigs undergoing laparoscopy with CO_2 at 15 mmHg there was a 75% increase in $\dot{V}ECO_2$ and development of significant acidosis (Ho et al 1995).

In healthy adults undergoing laparoscopic cholecystectomy who were given a constant minute ventilation of 80 ml/kg/min, end tidal CO_2 levels were statistically elevated after CO_2 insufflation (Monagle et al 1993). Gehring *et al* (Gehring et al 1998) measured end-tidal CO_2 and arterial blood gases during laparoscopic cholecystectomy, and also showed elevation of end-tidal CO_2.

Few studies have examined the effect of maintaining pre-insufflation ventilatory settings on CO_2 metabolism in infants and children, as most investigators prefer to keep end-tidal CO_2 in the normal (or pre-insufflation) range during laparoscopy in children. Manner studied children undergoing laparoscopy and found that if minute ventilation was unchanged, end tidal CO_2 increased from a baseline value of 4.3 kPa to 5.4 kPa (33-42 mmHg) (Manner et al 1998). These values were similar to the peak values in this chapter.

3.4.5 Interpretation of carbon dioxide elimination measured during laparoscopy.

Pre-insufflation, baseline $\dot{V}ECO_2$ measured represents the metabolically produced CO_2 only. Thereafter, the increase in $\dot{V}ECO_2$ is assumed to be that absorbed from the abdominal cavity. However it remains undetermined whether the increase in $\dot{V}ECO_2$ could partially be the result of metabolic changes. There have been no studies to directly quantify the amount of CO_2 absorbed from the abdominal cavity. Although the studies quoted have documented an increase in $\dot{V}ECO_2$ post insufflation, the increase in $\dot{V}ECO_2$ can only be assumed to result from peritoneal absorption of CO_2. While this assumption seems natural, studies are required to directly quantify the amount of CO_2 actually absorbed from the abdomen.

In any event, the increase in $\dot{V}ECO_2$ seen may not wholly be due to absorption. It is possible that some of this increase may be due to an increase in whole body metabolism (see Chapter 4), with more metabolically produced CO_2 being eliminated. It is unlikely that this increase in metabolism is solely responsible for the increase in $\dot{V}ECO_2$ as during laparoscopy there was a definite change at the time of insufflation to suggest that this was primarily due to absorption.

3.4.6 Carbon dioxide elimination differs in children compared to adults

Respiratory elimination of insufflated CO_2 has been characterised in adults. Mullet *et al* investigated diffusion and excretion of CO_2 in adults during laparoscopic and pelviscopic surgery (Mullett et al 1993). They found that $\dot{V}ECO_2$ and end-tidal CO_2 increased after creation of pneumoperitoneum. There was a plateau in $\dot{V}ECO_2$ after 15 minutes, at which point a steady state was reached. This pattern of an early rise in

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$\dot{V}ECO_2$ and end-tidal CO_2 that reaches a plateau is mirrored in other studies. Contrary to these findings, there was a continuous rise in $\dot{V}ECO_2$ during laparoscopic surgery in infants and children after the creation of a pneumoperitoneum. Before peritoneal insufflation in the laparoscopy group, $\dot{V}ECO_2$ was stable. Importantly, there was no rise in $\dot{V}ECO_2$ in children undergoing open surgery. This control group of patients served not only as a comparison group, but also a means of further validating the stability of the indirect calorimeter in an intraoperative setting.

3.4.7 Possible reasons for the carbon dioxide absorption profile in children

Mullet et al (Mullett et al 1993) found that extra-peritoneal insufflation during pelviscopic surgery in adults was associated with a different CO_2 absorption profile, with a steady rise in $\dot{V}ECO_2$ throughout the period of pneumoperitoneum. Glascock also found that there was significantly faster and greater absorption, as reflected by changes in $\dot{V}ECO_2$ and end-tidal CO_2, between intraperitoneal and extraperitoneal insufflation (Glascock et al 1996). Therefore CO_2 absorption is greater, and shows a continuous rise, with extra-peritoneal insufflation.

The continuous rise in $\dot{V}ECO_2$ with intraperitoneal CO_2 insufflation seen in this study has not been previously documented. Children may handle intraperitoneal CO_2 differently. There may be a longer time to steady state in children. Also the rate of absorption appeared to be age related. The peritoneal surface in children may have different characteristics at different ages that alter CO_2 absorption. This theory may be also reflected in the findings of Hsing et al, who found that there were different rates of absorption of CO_2 (as reflected in end-tidal CO_2) during laparoscopy in children of different ages (Hsing et al 1995). In their study, younger children had more rapid

changes in end-tidal CO_2 compared to older children. They did not measure $\dot{V}ECO_2$ in their patients. Findings in this chapter demonstrate a negative correlation between maximum increase in $\dot{V}ECO_2$ and both age and weight. Thus smaller, younger children absorbed relatively more CO_2 than older, heavier children. This may be due to the greater relative surface area of the peritoneum in the smaller child. Also, as CO_2 is fat soluble, intraperitoneal fat may act as a buffer for CO_2 and lessen the CO_2 load to the blood in adults and older children. Alternatively, the continuous rise in $\dot{V}ECO_2$ in this study may be a reflection increasing amounts of metabolically produced CO_2 as part of an intraoperative hypermetabolic response (see Chapter 4).

Whatever the mechanism there is a difference in metabolism in infants and children compared to adults. The absorption of CO_2 is also age related, with infants and smaller children being relatively more susceptible to absorption.

3.4.8 CO_2 absorption is increased in the immediately post desufflation

Surprisingly there was a sharp, transient increase in $\dot{V}ECO_2$ immediately after desufflation in seven patients. One patient undergoing a laparoscopic assisted pull-through, also exhibited this phenomenon at the time his anal incision was made, thereby effecting peritoneal desufflation. This transient increase waas not seen in all patients, and did not appear to be related to patient age, length of pneumoperitoneum, abdominal pressure or type of operation. It was not related to the procedure performed. It is difficult to sspeculate on the reason for the differences in the response between patients.

It has been shown that pneumoperitoneum causes lower limb venous stasis (Giebler et al 1997, Marshall et al 2000, Ortega et al 1996b). One possible explanation for this

short-lived and rapid rise in $\dot{V}ECO_2$ may be the sudden release of this tamponade effect

from the pneumoperitoneum on lower limb venous return. The CO_2 rich blood draining

from the lower limbs may cause the sudden rise in $\dot{V}ECO_2$. It is possible that this

phenomenon is associated with a transient increase in lower limb acidosis as a result of

the pneumoperitoneum-related tamponade.

Other authors speculate that the pneumoperitoneum itself may limit CO_2 absorption by

compressing capillaries and limiting blood flow in the peritoneum. In the immediate

post-desufflation period this factor may be removed. Another possible explanation for

this rise may be a sudden increase in minute ventilation as the intra-abdominal pressure

is suddenly reduced.

Although this increase in $\dot{V}ECO_2$ post desufflation was not seen in all patients, $\dot{V}ECO_2$

did not reach pre-insufflation values at the end of the operation. Kazama *et al* has shown

that there is an excess of CO_2 excretion above baseline 30 minutes after cessation of the

pneumoperitoneum (Kazama et al 1996). Blobner reported an elevated $\dot{V}ECO_2$ that

lasted 30 minutes post desufflation but returned to pre-insufflation values thereafter

(Blobner et al 1994). These findings suggest that increased $\dot{V}ECO_2$ is present up to 30

minutes post desufflation, but not thereafter. This may have important implications for

preventing hypercapnia in the recovery period.

3.5 Conclusions

In summary the results in this chapter show that in children CO_2 elimination increases

throughout the period of pneumoperitoneum. There was an immediate increase in CO_2

elimination after desufflation. CO_2 elimination is age related, as younger children

eliminated relatively more CO_2 than older children. This suggests that there may be a different CO_2 absorption and elimination between infants, children and adults. These differences may be of clinical importance as the peritoneal reaction may, to some extent, determine the overall metabolic and inflammatory response to operative stress.

Further studies are required to characterise the absorption of CO_2 from the peritoneal cavity. One possible means of distinguishing metabolically produced from absorbed CO_2 from the peritoneal cavity is by insufflation of a stable isotope of $^{13}CO_2$, and measuring enrichment in breath samples. The findings of an increase in $\dot{V}ECO_2$ with time can also be confirmed by mass spectroscopy.

The intraoperative absorption of CO_2 may modify the intraoperative metabolic response. Also, it is possible that the different CO_2 elimination profile seen is due to a difference in the intraoperative metabolism during laparoscopy in children. The intraoperative metabolic response was therefore investigated in the next chapter. This may give insights into the mechanisms controlling the intraoperative response to surgery and laparoscopy in children.

	Open		Laparoscopic		95% C.I for difference	p value
	Mean (S.D.)	Range	Mean (S.D.)	Range		
Age (months)	47.3 (59.53)	0.5 – 208	63.4 (48.0)	1 – 150	– 18.9, 51.1	0.36
Weight (Kg)	14.4 (11.8)	4 – 49	19.9 (13.9)	3 – 53	– 2.8, 13.9	0.19
Duration of operation (min)	97.3 (43.7)	43 – 195	163.3 (58.3)	59 – 300	32.4, 99.5	< 0.01
Operative stress score	7.9 (1.49)	6 – 11	7.2 (1.1)	6 – 11	– 0.32, 1.4	0.22

Table 3.1 *Demographics in patient undergoing intraoperative metabolic study.*

Open (n=19)		Laparoscopy (n=20)	
Nissen Fundoplication	8	Nissen Fundoplication	11
Colostomy Closure	3	Nephrectomy	5
Duhamel's Pullthrough	2	Duhamel's Pullthrough	2
Neuroblastoma excision	2	Cholecystectomy	1
Pyeloplasty	1	Pyeloplasty	1
ACE	1		
Pyloroplasty	1		
Colectomy	1		

Table 3.2 *Type of operation performed in each study group.*

(ACE; antegrade continent enema procedure)
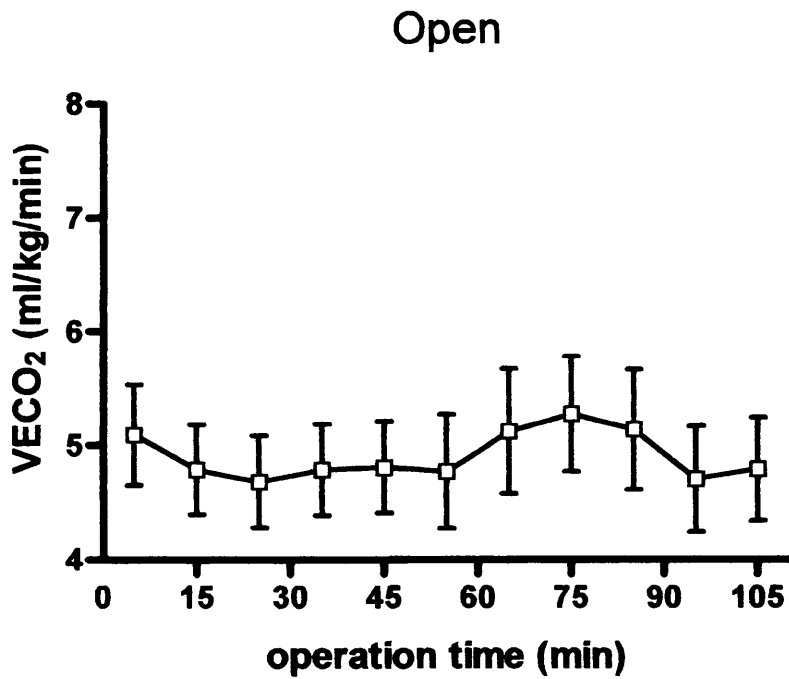
Open

Figure 3.1 $\dot{V}ECO_2$ in children undergoing open surgery.
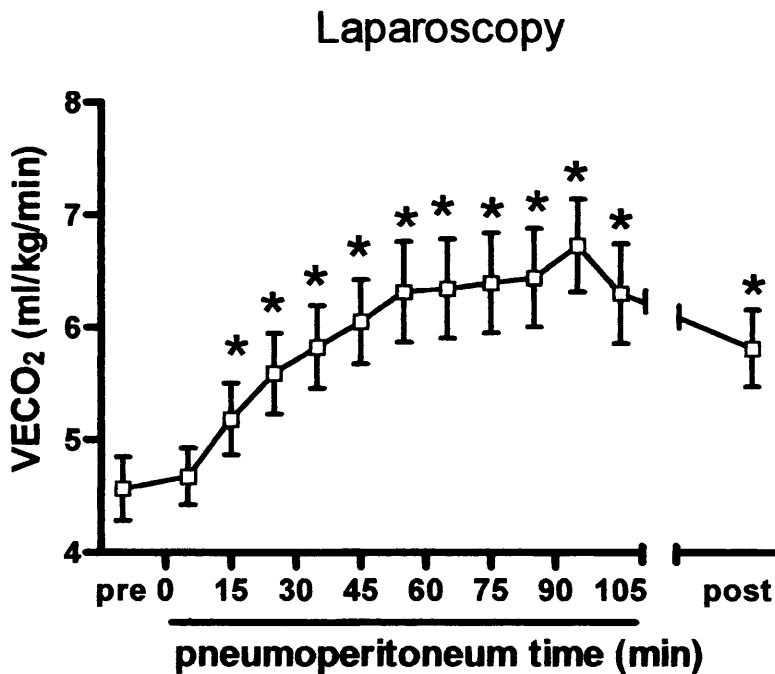
Laparoscopy

Figure 3.2 $\dot{V}ECO_2$ in children undergoing laparoscopic surgery.

# p<0.05 versus pre insufflation; * p<0.01 versus pre insufflation
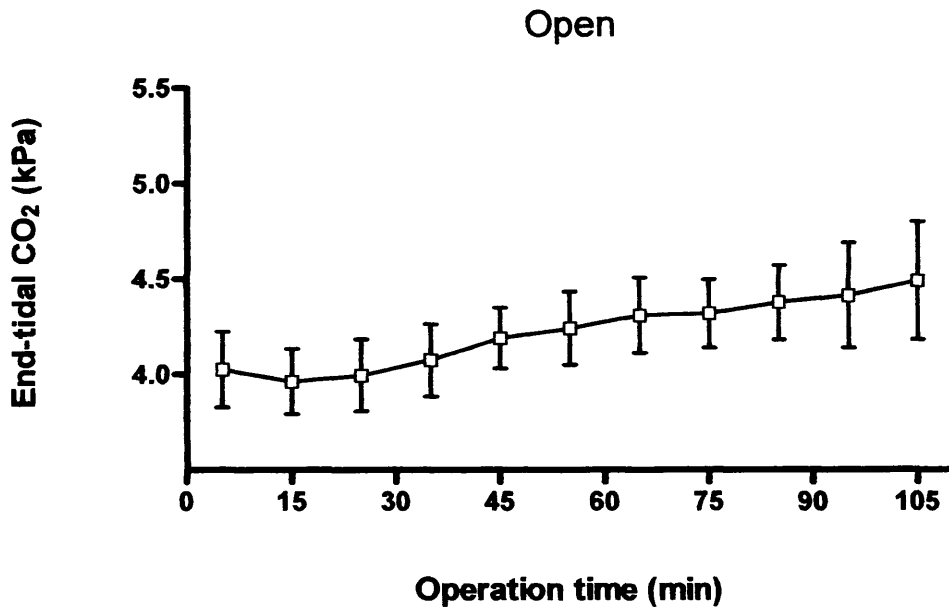
Open

Figure 3.3 *End-tidal CO_2 in children undergoing open surgery*
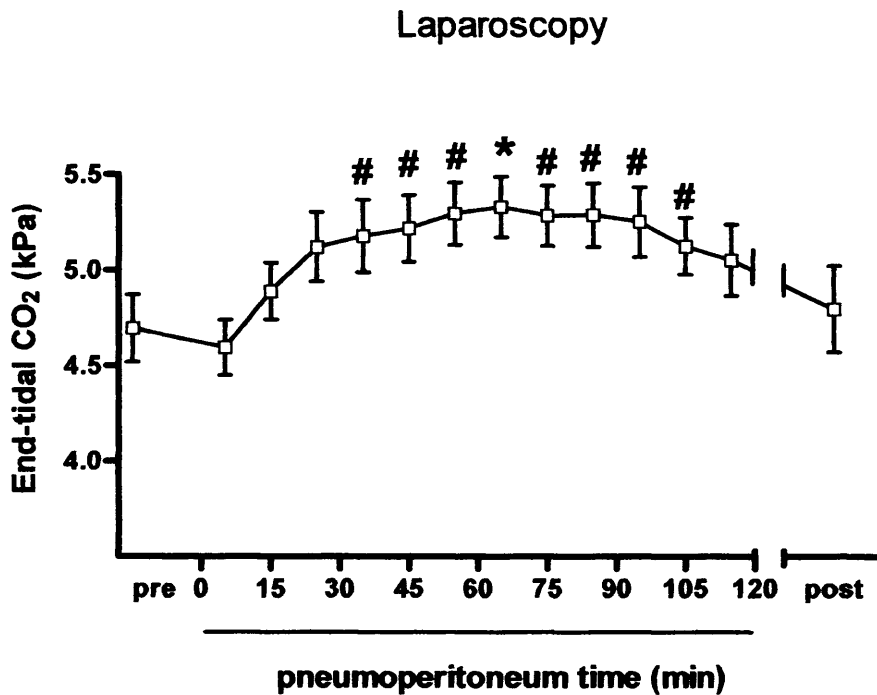
Laparoscopy

Figure 3.4 *End-tidal CO_2 in children undergoing laparoscopic surgery.*
*# $p<0.05$ versus pre insufflation; * $p<0.01$ versus pre insufflation*
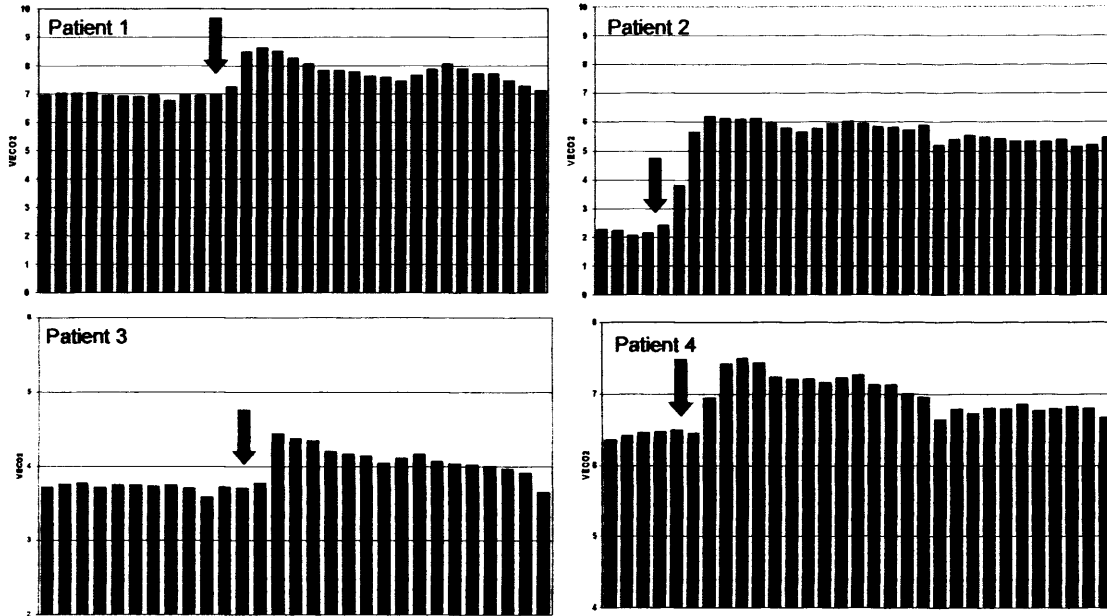
Figure 3.5 *Minute-by-minute* $\dot{V}ECO_2$ *during the phase immediately preceding and following peritoneal desufflation in 4 children.*

Each bar represents one minute recording. Y axes represent $\dot{V}ECO_2$ (ml/kg/min). The arrows indicate the time of peritoneal desufflation. Patient 1: age 9 months.; Nephrectomy; patient 2: age 105 months.; Nephrectomy; patient 3: age 150 months; Nissen Fundoplication; patient 4: age 3 months; Duhamel Pull Through.
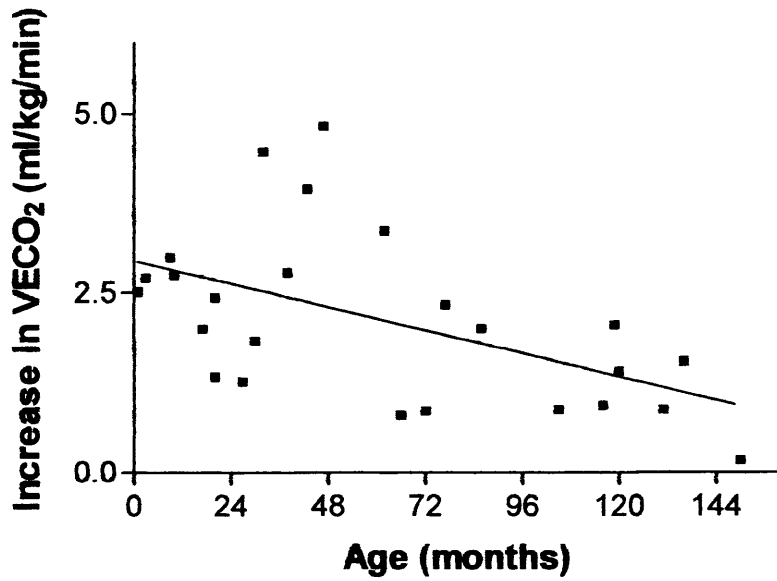
Figure 3.6 *Linear correlation between maximum increase in* $\dot{V}ECO_2$ *and patient age*
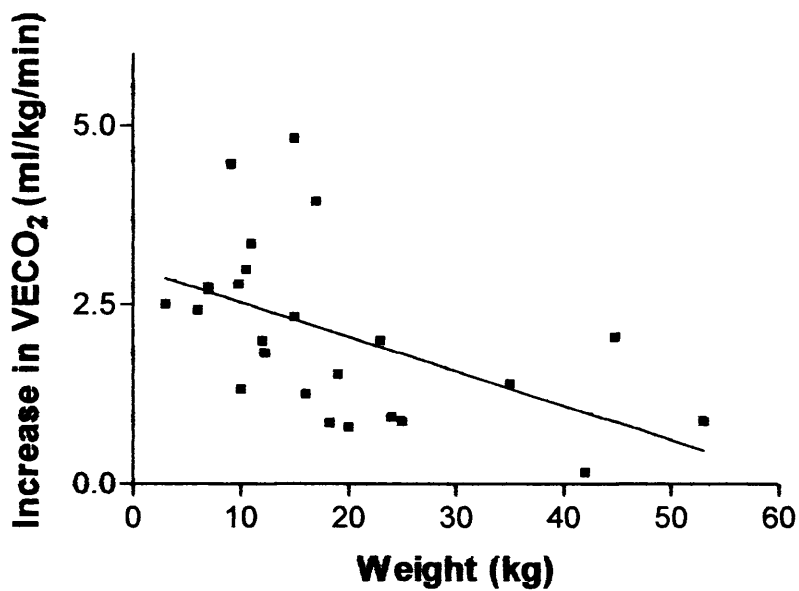
Figure 3.7 *Linear correlation between maximum increase in* $\dot{V}ECO_2$ *and patient weight*

CHAPTER 4

Effect of Laparoscopic surgery on Intraoperative Oxygen Consumption and Core Temperature

4.1   Introduction

Reviews of large adult series have concluded that there is a slight reduction in the inflammatory response and little difference in the metabolic response with laparoscopy compared to open surgery (Gupta & Watson 2001, Kehlet 1999). This has been assumed to be the case in children. Few studies have investigated the metabolic response to laparoscopic surgery in children (Bozkurt et al 2000, Fujimoto et al 1999a, Fujimoto et al 1999b). Results in the previous chapter demonstrated an exacerbated CO_2 response to laparoscopy in children compared to that described in adults. This response may be due to differences in intraoperative metabolic response to laparoscopy in children.

The metabolic response to surgery begins intraoperatively and is proportional to the magnitude of the operative trauma (Anand et al 1985b, Jones et al 1994). Infants and children mount a metabolic and endocrine response to surgery (Anand et al 1985b, Anand et al 1988, Jones et al 1993, Jones et al 1994, Khilnani et al 1993). This response is age related (Jones et al 1993, Pierro 2002, Ward Platt et al 1990) and differs substantially from that in adults (Pierro 2002). It is assumed that laparoscopic surgery is associated with blunting of the metabolic response to surgery.

Measurement of respiratory gas exchange is an indirect measure of mitochondrial metabolism. Indirect calorimetry has been validated as an appropriate means of measuring respiratory gas exchange in ventilated children (Behrends et al 2001, Powis et al 1999). Moreover, measurement of $\dot{V}O_2$ intraoperatively using indirect calorimeter has been used to quantify the metabolic response to surgery both in adults (Lind 1994, Pestana et al 1996) and children (Sandstrom et al 1999). Using the Deltatrac® II indirect calorimeter, Lind (Lind 1994) found a more significant rise in $\dot{V}O_2$ after skin

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incision during gynaecological laparotomy compared to laparoscopy, while Luiz et al (Luiz et al 1992) found that $\dot{V}O_2$ was stable throughout both laparoscopic and open cholecystectomy. Whole body $\dot{V}O_2$ reflects the overall changes in metabolism at the cellular level. Indeed most cellular reactions are associated with oxidative reactions. Furthermore, many of the stress responses to surgery can be associated with an oxidative (respiratory) burst (Kono et al 1995).

Metabolic reactions are associated with transfer of chemical energy to heat, playing an important role in thermoregulation. Therefore changes in $\dot{V}O_2$ are usually associated with changes in heat production and body temperature. Also, changes in $\dot{V}O_2$ on its own are a sensitive measure of energy metabolism as it is the major contributor to energy expenditure.

4.1.1 Aim

The aim of this chapter was to characterise the whole-body energy metabolism during laparoscopy and open surgery, by characterising intraoperative whole body oxygen consumption and core temperature.

4.2 Methods

Thirty nine children undergoing elective intra-abdominal surgery were studied. Patients in this chapter were the same as those studied in Chapter 3. Laparoscopic operations were performed in 20 patients; 19 patients had open surgery. All patients were classified as American Society of Anaesthesia status 1 to 3. Premature infants and patients with metabolic, renal and cardiac disease were excluded from the study.

4.2.1   Anaesthesia

One child in the laparoscopic group and 2 children in the open group received premedication with oral midazolam 0.5 mg/kg. Five out of the 19 patients in the open group had an epidural, compared to none in the laparoscopic group. Anaesthesia was standardised as outlined in the previous chapter. General anaesthesia was maintained with a mixture of air, O_2 and isoflurane inhalation. Anaesthetic inhalation was maintained at a constant proportion throughout the operation. Oxygen supplementation was in the range of 30 to 60% FiO_2, with no significant differences between patient groups.

Both laparoscopic and open surgery groups were treated in the same manner and the same principles for temperature management. Room temperature was in the thermo-neutral range throughout all procedures. A standard paediatric Bair Hugger® warming blanket was used in all patients to prevent intraoperative hypothermia. This was discontinued if core temperature reached 37°C at any time.

4.2.2   Laparoscopy

Unheated (room temperature) CO_2 was used for peritoneal insufflation, maintaining intra-abdominal pressure between 8 and 12 mmHg. Laparoscopic procedures were performed using standard techniques. Intraoperative dissection was carried out using a harmonic scalpel or diathermy.

4.2.3   Respiratory gas exchange

$\dot{V}O_2$ was measured throughout the operation, using the computerised indirect calorimeter, Deltatrac® II as described in Section 2.3. $\dot{V}O_2$ was recorded minute by minute and analysed in ten minute intervals, using the mean of each five minute period

before and after each time point. In patients undergoing laparoscopy, $\dot{V}O_2$ was assessed at the following times: (1) after induction of anaesthesia and before peritoneal insufflation, (2) during insufflation and (3) after cessation of pneumoperitoneum, until the end of anaesthesia.

4.2.4   Core temperature

Core temperature was measured using an oesophageal temperature probe placed at the induction of anaesthesia. Core temperature was recorded every ten minutes.

4.2.5   Operative Stress Score

To assess the operative trauma, the Operative Stress Score was calculated according to the criteria of Anand *et al* (Anand et al 1985b). The operative visceral and tissue trauma were assessed, and the amount of blood loss was noted. The presence of any intraoperative hypothermia was assessed by core temperature recordings. Other factors (length of operation, presence of infection) were recorded. The scoring system is shown in Table 4.1.

4.2.6   Assessment of the effect of CO_2 insufflation on $\dot{V}O_2$ measurement

CO_2 insufflation may influence the metabolic response and alter $\dot{V}O_2$. In order to assess the effect of CO_2 insufflation on $\dot{V}O_2$ measurement individual curves of patients were analysed to determine the correlation between CO_2 insufflation, $\dot{V}CO_2$ and $\dot{V}O_2$ responses. If the rise in $\dot{V}O_2$ was caused by CO_2 insufflation it was assumed that these changes will be related in time.

4.2.7   Calculations and statistical analyses

Changes in $\dot{V}O_2$ ($\Delta\dot{V}O_2$) and core temperature (ΔCT) were calculated as the difference between baseline measurements and subsequent time points. Baseline in the open group was defined as the first ten minutes of anaesthesia. Baseline in the laparoscopic group was defined as the pre-insufflation period. Age, weight, length of operation and operative stress scores were compared between open and laparoscopic groups using Mann-Whitney U-tests since the data was non-normally distributed. Changes in $\dot{V}O_2$ and temperature within patients were compared between the two groups, whilst accounting for differences in weight and age as necessary, using a series of multilevel models (MLwiN®; Centre for Multilevel Modelling, Institute of Education, University of London). All differences are presented with 95% confidence intervals. Correlations were analysed using linear regression.

4.3   Results

The patient characteristics and operations performed were already described in Chapter 3 (Table 3.1 and 3.2). There were no significant differences in age and weight between study groups (Table 3.1), although some of the confidence intervals are wide and there was a tendency for those in the open group to be younger and lighter. The operative stress score was similar in both groups (Figure 4.1).

4.3.1   Oxygen consumption during laparoscopy and open surgery

Oxygen consumption was stable in the group undergoing open surgery (Figure 4.2). Baseline $\dot{V}O_2$ in the laparoscopic group was initially lower than open surgery but showed a steady rise throughout the operation (Figure 4.2). The younger children tended to have higher baseline $\dot{V}O_2$, which rose faster throughout the operation.

When the results were expressed as $\Delta \dot{V}O_2$, these intraoperative changes were more obvious (Figure 4.3). After accounting for weight and age, $\dot{V}O_2$ rose faster amongst the laparoscopic group; increasing an average of 0.017 ml/kg/min more than the open group (95% confidence interval 0.005, 0.03; p=0.00002).

4.3.2 Core temperature during laparoscopy and open surgery

There were no initial differences in core temperature between groups (Figure 4.2). There was no significant change in CT during open surgery (Figure 4.2). There was a steady increase in CT in the laparoscopy group (Figure 4.2).

The changes were again more obvious when expressed as ΔCT (Figure 4.3). After accounting for weight and age, core temperatures rose 0.005°C faster per minute in the laparoscopic group (95% confidence interval 0.003, 0.007; p< 0.000001).

4.3.3 Correlation between $\dot{V}O_2$ and core temperature

There was a positive correlation between $\dot{V}O_2$ and core temperature in the laparoscopic group. The correlation became increasingly stronger throughout the operation. The best correlation was at 1 hour as most operations lasted around 1 hour; thereafter less patients were available for analysis. The relationship at 1 hour post insufflation is shown in Figure 4.4 ($r^2 = 0.35$; p = 0.017; n = 19). The relationships at other times are as follows: 45 minutes, n = 19, $r^2 = 0.26$, p = 0.26; 75 minutes, n = 15, $r^2 = 0.20$, p = 0.07.

4.3.4 Comparison between $\dot{V}CO_2$ and $\dot{V}O_2$ responses

Examination of the temporal relationship between CO_2 insufflation, $\dot{V}CO_2$ and $\dot{V}O_2$ in individual patients revealed that a different time course in the responses (Figures 4.5

and 4.6). $\dot{V}CO_2$ increased at the time of CO_2 insufflation. However $\dot{V}O_2$ did not increase synchronously with CO_2 insufflation, but increased in a gradual fashion that was not related to CO_2 insufflation.

4.4 Discussion

The widespread implementation of laparoscopic surgery has been fostered by the assumption that it is associated with a dampening of the stress response to surgery. This assumption has not been previously tested in children. The metabolic response to open surgery in children differs from adults (Jones et al 1993), and this may also hold true for laparoscopic surgery. These results show that laparoscopic surgery in infants and children is associated with an elevation of core temperature and $\dot{V}O_2$ intraoperatively that suggests hypermetabolism.

4.4.1 Correlation between $\dot{V}O_2$ and core temperature as measures of whole body metabolism

Interestingly, there was a significant rise in $\dot{V}O_2$ throughout the laparoscopic operations. The rise in $\dot{V}O_2$ was associated with a corresponding and parallel increase in core temperature in the laparoscopic group. There is a metabolic link between body temperature and oxygen consumption (Bacher et al 1997, Matthews et al 1995), both being indices of whole body metabolism. Cellular metabolism increases $\dot{V}O_2$ as oxygen is consumed in energy producing reactions. Conversion of chemical energy to heat increases body temperature. $\dot{V}O_2$ is thus a sensitive measure of whole body energy expenditure and heat production (Head et al 1984, Heim 1985). The rise in both $\dot{V}O_2$ and core temperature suggests the presence of relative hypermetabolism during laparoscopy. This link in intraoperative temperature regulation and metabolism is

supported by the positive correlation between $\dot{V}O_2$ and core temperature (Figure 4.4), which increased throughout the operation.

There are many studies that have confirmed the link between $\dot{V}O_2$ and body temperature in various clinical settings (Bacher et al 1997, Joosten et al 1999, Matthews et al 1995, Steinbrook & Seigne 1997, Valencia et al 1992). In ventilated children in an intensive care setting, there seems to be an increase in $\dot{V}O_2$ between 5 and 8% per °C rise in body temperature (Joosten et al 1999, Matthews et al 1995).

Therefore the increase in both $\dot{V}O_2$ and CT in the laparoscopic group, suggest an alteration of either intraoperative thermoregulation or the metabolic response during laparoscopic surgery in children. The increase in intraoperative metabolic processes may either be a primary or secondary event.

4.4.2 Oxygen consumption in adults undergoing laparoscopy

In an animal model (adult pigs) there was no increase in $\dot{V}O_2$ during CO_2 pneumoperitoneum (Ho et al 1995). Studies investigating $\dot{V}O_2$ during laparoscopic surgery in adults have yielded conflicting results. Using respiratory mass spectrometry, Mullet et al (Mullett et al 1993) found no significant rise in $\dot{V}O_2$ in adults undergoing laparoscopic cholecystectomy, gynaecological surgery and pelviscopy. Kazama et al (Kazama et al 1996) used mass spectrometry and also found that $\dot{V}O_2$ was stable in adults undergoing laparoscopic cholecystectomy. Using the Deltatrac® II indirect calorimeter, Lind found a more significant rise in $\dot{V}O_2$ after skin incision in gynaecological laparotomy compared to laparoscopy (Lind 1994), while Luiz et al (Luiz

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et al 1992) found $\dot{V}O_2$ was stable throughout both laparoscopic and open cholecystectomy. Blobner *et al* (Blobner et al 1993) found that $\dot{V}O_2$ was stable throughout laparoscopic cholecystectomy. The results of these studies in adults contrast with findings in this chapter, which show an intraoperative increase in $\dot{V}O_2$ during laparoscopy but not during open surgery in children.

4.4.3 Effect of insufflation of CO₂ at different temperatures on core temperature during laparoscopy.

There have been varying reports on the effects of CO_2 insufflation on body temperature. Holland and Ford (Holland & Ford 1998) found that infants undergoing laparoscopic pyloromyotomy had a greater fall in body temperature compared to infants undergoing open pyloromyotomy. However, the drop in temperature was not statistically significant. Other investigators have found that the use of unheated CO_2 for peritoneal insufflation can be associated with a drop in body temperature, and that the use of warm CO_2 prevented this hypothermia and blunted the cytokine response in adults (Puttick et al 1999). Another study in adults showed that nasopharyngeal temperature increased after 1 hour of pneumoperitoneum with heated CO_2 (Wills et al 2001). Despite insufflation of unheated CO_2 in the present study, body core temperature rose from a preoperative mean of 36.4°C to a mean of 37.4°C after 90 minutes of pneumoperitoneum. To our knowledge, a rise in core temperature during laparoscopy with unheated CO_2 has not been reported. The increase in core temperature in the laparoscopic group could not be due to any heating effect of the insufflation gas.

4.4.4 Effect of body temperature on oxygen consumption.

During laparoscopy, body temperature can also rise in response to the heat generated by the use of intra-abdominal diathermy or harmonic scalpel, and/or the lack of heat loss from the closed abdominal cavity. An increase in core temperature could be responsible for a secondary increase in $\dot{V}O_2$. However, studies in animals and humans (Kleiber 1975) have shown that there is no increase in heat production and energy expenditure in response to variations in environmental temperature in the thermo-neutral range (26 to 39 °C in adults). The effect of environmental temperatures on $\dot{V}O_2$ was found to affect the active portion of TEE, as opposed to REE (Valencia et al 1992), suggesting that environmental temperature caused a modification of behaviour as apposed to an effect on basal metabolism.

The effect of changes in core temperature on whole body energy metabolism may however be different to that caused by a change in environmental temperature. There are few studies on the effect of core temperature on $\dot{V}O_2$. In anaesthetised children there is a linear correlation between core temperature and $\dot{V}O_2$ (Joosten et al 1999, Matthews et al 1995). These studies were performed on children who were not artificially heated or cooled. Therefore cause and effect can not be ascertained. In patients actively cooled, $\dot{V}O_2$ decreased between temperatures of 35 to 32 °C (Bacher et al 1997). The direct effects of active elevation of core temperature on $\dot{V}O_2$ have not been specifically investigated. However, it can be proposed that the increase in body temperature is, at least, partially secondary to an increase in metabolic rate and heat production. This theory requires further evaluation in an animal model.

4.4.5   Age modulates the metabolic response

Whatever the mechanism for the increase in core temperature and $\dot{V}O_2$, changes were more marked in smaller and younger children. Age therefore modulates the intraoperative metabolic response to laparoscopy. If artificial heating from intraoperative manoeuvres was responsible for the rise in core temperature this suggests that the smaller children are more susceptible to this effect. This is possible, as smaller children have a relatively larger body surface area to weight ratio, and may be more susceptible to this heating. Also younger and smaller children, have less efficient thermoregulatory mechanisms.

If the metabolic rate is the primary event, then the smaller children have a proportionally larger increase in metabolic rate than the older child. As younger children have a proportionally higher metabolic rate, this will explain this more pronounced effect in younger children. These findings again confirm that age is a significant determinant of the magnitude of the metabolic response to operative stress.

4.4.6   Factors that could confound the interpretation of the results

This was not a randomised study and compounding factors may have influenced the results. These include anaesthetic technique, type of operation, length of operation, magnitude of the operative trauma, patients' age and weight. Anaesthesia was standardised between groups and there was little variation in the anaesthetic technique used. The type of operation did not seem to determine the increase in core temperature, as ΔCT in patients undergoing a laparoscopic Nissen fundoplication was higher than those undergoing an open Nissen fundoplication. $\dot{V}O_2$ was also higher in the laparoscopic Nissen group.

The choice between a laparoscopic or open approach was based on the individual surgeon's preference. One surgeon performed only open surgery and his patients were included in this study. The other four surgeons performed both the laparoscopic and open operations. With respect to magnitude of the surgical insult, we found that the operative stress score was similar in both groups. The operative stress score has been shown previously to correlate with the magnitude of the stress response (Jones et al 1994). It is therefore unlikely that differences in the metabolic response can be attributed to a variation in operative trauma. The age and weight of the two groups were not significantly different, and after accounting for any differences in our multilevel model statistical program, there were significant differences in $\dot{V}O_2$ and CT between laparoscopy and laparotomy. Although the operation length was significantly longer in the laparoscopic group, this difference was accounted for in the multilevel model analysis and the differences between groups were still significant. The difference in response was therefore not due solely to differences in operative time.

4.5   Conclusions

In summary, results of this chapter have shown that $\dot{V}O_2$ and core temperature steadily increased in children undergoing laparoscopic surgery. This increase was more significant in younger children compared to older children. Open surgery was not associated with a rise in CT and $\dot{V}O_2$. This data suggests that children have an intraoperative hypermetabolic response to laparoscopic surgery not observed in adults. This intraoperative response may be related to intraoperative heating, alteration in intraoperative thermoregulation, or an independent effect of laparoscopy on the metabolic response.

This altered intraoperative thermoregulation and metabolism may modify postoperative metabolism in children undergoing laparoscopy. Whole body $\dot{V}O_2$ in the postoperative period may be altered by this metabolic response to surgery. As changes in energy metabolism are linked to protein metabolism, the latter may be another significant metabolic pathway that differs in the postoperative period. The next chapter investigated the link between the intraoperative findings and the postoperative metabolic responses in children undergoing laparoscopy.

PARAMETER	DESCRIPTION	SCORE
SITE OF SURGERY	SUPERFICICAL	0
	INTRA- ABDOMINAL	1
	INTRA-THORACIC	2
TISSUE TRAUMA	MINIMAL	1
	MODERATE	2
	MAXIMUM	3
VISCERAL TRAUMA	PROLONGED HANDLING	2
	MINOR RESECTION	3
	MAJOR RESECTION	4
LENGTH OF OPERATION	0-30 MINUTES	1
	30-90 MINUTES	2
	90-180 MINUTES	3
	> 180 MINUTES	4
BLOOD LOSS	< 5%	0
	5-10%	1
	10-15%	2
	>15%	3
ASSOC FACTORS HYPOTHERMIA	1.5-3 °C	1
	> 3 °C	2
INFECTION	LOCALISED	1
	GENERALISED	2
	CARDIAC BYPASS	4

Table 4.1 *Operative Stress Score system by Anand et al (Anand et al 1985b).*

Operative Stress Score
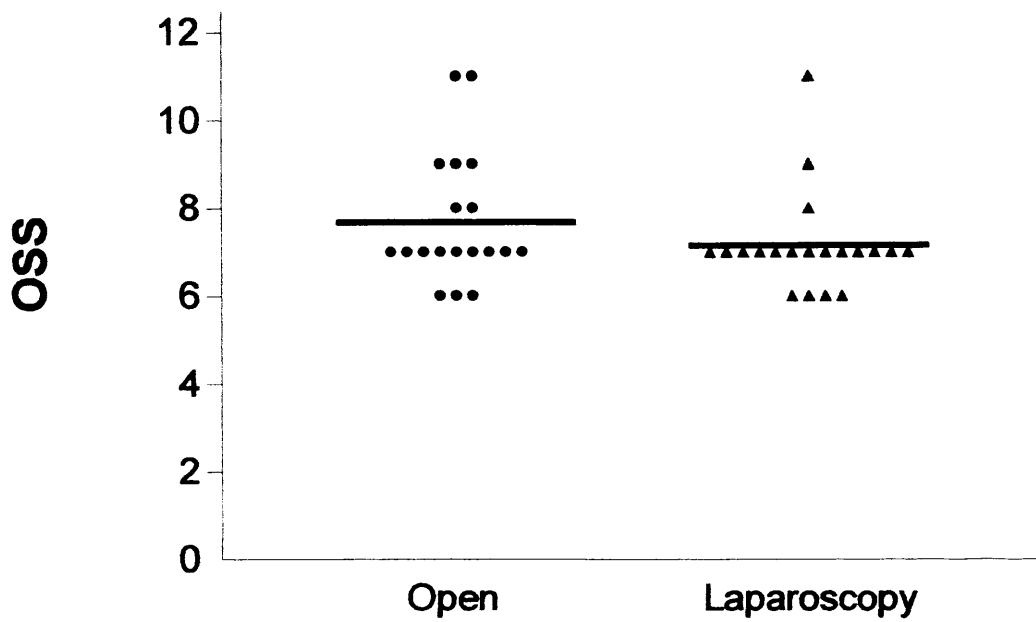
Figure 4.1 *Operative Stress Score (OSS) as devised by Anand et al (Anand et al 1985b) was similar between the two study groups. Lines represent means.*
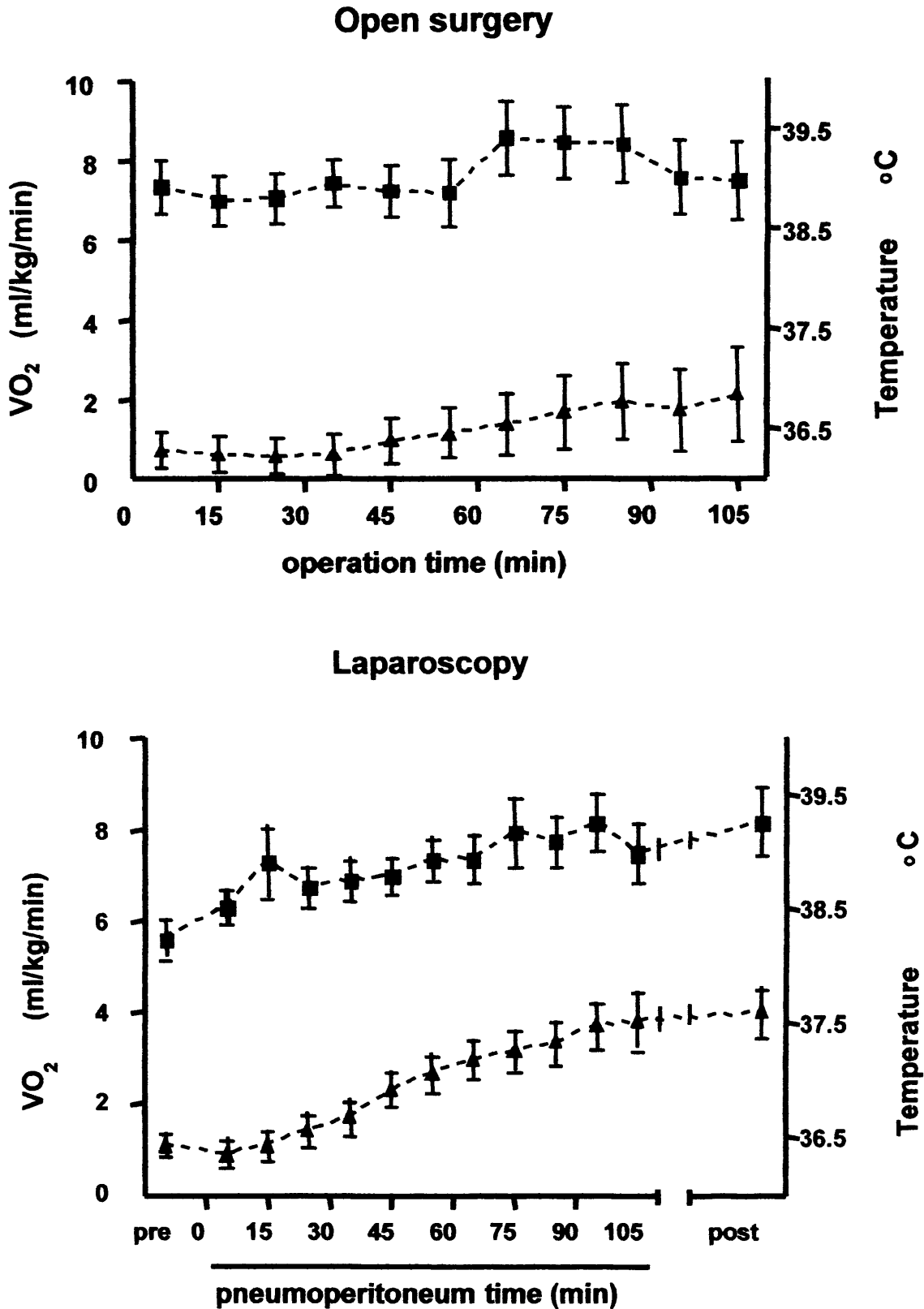
Figure 4.2 *Oxygen consumption (V̇O₂ ; ■), and core temperature (▲)during open and laparoscopic procedures.*

Error bars represent standard error of the mean.

Open surgery
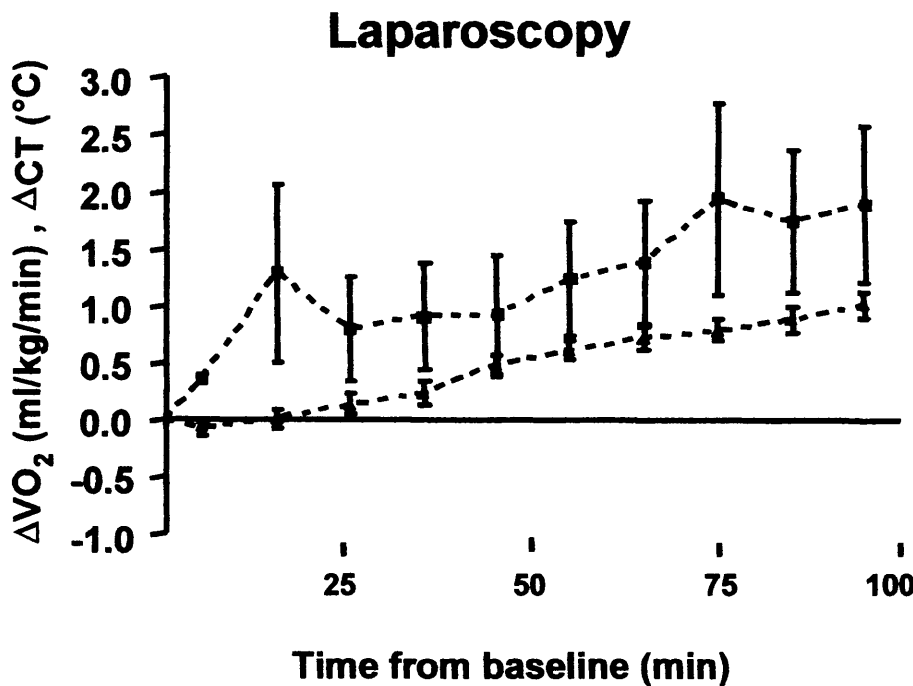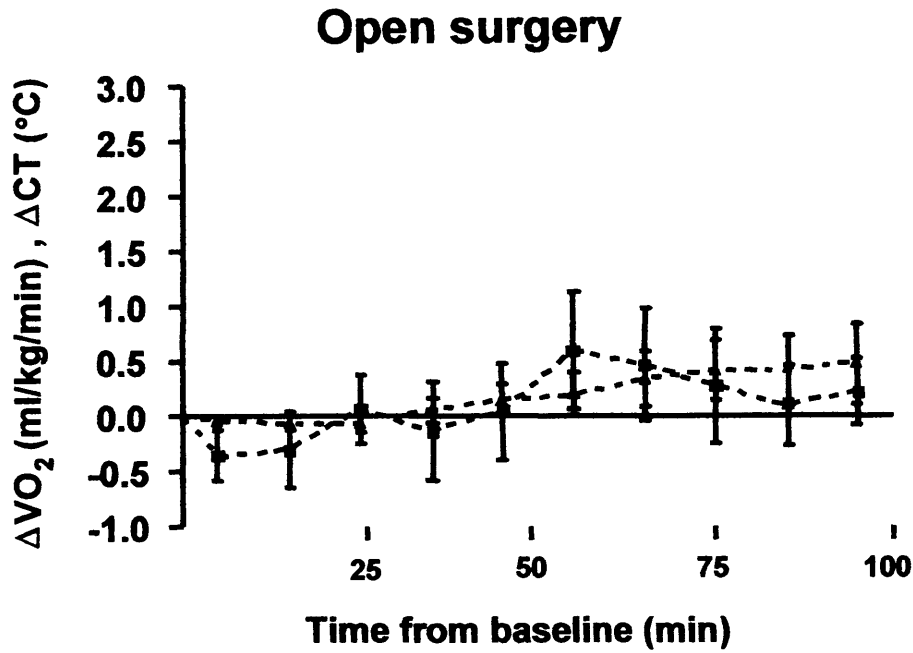
Laparoscopy

Figure 4.3 *Changes from baseline in oxygen consumption (ΔVO_2; ■) and core temperature (ΔCT; ▲) during open and laparoscopic procedures.*

Error bars represent standard error of tne mean
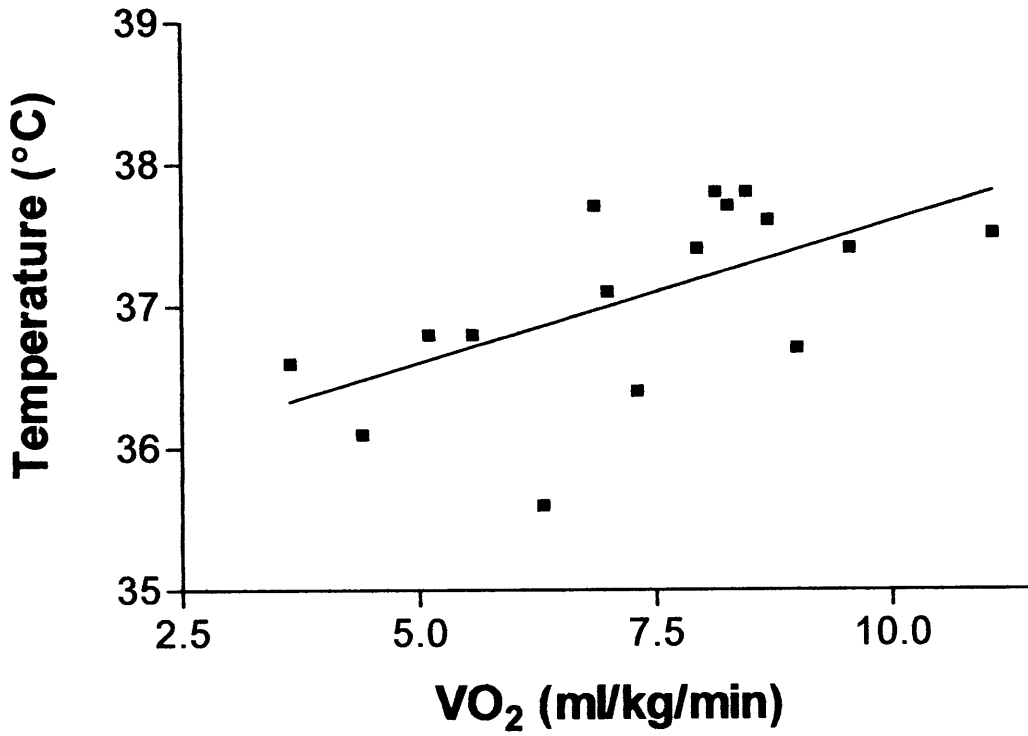
Figure 4.4 *Correlation between body core temperature and oxygen consumption ($\dot{V}O_2$) 1 hour post insufflation in the laparoscopy group*
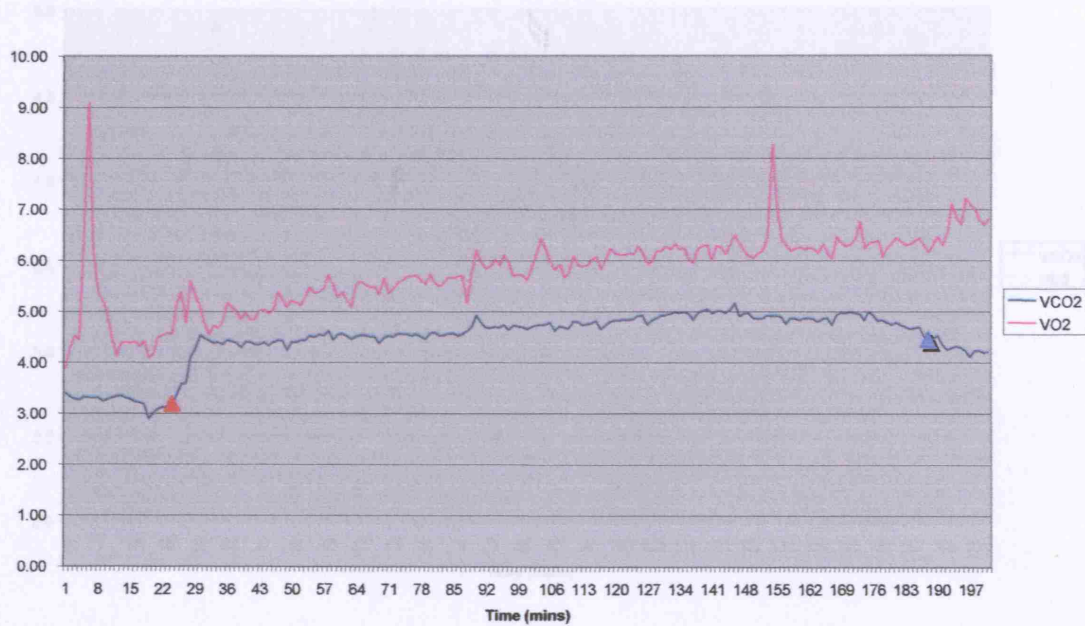
Figure 4.5 *Minute-by-minute $\dot{V}CO_2$ and $\dot{V}O_2$ reading in a patient undergoing laparoscopic nephrectomy.*
Red arrow ▲ indicates beginning of insufflation; the blue arrow ▲ indicates time of desufflation.
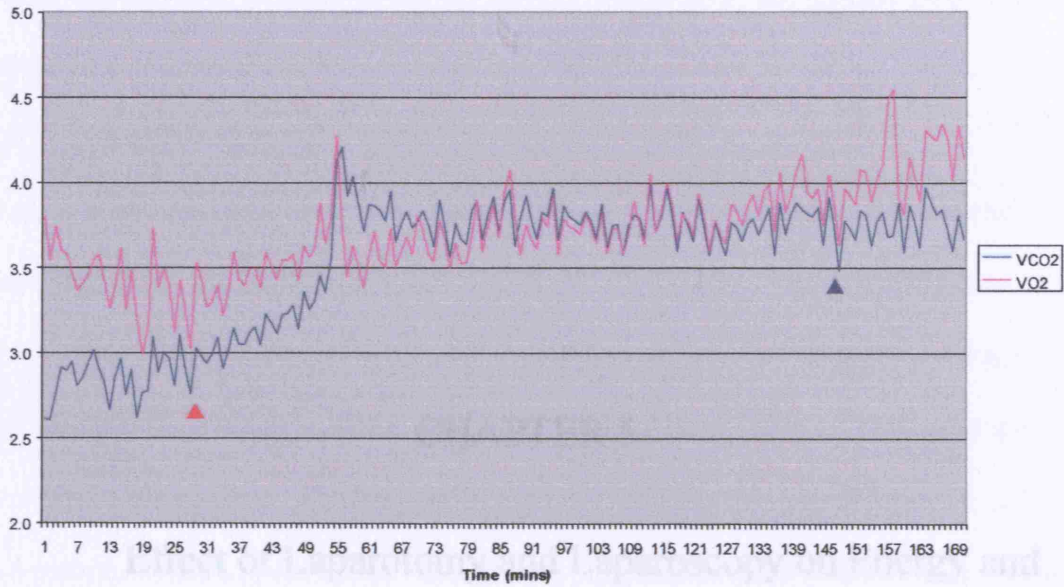
Figure 4.6 *Minute-by-minute $\dot{V}CO_2$ and $\dot{V}O_2$ reading in a patient undergoing laparoscopic fundoplication.*

Red arrow ▲ indicates beginning of insufflation; the blue arrow ▲ indicates time of desufflation.

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CHAPTER 5

Effect of Laparotomy and Laparoscopy on Energy and

Protein Metabolism: Randomised Controlled Trial

5.1   Introduction

The inflammatory response to surgery induces physiological changes that influence whole body metabolism. Measurement of respiratory gas exchange, and therefore energy expenditure, allows the opportunity to characterise the overall (whole body) metabolic responses to surgery.

Oxygen consumption and energy expenditure are appropriate tools for assessing the magnitude of the metabolic response, as they reflect the sum of all metabolic processes at the cellular level (Heim 1985). Most metabolic reactions in the body are associated with oxidative reactions and/or usage of high energy bonds. Thus changes in metabolism are reflected in changes in oxygen consumption and energy expenditure. The previous chapters described modification of intraoperative thermoregulation and metabolic response to laparoscopy in children. The influence of this altered intraoperative response on the postoperative metabolic processes is investigated here.

5.1.1   Partition of energy metabolism

Basal metabolic rate is the partition of whole body energy expenditure altered by metabolic processes. As REE includes a relatively small increase (5 to 10% due mostly to diet induced thermogenesis) it closely mirrors the basal metabolic rate. TEE includes energy requirement of activity which contributes a further increase of between 10 to 30%; it is subject to large individual variation. TEE is thus a less useful tool for assessing the metabolic response to stress.

5.1.2   Energy metabolism and relation to various physiological states

Surgery affects basal energy metabolism. In the postoperative period there is an inflammatory response that can affect metabolic pathways and alter oxygen

consumption. Metabolic changes in white cells are one of these responses. Neutrophils, for instance, have been shown to have a respiratory burst associated with the inflammatory response (Redmond et al 1994), which is associated with production of powerful toxic anti-bacterial defensins, e.g. oxygen free radicals. These reactions proceed through NADPH oxidase, which is responsible for the respiratory burst (Ganong 1995a, Henderson & Chappel 1996).

The liver produces many acute phase reactive proteins that are involved in the inflammatory response. This acute phase response in the liver is associated with an increase in hepatic oxygen consumption (Dahn et al 1995). Liver metabolism contributes a significant quantity to whole body energy expenditure.

Whole body energy expenditure is related to cardiac output (Hersio et al 1993). Heart rate, and therefore cardiac output, is elevated in the postoperative period and increases myocardial oxygen consumption. Though this is a minor percentage of whole body oxygen consumption, it is physiologically one of the most important. Indeed one of the major determinants of postoperative recovery after major surgery is the balance between myocardial oxygen consumption and supply.

The metabolic processes and tissues affected by the stress response are many, and cannot all be listed. However, they can all impact on metabolic rate. Indeed, whole body oxygen consumption is affected by surgery and critical illness (Brandi et al 1996, Frankenfield et al 1997, Hersio et al 1993, Saffle et al 1985). Many of the clinical interventions in postoperative and critically ill patients are directed at increasing oxygen delivery in the face of an increased oxygen demand, stressing its importance in overall physiology and homeostasis. Thus, a useful tool for measuring the overall whole body

metabolic changes is characterising changes in oxygen consumption. Energy expenditure has been shown to be a sensitive measure of the severity of illness in children (Chwals 1994).

5.1.3 Changes in energy expenditure in response to operative stress

In adults undergoing elective abdominal surgery of moderate intensity there is an increase in REE that usually lasts for up to 5 days, but can be more prolonged after very severe surgical stress (Brandi et al 1996, Hakanson et al 1984, Steinbrook & Seigne 1997). Neonates have however been shown to have a different pattern to that of adults after major laparotomy. There is a short-lived increase in REE that peaks at 4 hours postoperative, with a decrease to preoperative values within 24 hours (Jones et al 1993). This response is related to the magnitude of the operative trauma as measured by the operative stress score (Anand et al 1985b, Jones et al 1994).

There is a link between body temperature, metabolism and $\dot{V}O_2$ (Bacher et al 1997, Li et al 2000, Matthews et al 1995). Results in Chapter 4 revealed an alteration of intraoperative thermoregulation during laparoscopy in children resulting in an increase in core temperature and $\dot{V}O_2$ in children undergoing laparoscopy. This increase in intraoperative metabolism can possibly modulate the postoperative response. This chapter investigates the possible consequences of this alteration in intraoperative metabolism on postoperative energy metabolism. There are no randomised controlled trials on the effect of laparoscopy on postoperative metabolism in children.

5.1.4 Intermediary metabolism postoperatively

There are changes in intermediary metabolism that accompany the changes in metabolic rate (Wolfe & Martini 2000). Postoperatively there is often a switch from

predominantly glucose metabolism towards greater fat metabolism. This is reflected in a decrease in the RQ postoperatively (Gebara et al 1992), although this has not been a universal finding (Groner et al 1989). This change in substrate utilisation has been shown to begin in the intraoperative period in children, and to be only partially reversed by the administration of glucose intraoperatively (Sandstrom et al 1999).

Little is known about the postoperative changes in protein catabolism in children; or the effect of laparoscopy on the response. In adults, operative stress causes marked changes in whole body protein metabolism characterised by enhanced protein turnover (Carli & Halliday 1996, Clague et al 1983), an increase in protein (in particular muscle protein) catabolism (Essen et al 1995), and a negative nitrogen balance (Carli et al 1991b). The protein catabolism results in marked skeletal muscle wasting as substrate is mobilised to other viscera. Changes in protein metabolsism may depend on the severity of the surgical trauma.

5.1.5   Aim

The aim of this chapter was to compare the changes in energy and protein metabolism in children undergoing Nissen fundoplication who were randomised to laparotomy or laparoscopy.

5.2   Methods

The protocol and patients used for this randomised controlled trial has already been outlined in Section 2.2.1. Children were fasted for 6 hours preoperatively. Last clear fluid intake was allowed 4 hours before anaesthesia. Anaesthesia was standardised as outlined in Section 2.2.1.4. General anaesthesia was maintained with a mixture of air, O_2 and 0.5 to 2% isoflurane inhalation. Nitrous oxide was not employed during

anaesthesia as it has the same molecular weight as $^{13}CO_2$. A heating blanket was used in all patients to prevent intraoperative hypothermia. The heating blanket was discontinued in all patients if core temperature was $37^{\circ}C$. Intraoperative oxygen consumption and core temperature were measured as described in the previous chapter (Sections 4.2.3 and 4.2.4).

5.2.1 Measurement of EE by Indirect Calorimetry

The principles of indirect calorimetry have already been outlined in Section 2.3.1. Respiratory gas exchange was measured using the indirect calorimeter Deltatrac II in the canopy mode (Figure 5.1).

Measurements were made over a 1 hour period. The accuracy of 1 hour recordings for measurement of EE was validated. The shortest period suggested for accurate assessment of energy metabolism has been 30 minutes in the literature (Wells & Davies 1998). To assess the validity of this assumption, periods of 30 to 60 minute recordings were compared to recordings made over a continuous 2 hour period in patients who allowed lengthy measurements. The first 30 minutes of recording were compared to the entire period of measurement. The EE measured using these 2 lengths of recordings were then compared.

Preoperatively, indirect calorimetry was performed at the same time of day for all patients. This was done early on the morning of the operation; 4 hours after a meal. Patients were studied lying supine in bed after at least 4 hours rest. Room temperature was in the thermoneutral range, and patients were lightly clothed. Periods of activity and crying were excluded from the analysis to calculate REE. REE was assessed at 4 and 24 hours after the end of the operation, in the same environmental conditions to the

preoperative period. Calculation of EE and assessment of substrate utilisation (RQ) by respiratory gas analysis was described in the methods (Section 2.3.1).

To assess any effect of continuing absorbed CO_2 on the calculation of EE in the postoperative period in patients undergoing laparoscopy, the correlation between $\dot{V}O_2$ and REE was analysed. Also the $\dot{V}O_2$ curve was compared to the REE curve to rule out any differences between these two parameters.

5.2.2 Measurement of protein turnover

Protein turnover was measured using a stable isotope infusion of ^{13}C leucine, using a simplified two pool model of protein dynamics (Wolfe 1992), as described in Section 2.4.

Protein turnover was assessed in the immediate preoperative period and in the immediate (4 hours) postoperative period. In contrast to most studies that use a continuous infusion across the intraoperative period, a time lapse of six hours was allowed between the pre- and postoperative arms of the study to reduce the effect of any isotope recycling. Therefore a repeat baseline sample in the postoperative period was compared to preoperative baseline enrichment.

5.2.3 Calculations and statistical analysis

Patient characteristics between open and laparoscopic groups are presented as median and range and are compared using independent t-tests. Data are presented as mean ± SD. Correlation between data was assessed using linear regression. Protein turnover data are compared between pre- and postoperative time points using paired t-test. Repeated data at time points within groups were compared using repeated measures ANOVA, and Tukey's multiple comparison test to analyse individual time points. Non-

normally distributed repeated data was analysed using Wilcoxon matched pairs test.

Data for curves were also analysed using a series of multilevel models (MLwiN®;

Centre for Multilevel Modelling, Institute of Education, University of London).

5.3 Results

5.3.1 Patient demographics

Sixty-eight patients were assessed for entry into the trial (see flow chart Figure 5.2).

Thirteen patients met exclusion criteria (O_2 requirement n=6; cardiac abnormality n=2;

metabolic disease n=3; chronic renal failure n=1; unsuitable for laparoscopy n=1) and

were therefore excluded. Ten patients/parents declined entry into the trial; one other

missed the date of admission. Forty-four patients were randomised (open=21,

laparoscopy=23). Three patients did not complete the trial after randomisation. One

patient (laparoscopic) developed pneumonia preoperatively and the operation was

cancelled; two other patients (1 open, 1 laparoscopic) operations were cancelled

preoperatively for parental reasons. Forty-one patients proceeded through the trial

protocol. Of these, 2 randomised to laparoscopic were converted to open. One patient

was converted preoperatively, due to clinical decision by the surgeon. The other patient

was converted intraoperatively because of operative difficulties (10% conversion rate).

These patients were excluded from further analysis. This left 20 patients in the open and

19 patients in the laparoscopic group.

5.3.2 Intraoperative core temperature and $\dot{V}O_2$ were higher in the laparoscopic group

Intraoperative core temperature increased significantly more in the laparoscopic group

compared to the open group (Figure 5.3). Core temperature in the laparoscopic group

rose 0.003 °C per minute more than the open group (p=0.0008)

Intraoperative $\dot{V}O_2$ increased in the laparoscopic group but not in the open group (Figure 5.4). Multi-level analysis revealed that this difference was significant (p=0.04). On average $\dot{V}O_2$ in the laparoscopic group increased was 0.04% more per minute than the open group.

5.3.3 REE measurements were stable using indirect calorimetry

EE was found to be stable during the periods of measurements used to calculate REE. The coefficient of variation during measurements was less than 10%. A representative calorimetric reading in a patient is shown in Figure 5.5.

There was a good correlation between measurements taken over short periods (30 to 60 minutes) compared to that over longer periods (90 to 120 minutes). Values of REE calculated over these two different periods of time were highly significantly correlated (Figure 5.6; $r^2 = 0.95$, p<0.001).

Absolute EE (kcal/day) was positively correlated with patient weight (EE = 145.7 + 18.7 x weight; r^2=0.76; p<0.001) and age (EE = 240.51 + 3.2 x weight; r^2=0.63; p<0.001). Baseline REE (kcal/kg/day) was negatively correlated to patient age (REE = 34.7 − 0.06 x age; r^2=0.19; p=0.01; Figure 5.7) and weight (REE = 36.56 − 0.04 x weight; r^2=0.26; p=0.03; Figure 5.8).

5.3.4 REE after open and laparoscopic surgery

REE was available in 16 patients in the open group and 16 patients in the laparoscopy group. Patient demographics for these patients are shown in Table 5.1. Groups were comparable with respect to age, weight and presence of neurological impairment.

Operative time was significantly longer in the laparoscopic group (p<0.001, Table 5.1). Patients' weights for the two groups are shown in Figure 5.9.

There was no difference in REE between groups in the preoperative period (p=0.36). There were different patterns of changes in REE between patients undergoing laparoscopy compared to open surgery (Figure 5.10). Open surgery caused a significant decrease in REE in the postoperative period (p = 0.04). In the open surgery group, REE decreased from 31.5 ± 10.3 kcal/kg/day preoperatively to 27.3 ± 4.6 (p<0.05) at 24 hours postoperatively. Laparoscopy did not cause any significant overall change in REE (preoperative 28.4 ± 8.2 vs. 24 hours 27.7 ± 9.9; p = 0.11). Laparoscopic patients exhibited a consistent increase in REE at 4 hours postoperatively. Multilevel models showed that REE at 4 hours postoperatively in the laparoscopic group were on average 15% higher (95% C.I.; 1, 30%) than in the open group.

The area under the curve was calculated for each group using the preoperative value for each patient as baseline. There was a significant difference between open (AUC= 1.574 ± 3.379) and laparoscopic groups (AUC= 5.596 ± 5.846; p=0.03).

There was a positive linear correlation between REE and $\dot{V}O_2$ at all time points (r^2=0.98; p<0.001) with no differences between open and laparoscopic groups (Figure 5.11). The pattern of the postoperative changes in $\dot{V}O_2$ paralleled the response in REE (Figure 5.12). This is consistent with the assumption that there was no significant excess exogenous (absorbed CO_2) that contributed to erroneous EE calculations in postoperative laparoscopic patients; although the contribution of CO_2 to REE is minimal compared to $\dot{V}O_2$ (see equation in Section 2.3.1).

5.3.5 Postoperative substrate utilisation

There was no significant difference in RQ in the preoperative period between groups (p=0.11). In both study groups RQ fell postoperatively (Figure 5.13), indicating an increase in fat utilization (open surgery: preoperative 0.80 ± 0.04, 24 hours 0.70 ± 0.02; p<0.001; laparoscopy: preoperative 0.76 ± 0.06, 24 hours 0.71 ± 0.05; p=0.004). There was no significant difference in the response between groups (p=0.30).

5.3.6 Protein metabolism

Plasma ^{13}C-KIC enrichment in the postoperative baseline samples was equivalent to pre-infusion enrichment (example shown in Figure 5.14). Steady state conditions during leucine infusion were achieved in 20 patients in the preoperative period and 22 patients in the postoperative period. A typical patient in which steady state was achieved is shown in Figure 5.14. The APE of all patients in steady state is shown in Figure 5.15. The coefficient of variation in enrichment was less than 5%.

Baseline leucine flux and protein catabolism negatively correlated with patient weight (r^2=0.21, p=0.04; Figure 5.16). Overall, there was individual variation in leucine flux (Table 5.2). As a whole group, there was no change in protein catabolism (g/kg/hr) between preoperative (0.37 ± 0.20) and postoperative (0.33 ± 0.17) periods (p=0.12; Figure 5.17).

Paired steady state conditions (i.e. both preoperative and postoperative leucine flux) were available in 15 patients (open n = 6; laparoscopic n = 9). In this sub-group, patient demographics were similar in the 2 arms of the study, and are shown in Table 5.3. All patients in the open group exhibited a decrease in protein catabolism (Figure 5.18), which was significant (Table 5.4; p=0.03). In the laparoscopic group, there was no

consistent pattern of individual change in protein catabolism postoperatively (Figure 5.19); with no significant difference in whole body protein catabolism in this group (Table 5.4; p=0.51). The overall change in protein catabolism in each group is shown in Figure 5.20.

5.3.7 Correlation between energy metabolism and protein catabolism

There was a significant negative correlation between the change in protein catabolism and change in REE over 24 hours in the laparoscopic group (r^2=0.53; p=0.03; Figure 5.21). This suggests that the changes in protein metabolism and energy expenditure are linked in the laparoscopic group. This correlation was not present in the open group (r^2=0.15, p=0.53). The difference in the relationship between groups was not significant (p=0.65), as numbers in each group were small.

5.4 Discussion

The metabolic response to open surgery in infants differs from adults (Jones et al 1993), and this may also hold true for laparoscopic surgery. The results in Chapter 4 demonstrated that laparoscopic surgery in infants and children is associated with an elevation of core temperature and oxygen consumption that suggests hypermetabolism intra-operatively. The results in this chapter demonstrate that postoperative energy metabolism and protein catabolism also differs between laparoscopy and laparotomy in children.

5.4.1 Accuracy and validation of indirect calorimetry for measuring energy expenditure

Indirect calorimetry has been used extensively in metabolic studies of energy expenditure since the 1920s, and has been validated extensively in children (Chwals et al 1992, Wells 1998). Wells and Fuller (Wells & Fuller 1998) tested the reliability and reproducibility of the Deltatrac II calorimeter in a laboratory setting. They found them to be very reliable and accurate within 3% for gas exchange and EE. Furthermore they found that between study variability for each individual machine was < 0.2% for RQ, < 1% for $\dot{V}CO_2$, and < 2% for $\dot{V}O_2$ and EE. They tested the effect of time elapsed following gas calibration on measurement accuracy and found that there was minimal effect on $\dot{V}O_2$ and EE, with decreasing accuracy for $\dot{V}CO_2$ and RQ with increasing time. They agreed with manufacturer's recommendations for gas calibration before every measurement. During this study gas calibration was performed before each measurement; flow calibrations were performed monthly.

Other means of measuring EE include infusion of isotopically labelled H_2O (Wells 1998, Wells & Davies 1998). However this method is more costly and more invasive and requires a long period (days) for equilibration and therefore cannot be used to assess acute changes. It is also not possible to distinguish the various partitions contributing to TEE by this method. In children 30 minutes of measurements, with physical activity kept to a minimum, is sufficient for accuracy (Wells 1998). The reliability of 30 minute recordings was confirmed by comparisons performed in this study. Energy expenditure was found to be relatively stable in the absence of any major physical activity. There was little variation in EE during this period. There was a high degree of correlation between REE measured over 30 minutes or over 2 hours (r^2=0.95).

There was a decrease in RQ postoperatively in both groups, indicating increased fat metabolism. This did not differ between groups. Measurement of EE from respiratory gas exchange has been shown to be relatively reliable even in a setting of altered substrate utilisation (Ferrannini 1988, Heim 1985). It has been shown that allowing for different substrate utilisation, there is little error in calculating EE from measured $\dot{V}CO_2$ and $\dot{V}O_2$. Errors of around 1 to 2% have been estimated to occur if different physiological states such as lipogenesis and glycogenolysis are not factored into equations for EE (Ferrannini 1988).

5.4.2 The metabolic response in REE to abdominal surgery in children differs to that in adults and neonates

There is an increase in energy expenditure in adults postoperatively. This increase may be proportional to the degree of operative stress. After thoracic surgery in adults with carcinoma of the oesophagus, EE increased up to 7 days postoperatively (Sato et al 1997). Brandi *et al* found that EE increased 24 hrs post thoracic surgery for lung resections (Brandi et al 1996).

The response to abdominal surgery in adults has also been characterised. Tannus *et al* found no significant increase in EE in patients after 2 to 4 hour operations of mild severity (Tannus et al 2001). Their lack of a response may be due to the fact that the operations performed in this study were hernia repair and cholecystectomy, which are associated with minimal metabolic response. Also they measured TEE, which is a poor indicator of the metabolic response to surgery, as primarily BMR is affected by surgery. Discriminating between TEE and REE is critical in assessing the meaningfulness of any change in energy metabolism postoperatively (Bodamer et al 1997). Indeed, when REE was used as the measure of the metabolic response in patients undergoing

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cholecystectomy, Luo *et al* (Luo et al 2003) found an increase 24 hours postoperatively. Watters *et al* confirmed an increase after patients undergoing elective resections of the colon and/or rectum (Watters et al 1993). Also Fredrix *et al* (Fredrix et al 1991) documented an increase in REE 7 days after abdominal surgery of severe intensity (colorectal and gastric surgery).

In this randomised study the operation performed was Nissen fundoplication, an operation associated with moderate operative stress. In addition the outcome used was REE, which is more meaningful than TEE in monitoring changes in energy metabolism (Bodamer et al 1997). There was a decrease in REE in the open group in the postoperative period. This has not been previously described in children. Decreased metabolism in the intraoperative period is well documented (Brundin, et al. 1994) and is contributed to by the effects of anaesthesia, and decreased thermoregulation (Motamed et al 1998). The postoperative decrease in REE in the immediate postoperative period may represent an extension of the decrease in metabolism of the intraoperative period.

This contrasts to the data in neonates, in whom there was a short-lived increase in REE 4 hours after abdominal surgery of moderate severity (Jones et al 1994, Jones et al 1995). This difference in responses may confirm that age modulates the pattern of the metabolic response to operative stress. Neonates are relatively hypermetabolic compared to older children and adults, and have a substantial proportion of energy metabolism dedicated to growth. The early postoperative increase in REE may be confined to this period of hypermetabolism. No patients studied in this chapter were in the neonatal period.

There may also be an effect of vagal stimulation on the metabolic response noted. Studies have shown that there is an alteration of the postoperative inflammatory response with vagal stimulation (Borovikova et al 2000). Consequently there may be an effect on the metabolic response after Nissen fundoplication due to stimulation of the vagus nerve in the postoperative period. This may partially contribute to the metabolic response seen.

5.4.3 Laparoscopy alters the metabolic response to operative stress compared to open surgery

Compared to open surgery in this group of patients, there was no significant change in REE up to 24 hours after laparoscopy. The overall pattern of the response was also different between groups. The laparoscopic group had a consistent rise in REE at 4 hours postoperatively. The increase in REE at 4 hours was not an artificial finding resulting from increase CO_2 absorption from the abdominal cavity at 4 hours, since when only VO_2 was examined, the pattern of change was identical. The contribution of CO_2 to REE is minimal, and the effect of any absorbed CO_2 is negligible. Furthermore previous studies have found that 30 minutes after laparoscopic surgery there is no significant CO_2 excretion above baseline (Blobner et al 1994), indicating complete excretion of any exogenous CO_2 from the peritoneal cavity.

Operative time was significantly longer in the laparoscopic group. However, after accounting for the differences in operative time in the multilevel model analysis, the pattern of change in the metabolic responses between groups was significant. This suggests that these differences were independent of the operation length.

Pain may be one of the stimuli for the post operative metabolic responses to operations. It has been shown that the degree of sedation can influence energy metabolism postoperatively (Terao et al 2003). This is not a consistent finding, as Watters *et al* (Watters et al 1993) found no difference in the hypermetabolism of adults undergoing colorectal resection randomised to have epidural anaesthesia. Despite significantly higher analgesic requirements and pain scores in the group without epidural anaesthesia, both groups in their study displayed an increase in EE up to 2 days postoperatively.

The possibility exists that the metabolic response to pain may be a determinant of postoperative EE. However, postoperative pain relief was standardised and there was no difference in morphine doses or pain scores between groups in this study (see Chapter 8 for details) to account for the significant difference in the metabolic response in the two groups of patients.

Although there are many studies on other aspects of the metabolic response to laparoscopy, only one study has directly compared the postoperative changes in REE between open and laparoscopic surgery. Luo *et al* compared the changes in REE after laparoscopic and open cholecystectomy in adults (Luo et al 2003). There was an increase in REE at 24 hours in both groups; the increase was significantly higher in the open group compared to the laparoscopic group. However the pattern of change was similar in the two groups.

This decrease in REE seen in patients in the open group in this chapter may represent the 'ebb phase' of Cuthbertson's original description of metabolic response to injury and surgery (Cuthbertson 1970, Cuthbertson 1932, Cuthbertson 1942). Cuthbertson also highlighted the importance of thermoregulation in modifying the metabolic response to

surgery in the postoperative period (Cuthbertson et al 1968, Cuthbertson et al 1972a). He found that alterations in postoperative temperature altered the observed metabolic response. The intraoperative increase in core temperature and subsequent maintenance of postoperative REE may represent a blunting of the 'normal' metabolic response seen in the open group by alteration in intraoperative metabolism and/or thermoregulation in children during laparoscopy.

The alteration of intraoperative metabolic responses may be an important contributor to the pattern of postoperative energy and protein metabolism seen. Intraoperative anaesthesia alters thermoregulation and can give rise to intraoperative hypothermia (Sellden 2002). Children are more susceptible to the effects of intraoperative hypothermia due to their poor insulation and decreased thermoregulatory reserve. Laparoscopy preserves body heat compared to open surgery, as there is no open wound from which heat can be lost. This preservation of body heat translates into an alteration in thermoregulation that may be the stimulant for the relative hypermetabolism seen during laparoscopy. This increase in intraoperative metabolism was reflected by the increase in $\dot{V}O_2$ seen in patients during laparoscopy.

5.4.4 Protein metabolism in the early postoperative period

In adults, surgical insult has been shown to result in an increase in whole body protein turnover and catabolism, with a decrease in whole body synthesis in the days following surgery (Carli & Halliday 1996, Clague et al 1983). This lasts between 2 to 7 days, and may extend into weeks postoperatively. There is the reciprocal relationship between visceral and acute phase proteins in the postoperative period (Chwals et al 1993); the postoperative protein catabolism particularly affects muscle protein. Protein metabolism

can be a determinant of postoperative outcome and morbidity in children (Chwals et al 1993).

Few studies have investigated protein metabolism in the immediate postoperative period. Lattermann *et al* demonstrated a decrease in protein synthesis and catabolism along with an increased oxidation 2 hours postoperatively in adults undergoing colectomy (Lattermann et al 2002). Another study found that protein catabolism decreased intraoperatively and in the first 2 hours postoperatively (Carli et al 1990). The findings in this chapter concur with the decrease in protein catabolism in the immediate postoperative period.

In a study of neonates operated on for necrotising enterocolitis, there was no difference in protein metabolism during the acute and convalescent period (Powis et al 1999). In that study it is possible that the findings are influenced by the disease process as well, rather than a measure of the influence of operative stress only. In another study in infants and children (Powis et al 1998), there was no significant change in protein catabolism in the early postoperative period (6 hours), although there was large individual variation in the response. Concomitantly, they found no significant change in EE. Their study group consisted of patients undergoing operations of varying severity, which could account for the degree of variability in their results and the difference to the findings in this chapter. This study consisted of a controlled group of children, undergoing a standardised operation and anaesthesia and demonstrated a consistent and early decrease in protein catabolism after open surgery.

Values for protein catabolism in this chapter are similar to values found in other studies in children (Schutz et al 1999). The values for baseline protein catabolism were, as

expected, inversely proportional to weight. This is in keeping with the correlation between energy metabolism and weight. Protein metabolism is a major contributor to overall energy metabolism. The energy requirement of protein synthesis, for example, has been estimated to account for between 1/3 and 1/5 of REE (Schutz et al 1999).

5.4.5 Laparoscopy altered protein catabolism compared to open surgery

In the laparoscopic group there were no set changes in protein catabolism. In contrast to the open group, there was no significant change in whole body catabolism in the laparoscopy group. This maintenance of protein catabolism after laparoscopy has not been previously described.

In one study on the effects of cholecystectomy on muscle protein synthesis (Essen et al 1995), there was a decrease 24 hours postoperatively. However, there was no difference between laparoscopy and laparotomy in this regard. In that study, protein metabolism was assessed at the same time as a hyperinsulinaemic normoglycaemic clamp. Therefore, muscle protein synthesis in their study may reflect the response or sensitivity to insulin, rather than a natural response. Using different methodology, Hammarqvist *et al* demonstrated no significant difference in muscle protein catabolism 48 hours after laparoscopic *versus* open cholecystectomy (Hammarqvist et al 1996). There have been no studies comparing whole body protein metabolism after open and laparoscopic surgery.

5.4.6 Protein catabolism correlates with body temperature and energy metabolism

A study by Carli *et al* found that protein breakdown on the 2nd postoperative day was reduced in adults who were kept warmed (intra- and postoperatively) compared to those

who were cold (Carli et al 1991a). Warming also inhibited the degree of leucine oxidation. Carli *et al* also demonstrated that there was a greater decrease in protein catabolism in elderly patients undergoing hip surgery that were cold compared to those kept at normothermia (Carli et al 1989). This link between intraoperative temperature regulation and protein metabolism offers one possible explanation for the observed differences in protein catabolism between groups in this chapter. General anaesthesia causes hypothermia due to decreased metabolic rate and impaired thermoregulation (Sellden 2002). The increase in core temperature during laparoscopy noted in the intraoperative period may maintain protein turnover in the early postoperative period in children. Maintaining thermo-neutrality may alter the effect of surgery on protein metabolism in children.

In this chapter protein catabolism generally mirrored the changes in energy expenditure. Associated with a decrease in REE in the open group at 4 hours, there was a decrease in protein catabolism. In the laparoscopy group both REE and protein catabolism were preserved at 4 hours. Also the overall change in REE significantly correlated with the change in protein metabolism in the laparoscopic group. Protein metabolism may contribute up to 1/3 to REE. Overall 53% of the changes in REE could be accounted for by the change in protein catabolism (r^2=0.53). This suggests that after laparoscopy protein metabolism may be one of the factors involved in the overall metabolic response in children. Previous studies have found similar correlation between protein balance and energy balance in depleted patients (Elwyn et al 1979). The link between protein and energy metabolism was suggested by Cuthbertson's observation of the parallel changes in these two parameters in the postoperative or traumatised patient (Cuthbertson 1932).

Though the late postoperative changes have not been investigated in this study, it is probable that these changes may affect the metabolic response and protein metabolism beyond the 24 hour period. Whether there is a flow phase of hypermetabolic response after open surgery in children, and whether this is also blunted by laparoscopy is not known. This can be investigation in future studies.

5.4.7 CO_2 metabolism may alter protein catabolism after laparoscopy

CO_2 is absorbed from the abdominal cavity during laparoscopy. Results from Chapter 3 showed that the absorption profile in children is different to that of adults. The local reaction to this CO_2 may be responsible for driving a different local, and systemic, metabolic response in the laparoscopic group. Studies have shown that there is a local peritoneal acidosis during CO_2 laparoscopy (Wong et al 2004). This local acidosis may be a significant determinant of the metabolic response in the immediate postoperative period.

Interestingly studies of children (Boirie et al 2000) and adults (Mitch et al 1994, Price et al 1998, Reaich et al 1992, Reaich et al 1993) with chronic renal failure have shown that acidosis was strongly correlated to the rate of protein catabolism and leucine oxidation. Acidosis also increases protein catabolism in healthy volunteers (Reaich et al 1992). Children with increasing acidosis had higher rates of protein breakdown (Boirie et al 2000). Compared to their findings, baseline leucine appearance from protein (C_{leu}; μmol/kg/hr) in this study (167) was lower than the group of children who were acidotic (249) but higher than the children who were not (86). In the postoperative period, C_{leu} in the laparoscopic group (203) approached that of children who were acidotic, but was lower after open surgery (184).

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It can therefore be hypothesised that protein catabolism in the laparoscopic group may be driven by the local (and/or systemic) acid base balance. As this increased protein catabolism is mediated by an ATP-dependent pathway (Mitch et al 1994), this may also partially explain the link between protein and energy metabolism seen, and the maintained REE after laparoscopy.

5.5   Conclusions

In conclusion, laparoscopy was associated with an increase in intraoperative metabolism compared to open surgery. There was a decrease in energy metabolism 24 hours after open Nissen fundoplication in children. This response was blunted by laparoscopic surgery. There was a decrease in protein catabolism after open surgery; laparoscopy was associated with blunting of this response. Protein catabolism significantly correlated with changes in REE in the laparoscopy group. These findings suggest that laparoscopy alters intraoperative thermo-regulation, energy metabolism and protein catabolism after Nissen fundoplication in children. In both groups there was a shift to increased fat oxidation postoperatively.

The link between REE in the immediate postoperative period and intraoperative thermoregulation and energy metabolism can be investigated in an animal model and/or a clinical setting. There have been no studies directly investigating the link between intraoperative core temperature and energy metabolism, and the effect this may have on postoperative metabolism.

Although the results in this chapter have shown differences in protein catabolism between laparotomy and laparoscopy, net protein balance was not available. Further

studies on whole body protein catabolism, oxidation and synthesis should be undertaken to assess the effect on overall protein balance.

The link between intraoperative acid base balance and protein metabolism also needs investigation. Measurements of acid base status, along with postoperative protein turnover studies, may shed light on the mechanisms controlling immediate postoperative protein metabolism. The local acid base status in the peritoneal cavity in children should also be investigated to identify any local and systemic acidosis that might drive the metabolic response.

The late postoperative response in energy and protein metabolism should also be investigated to gain an insight into the late responses. There may be a rebound increase in metabolic changes similar to the 'flow phase' described by Cuthbertson. The late response may also differ between open and laparoscopic groups.

The changes in the postoperative metabolic responses may be due to alterations in the postoperative hormonal milieu. Alternatively, if the changes in metabolism are due to alteration in intraoperative thermoregulation, there may be dissociation between the hormonal environment and metabolic responses. This possibility is investigated in the following chapter.

Figure 5.1 *Indirect calorimetry using the canopy mode to measure REE.*
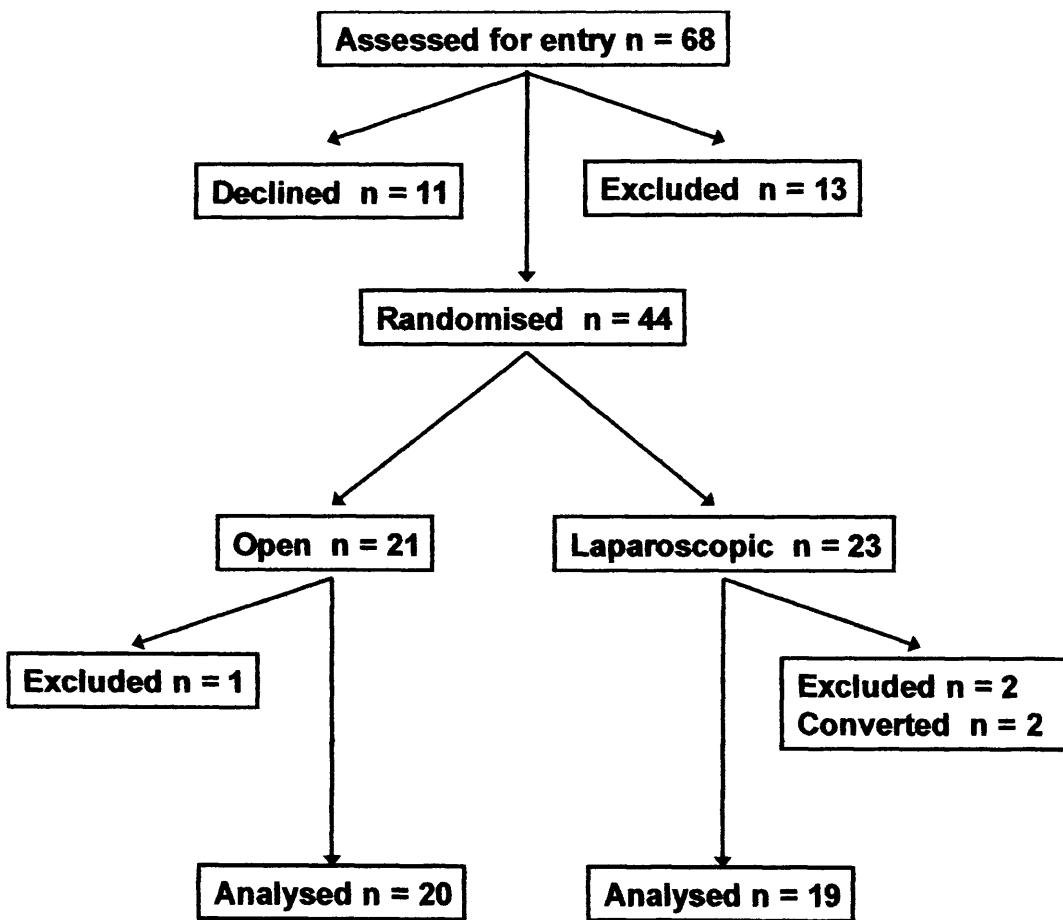
Figure 5.2 *Patient flow chart for randomised control study*

	Open surgery (n=16)		Laparoscopy (n=16)		p value
	Median	Range	Median	Range	
Age (months)	53.9	3.9 – 199.3	67.0	3.8 – 236.4	0.77
Weight (kg)	15.0	4.1 – 60.0	13.7	4.5 – 43.0	0.81
Neurologically impaired (%)	75		81		0.67
Operation length (minutes)	80.0	45 – 120	163	126 – 210	< 0.001

Table 5.1 *Patient demographics in patients with REE data.*

	Preoperative (n=20)		Postoperative (n=22)	
	Mean	S.D.	Mean	S.D.
Leucine Flux (μmol/kg/hr)	232.48	118.21	207.22	101.13
Protein catabolism (g/kg/hr)	0.37	0.19	0.33	0.17

Table 5.2 *Protein kinetics in the preoperative and postoperative phase in all patients*

	Open surgery (n=6)		Laparoscopy (n=9)		p value
	Median	Range	Median	Range	
Age (months)	44.4	3.9 – 108.8	90.1	4.2 – 236.4	0.24
Weight (kg)	13.8	6.8 – 22.2	14.5	4.7 – 39.0	0.77

Table 5.3 *Demographics for patients with paired protein turnover data.*

	Preoperative		Postoperative		p value
	Mean	S.D.	Mean	S.D.	
Open n=6	0.39	0.17	0.28	0.16	0.03
Laparoscopy n=9	0.37	0.20	0.32	0.19	0.51

Table 5.4 *Paired protein catabolism (g/kg/hr) in the pre- and postoperative periods after open and laparoscopic surgery.*
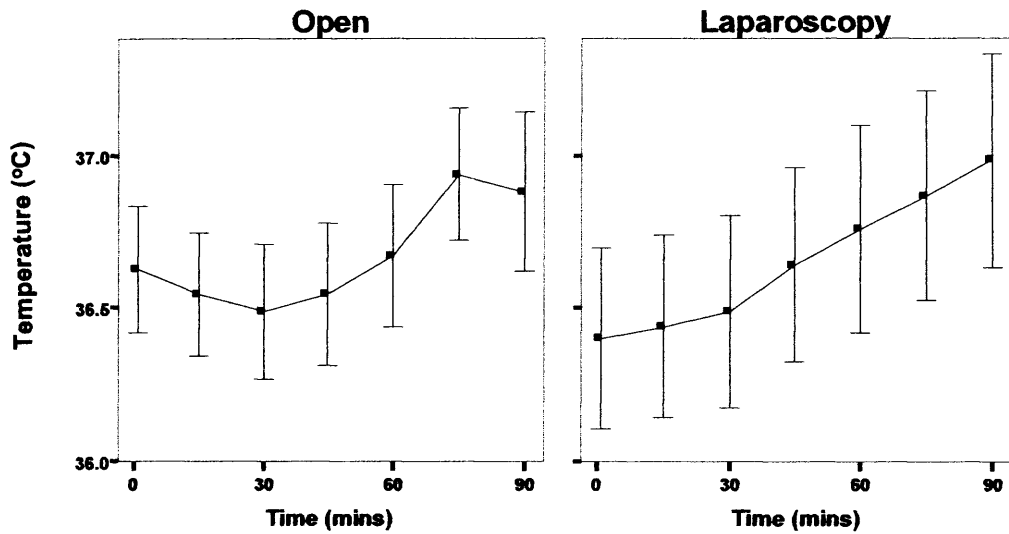
Figure 5.3 *Intraoperative core temperature in patients randomised to open and laparoscopic Nissen fundoplication.*
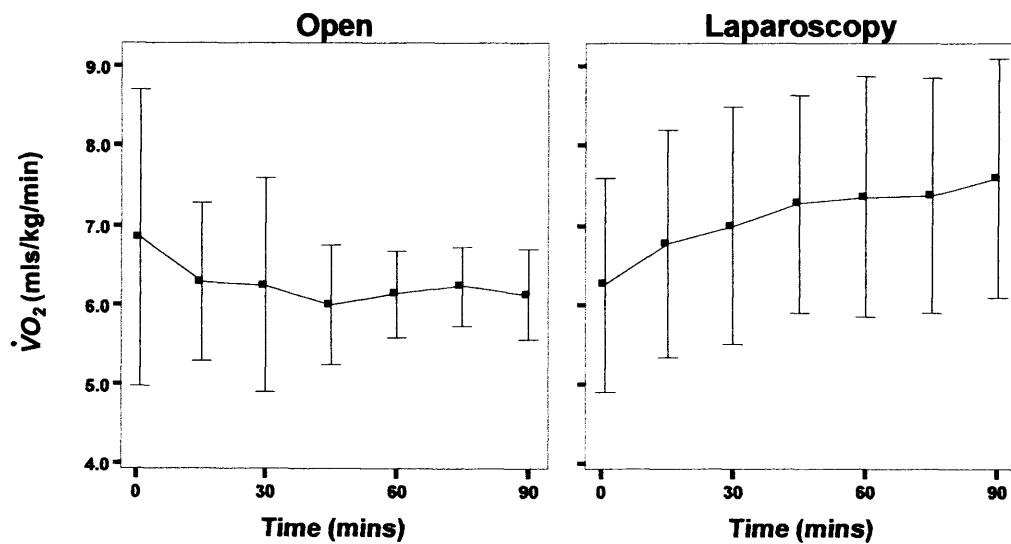
Figure 5.4 *Intraoperative $\dot{V}O_2$ in patients randomised to open and laparoscopic Nissen fundoplication.*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
EE	27.4	24.7	24.8	25.4	24.6	23.5	20.5	29.1	22.0	26.8	24.3	25.4	25.0	24.3	29.7	25.9	24.1	24.6	24.3	24.2	29.1	30.8	26.3	29.2	27.0	23.7	23.0	20.6	26.5	25.3
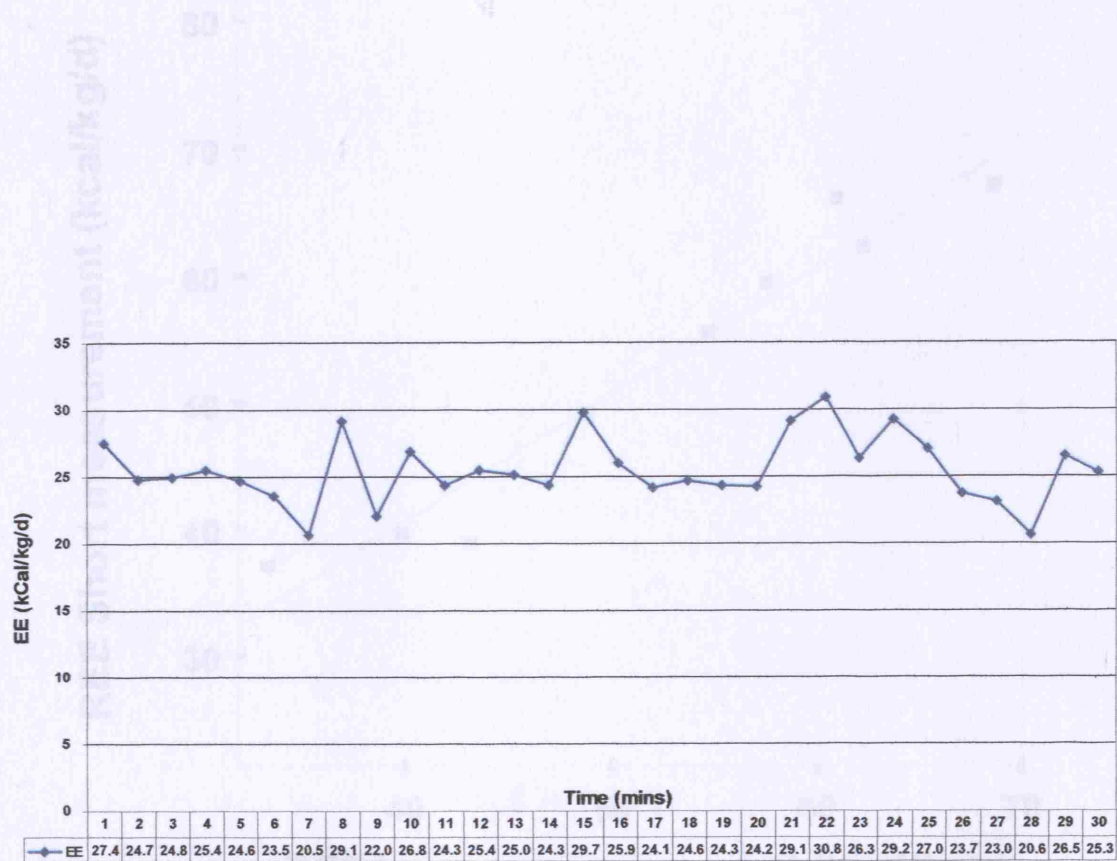
Figure 5.5 *Minute by minute calorimetry recording for REE in one patient.*
Measurements were stable during recordings.
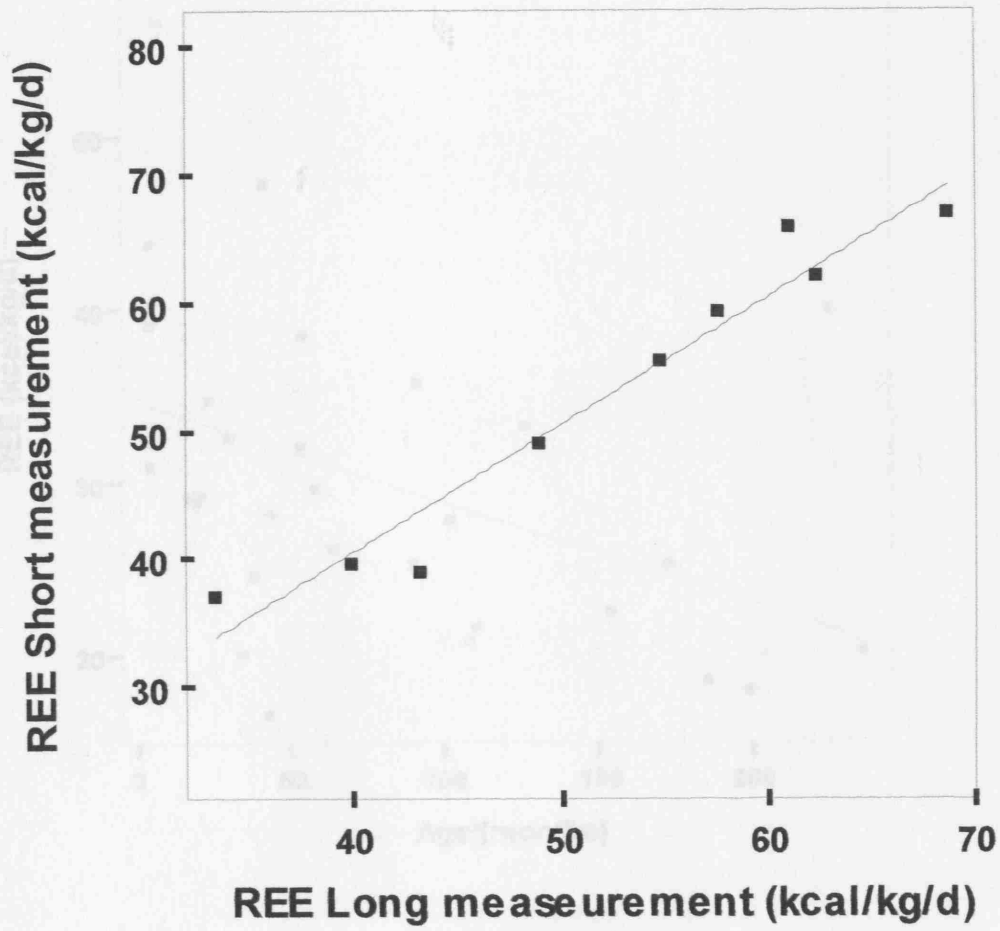
z
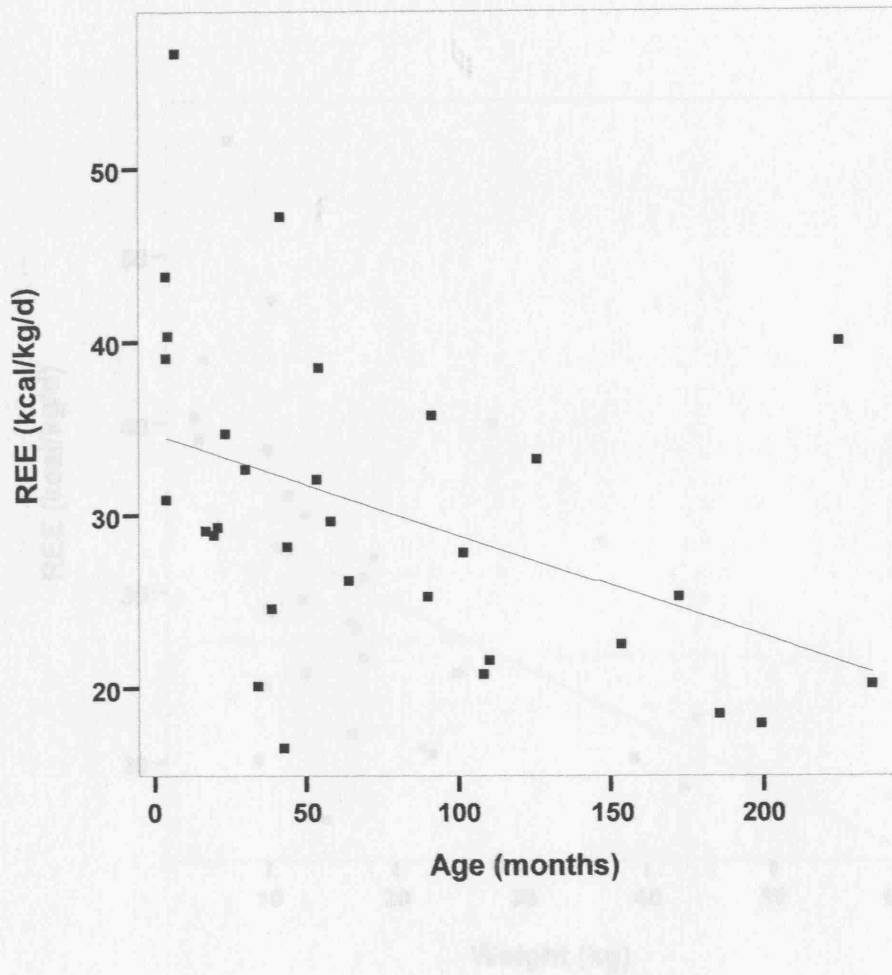
Figure 5.7 *Correlation between baselines REE and patient age.*

Figure 5.8 *Correlation between baselines REE and patient weight.*
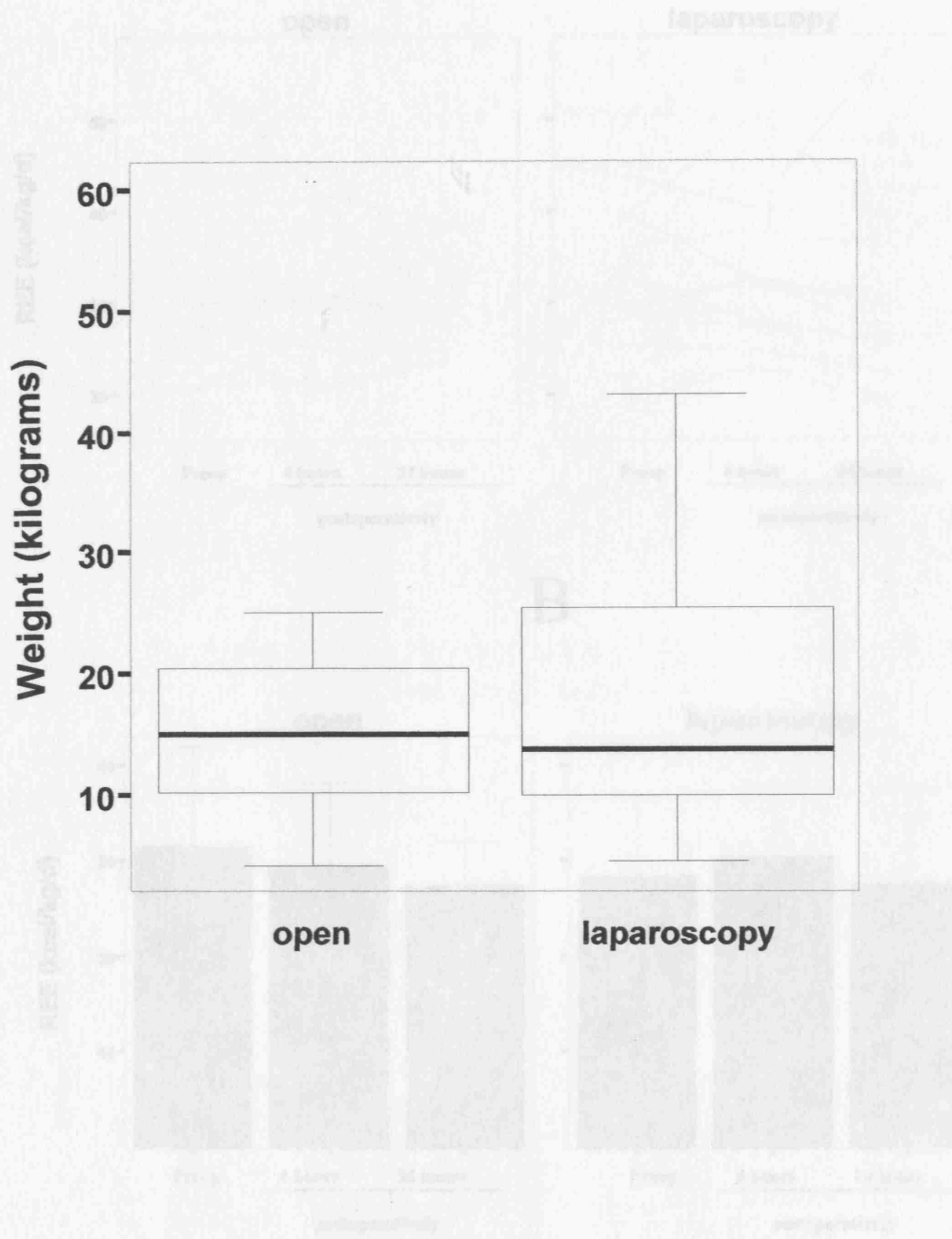
Figure 5.9 *Weight in patients with REE data*
(Median, interquartile range and 95% centile)

A

B
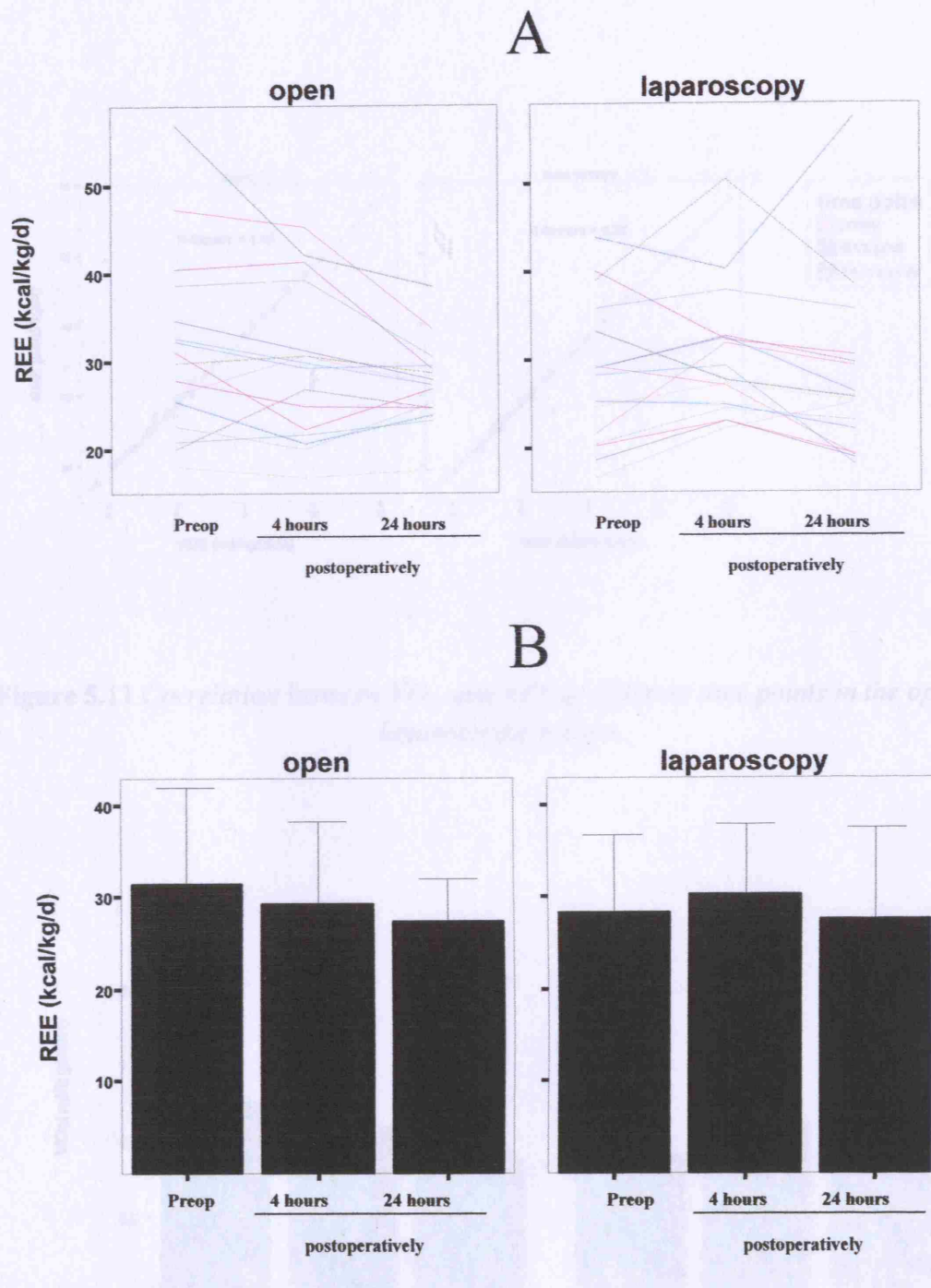
Figure 5.10 *Changes in REE in the postoperative period in the open and laparoscopic groups.*
(A: individual lines for each patient, B: mean + SD)
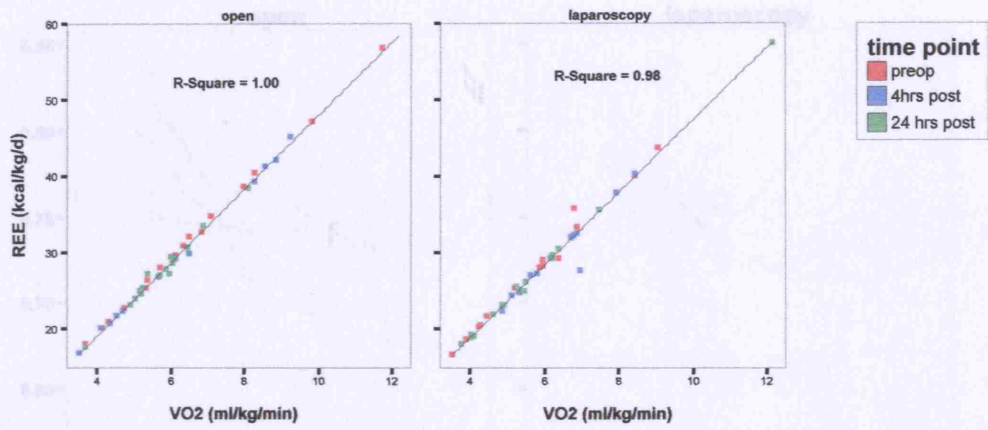
Figure 5.11 *Correlation between* $\dot{V}O_2$ *and REE at different time points in the open and laparoscopic groups.*
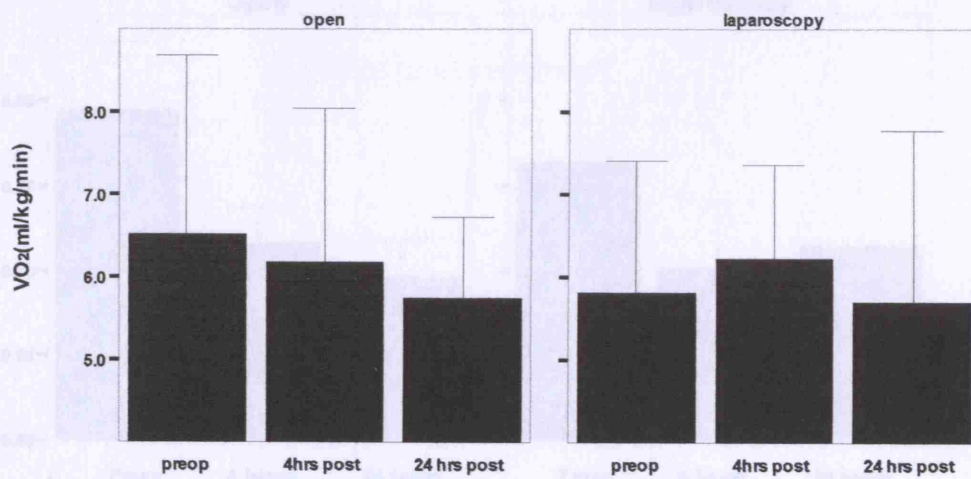
Figure 5.12 *Pattern of change in* $\dot{V}O_2$ *after open and laparoscopic surgery.*
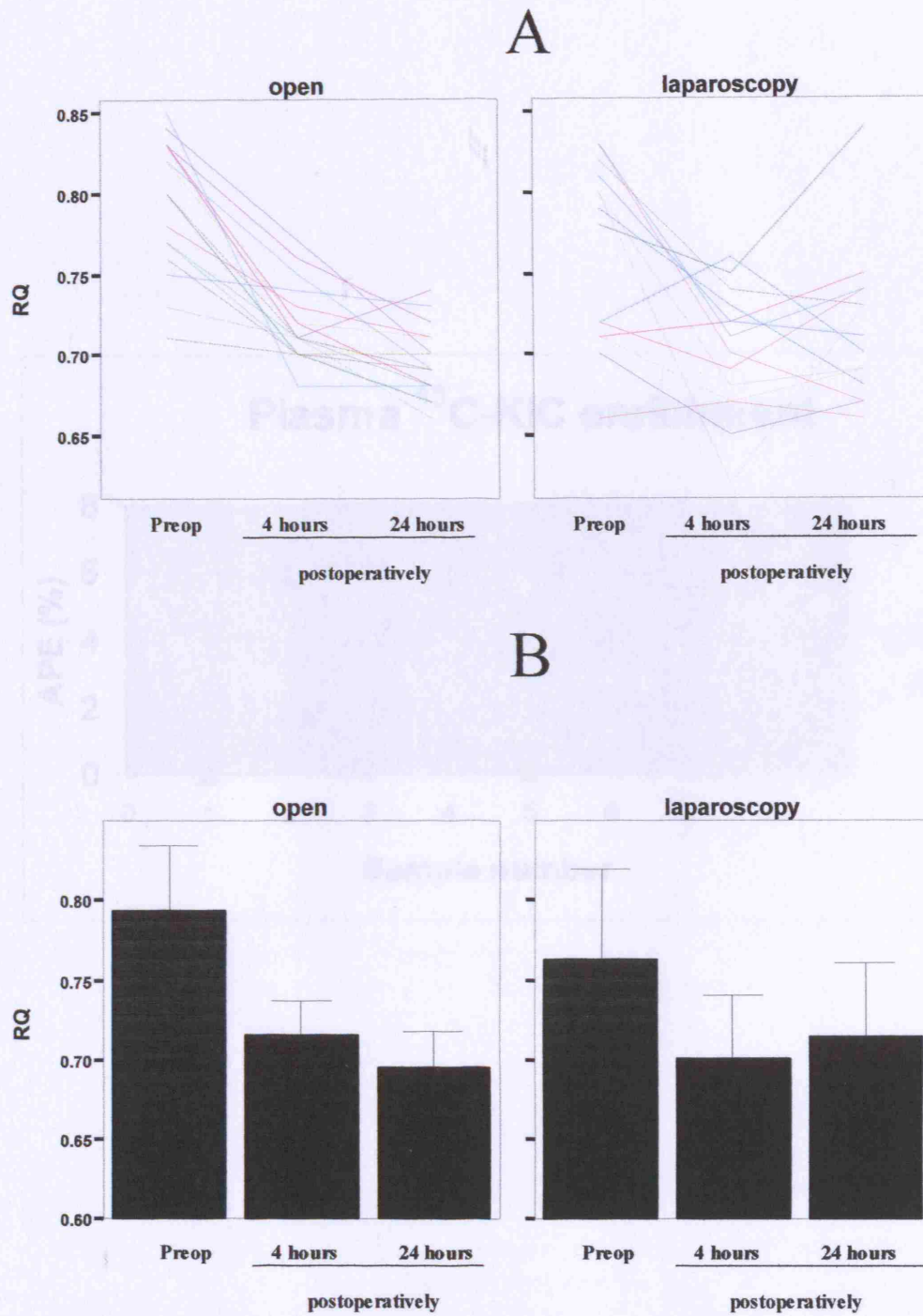
Figure 5.13 *Change in RQ in the postoperative period in the open and laparoscopic groups*
(A: individual lines for each patient, B: mean + SD)
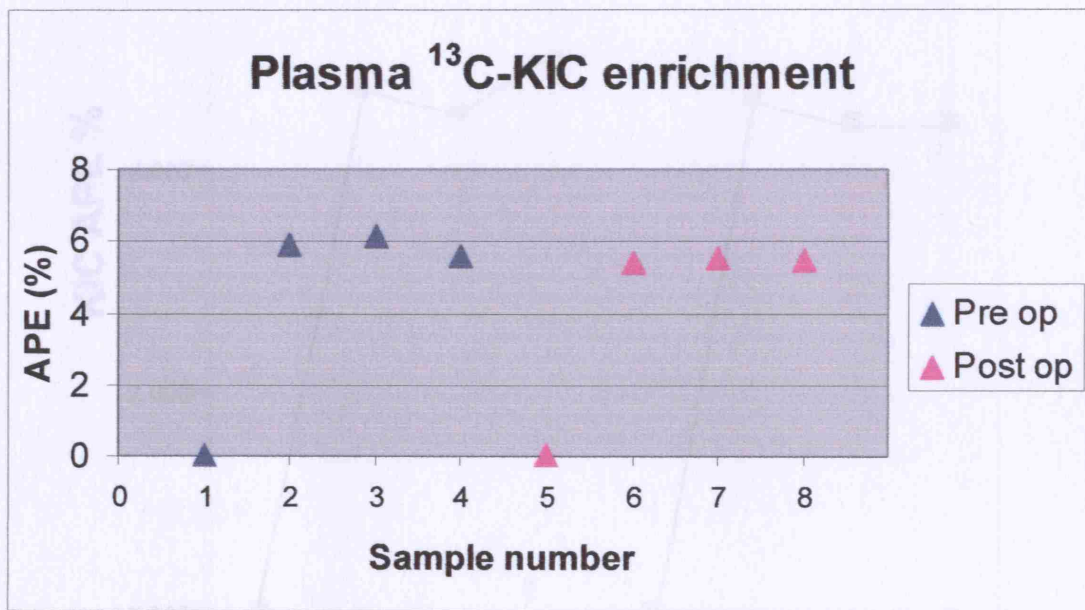
Figure 5.14 *Plasma ^{13}C-KIC enrichment during steady state conditions in one patient in the preoperative and postoperative period*
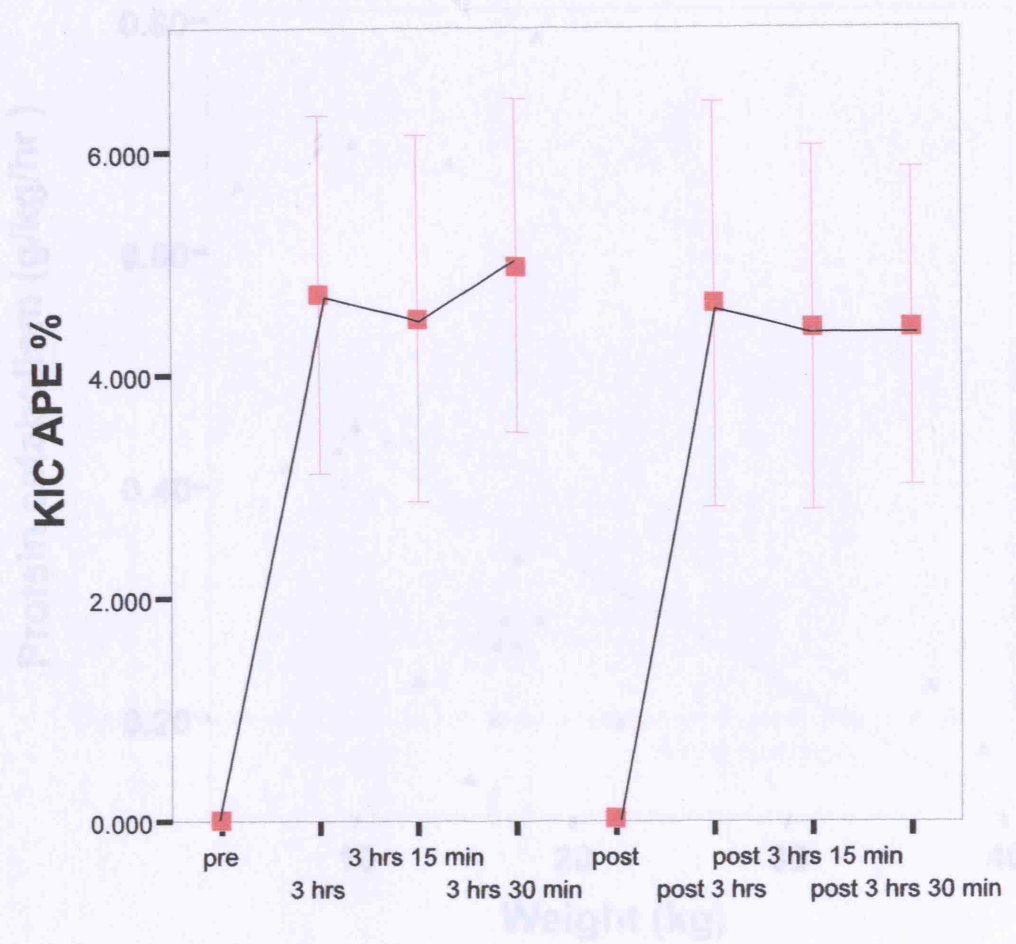
Figure 5.15 *Plasma ^{13}C-KIC enrichment at plateau in all patients in the pre- and postoperative period.*
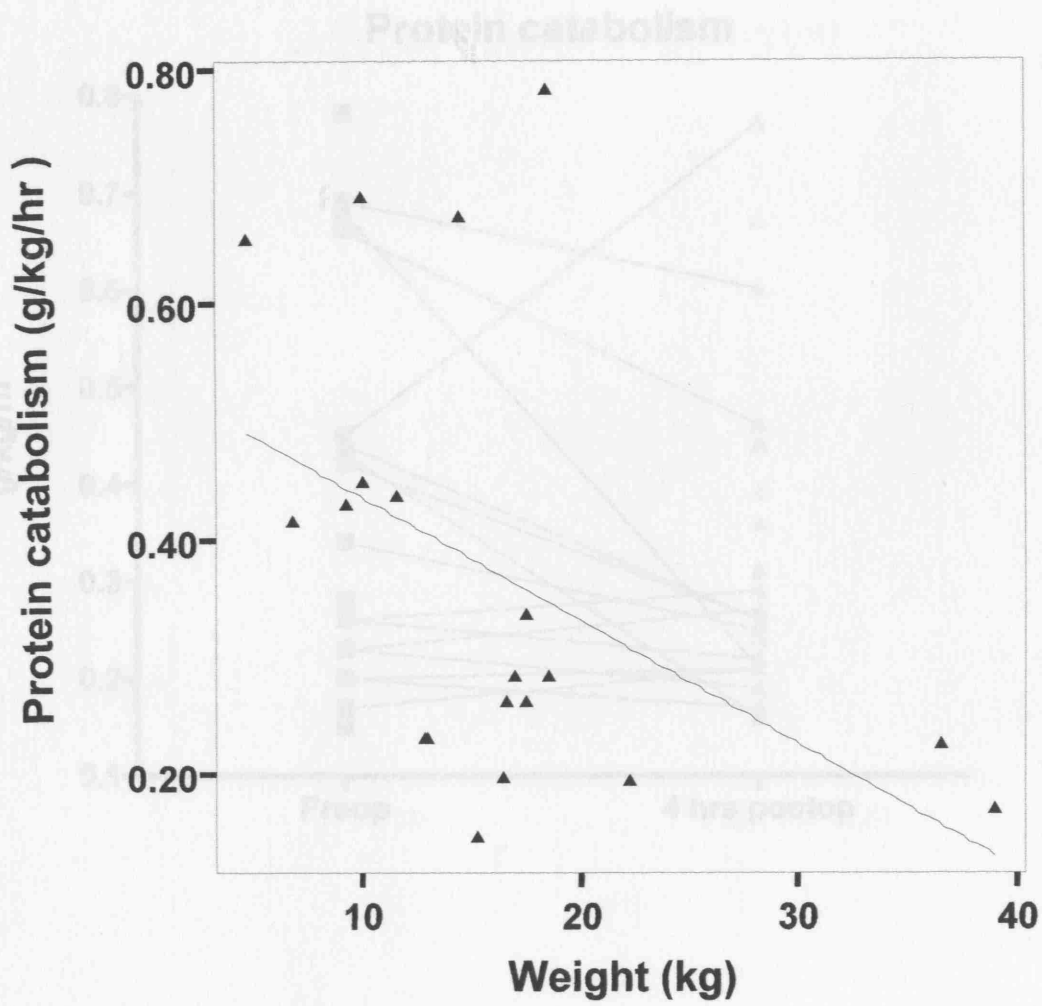
Figure 5.16 *Correlation between baseline protein metabolism and patient weight.*
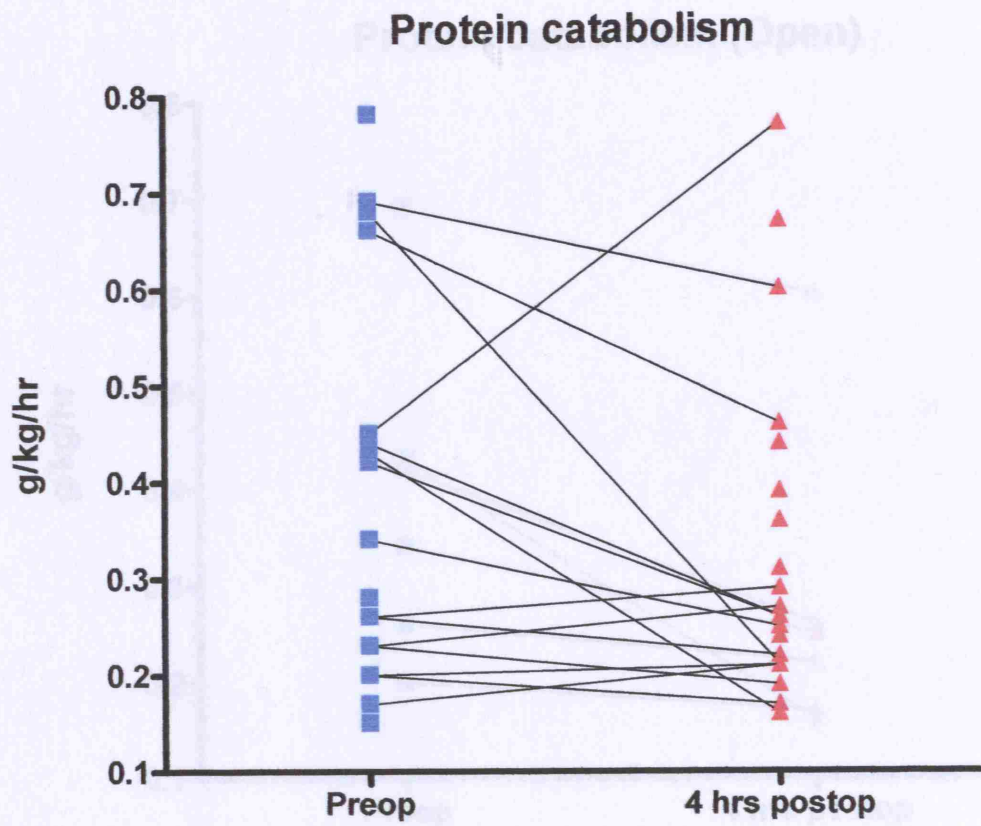
Figure 5.17 *Whole body protein catabolism in all patients pre- and postoperative. Paired data between patients are connected.*
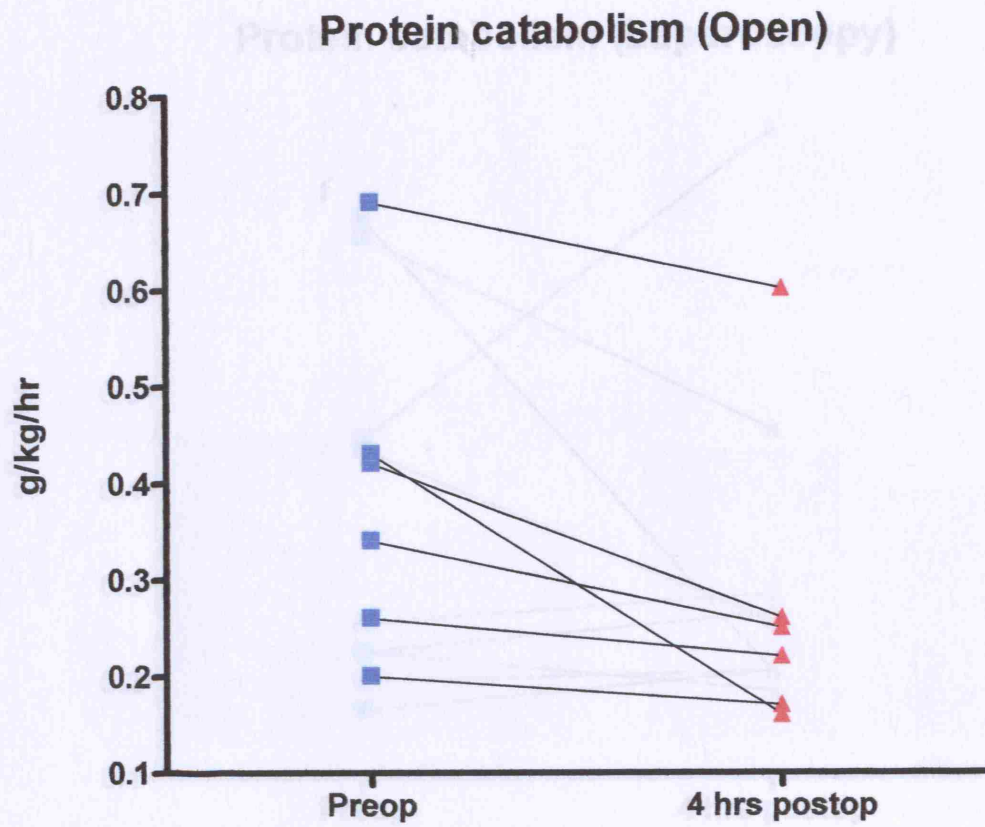
Figure 5.18 *Protein catabolism in the pre- and postoperative periods in the open group*
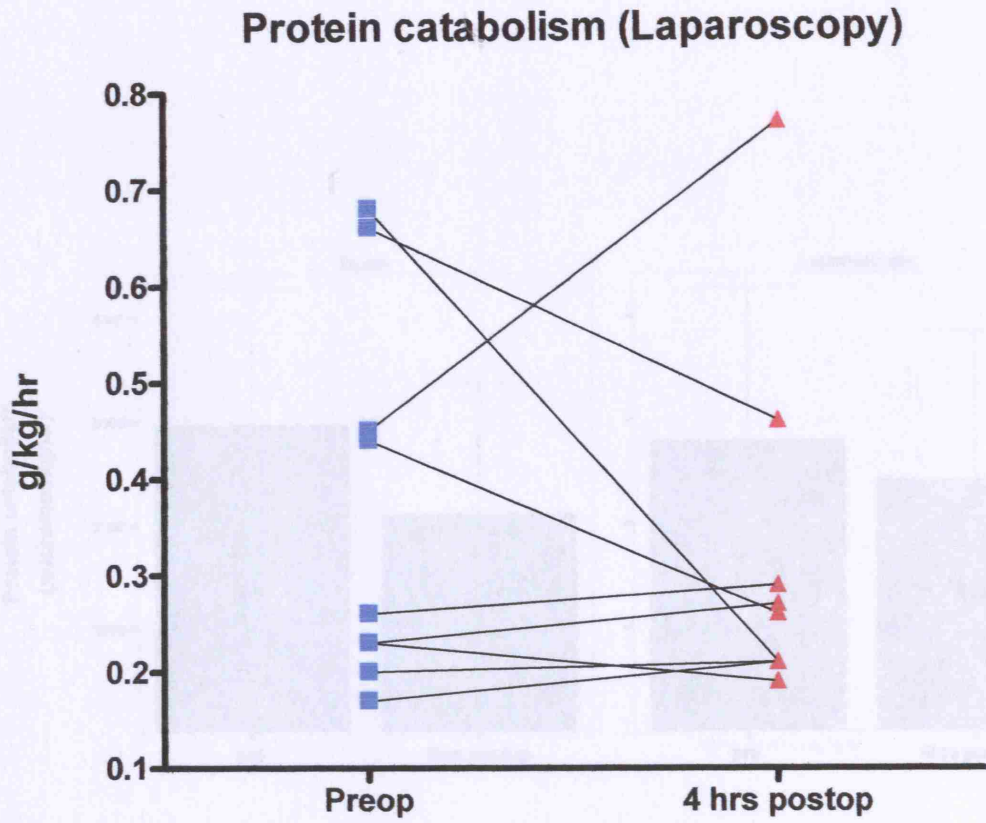
Figure 5.19 *Protein catabolism in the pre- and postoperative periods in the laparoscopy group.*
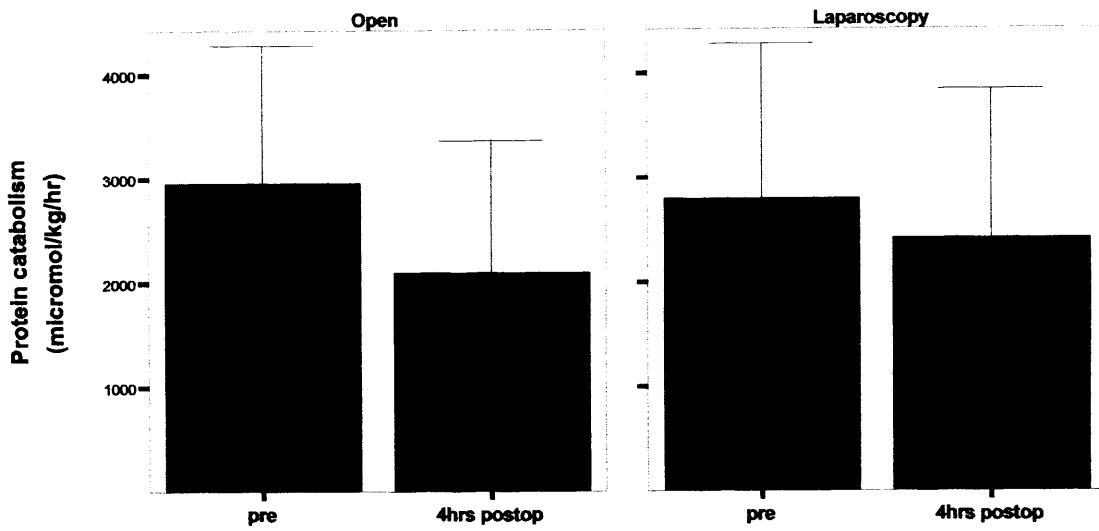
Figure 5.20 *Protein catabolism in the pre- and postoperative phase after open and laparoscopic surgery.*
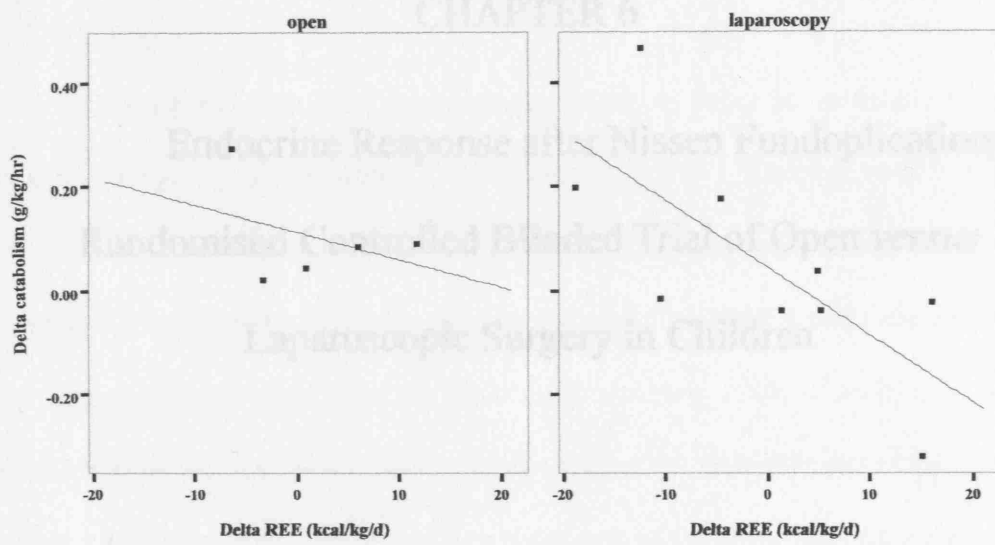
Figure 5.21 *Correlation between changes in protein catabolism and REE*

CHAPTER 6

Endocrine Response after Nissen Fundoplication;

Randomised Controlled Blinded Trial of Open *versus*

Laparoscopic Surgery in Children

6.1 Introduction

The hormonal response to operative stress drives some of the postoperative metabolic responses. Although it is assumed that laparoscopic surgery is associated with a decreased hormonal response compared to open surgery, no randomised control studies have been performed to investigate this assumption in children. The alteration in metabolic responses after laparoscopy that were described in the previous chapters may also be related to the hormonal response.

There are substantial hormonal responses to operative stress in neonates, infants and children undergoing major surgery (Deshpande et al 1993, Khilnani et al 1993, Ward Platt et al 1990). Glucose, insulin, catecholamines, lactate and cortisol have all been shown to altered postoperatively in children (Anand et al 1985b), with some correlation to the degree of operative stress (Anand & Aynsley-Green 1988, Ward Platt et al 1990). The changes in the hormonal milieu may be one of the determinants of the metabolic processes postoperatively. It is therefore important to correlate the metabolic response with the endocrine response in an attempt to elucidate the physiological basis of the altered postoperative responses.

6.1.1 Aim

The aims of this chapter are to:

1. Compare the hormonal response in a randomised controlled blinded trial of open *versus* laparoscopic Nissen fundoplication in children and

2. Investigate any association between the endocrine response and the metabolic changes seen in the postoperative period.

6.2   Methods

6.2.1   Patient selection, inclusion and randomisation

The patient group in this chapter consists of the same group as the previous chapter and described in Section 2.2.1. The protocol for this randomised trial has been outlined in Section 2.2.1.1. Parents of patients admitted for Nissen fundoplication were approached for inclusion in this study. Parents were given full informed consent. This study was approved by the Research Ethics Committee of The Institute of Child Health and Great Ormond Street Hospital. Patients were randomised to either laparotomy or laparoscopy by minimisation program, as detailed in Section 2.2.1.1.

6.2.2   Anaesthesia and postoperative management

Children were fasted for six hours preoperatively. Last clear fluid intake was allowed 4 hours before anaesthesia. A standard infusion of dextrose-saline at maintenance rate was commenced at the time of fasting. Anaesthesia was standardised as outlined in Section 2.2.1.4.

Preoperative and postoperative management, fluid management, postoperative feeding were standardised. There was a standardised postoperative pain management protocol used in all patients. These were described in Section 2.2.1.4 and 2.2.1.6.

6.2.3   Measurement of blood glucose, lactate cortisol and insulin

Blood was taken for markers of the endocrine response at the following time points:

- Preoperatively,

- Immediately postoperatively,

- 4 hours postoperatively,

- 24 hours postoperatively and

- 48 hours postoperatively.

Because of ethical considerations and logistical reasons the preoperative blood sample was not taken after overnight fasting, as this would require an additional venipuncture. Baseline sample was taken on admission the evening before the operation, at the same time that other bloods were taken for clinical reasons, and an intravenous cannula placed for preoperative fluid infusion. This was generally taken 4 hours after a meal.

Blood for glucose was collected into fluoride tubes and analysed by dry slide chemistry colorimetric reaction by the Chemical Pathology laboratory at Great Ormond Street Hospital. Glucose is converted by glucose oxidase to gluconate and hydrogen peroxide. In the presence of oxygen, the hydrogen peroxide reacts with 4-aminophenazone and phenol to yield a coloured complex. The intensity of the complex is measured at 505 nm and is proportional to the concentration of glucose.

Blood for cortisol and insulin levels were collected in heparin and immediately sent to the Chemical Pathology laboratory, and analysed by Immulite competitive immunoassay (chemiluminescence) technique. In this assay the amount of light emitted is proportional to the amount of analyte present and is detected using a photomultiplier tube.

Blood for lactate levels were collected into fluoride tubes and analysed by dry slide chemistry colorimetric reaction by the Chemical Pathology laboratory. Lactate is converted by lactate oxidase to pyruvate and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and the 1,7 dihydronapthalene dye system in a horse

radish peroxidase catalysed reaction to yield a coloured complex. The intensity of the complex is measured at 540 nm and is proportional to the concentration of lactate.

Blood catecholamines were measured at the Chemical Pathology department of the Royal Brompton Hospital by High Pressure Liquid Chromatography (HPLC).

6.2.4 Blinding

Laboratory technicians performing the analysis of blood endocrine levels were unaware of which operative approach was performed. All samples were labelled with a study code, which was identical in both groups, along with the time sample was taken. No other clinical data was included in the laboratory request forms.

6.2.5 Calculations and statistical analysis

Data are presented as mean ± SD. Correlation between data was assessed using linear regression. Repeated data at time points within groups were compared using repeated measures ANOVA, and Tukey's multiple comparison test to analyse individual time points. Differences in response were analysed using repeated area under the curve. curves were also analysed using a series of multilevel models (MLwiN®; Centre for Multilevel Modelling, Institute of Education, University of London). Correlations were analysed using linear regression. Differences in correlations between groups were tested using linear regression with laparoscopic and open interactions built into the model.

6.3 Results

6.3.1 Blood glucose

Blood glucose levels were significantly elevated in the postoperative period in both groups (Figure 6.1; n=17, $p<0.001$ in both groups). Levels peaked immediately

postoperatively. There was no significant difference between open and laparoscopic groups with respect to glucose response (p=0.25).

6.3.2 Plasma Insulin

Plasma insulin was measured in 17 open and 14 laparoscopic patients. Contrary to the increase in glucose levels, there was a decrease in blood insulin levels in the postoperative period (Figure 6.2). Levels were lowest 24 hours postoperatively in both groups. Multi-level analysis revealed that the fall was greater in those undergoing laparoscopy (p=0.025). In the open group, plasma insulin 24 hours postoperatively were on average 47% (95% C.I. 33%, 67%) of the preoperative values and the 24 hour measures were reduced by a further 54% (25, 72%) amongst the group undergoing laparoscopy.

There was a significant correlation between glucose and insulin levels in the immediate postoperative period in the laparoscopic group (r^2=0.68, p<0.001) but not the open group (r^2=0.04, p=0.45, Figure 6.3). The difference in the relationship between groups was significant (p=0.03). At 4 and 24 hour postoperatively there was no correlation in either group. At 48 hours blood glucose and insulin significantly correlated with each other in both the open (r^2=0.37, p=0.047) and the laparoscopic group (r^2=0.71, p=0.009, Figure 6.4); with no significant difference between groups at this time (p=0.48).

The insulin/glucose ratio (IU/mol) was calculated (Figure 6.5). Overall there was a decrease in the ratio in the postoperative period in the open group (p<0.05) and the laparoscopic group (p<0.0001). In both groups the ratio decreased to a minimum at 24 hours postoperatively, but was much more significant in the laparoscopic group (24 hours p<0.001) compared to open group (p< 0.01).

6.3.3 Plasma lactate

Lactate levels were significantly altered in the postoperative period in both the open (n=17, p=0.005) and laparoscopy (n=16, p=0.003) groups (Figure 6.6). Levels peaked immediately postoperatively, but then decreased at 4 hours postoperatively. Multi-level analysis confirmed a significant change in lactate in the postoperative period (p<0.001). Overall, lactate levels were lower in the laparoscopy group (p=0.025).

6.3.4 Plasma cortisol

Plasma cortisol was measured in 17 open and 14 laparoscopic patients. Cortisol levels were significantly elevated in the immediate postoperative period in both groups (p<0.001; Figure 6.7). At 4 hours postoperatively levels were still significantly higher than preoperative in the open (p=0.07), but not the laparoscopy group (p=0.37). Levels at all other time points were not significantly different to preoperative values in either group. There was no significant difference in AUC between groups (p=0.22). Multi-level analysis revealed no significant difference between open and laparoscopic groups (p=0.10).

There was no correlation between plasma cortisol and glucose in the immediate postoperative period in either group (r^2=0.06, p=0.64), or at any other time point.

6.3.5 Plasma catecholamines

Adrenaline (Figure 6.8) and noradrenaline (Figure 6.9) levels were measured in 10 patients in the laparoscopy group and 7 patients in the open group. There was no significant change in levels of either catecholamines postoperatively (p=0.1) and no significant difference between open and laparoscopic groups.

6.3.6 Correlation between hormone response and energy metabolism

There was a significant correlation between REE and cortisol at 4 hours postoperatively in the open group (r^2=0.34, p=0.04) but not the laparoscopic group (r^2=0.08, p=0.37 Figure 6.10). There was a significant difference in the correlation between groups (p=0.04). Similarly there was a correlation at 24 hours in the open group (r^2=0.37, p=0.04) but not the laparoscopic group (r^2=0.00, p=0.90, Figure 6.11). The difference was not significant at 24 hours (p=0.38).

6.3.7 Correlation between hormone response and protein metabolism

Protein catabolism in the postoperative period negatively correlated with plasma insulin levels in the open group (r^2=0.44; p=0.04) but not the laparoscopy group (r^2=0; p = 0.9; Figure 6.12). However, the difference was not significant (p=0.36). There was no significant correlation in the preoperative period in either group.

Protein catabolism did not correlate with plasma cortisol at any time point in either group (4hrs data: open r^2=0.03, p=0.065; laparoscopy r^2=0.11, p=0.32). There was also no correlation between protein catabolism at 4 hours postoperatively and plasma cortisol levels measured immediately postoperatively ($r^2$0.00, p=0.86). Protein catabolism did not correlate to plasma catecholamine levels.

6.4 Discussion

This chapter describes the endocrine response to open and laparoscopic Nissen fundoplication in children. The laparoscopic approach did not alter the responses with respect to plasma glucose, cortisol or catecholamines. There was a significant difference in the insulin response between open and laparoscopic groups. Laparoscopy also

significantly altered the hormonal control of intraoperative glucose metabolism, and of energy and protein metabolism in the postoperative period.

6.4.1 Postoperative hyperglycaemia was not altered by laparoscopy

The hyperglycaemic response to operative stress is well documented both in children and adults. The degree of hyperglycaemia can be a significant determinant of postoperative outcome (Zerr et al 1997). This study confirmed a postoperative hyperglycaemic response in children undergoing Nissen fundoplication. This hyperglycaemia was not significantly altered by laparoscopy in this study. Postoperative fluid and glucose administration was standardised in both arms of the study, therefore allowing accurate comparisons between groups.

6.4.2 The insulin and glucose relationship differed between laparoscopy and open surgery

This trial demonstrated a decrease in insulin levels in the postoperative period. This contrasts with the documented increase in insulin secretion in the postoperative period in some studies (Anand et al 1985a, Anand et al 1990). However, this is not universal (Anand et al 1985b, Anand & Aynsley-Green 1988). One of the reasons for the difference in the findings of this trial, to that of other studies in children, may be due to the timing of the baseline blood sample. Whereas in previous studies the baseline sample was taken after an overnight fast, in this trial, because of ethical considerations, the baseline sample was taken on the night before operation. This was before the overnight fasting was initiated, but generally 4 hours after any previous feed. Therefore baseline insulin levels in this study probably reflect more 'physiological' levels than a fasting level.

Interestingly, laparoscopy was associated with a significantly larger decrease in insulin levels in the postoperative period compared to open surgery. At 24 hours postoperatively levels in the laparoscopic group were on average 50% that of the open group. This decrease in insulin levels in the laparoscopic group compared to open group is not previously documented. The cause of this difference is not known. However, laparoscopy was associated with a significant correlation between glucose and insulin in the immediate postoperative period, whereas this relationship was not present in the open group. Therefore laparoscopy maintained some homeostatic glucose control mechanisms intra-operatively, despite similar glucose responses postoperatively. At 48 hours postoperatively the expected relationship between insulin and glucose returned in both groups.

Anand *et al* (Anand et al 1985b) found that neonates exhibited an initial decrease in the insulin/glucose ratio in the immediate postoperative period that was restored by 6 hours. Ward-Platt *et al* (Ward Platt et al 1990) and Bouwmeester *et al* (Bouwmeester et al 2001) found a rapid and continuous rise in the ratio in older infants and children. In contrast there was a continuous fall in the ratio up to 24 hours in the infants and children in this study. The decreased ratio translates into a failure of insulin secretion in the face of hyperglycaemia. Other than the possible influence of timing of baseline sample, it is difficult to speculate on the reason for this difference noted between studies. The study by Anand *et al* noted a difference in the insulin response between term and preterm neonates (Anand et al 1985b), suggesting that age can modulate the postoperative insulin response to hyperglycaemia. Bouwmeester et al also commented on the variation of the response between age groups (Bouwmeester et al 2001).

Preoperative glucose loading can alter or diminish the postoperative metabolic response to operative stress (Ljungqvist et al 1994, Nygren et al 1998). Most studies on the hormonal response to surgery in children performed previously have been performed on children after an overnight fast. It is possible that the differences noted are due to the infusion of glucose given in the preoperative period, and alteration of the response. These observations warrant further investigation of glucose turnover in children undergoing surgery.

6.4.3 Laparoscopy does not blunt the endocrine response to operative stress after Nissen fundoplication in children

In all other respects there was no significant difference in the postoperative endocrine response between the two groups. Plasma cortisol is one of the widely used hormonal markers of operative stress in both adults (Kehlet 2000) and children (Anand et al 1985b). There was a significant increase in both groups postoperatively. Laparoscopy did not blunt this response. It can therefore be concluded that laparoscopic Nissen fundoplication stimulates the pituitary-hypothalamic axis to the same extent as the open procedure in children. This lack of effect of laparoscopy on the endocrine response to surgery concurs with the general view in adults (Kehlet 2000).

6.4.4 Hormonal control of energy and protein metabolism in the postoperative period

Postoperatively, hormonal changes may be one of the factors affecting energy and protein metabolism. The correlation between the changes in protein metabolism and energy metabolism has already been noted in Chapter 5. It is possible that the alteration in protein metabolism in the laparoscopic group may drive the changes in REE, thereby dissociating the 'normal' hormonal drive in the postoperative period.

At 4 hours postoperatively there was a significant correlation between plasma cortisol and REE in the open group; this was not present in the laparoscopic group. Therefore, it seems that overall laparoscopy alters the correlation between the hormonal milieu and energy metabolism in the early postoperative period.

Cortisol increases protein catabolism (Simmons et al 1984), and decreases protein synthesis. However, there was no correlation between cortisol and protein catabolism in this study. This lack of a catabolic effect of cortisol in the early postoperative period concurs with a previous study in adults (Lattermann et al 2002). The catabolic effects of the increased cortisol may not manifest until after 4 hours after circulating levels, due to the delay that may be expected for its translational effects. However, there was no correlation between plasma cortisol immediately postoperatively and protein catabolism at 4 hours.

In contrast, protein catabolism negatively correlated with insulin levels in the open group in the postoperative period. Insulin may represent the major endocrine regulator of protein metabolism postoperatively. Insulin is principally anabolic in nature, increasing protein synthesis and decreasing catabolism (Abu-Lebdeh & Nair 1996, Butte et al 1999, Petrides et al 1994). Therefore maintained insulin secretion in the postoperative period may be crucial to maintaining protein homeostasis.

Laparoscopy altered this relationship between protein metabolism and insulin levels in the postoperative period. In contrast to the situation after open surgery, protein catabolism did not correlate with insulin in the laparoscopic group. Laparoscopy was associated with a significantly lower insulin levels (on average 50% less than the open group) 24 hours postoperatively; however insulin levels were not significantly different

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at 4 hours to explain the difference in its relationship to protein metabolism between groups.

It is possible that the metabolic effect of CO_2 insufflation may be a stimulus for protein metabolism in the laparoscopic group. The increased CO_2 absorption during laparoscopy (see Chapter 3) may cause local and/or systemic acidosis that drives protein catabolism. Children with increasing acidosis have been shown to have higher rates of protein breakdown (Boirie et al 2000). This acidosis may be the primary stimulus for protein catabolism in the immediate postoperative period, thereby dissociating the hormonal influence of protein and energy metabolism seen in the open group. The metabolic demands of protein catabolism may also partially explain the dissociation of the hormonal response to the observed changes in energy metabolism, as this increased protein catabolism is mediated by an ATP-dependent pathway (Mitch et al 1994).

6.5   Conclusions

Both open and laparoscopic approaches caused similar postoperative hyperglycaemia and hypercotisolaemia, but laparoscopy decreased insulin levels to a greater extent compared to open surgery. Changes in the other endocrine markers were not significantly different between the two groups.

Laparoscopy significantly altered the hormonal control of energy metabolism in the postoperative period. Laparoscopy also tended to alter the insulin control of protein metabolism in the postoperative period. Overall the metabolic processes correlated to hormone levels in the open group but not the laparoscopic group postoperatively.

The mechanism behind the differences in insulin homeostasis is not clear. Further studies are required to validate these findings and elucidate the mechanisms behind it. Energy metabolism may be the primary stimulus for protein metabolism in the laparoscopic group, thereby altering the hormonal control. The effect of CO_2 absorption and acid base balance on protein metabolism in the early postoperative period after laparoscopy needs investigation.

Alterations in protein metabolism may alter postoperative substrate availability, and thereby alter the postoperative immune and inflammatory response. Postoperatively, substrate for immune cells in the fasting state may be derived from protein catabolism in the postoperative period. The hormonal response has also been shown to affect the postoperative immune response to surgery. There may therefore be a difference in the immune response in children undergoing laparoscopy. The differences in whole body metabolic response may also be due to differences in the inflammatory response; this may be reflected in the free radical response. The immune and inflammatory responses were examined in the following chapter.
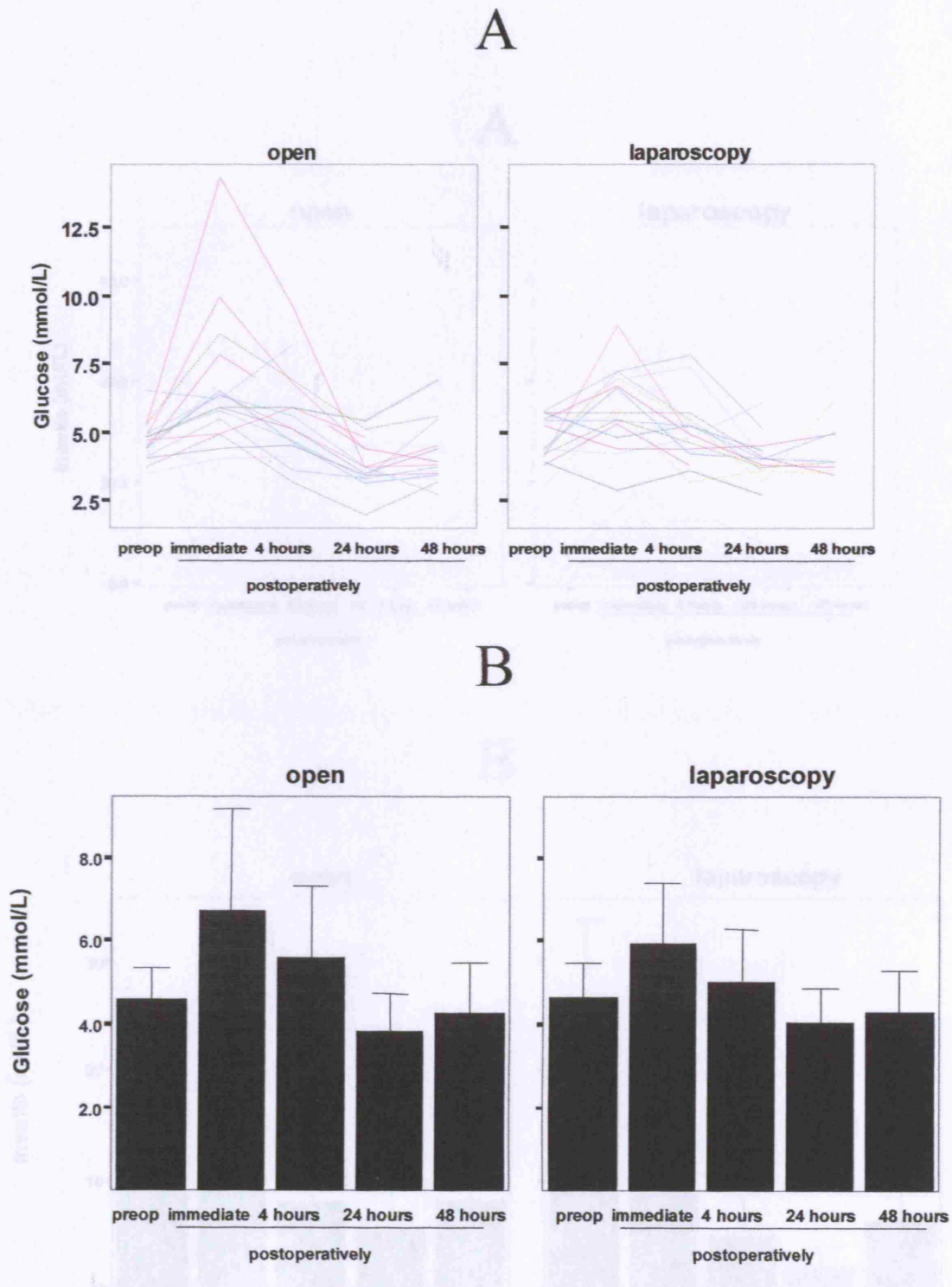
Figure 6.1 *Plasma glucose after open and laparoscopic surgery*
(A: individual lines for each patient, B: mean + SD)
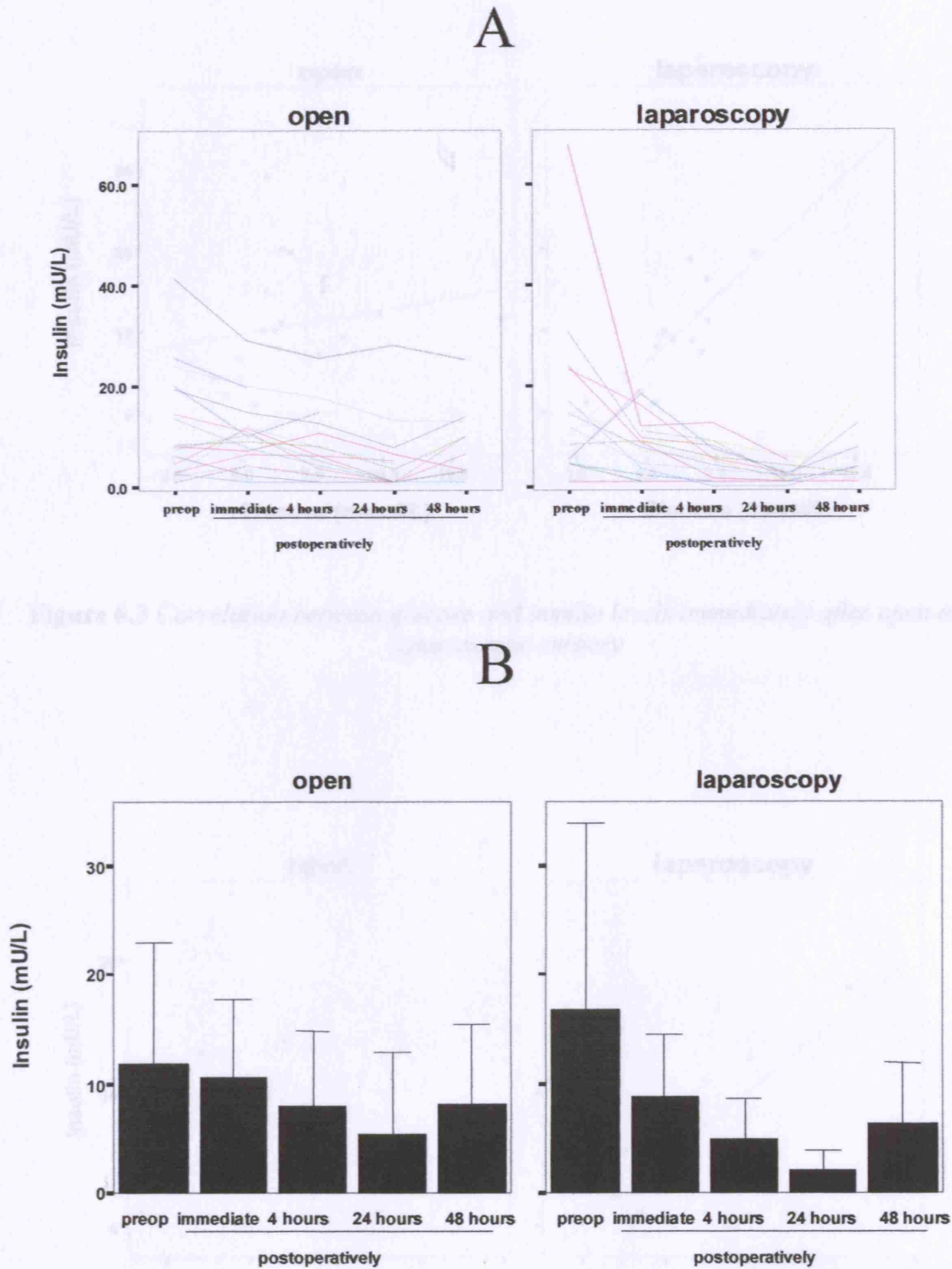
Figure 6.2 *Plasma levels of insulin after open and laparoscopic surgery*
(A: individual lines for each patient, B: mean + SD)
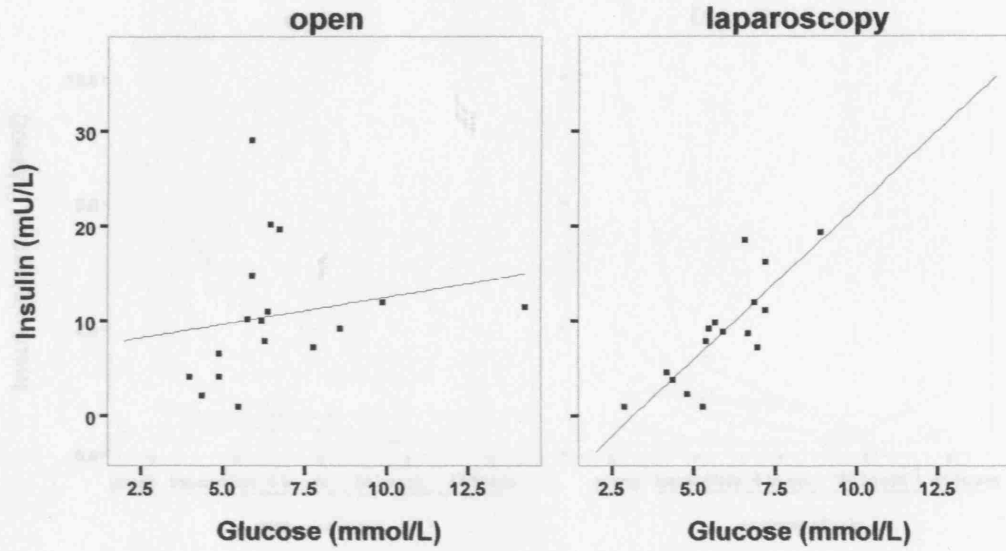
Figure 6.3 *Correlation between glucose and insulin levels immediately after open and laparoscopic surgery*
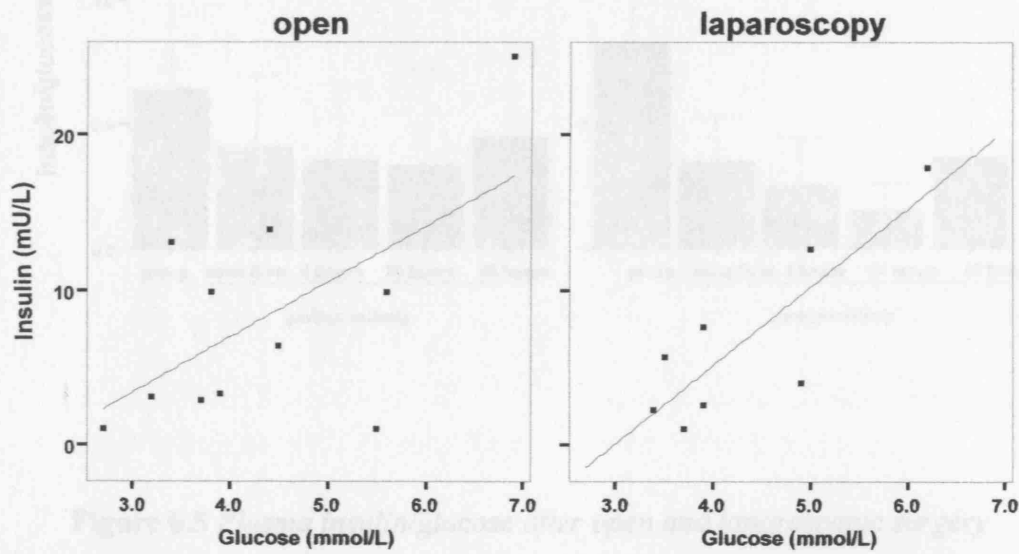
Figure 6.4 *Correlation between glucose and insulin levels 48 hours after open and laparoscopic surgery*
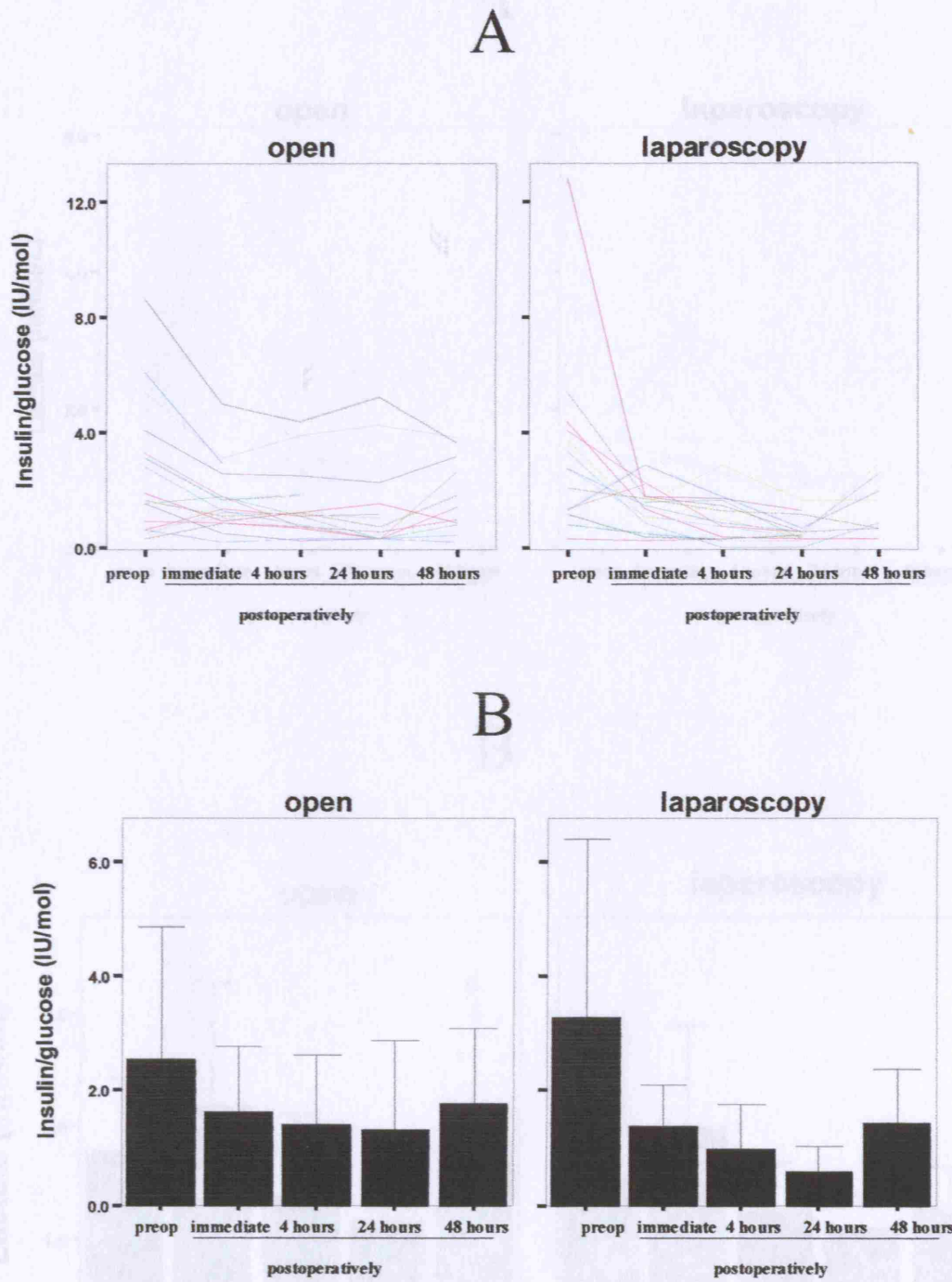
Figure 6.5 *Plasma insulin/glucose after open and laparoscopic surgery*
(A: individual lines for each patient, B: mean + SD)
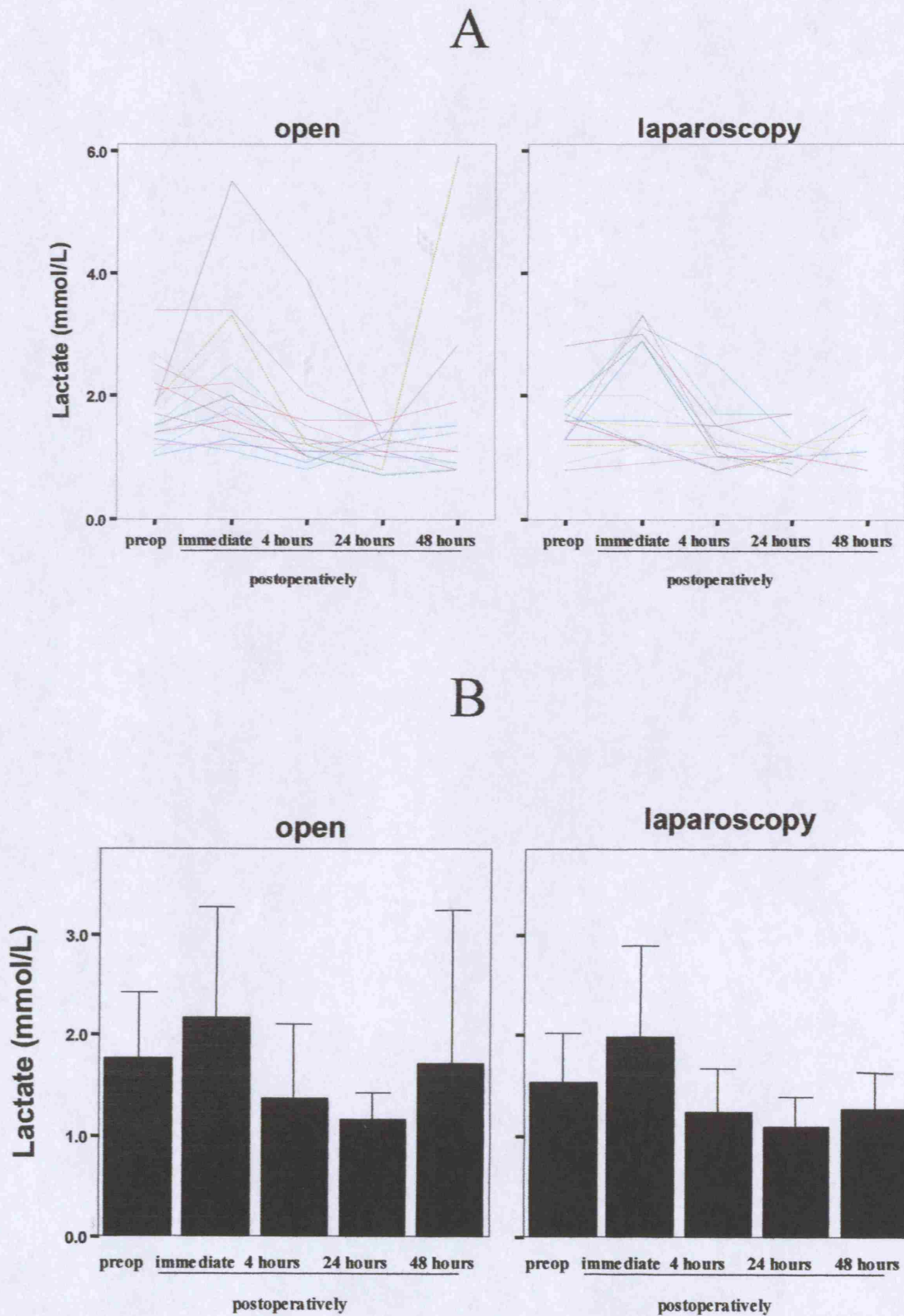
Figure 6.6 *Plasma lactate levels after open and laparoscopic surgery*
(A: individual lines for each patient, B: mean + SD)

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A
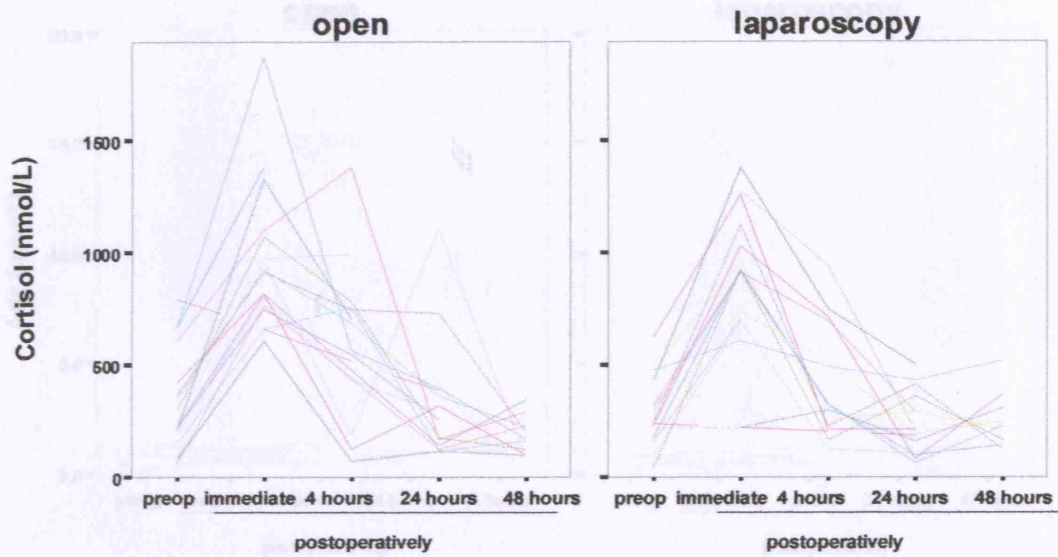
B
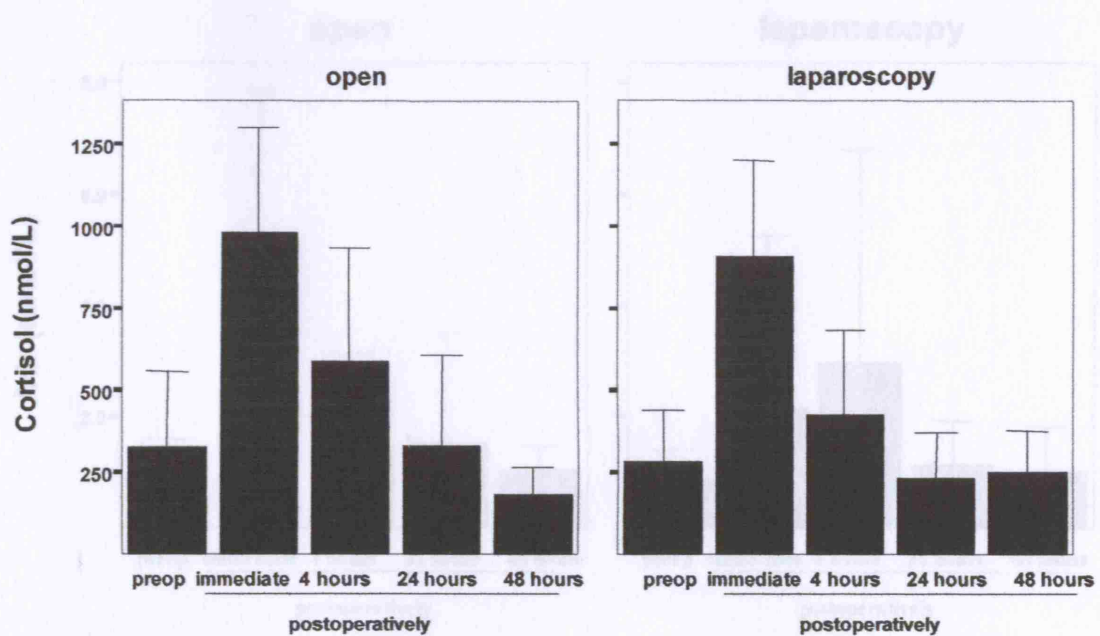
Figure 6.7 *Plasma cortisol levels after open and laparoscopic surgery*
(A: individual lines for each patient, B: mean + SD)

A

B
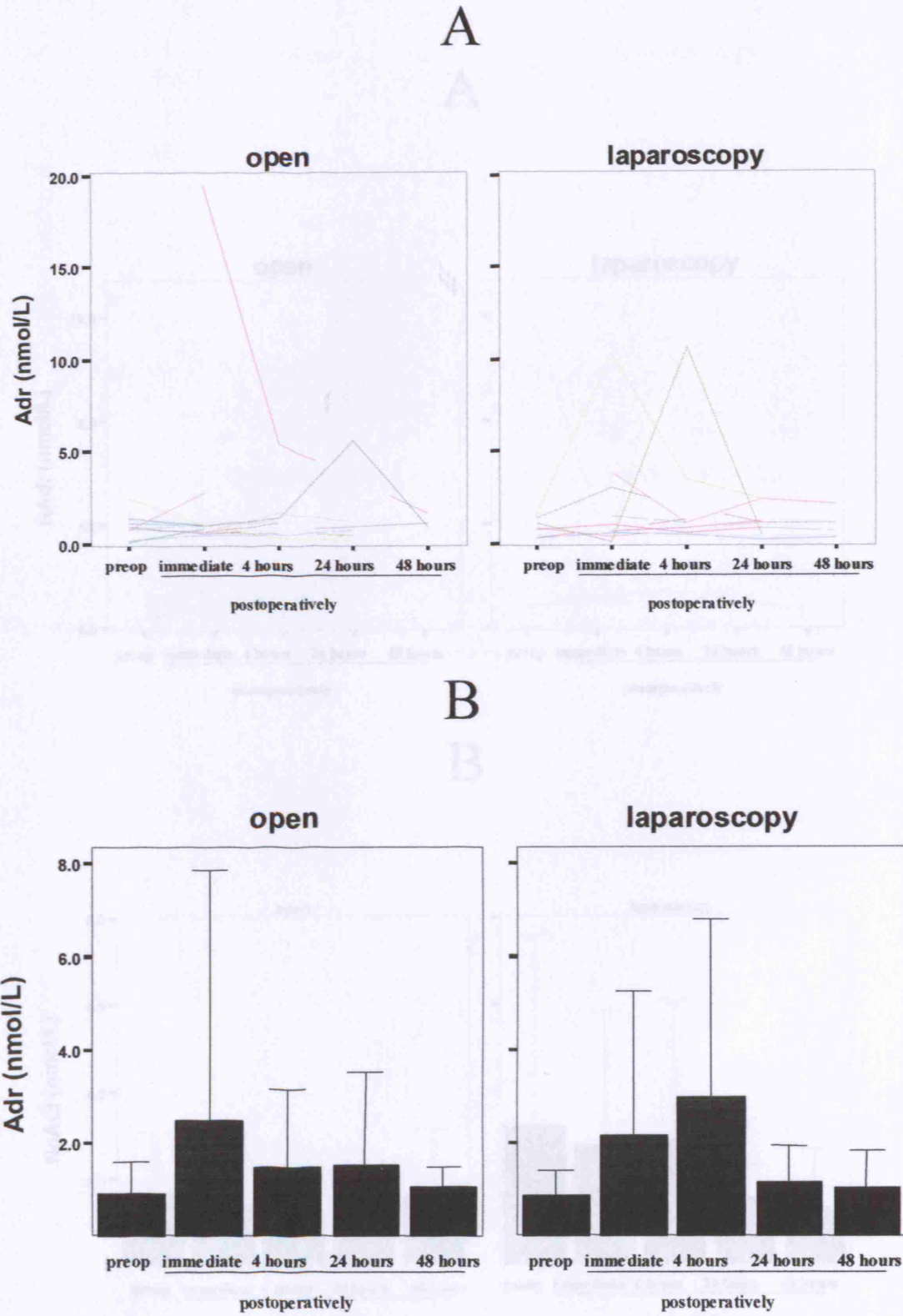
Figure 6.8 *Plasma adrenaline levels after open and laparoscopic surgery (A: individual lines for each patient, B: mean + SD)*
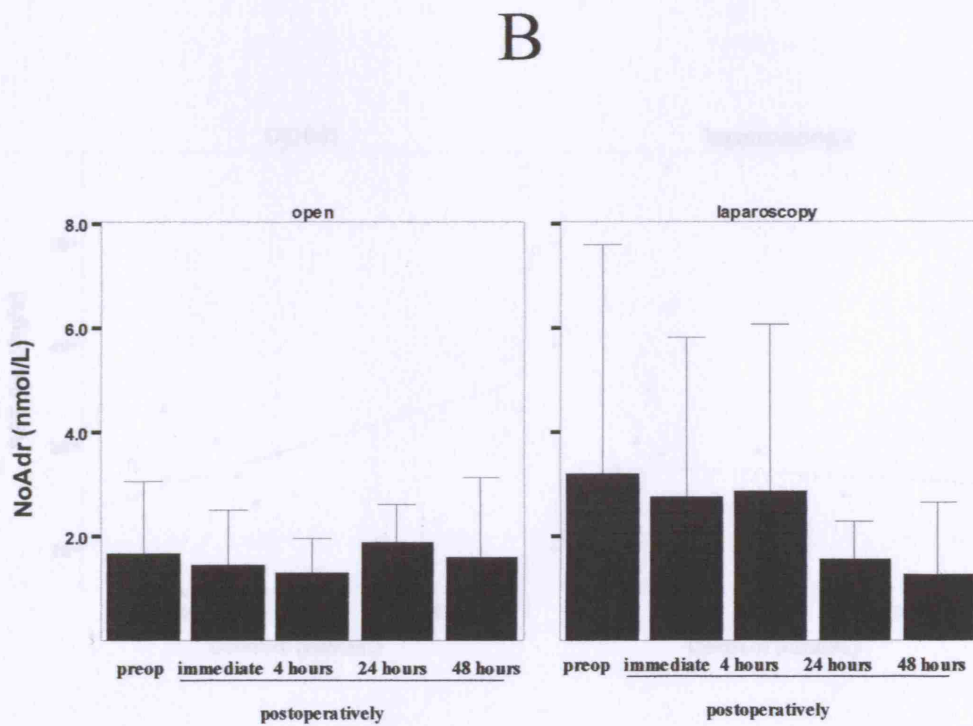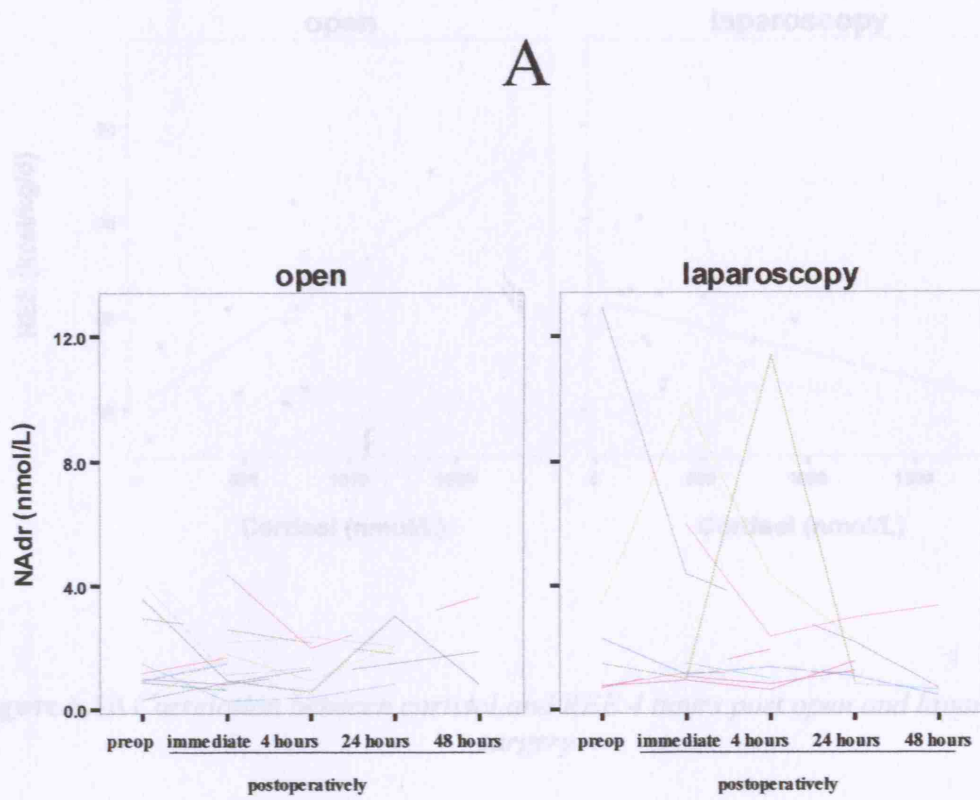
Figure 6.9 *Plasma noradrenaline levels in the open and laparoscopic groups*
(A: individual lines for each patient, B: mean + SD)
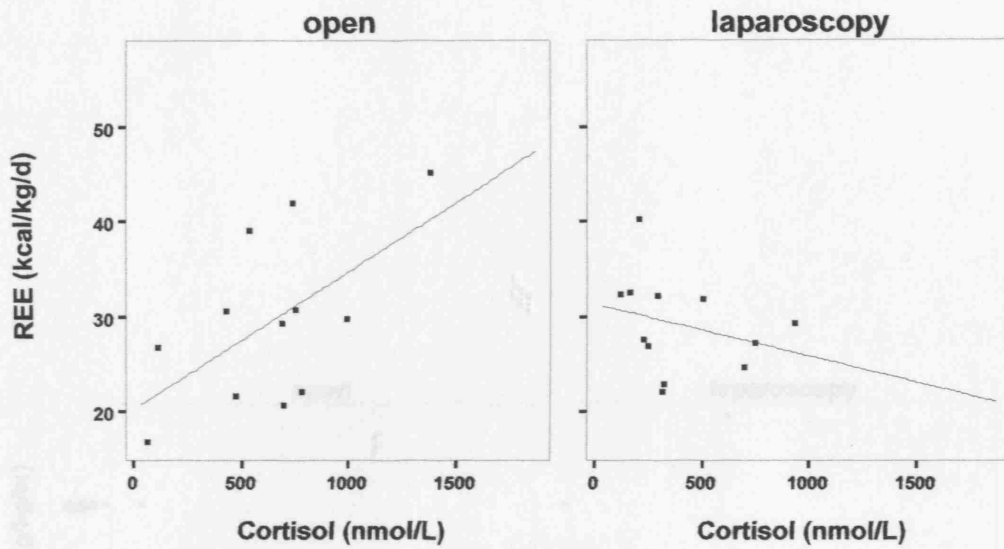
Figure 6.10 *Correlation between cortisol and REE 4 hours post open and laparoscopic surgery*
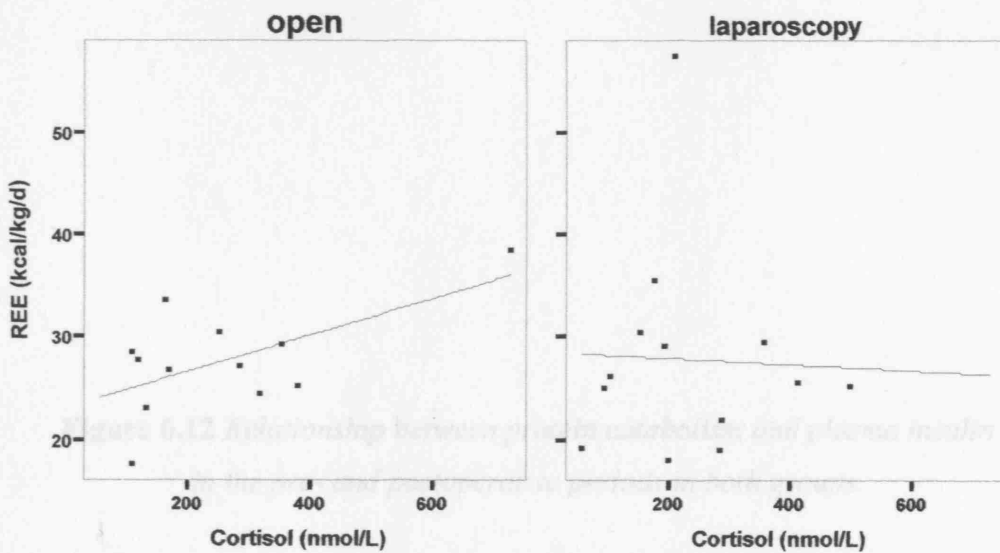
Figure 6.11 *Correlation between cortisol and REE 24 hours post open and laparoscopic surgery*
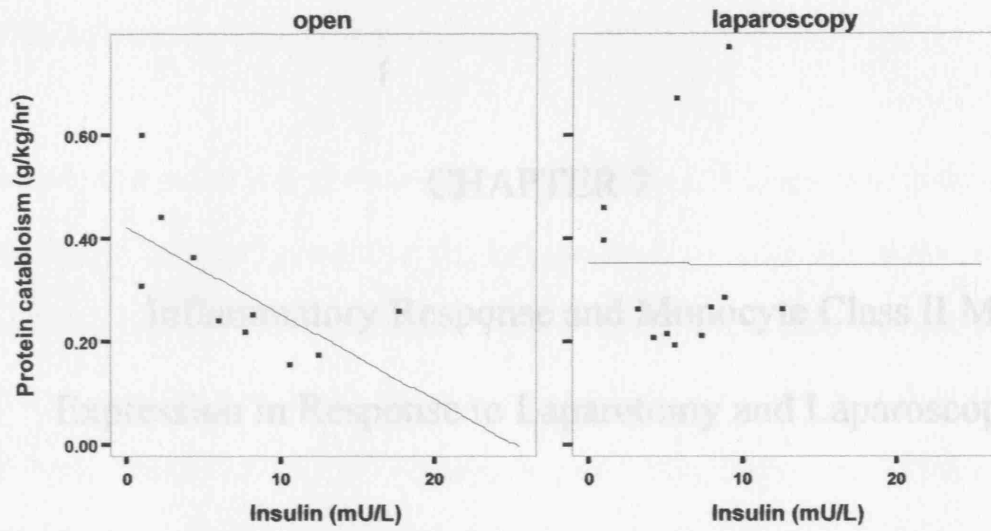
Figure 6.12 *Relationship between protein catabolism and plasma insulin*
in the pre- and postoperative periods in both groups.

CHAPTER 7

Inflammatory Response and Monocyte Class II MHC

Expression in Response to Laparotomy and Laparoscopy

7.1 Introduction

This chapter describes the inflammatory and immune response to laparoscopy and laparotomy in children. Several differences in the intra- and postoperative metabolic response to laparoscopy have been described in Chapters 3, 4 and 5. These differences may drive, or be due to a different inflammatory response to laparoscopy. The altered metabolic response may also influence the postoperative immune response by alteration of the substrate availability in the postoperative period.

Several moderators and markers of the inflammatory response have been shown to circulate in the postoperative period. Inflammatory cytokines have been extensively studied as markers of operative stress, and can be used to grade the response to surgery. IL-6 is one of the most consistently (Akhtar et al 1998, Cruickshank et al 1990, Jones et al 1994, Shenkin et al 1989, Tsang & Tam 1994, Zieren et al 2000), but not unequivocally (Hansen et al 1998), elevated circulating cytokines in the postoperative period. IL-6 peak levels often reflect the severity of operative insult (Cruickshank et al 1990, Jones et al 1994). There is a recognized cytokine response to operative stress that may be altered by laparoscopy.

Oxidative stress with free radical induced injury occurs in the perioperative period, and can be assessed by circulating MDA (Bentes de Souza et al 2003, Soong et al 1996, Valer et al 1994). The concentrations of serum nitrate and nitrite (which are stable end products of nitric oxide synthesis) have been used to assess the change in NO production in the postoperative period (Kalff et al 2003, Satoi et al 1998, Zhou et al 2000). CO_2 has been shown to alter peroxynitrite metabolism (Uppu et al 1996, Uppu & Pryor 1996, Zhang et al 1997). Laparoscopy, by altering local CO_2 concentration, may alter postoperative free radical production.

The inflammatory response also induces changes in the immune system. Cytokines produced during the inflammatory response have functional effects on the immunocompetent cells. Inflammatory cytokines induce changes in leukocyte gene expression thereby affecting immunocompetence, and release of counter-regulatory hormones (Chwals WJ 1998). The pro-inflammatory response is balanced by an anti-inflammatory response. This balance is thought to affect specialised antigen presenting cells like monocytes (de Waal et al 1991b). Monocytes play an important role in the stimulation of specific immunity by antigen presentation to helper T cells. Class II major histocompatibility complex (MHC) surface expression is crucial to monocyte antigen presentation capacity. Monocyte MHC surface expression is decreased after major surgery (Klava et al 1997b, Kono et al 1995, Smith et al 2000, Spittler et al 2001, Zieren et al 2000). Decreased surface monocyte human lymphocyte antigen DR (HLA-DR) expression is associated with defective antigen presentation and stimulation of T-cells (de Waal et al 1991b, Rhodes et al 1986). Decreased monocyte MHC/HLA-DR expression is associated with (Cheadle et al 1991, Hershman et al 1990, Kawasaki et al 2001, Schinkel et al 1998) and indeed predictive of (Allen et al 2002, Hallwirth et al 2002a) post-operative infection. Children who require prolonged postoperative periods of intensive care following cardiac surgery have significantly reduced levels of monocyte HLA-DR expression (Allen et al 2002).

The effect on laparoscopy on postoperative monocyte class II expression in children has not been previously investigated.

7.1.1 Aim

The aims of this chapter were to:

1.   Characterise the inflammatory and immune response to operative stress in children;

2.   Characterise monocyte function in children following abdominal surgery, and

3.   Test the hypothesis that laparoscopy is associated with blunting of the inflammatory response to surgery.

7.2   Methods

The patient group in this chapter consists of the same group described in Section 2.2.1. The protocol for this randomised trial has already been outlined in Section 2.2.1. Patients undergoing Nissen fundoplication were randomised to either laparotomy or laparoscopy as already detailed. Blood was taken for markers of inflammation and immune response at the five time points mentioned (Figure 2.2).

7.2.1   Measurement of plasma MDA levels

Blood was taken for MDA analysis in EDTA tubes, centrifuged at 3000 rpm and the supernatant stored at $- 70^{\circ}C$ until analysis. Plasma MDA was analysed by the candidate using the following protocol based on a thiobarbituric acid (TBA) releasing reaction and HPLC analysis.

Standards were prepared as follows;

0.031353g MDA in 1ml distilled H_2O	100mM
100µl 100mM made up to 1000µl (1ml)	10mM
10µl 10mM made up to 1000µl (1ml)	100µM
100µl of 100µM + 900µl total 1ml	10µM
75µl of 100µM + 825µl total 1ml	7.5µM

50µl of 100µM + 950µl total 1ml	5µM
25µl of 100µM + 975µl total 1ml	2.5µM
10µl of 100µM + 990µl total 1ml	1µM
H₂O	0µM

The following steps were then performed:

1. 25µl of plasma and standards in duplicate plus 2.5µl of fresh Butylated Hydroxytoluene (0.002g in 1ml ethanol) and 375µl (1% v/v) phosphoric acid were incubated at room temperature for 10 minutes.

2. 345µl fresh TBA solution (0.0435g TBA in 20ml water) added, mixed and incubated in a heating block at 100°C for 60 minutes.

3. Samples were then cooled to 50°C in a pre-heated block

4. 200µl was transferred to a new eppendorf containing 200 µl methanol preheated in an oven at 50°C.

5. To each eppendorf was added 15 µl KH₂PO₄ 1M buffer and 4 µl KOH/KHCO₃ 2M/2.4M

6. Samples centrifuged (13,000 rpm x 10 minutes), 400µl of supernatant were added to autosampler vial of the HPLC.

7. Samples were then read by HPLC (Jasco): C18 Column, solvent B = 100% Methanol, solvent C = 50mM NaPO₄, pH 7.0; flow rate 1.0 ml/min; isocratic 65% C, 35% B. Detection: fluorescence excitation 515, emission 553nm.

8. Data was acquired using BORWIN® software program. A calibration curve was created from the area under the peak of absorbance of the standards (Figure 7.1).

MDA values for plasma samples were read of this standard curve using linear regression

7.2.2 Measurement of plasma nitrates and nitrites

The plasma concentration of nitrate plus nitrite, which reflects systemic production of NO, was measured by HPLC using a highly sensitive method (Stefanutti, Pierro and Eaton, submitted for publication) based on reduction of nitrate to nitrite by incubation with NADPH and nitrate reductase, followed by derivatization with N-methyl-4-hydrazino-7-nitrobenzofurazan and HPLC analysis.

7.2.3 Measurement of plasma cytokine levels

Blood for cytokine was taken in heparin and was spun within 15 minutes, at 3000 rpm and plasma frozen at $-70^{\circ}C$ until analysis. Plasma cytokine levels were measured by the candidate using ELISA method as described in Section 2.5. All samples form each patient was analysed on the same plate. Samples were, whenever possible, only thawed once and all cytokines measured on the same occasion.

7.2.4 Effect of double gating on monocyte class II MHC expression

Monocyte class II MHC expression was measured by the candidate using flow cytometry as described in Section 2.6. Whole blood was processed within 20 minutes to minimise artefacts from monocyte adherence. Monocytes were identified using CD14 expression *vs*. SSC as described.

In addition to the single gating method, monocytes were identified using a double gate method, to asses the accuracy of single gating on the level of class II MHC expression. This was compared in 22 samples. Monocytes were firstly identified on a gate based on

SSC vs. FSC properties (Figure 2.14). This population of monocytes thereby identified, was then plotted on a CD14 vs. SSC box plot (as in Figure 2.16), as used for the single gating method. Monocytes were identified in this second population as being CD14 positive cells, and then analysed for class II expression as described in Section 2.6.2. Monocyte class II expression was then compared between these two methods. This double gating method may give a purer population of monocytes compared to the single gating method, and may theoretically increase the sensitivity of class II expression.

7.2.5 Comparison of surface monocyte class II expression using CD14 or CD64 as a marker for monocytes

To assess any possible effect of surgery on monocyte CD14 expression and the effect this may have on subsequent analysis of class II molecules, expression was determined using CD64 as well as CD14 as marker for monocytes in a subset of patients.

In addition to the 2 samples stained with R-PE CD14 (as described in Section 2.6.2), 2 additional samples were prepared. Cells were stained using 5µl saturating concentrations of R-Phycoerytherin Cy5 (R-PE Cy5) conjugated monoclonal antibody to CD64. HLA expression was determined using FITC conjugated monoclonal antibody to HLA-DR/DP/DQ as described in Section 2.6.2. R-PE Cy5 emission was detected in FL3 on the cytometer. Similar monocyte regions, based on CD14 or CD64 vs. SSC expression respectively, were then compared for monocyte class II MHC expression.

7.2.6 Validation and assessment of the effect of operative stress score on monocyte class II expression after abdominal surgery

Monocyte class II expression was also measured in a sub-group of patients undergoing major open surgery of varying severity, to assess the use of monocyte class II

expression to quantify the effect of the magnitude of the operative stress on the immune response. Patients with, metabolic disorders, sepsis, and disorders of the immune system were excluded. Six patients undergoing operation listed (adhesiolysis n=1, Neuroblastoma excision n=4, excision of ovarian tumor n=1) were studied, and compared to patients undergoing open Nissen fundoplication. Operative stress score (OSS) was calculated according to the system by Anand *et al* (Anand & Aynsley-Green 1988) (Table 4.1). Patients were classified as having either moderate (OSS < 10) or severe (OSS ≥ 10) operative stress based on this scoring system.

7.2.7   Calculations and statistical analysis

Data are expressed as mean and standard deviation, except for monocyte MHC expression, IL-6 and IL-1ra, which were not normally distributed, and are presented as median and interquartile range. Independent sample t-tests were used to compare data between groups of patients. Bland-Altman analysis was used to compare differences in MHC class II expression between two methods of measurement (Bland & Altman 1986). Data between time points within each group were compared using repeated measures ANOVA test with Tukey's multiple post hoc tests. Monocyte percentage expression was analysed using Friedman test with Dunn's multiple comparison between time points. Differences between open and laparoscopic surgery were compared using area under the curve (AUC). Time series data for curves were also analysed using a series of multilevel models (MLwiN®; Centre for Multilevel Modelling, Institute of Education, University of London). Differences are presented with confidence intervals wherever possible.

7.3   Results

7.3.1   Plasma MDA

MDA was measured in 11 patients in the open group and 6 patients in the laparoscopy group. There was no significant change in MDA levels in the postoperative period in either group (Figure 7.2).

7.3.2   Plasma nitrate plus nitrite (systemic NO production)

Plasma nitrate plus nitrite (NOx) levels were available in 14 and 12 patients respectively. There was no significant change in NOx after either open or laparoscopic surgery (Figure 7.3).

7.3.3   Plasma cytokine response

IL-10 was measured in 15 open patients and 14 laparoscopic patients. There was no significant response in IL-10 (Figure 7.4). Plasma TNF-α was measured in 14 and 13 patients respectively, with no significant change in the postoperative period in either group (Figure 7.5).

IL-6 levels were measured in 14 patients in each group. Levels increased significantly over time in both the open and laparoscopic groups (p<0.0001) and with no difference between open and laparoscopic groups (p=0.08; Figure 7.6). There was no correlation between plasma IL-6 and REE (r^2=0.03, p=0.20).

IL-1ra was measured in 15 patients in the open and 14 patients in the laparoscopic group. Values rose significantly postoperatively in both groups (Figure 7.7). Levels peaked in the immediate postoperative period (p=0.007 vs. preoperatively). The

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postoperative rise in IL-1ra was lower in the laparoscopic group, although this difference did not reach significance (p=0.10).

7.3.4 Measurement of MHC class II expression using single and double gating for monocyte identification

There was no difference between monocyte class II expressions based on single gating and double gating methods (HLA% p=0.90, MFI p=0.81). Monocyte class II expression significantly correlated with each other, both in terms of % monocytes expressing HLA (r^2=0.97, p<0.001; Figure 7.8) and the MFI (r^2=1.0, p<0.001; Figure 7.9). Therefore, all subsequent results were read from the monocyte region defined on CD14 vs. SSC dot plot.

7.3.5 Comparison of monocyte MHC class II expression using CD14 or CD64 as a marker for monocyte identification in the postoperative period

There was a good correlation between class II expression measured with CD14 and CD64 markers (r^2=0.91, p<0.001; Figure 7.10). The Bland-Altman plot of difference in expression against mean expression showed that there was no significant bias between the two methods of measurements (r^2=0.002, p=0.87; Figure 7.11). Therefore, there was no significant difference in % monocyte class II expression between gating with CD14 or CD64 as monocyte markers.

7.3.6 Monocyte MHC class II expression after open and laparoscopic surgery

Monocyte class II MHC expression was measured in 16 patients in the open group and 17 in the laparoscopic group. Baseline MHC expression was not different between groups (p=0.67). Postoperatively there was a significant decrease in monocyte class II % expression (Figure 7.12) after both open and laparoscopic surgery (p<0.00001).

Lowest levels were seen at 24 hours (p<0.001 *vs.* preoperative in both groups). At 48 hours there was partial recovery in class II expression, but values were still significantly lower than preoperatively (p<0.05 *vs.* preoperative in both groups). Overall, MHC % expression was lower throughout the postoperative period in the open group (p<0.05). In the multilevel model analysis there was a less pronounced postoperative drop in MHC expression in patients undergoing laparoscopic Nissen, although this approached, but did not reach significance (p=0.09). The fall in % of monocytes expressing class II MHC between preoperative and 24 hours (delta MHC class II) tended to be larger in the open group (32.0, 11.5 – 42.8) than the laparoscopy group (12.6, 4.3 – 20.0). This difference also approached statistical significance (Figure 7.13, p=0.07).

In addition, at 24 hours postoperatively the proportion of patients with MHC expression less than 60% was lower in the laparoscopic group (3/15 = 20%) compared to the open group (7/15 = 47%) although this difference (27%, 95% C.I. -13% to 60%) did not reach significance (p=0.12). This level of immune suppression has been previously predictive of postoperative infection in children (Allen et al 2002).

There was also a significant decrease in MFI in the postoperative period in both the open (p=0.002) and laparoscopy (p=0.009) groups (Figure 7.14). Lowest levels were seen at 24 hours in both open (p=0.001) and laparoscopy groups (p=0.006). Levels recovered at 48 hours and were not significantly lower than preoperative levels (p=0.34 in both groups). Overall, MHC MFI expression was lower throughout the postoperative period in the open group (p<0.05). There was a greater fall in monocyte MHC MFI in patients in the open group, which approached, but did not reach significance (p=0.09). At 24 hours monocyte MFI expression were on average 37% (9, 74%) higher in the laparoscopy group.

7.3.7   Effect of operative stress score on monocyte class II expression

Six patients with an OSS ≥10 (severe surgical stress) undergoing open surgery had class

II expression measured. In these 6 patients, class II expression continued to decrease at

48 hours postoperatively (48 hours MHC % p<0.01 vs. preoperatively; Figure 7.15 and

MFI 14.0 ± 0.1, p<0.01 vs. preoperatively; Figure 7.16). This contrasted with the

recovery in MHC expression between 24 and 48 hours in patients after open Nissen

fundoplication, all of whom had an OSS <10.

7.3.8   Postoperative infections

Postoperative infections occurred in 4 patients. One patient in the laparoscopy group

had a postoperative chest infection, secondary to aspiration that occurred during

anaesthesia. Another patient in the laparoscopic group, who had bronchiectasis, had a

postoperative chest infection. One patient each in both groups had a wound infection.

There was no significant difference in rates of infection between groups (p=0.60).

7.4   Discussion

The inflammatory response to surgery may determine postoperative course and

outcome. The immune response, and in particular the immuneparesis, associated with

surgery may predispose to postoperative infections. There was a similar cytokine

response after both open surgery and laparoscopy. Laparoscopy may be associated with

preservation of monocyte function, as measured by class II MHC expression, in the

postoperative period.

7.4.1   Lack of significant oxidative stress in the postoperative period

Although Nissen fundoplication has been shown to produce a significant increase in

circulating inflammatory markers in adults (Zieren et al 2000), the response in this

chapter was not as marked as previously published studies. There was only a modest IL-6 response. Plasma MDA, NOx, IL-10 and TNF-α were not elevated in the postoperative period in either group.

Investigators have studied the possible effects of CO_2 on the metabolism of peroxynitrite and its effect on the free radical cascade. Interaction of peroxynitrite with CO_2 is thought to result in the formation of the intermediate nitrosoperoxycarbonate and nitrocarbonate anions (Uppu et al 1996) that are involved in oxidation and nitration of biological compounds (Uppu & Pryor 1996). Therefore laparoscopy, by introducing an enhanced CO_2 environment, may alter free radical induced injury, independent of its effect on surgical trauma. However there was no significant increase in free radical production in the laparoscopic group.

There have been a few studies investigating the free radical induced injury after both open and laparoscopic surgery. In adults, cholecystectomy was shown to increase circulating MDA and NOx levels, with a significantly blunted response after laparoscopic approach (Bukan et al 2004). In another study lipid peroxidation in the postoperative period was also dampened by laparoscopy (Seven et al 1999). de Souza et al demonstrated a difference in effects on cyclooxygenase induction and MDA levels in the peritoneal cavity (Bentes de Souza et al 2003). Lipid peroxidation was observed in the peritoneum distant from the site of laparoscopic surgery, mediated through non-cyclooxygenase and lipoxygenase pathways, and appeared to be due to effects of CO_2 pneumoperitoneum. However, there was no systemic evidence of oxidative stress demonstrated in this chapter. Murayama et al detected no significant difference in MDA level in neonates in the postoperative period (Murayama 1991).

7.4.2 Plasma cytokines cascade after fundoplication may differ between children and adults

There was no elevation of IL-10 or TNF-α in the postoperative period in either group. There was an elevation of IL-6 in the postoperative period in both groups. However, the IL-6 response was dampened compared to the increase seen in infants in other studies (Bolke et al 2002), where postoperative levels were double that seen in this chapter. The inflammatory response to surgery is closely correlated to endotoxaemia. Postoperative IL-6 levels in infants and children has been shown to be associated with the degree of endotoxin levels (Bolke et al 2002), although this has not been a universal finding (Baigrie et al 1993). Upper and lower gastrointestinal surgery may have differing effects on the immune response. The colon is a potent source of endotoxin (Baigrie et al 1993). Colorectal surgery may be associated with a higher degree of endotoxaemia than Nissen fundoplication. Patients with perforated peptic ulcer do not have a significant endotoxaemia (Lau et al 1998), so endotoxaemia is also unlikely to occur after uncomplicated fundoplication. It is plausible that the decreased pro-inflammatory response may be partially due to the lack of circulating endotoxin. The lack of endotoxaemia along with a lack of a significant pro-inflammatory cytokine response may partially explain the lack of free radical response noted.

One other possible explanation for the blunted inflammatory response in this group of patients may be vagal inhibition of the inflammatory response. It has been shown that there is a decrease in the inflammatory cytokine production to both endotoxin (Borovikova et al 2000) and surgically (Bernik et al 2002) induced stress in the presence of vagus nerve stimulation. It is possible that in children undergoing Nissen fundoplication, the stimulation of the vagus nerve in the intra- and postoperative period may attenuate the inflammatory response. The vagus nerve may be stimulated by the

dissection of the stomach and oesophagus. Thereafter the oesophageal wrap itself may continue to stimulate the vagus in the postoperative period.

The blunted IL-6 response may also partially explain the lack of an increase in REE in the postoperative period, as IL-6 has been shown correlate with the metabolic rate in the postoperative period (Jones et al 1994, Kotani et al 1996). It can be postulated that operations associated with a higher IL-6 response may be associated with an increase in the metabolic rate postoperatively.

7.4.3 Laparoscopy partially blunted the postoperative IL-1ra response

Despite the blunted pro-inflammatory response and lack of IL-10 response, plasma IL-1ra was significantly elevated in the immediate postoperative period. It has been shown that the postoperative induction of IL-1ra is not dependent on endotoxaemia (O'Nuallain et al 1995). The early induction of circulating IL-1ra confers with other studies in children (Hansen et al 1998, O'Nuallain et al 1993). The early induction of IL-1ra may act as a control mechanism to prevent overwhelming pro-inflammatory response with widespread inflammation.

Interestingly, the increase in IL1-ra was partially blunted by laparoscopy. The increase in IL-1ra in the laparoscopic group showed a tendency to be smaller than that of the open group.

7.4.4 Monocyte class II MHC response was relatively preserved by laparoscopy postoperatively

The results of studies on the impact of laparoscopy on monocyte function in adults have yielded mixed results. Some studies have shown no difference between laparotomy and

laparoscopy with respect to monocyte count and MHC class II expression in the postoperative period (Klava et al 1997a, Perttila et al 1999). In a randomised study of adults undergoing Nissen fundoplication there was also no difference in monocyte class II expression between approaches (Zieren et al 2000). Patients undergoing aortic aneurysm repair had similar class II MHC downregulation after open and endovascular approaches (Syk et al 1999). Conversely, in a randomised study of patients undergoing colorectal resection there was preservation of (Ordemann et al 2001), or an earlier return to normal (Wu et al 2003) monocyte HLA-DR expression after laparoscopy. Thus the impact of laparoscopy on the pattern of change in adults may be related to the degree of operative stress or the presence of endotoxaemia.

In this study, both the percentage and MFI of monocyte class II MHC expression decreased postoperatively. This indicates that postoperatively some monocytes lost all surface expression, while the remainder have relatively less class II molecules. This decrease in surface expression of class II antigen may translate to clinically important impairment of T cell stimulation. In contrast to the randomised trial in adults undergoing Nissen fundoplication (Zieren et al 2000), laparoscopy was associated with a tendency to preserve MHC class II relative to laparotomy at 24 hours, though this did not reach statistical significance. Levels were higher in the laparoscopic groups in the postoperative period.

During flow cytometry assessment of monocyte class II expression, CD14 was used as a surface marker for monocytes. Although there has been conflicting results, some studies have shown an alteration in monocyte CD14 expression after injury and surgery (Gotzinger et al 2000, Heinzelmann et al 1996, Kawasaki et al 2001). There was no difference in class II expression when CD64 was used to identify monocytes. The effect

on class II expression was therefore not an artefact secondary to monocyte selection bias. There was no selection bias across the range of measurements with respect to comparison with CD14 and CD 64. There was good correlation in results throughout the period of measurements.

7.4.5 Monocyte class II MHC expression is a relatively sensitive marker of the operative stress response to general surgery in children

The pattern of monocyte class II expression was significantly altered by operation type and severity. In patients who underwent open surgery of a larger magnitude there was a larger decrease in MHC expression and a more prolonged period of immuneparesis compared to the open Nissen group. In the group undergoing operations of a larger magnitude, this MHC expression continued to decrease at 48 hours postoperatively; whereas there was recovery in the open Nissen group at 48 hours. The different patterns of expression in patients undergoing open surgery of different magnitude support the notion that monocyte function may be a sensitive marker of the overall immune response to operative trauma.

7.4.6 Implications of the maintaining monocyte class II expression

Postoperative infections are one of the common causes of morbidity (Windsor et al 1995). The postoperative period of reduced monocyte class II expression may represent the time at greatest risk of infection (Cheadle et al 1991, van den Berk et al 1997). Dampened T cell response, in the face of decreased monocyte class II MHC expression, may be one of the mechanisms for the increase in infection in the postoperative period. In support of this concept the rates of postoperative infections have been found to be correlated to monocyte MHC class II/HLA-DR expression (Cheadle et al 1991, Hallwirth et al 2002a, Schinkel et al 1998). The results in this chapter indicate that the

degree of monocyte HLA-DR expression is proportional to the degree of operative trauma. Therefore minimising operative trauma may help decrease the risk of infection. Maintained expression may translate into a state of immune-readiness to react to any secondary infection. Also, defining the period of decreased class II expression may help define the period of postoperative immune suppression and the need for postoperative antibiotics.

There were no major postoperative infections and no marked pro-inflammatory response in this group of patients. This may be due to the lack of other predisposing factors. However, other operations associated with greater inflammatory response have a higher incidence of infection. It was not possible to test the postoperative deactivation of monocytes by investigating their cytokine production to LPS due to the limited blood volume that could be sampled from patients during these studies. Reduced response to LPS stimulation may provide further evidence of postoperative immuneparesis. Identification of the postoperative immuneparesis can lead to therapy that may be able to restore the postoperative immune function and prevent infections. The impact of laparoscopy on the immune response after operations of larger magnitude in children needs investigating.

7.4.7 Factors that may be responsible for the difference in monocyte function between laparoscopy and laparotomy

Sietses *et al* found no difference in the effects of three different types of laparoscopic procedures (cholecystectomy, Nissen fundoplication and colorectal resection) on monocyte function; but all laparoscopic procedures caused less alteration than open operations (Sietses et al 2000). Laparoscopy may significantly alter the immune response by decreasing the tissue trauma associated with abdominal access. One source

of the cytokines in the postoperative period may be from adipose tissue (Mohamed-Ali et al 2000) or probably muscle during abdominal wall dissection. By lessening the degree of abdominal wall trauma, laparoscopy only minimally affects the operative stress score, but can have significant impact on postoperative immune function.

7.4.8 Possible link between protein metabolism and monocyte function in the postoperative period

Glutamine is an important fuel for cells of immune system. The postoperative decrease in monocyte class II expression is minimised or abolished by the administration of glutamine in the postoperative period (Spittler et al 1995b, Spittler et al 2001). There was a difference in whole body protein catabolism between open surgery and laparoscopy in Chapter 6. Increased protein catabolism in the postoperative period increases the availability of amino acids for alternate purposes, one of which could be precursor fuel for monocytes. Glutamine accounts for a large percentage of amino acids released from muscles and provides fuel for enterocytes and cells of the immune system (Wilmore 2000). It can be speculated that the decrease in protein catabolism seen in the open group may decrease the supply of glutamine for monocytes, thereby inhibiting MHC/HLA-DR expression in the postoperative period. There was no significant change in protein catabolism in the laparoscopic group. Blood glutamine levels and flux were not measured in this study, and this theory could not be confirmed. This hypothesis needs investigating.

7.5 Conclusions

These results show that the circulating markers of inflammation and secondary oxidative stress in children undergoing either open or laparoscopic Nissen fundoplication were somewhat different to previously documented responses. The IL-6

response was somewhat blunted; with a lack of IL-10 response. The anti-inflammatory cytokine IL-1ra was significantly elevated in both groups, but only partially blunted by laparoscopy. There was a period of immune suppression (decreased monocyte class II MHC expression) in both groups, which was more pronounced in the open group. This immune suppression seems to be related to the degree of surgical stress.

There is a possible link between protein metabolism, as a determinant of glutamine in the postoperative period, and monocyte function. The relationship between glutamine levels after laparoscopy in children, and its relationship to monocyte HLA-DR expression, needs investigating in further studies. Documentation of postoperative glutamine levels along with measurement of postoperative protein catabolism and monocyte class II MHC expression may confirm this theory. Perioperative glutamine administration may decrease the postoperative immuneparesis and decrease postoperative infection in children.

Studies on the alteration in gene expression of monocytes in the postoperative period may also shed light on possible causes for the decreased in surface class II MHC expression. Genes controlling monocyte surface class II MHC expression (e.g. CIITA, HLA-DR) may be altered by open surgery but relatively maintained after laparoscopy. There may also be a link between IL-1ra expression and monocyte surface class II MHC expression that can be investigated on a molecular level or in cell culture.
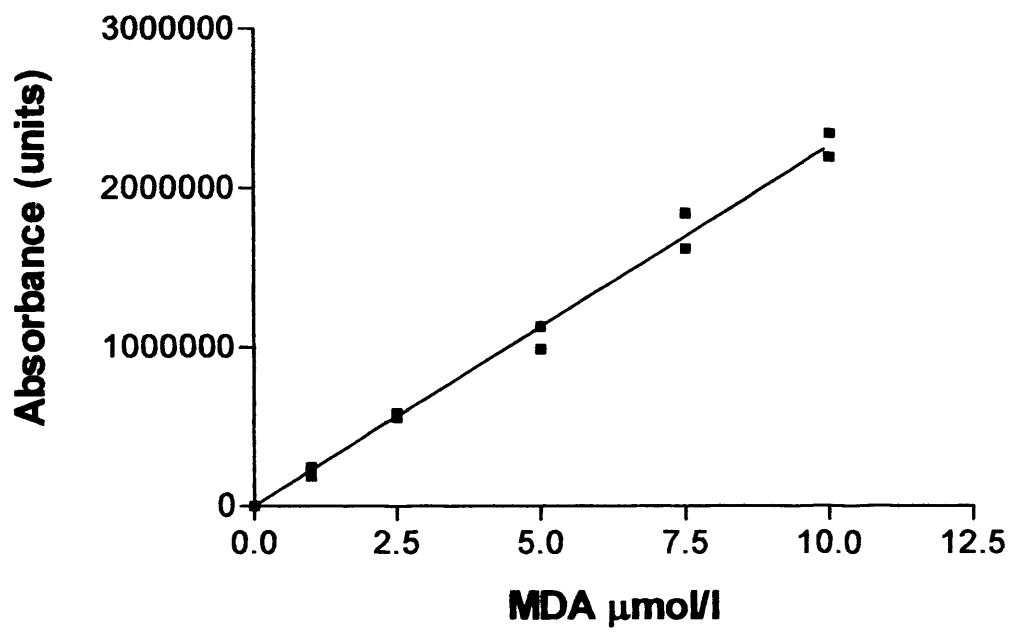
Figure 7.1 *Standard curve for measurement of plasma MDA by HPLC.*
In this example $r^2 = 0.99$.
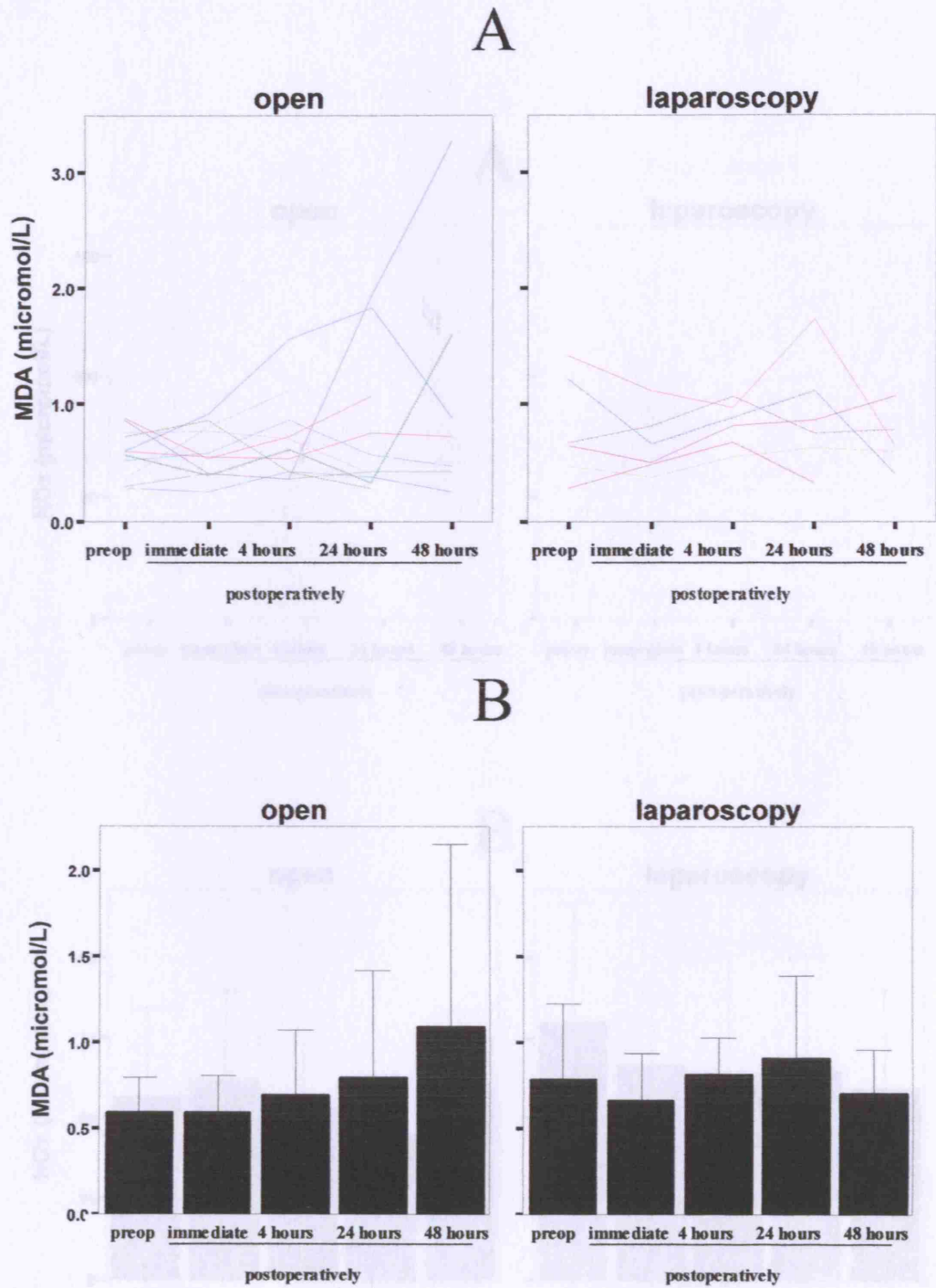
Figure 7.2 *Plasma MDA levels after open and laparoscopic surgery*
(A: individual lines for each patient, B: mean + SD)
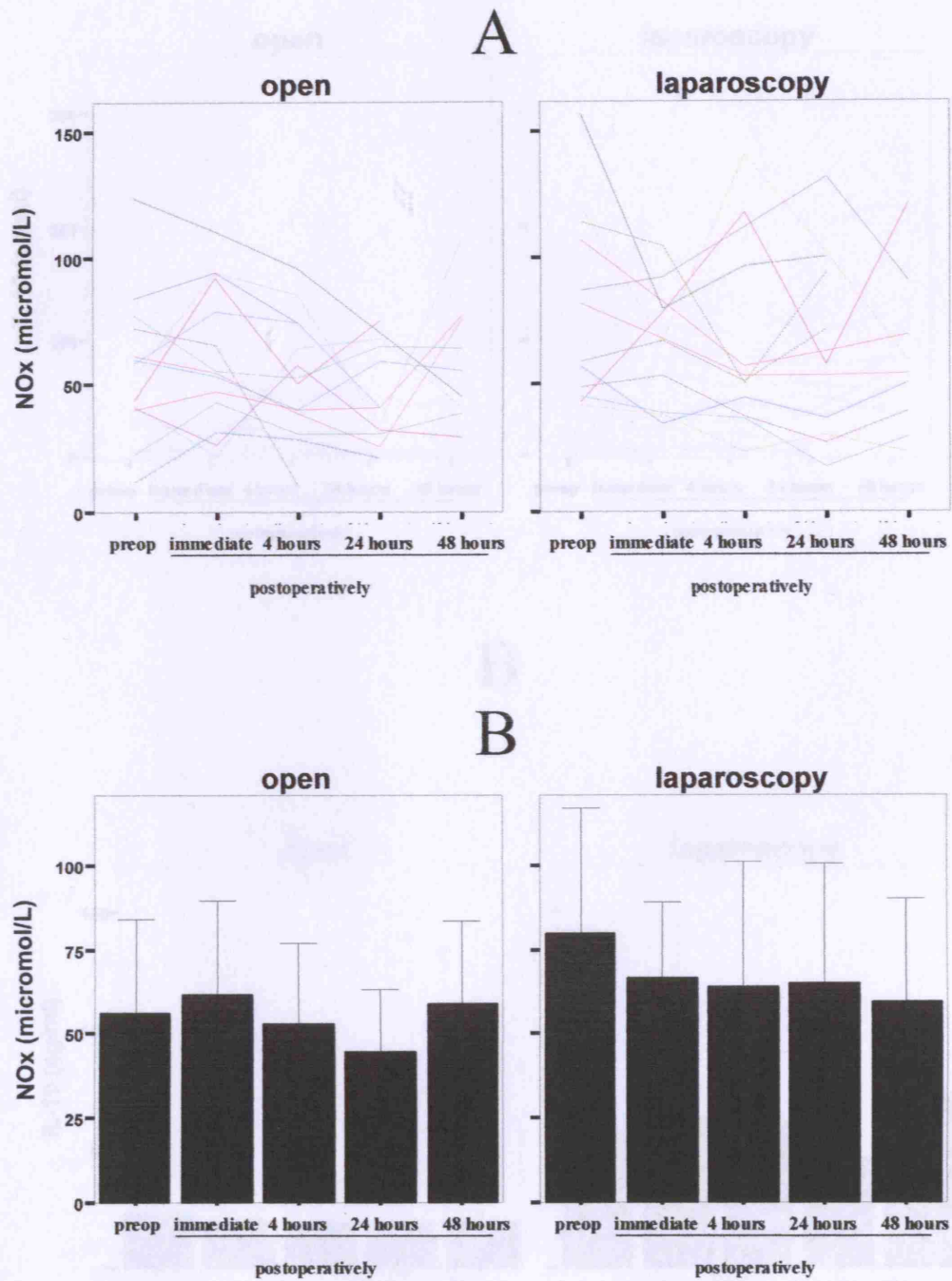
Figure 7.3 *Plasma NOx levels after open and laparoscopic surgery*
(A: individual lines for each patient, B: mean + SD)
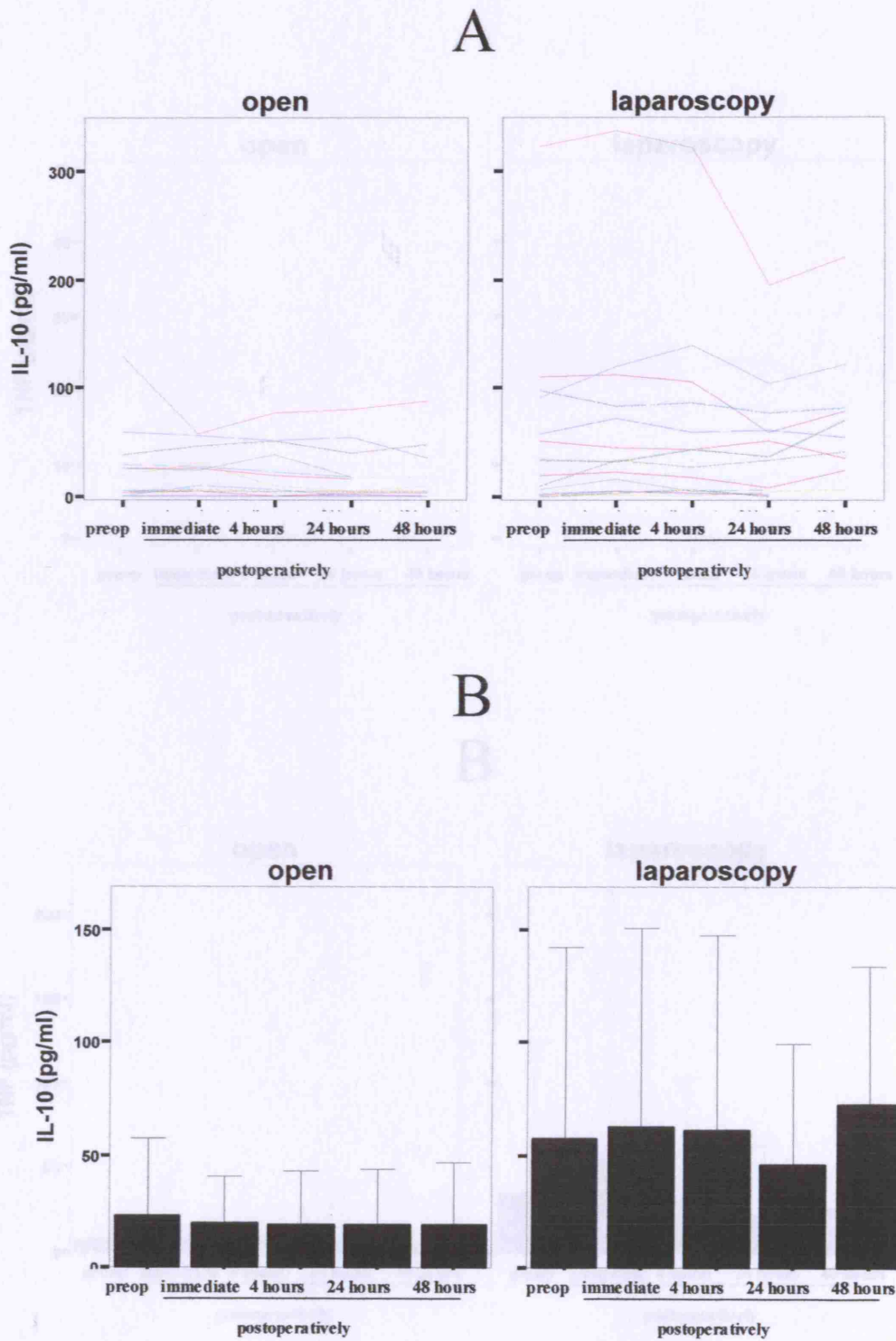
Figure 7.4 *Plasma IL-10 levels after open and laparoscopic surgery*

(A: individual lines for each patient, B: mean + SD)
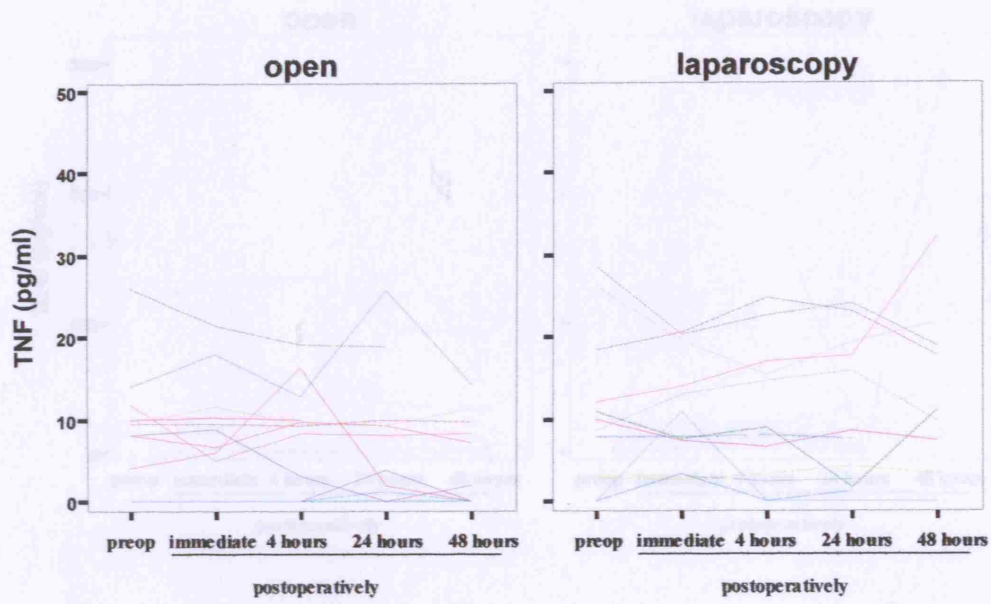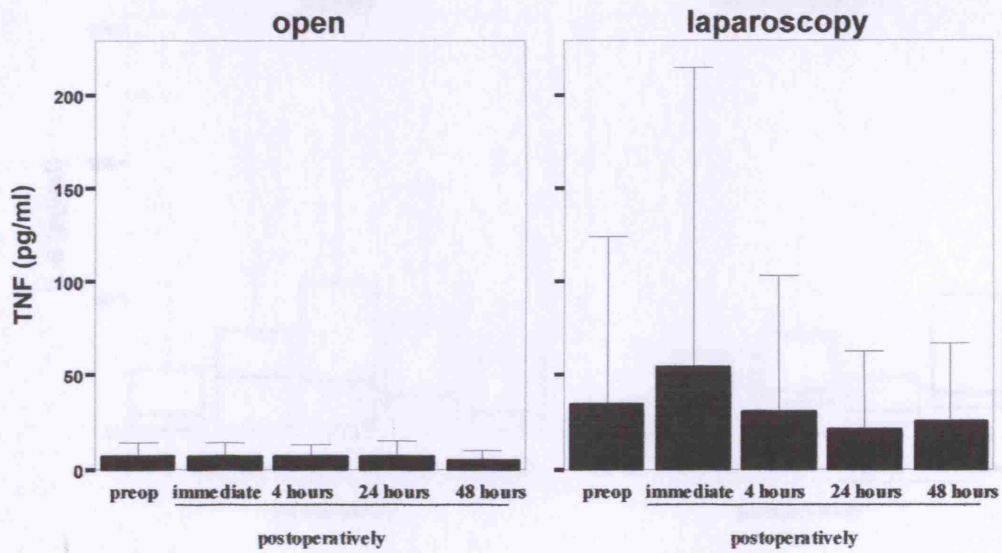
Figure 7.5 *Plasma TNF-α levels after open and laparoscopic surgery*
(A: individual lines for each patient, B: mean + SD)

A

B
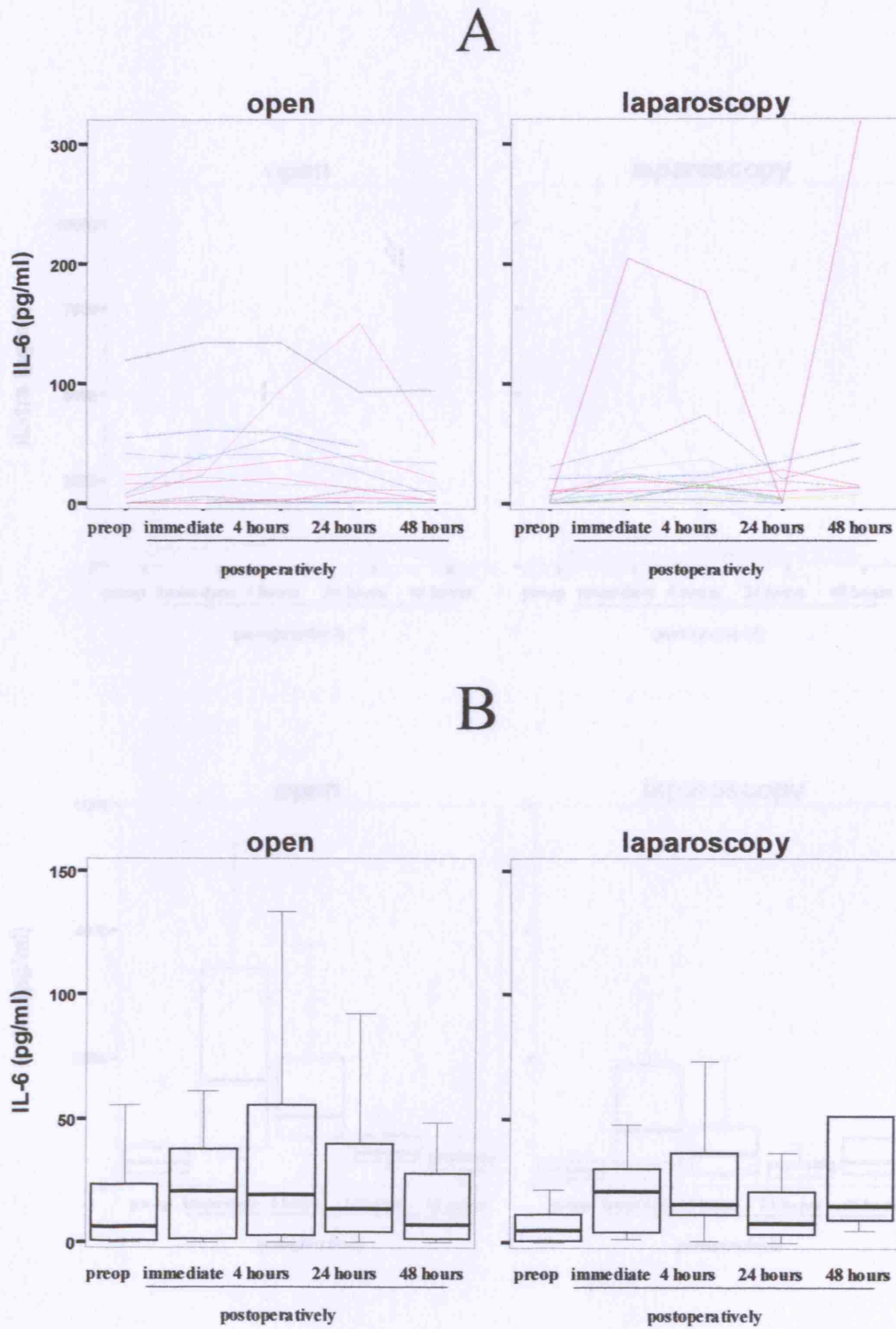
Figure 7.6 *Plasma IL-6 levels after open and laparoscopic surgery*
(A: individual lines for each patient, B: median + IQR)
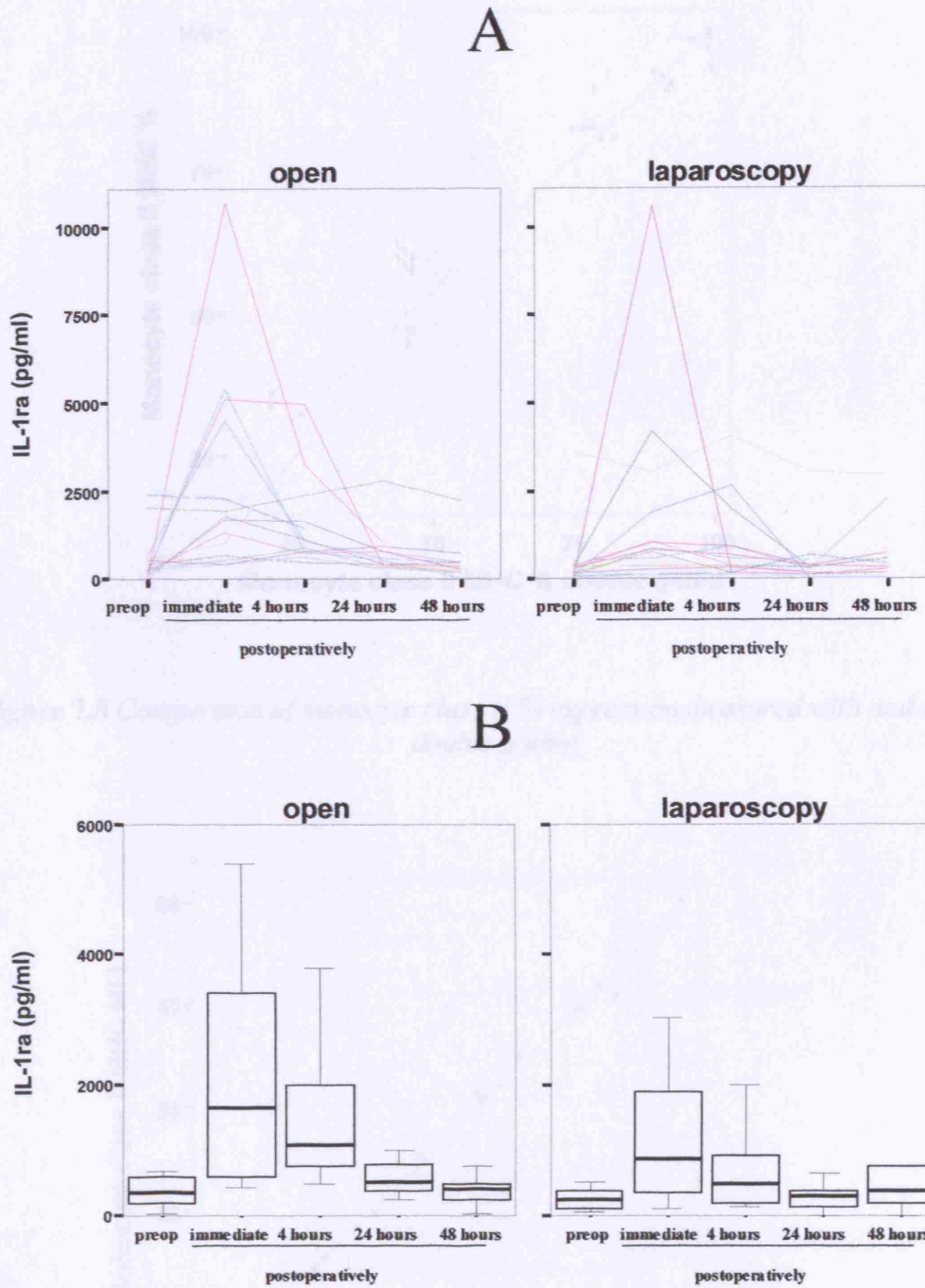
Figure 7.7 *Plasma IL-1ra levels after open and laparoscopic surgery*

(A: individual lines for each patient, B: median + IQR)
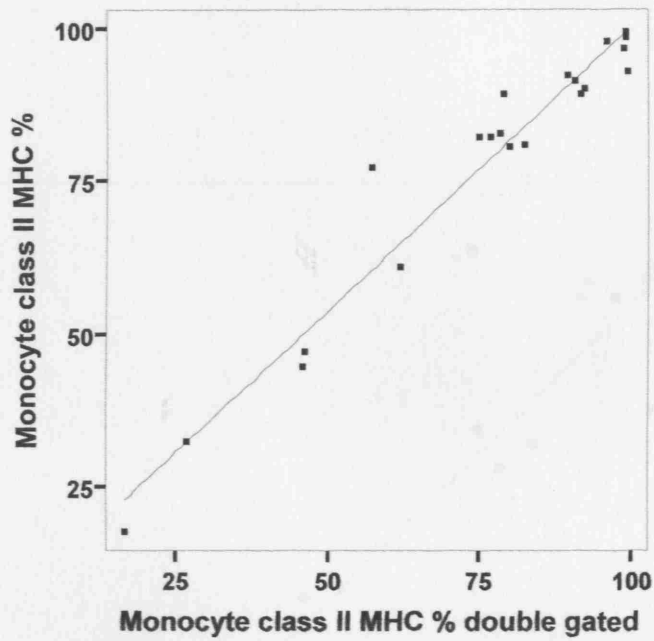
Figure 7.8 *Comparison of monocyte class II % expression measured with and without double gating*
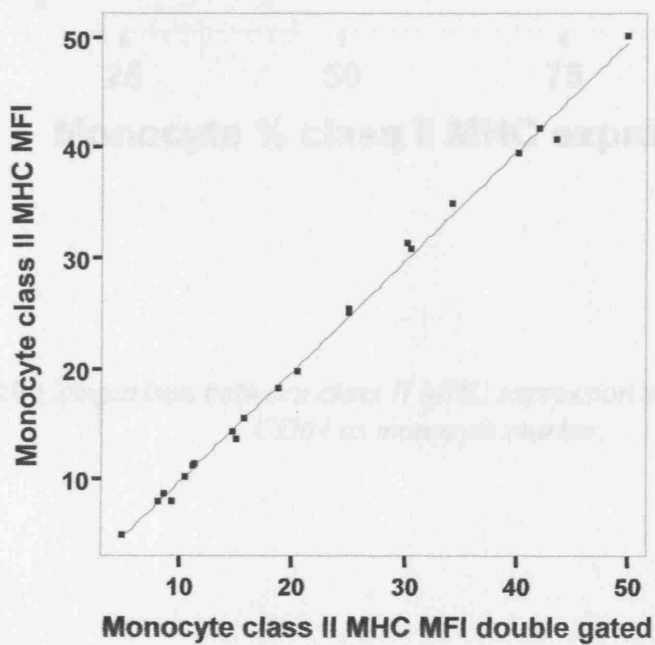
Figure 7.9 *Comparison of monocyte class II expression (MFI) measured with and without double gating*
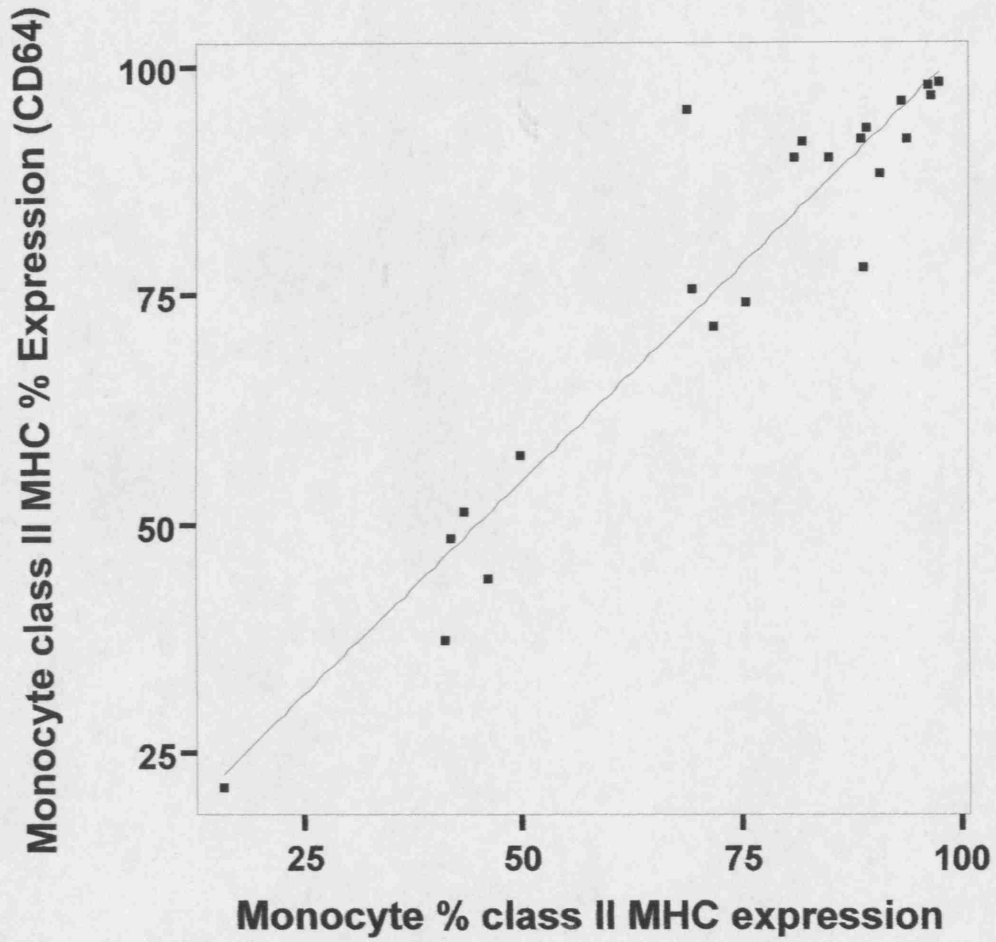
Figure 7.10 *Comparison between class II MHC expression measured using CD14 and CD64 as monocyte marker.*
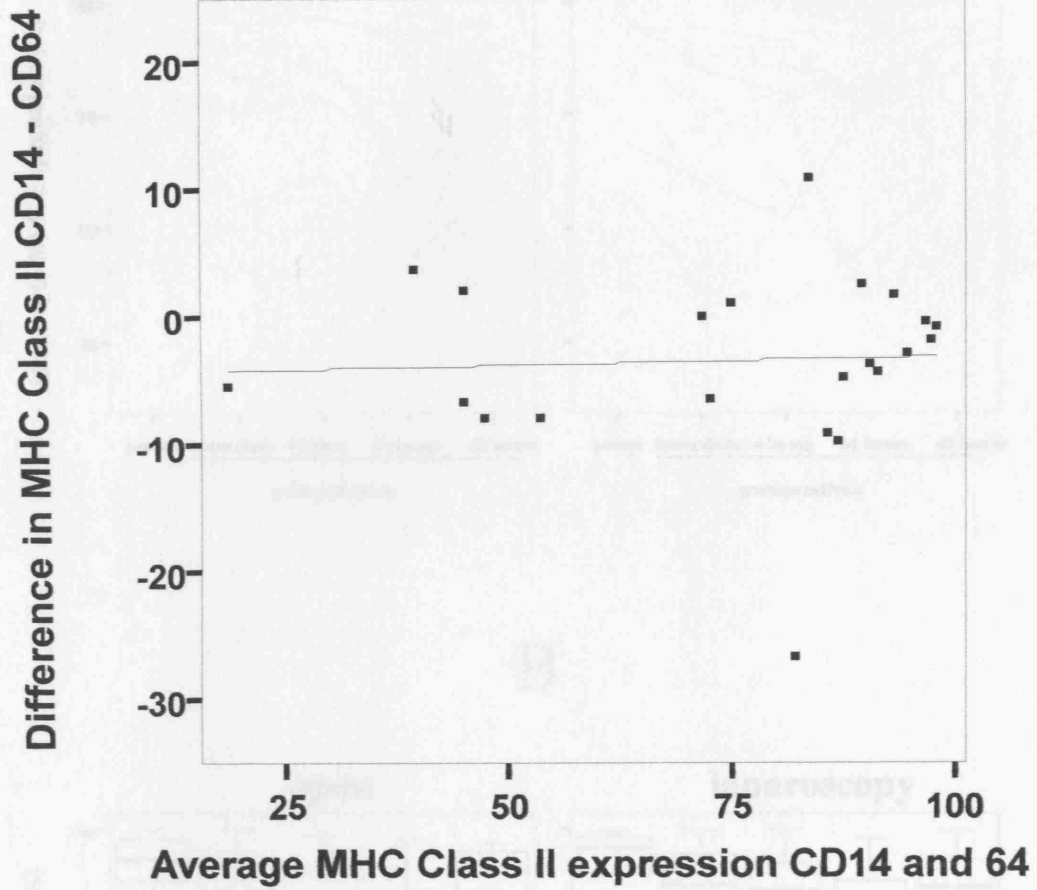
Figure 7.11 *Bland-Altman plot of difference vs. average for measurements of monocyte class II MHC expression using CD14 and CD64 as monocyte markers*

Figure 7.12 *Monocyte class II % expression after open and laparoscopic surgery.*
(A: individual lines for each patient, B: median + IQR)
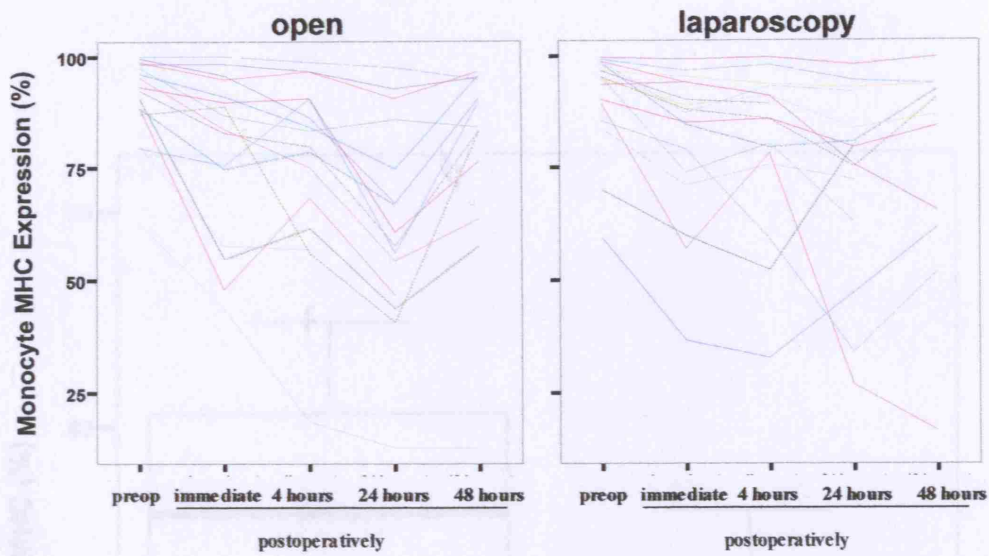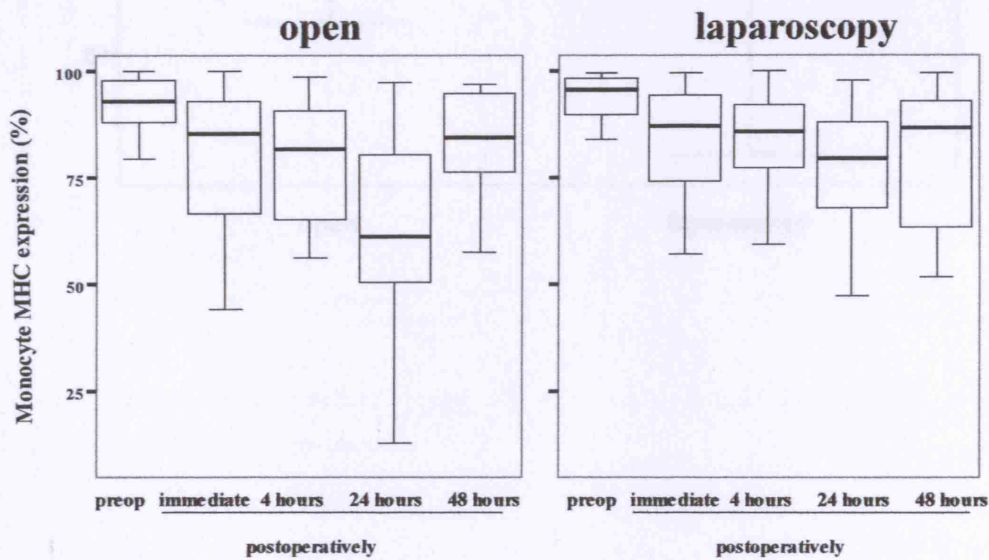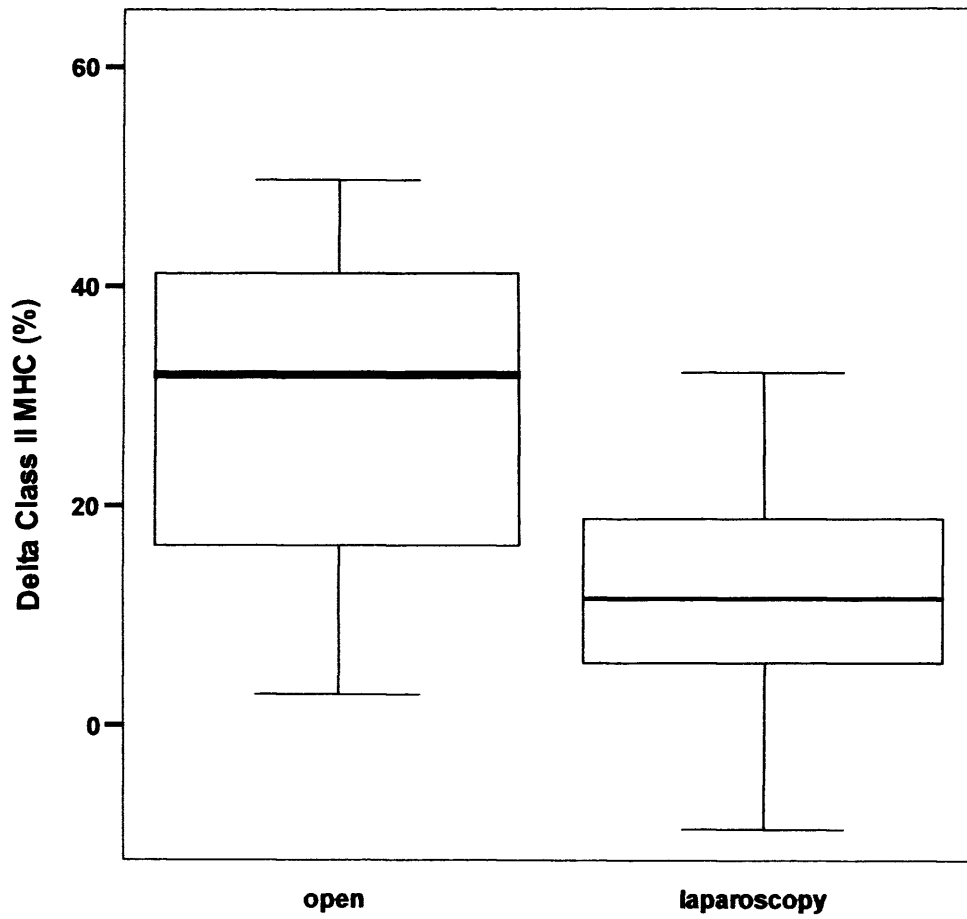
Figure 7.13 *Delta MHC class II % expression at 24 hours after open and laparoscopic surgery.*

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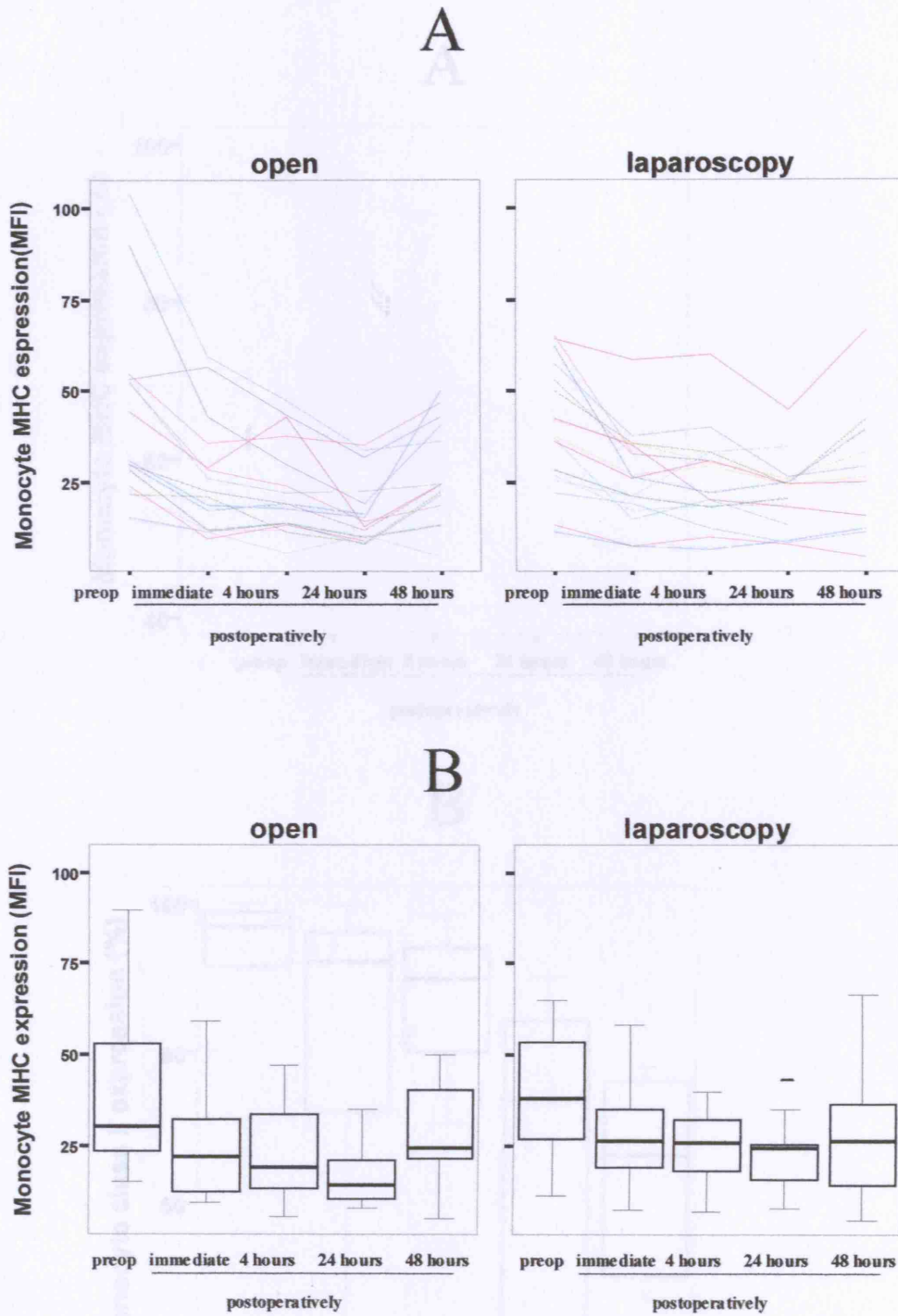
Figure 7.14 *Monocyte class II MHC expression (MFI) after open and laparoscopic surgery*

(A: individual lines for each patient, B: median + IQR)
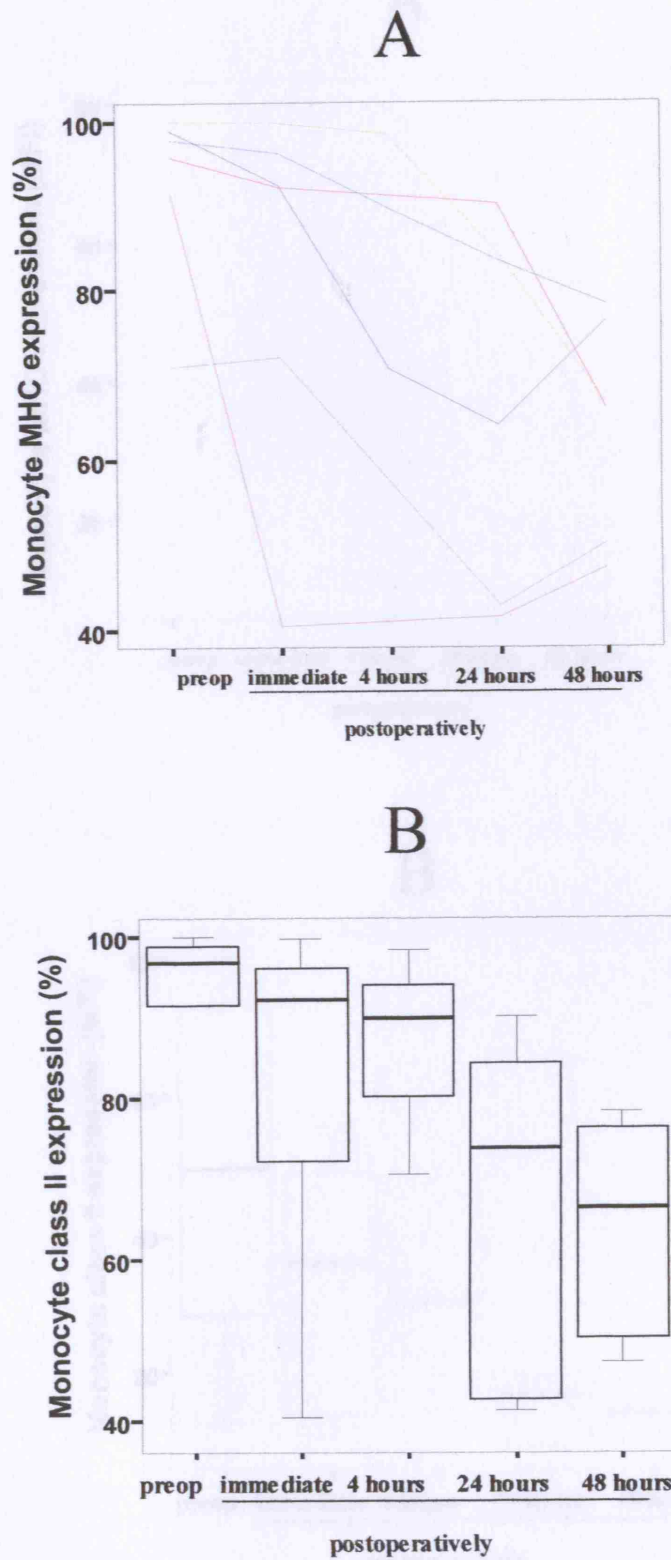
Figure 7.15 *Monocyte class II % expression in patients with operative stress score ≥10.*
(A: individual lines for each patient, B: median + IQR)
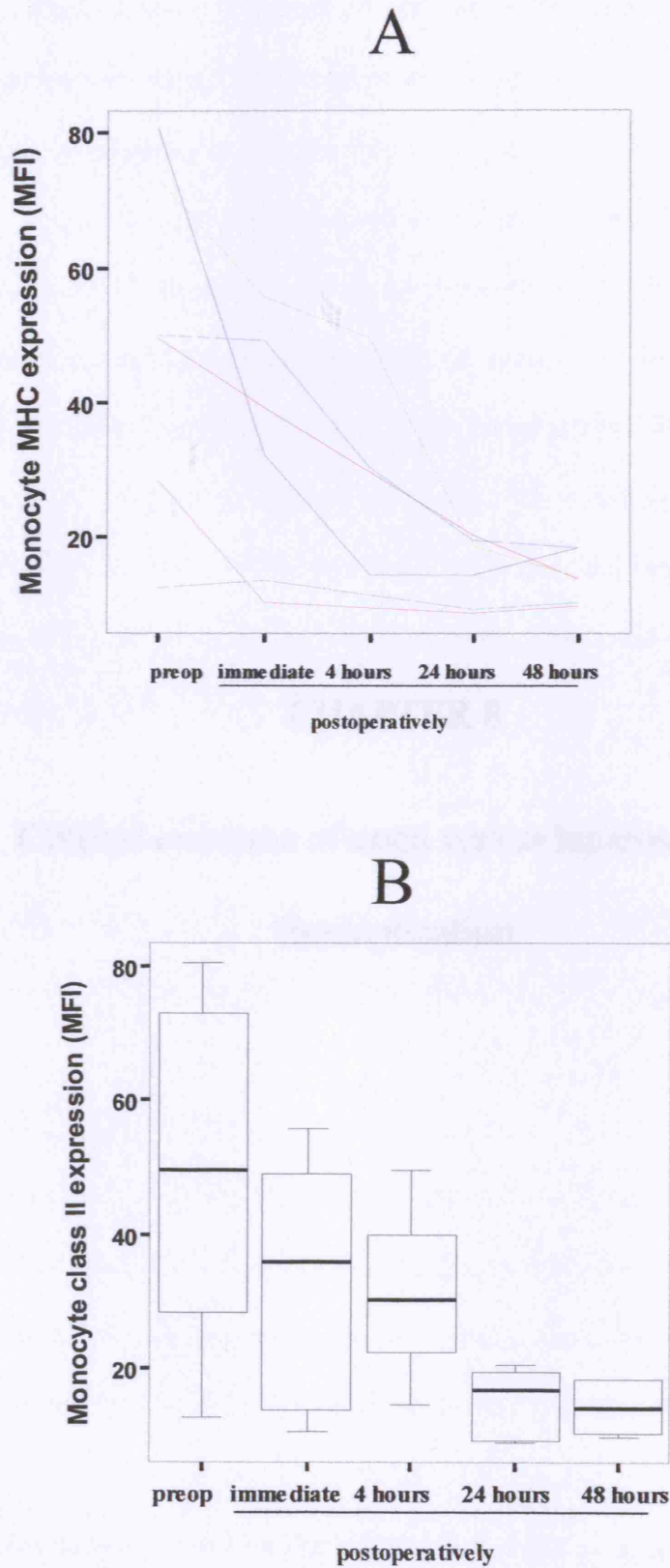
Figure 7.16 *Monocyte class II MFI in patients with operative stress score ≥10.*
(A: individual lines for each patient, B: median + IQR)

CHAPTER 8

Clinical outcome of open *versus* laparoscopic Nissen

fundoplication

Laparoscopic fundoplication is performed with the widely held belief that it is superior to open operation with regard to postoperative outcome and complications. Although it is assumed that laparoscopy is associated with less postoperative pain, quicker recovery, and better clinical outcome compared to open surgery no randomised control studies have been performed to investigate this in children. There have been many randomised controlled trials suggesting a beneficial affect of laparoscopy on postoperative course after cholecystectomy in adults (Cuschieri 1995, Kehlet 1999). There have been much fewer randomised controlled studies comparing the outcome between open and laparoscopic fundoplication in the literature. However, no meta-analysis has been performed to consolidate the findings of these trials. There have been no randomised controlled studied in children.

The altered metabolic responses described in the previous chapters may modify the postoperative clinical response in children. It is important to correlate metabolic studies with clinical outcome in an attempt to explore this possibility

8.1   Aims

1.   The first aim of this chapter was to present a meta-analysis of the published randomised controlled trials of open *versus* laparoscopic Nissen fundoplication.

2.   The second aim of this chapter was to compare the clinical outcome in children undergoing Nissen fundoplication randomised to open surgery or laparoscopy.

8.2   Meta-analysis of randomised controlled trials of open *versus* laparoscopic Nissen fundoplication

A meta-analysis of all randomised controlled trials comparing open (ONF) *versus* laparoscopic Nissen fundoplication (LNF) was undertaken.

8.2.1 Methods

A systematic review of the literature to identify randomised controlled trials investigating the clinical outcome after Nissen fundoplication was performed. MEDLINE and Cochrane databases were reviewed using the following search strategies:

1. Fundoplication AND laparoscopy AND trials

2. Fundoplication AND laparoscopy AND randomised controlled trials

3. Fundoplication AND trials

4. Fundoplication AND randomised controlled trial

All abstracts were read independently by two investigators to identify studies that fit the inclusion criteria. All papers were then retrieved. References within each paper were then scrutinised to identify any further randomised trials not identified in the original search. Any relevant papers were also retrieved.

Papers were included if they were randomised trials comparing defined clinical outcome after Nissen fundoplication. Exclusion criteria included papers comparing fundoplications other than Nissen, non-randomised trials, papers not comparing clinical outcome, and those with no data that could be included in the analysis.

Defined outcomes were

- Operative time

- Length of recovery

- Resolution of acid reflux on pH study

- Dysphagia

- Postoperative complications (systemic and abdominal)

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- Recurrence

Postoperative complications were divided into abdominal (slipped wrap, wound infection, adhesions, bleeding and splenectomy) and systemic (pneumonia, atelectasis and pleural effusion). Any data not forthcoming in the articles were requested from authors where possible.

195 abstracts were retrieved by the search criteria and these were reviewed. Review of the bibliography in relevant papers revealed no further studies.

8.2.2 Statistics

Retrieved data for the meta-analysis was entered into the Revman® 4.2 and Comprehensive Meta-analysis® programmes were used to analyse data. Relative risks (RR) was calculated for dichotomous variables and estimated mean differences (eWMD) were calculated for continuous variables.

8.2.3 Results of meta-analysis

Review of 195 articles identified 7 randomised controlled trials (Bais et al 2000, Chrysos et al 2002, Heikkinen et al 2000, Laine et al 1997, Luostarinen et al 2001, Nilsson et al 2000, Sietses et al 1999); none were in children.

Overall these 7 studies accounted for 233 patients in the open group and 223 in the laparoscopic. Mean conversion rate with LNF was 8.5% (C.I. 3.5-13). ONF was associated with shorter operative time (eWMD = 23.4 mins, p=0.005)

Post-operative pH studies were performed in 4 trials. All four found that the two approaches improved acid reflux equally: mean % time pH<4.0 postoperatively was 3.4% (C.I. 2.3-4.1) after ONF and 1.3% (C.I. 0.27-0.46) after LNF (p=0.496).

ONF was associated with 35.9% less dysphagia (RR=0.62; C.I. 0.39-1.00; p=0.05; Figure 8.1). Persistent dysphagia that required dilatation or re-operation was 18% lower with ONF (RR 0.23; C.I. 0.06-0.93; p=0.03). One study (Bais et al 2000) was terminated early because of the higher incidence of dysphagia in the laparoscopic group.

However, ONF was associated with 77.2% more respiratory and systemic complications (RR=2.50; C.I. 1.43-4.38; p=0.001; Figure 8.2) and 88.1% more abdominal complications (RR=5.97; C.I. 2.82-12.67; p<0.00001; Figure 8.3). Patient recovery was also significantly more protracted in the open group, with longer hospital stay (eWMD=2.4 days, p=0.0005) and longer sick leave and time off work (eWMD=17.1 days, p=0.0188).

Data on operative failure rates were poorly reported so could not be analysed.

Heterogeneity in effect estimates were absent (p>0.05) amongst all variables thus excluding sample bias.

8.2.4 Discussion

8.2.4.1 Discussion of meta-analysis

Laparoscopy has been shown to reduce the postoperative complication and improve recovery in adults undergoing cholecystectomy in many randomised clinical trials. The outcome for operations of greater magnitude and of greater complexity is less

established and sometimes controversial (Cuschieri 1995). Nissen fundoplication is now performed laparoscopically in the belief that it is of similar efficacy to the open procedure and associated with less postoperative complications. This meta-analysis demonstrated a reduction in postoperative complication and quicker postoperative recovery after laparoscopic *versus* open Nissen fundoplication, but a higher incidence of dysphagia.

Laparoscopic approach resulted in 2.5 fewer inpatient days and 17 fewer days off work with sick leave. The hospitalisation cost is therefore reduced by the laparoscopic approach. In addition there was quicker return to work and therefore less loss of earnings. Although equipment and operative cost of laparoscopic surgery are possibly higher than the corresponding open operation, this is probably offset by the benefits of less time recovering in hospital and at home. Although no direct cost analysis was done in this meta-analysis, this can represents a huge cost benefit. Heikkinen et al (Heikkinen et al 1999) found that the short term cost (including operative costs, hospitalisation and recovery) favoured laparoscopy.

There was however a higher incidence of postoperative dysphagia with the laparoscopic approach. Postoperative dysphagia was 1.6 times more common after laparoscopic fundoplication. Dysphagia is a significant clinical problem for patients, as this can severely affect quality of life. The dysphagia may be transient in some, or became less symptomatic in others, as not all patients required intervention for their dysphagia. This translated to an 18% higher risk of reoperation for dysphagia in the laparoscopic group.

Overall both procedures were equally efficacious in improving acid reflux. Pre- and postoperative pH studies showed similar reduction in acid reflux index.

8.3 Blinded randomised control trial of open *vs.* laparoscopic Nissen fundoplication in children

It assumed that laparoscopic surgery is associated with improved clinical outcome compared to open surgery in children. Laparoscopic fundoplication is performed in the belief that it improves postoperative outcome. However, there are no randomised controlled studies in children undergoing laparoscopic or open Nissen fundoplication. An analysis of the clinical outcome of patients in the randomised controlled blinded trial is presented in this section.

8.3.1 Methods

The protocol and patient group for this randomised trial has already been outlined in Section 2.2. All patients were investigated for reflux depending on clinical presentation. Reflux was documented by pH study, contrast study, endoscopy or a combination of the three. Parents were given full informed consent, and this study was approved by the Research Ethics Committee of The Institute of Child Health and Great Ormond Street Hospital. Patients were randomised to either laparotomy or laparoscopy by minimisation, as detailed in Section 2.2.1.1.

8.3.1.1 Anaesthesia

Children were fasted for six hours preoperatively. Last clear fluid intake was allowed 4 hours before anaesthesia. A standard infusion of dextrose-saline infusion was commenced at the time of fasting. Anaesthesia was standardised as outlined in Section 2.2.1.4.

8.3.1.2   Operative procedure

Operative technique was standardised between both limbs of the trial. Nissen fundoplication, with or without gastrostomy, was performed using routine techniques. Details of intraoperative management are described in Section 2.2.

8.3.1.3   Blinding

Postoperatively parents, ward nurses, acute pain team nurses and nurses involved in feeding were blinded to patient allocation. An occlusive dressing was used to hide the operative site in the postoperative period (Figure 8.4). Standardised operative notes which did not disclose the arm of the trial until discharge were provided for patients records.

8.3.1.4   Postoperative analgesia

The following pain assessment tools that have been validated in children were used:

- FLACC

- Linear visual analogue scale

as described in Section 2.2.1.6. Pain assessment was done by nurses and the acute pain assessment team, who were blinded to patient allocation. All hourly scores over each 24 hour period were averaged to calculate a daily score. Daily pain scores were then analysed between groups.

Unless contraindicated, all patients received regular identical doses of non-steroidal anti-inflammatory analgesia as follows;

- Paracetamol 15mg/kg/6hr for 48 hours then as needed

- Diclofenac 1mg/kg/8hr for 48 hours then as needed

One patient with raised liver enzymes in the open group did not receive Paracetamol and another patient in the laparoscopic group did not receive Diclofenac.

All patients had a standardised morphine infusion for postoperative pain relief, either as a nurse controlled analgesia (NCA) or patient controlled analgesia (PCA), as outlined in Section 2.2.1.6. Daily morphine requirements (μg/kg/d) were compared between groups.

8.3.1.5   Postoperative management

All patients were managed by a standardised postoperative management as described in Section 2.2.1.7. The standardised feeding regimen is shown in Figure 8.5. Patients that were unable to establish feeds as per protocol were treated as clinically indicated.

8.3.1.6   Clinical outcome in the early postoperative period

All patients were prospectively followed up. The postoperative complications of infection, adhesions, ileus and pulmonary complications were recorded. Any patient with signs of infection was investigated and treated appropriately. Any positive cultures were recorded.

8.3.1.7   Clinical outcome in the late postoperative period

Patients were prospectively followed in outpatients department after discharge from hospital. The impact of fundoplication on feeding was noted. Patient weight gain was assessed on each visit. Parents were asked about any recurrence of vomiting. Patients with recurrent vomiting were re-assessed for reflux using standard investigations. Patients with progressive vomiting, a positive barium, or pH study were defined as

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having recurrence. Anatomical findings of a 'slipped wrap' on barium meal was also noted. The need for re-do fundoplication was recorded.

Retching, defined as the action of vomiting without the appearance of any vomit, is due to activation of the emetic reflex. Parents were asked for the presence of retching and this was recorded. Postoperative retching that persisted beyond the first 6 weeks was considered clinically significant.

8.3.1.8 Statistics

Patient demographics and outcome measures were compared using independent sample t-tests. Differences in percentages were compared by chi squared. Data between time points were analysed using repeated measures ANOVA with post hoc tests. Time series data for curves were also analysed using a series of multilevel models (MLwiN®; Centre for Multilevel Modelling, Institute of Education, University of London).

8.3.2 Results

8.3.2.1 Patient demographics

Sixty-eight patients were assessed for entry into the trial (see Section 5.3.1; Figure 5.2). Patient demographics are shown in Table 8.1 Groups were comparable with respect to the characteristics used for randomisation. There was no significant difference in patient age (p=0.33; Figure 8.6) and weight (p=0.69; Figure 8.7) between groups. Operative time was significantly longer in the laparoscopic group (p<0.001, Figure 8.8).

8.3.2.2 Postoperative pain scores and analgesia requirements

Pain scores were highest in the first day postoperatively and fell throughout the study period in both groups. There was no significant difference between pain scores between

groups throughout the study period (p=0.56; Figure 8.9). Morphine requirements were highest in the first 24 hours postoperatively in both groups, and was needed for a median of 2 (mean 2.3) days in the open group and 3 (mean 2.5) days in the laparoscopic group (p=0.63). There was no significant difference in postoperative morphine requirements between groups (p=0.49; Figure 8.10).

8.3.2.3   Early postoperative clinical outcome

Table 8.2 shows the early postoperative outcome, including median time to full feed and length of hospital stay in both groups. There was no significant difference in time to full feed between groups (p=0.85, Figure 8.11). Median time to establish full feeds was 2 days in both groups. Three patients in the laparoscopic group and 2 patients in the open group developed gastric paresis (delayed gastric emptying clinically, without electrogastrographic or radiological conformation) in the postoperative period requiring institution of parenteral feeding. All five required two additional operations for insertion and removal of central lines. One patient with persistent gastrostomy aspirates had a pyloroplasty in the postoperative period. The paresis persisted in spite of this, and the patient slowly recovered and was fully fed on day 53 postoperatively.

Median hospital stay was 4.5 days in the open group and 5 days in the laparoscopic group (Figure 8.12), with no significant difference between groups (p=0.57).

Postoperative infections occurred in 4 patients. One patient in the laparoscopy group had a postoperative chest infection, secondary to aspiration that occurred during anaesthesia. Another patient in the laparoscopic group, who had bronchiectasis, had a postoperative chest infection. One patient each in both groups had a wound infection. There was no significant difference in rates of infection between groups (p=0.60).

8.3.2.4   Late postoperative outcome

Median length of follow-up was 22 (range 12-34) months. Incidence of dysphagia, recurrence of reflux and need for re-do fundoplication were not significantly different between groups (Table 8.3). At the time of last follow up the incidence of retching was higher after open surgery (56%) *versus* laparoscopy (6%; p=0.003).

8.3.3   Discussion of randomised controlled trial in children

The widespread implementation of laparoscopic surgery in children has been fostered by the assumption that it is associated with a quicker recovery, less pain, and less postoperative complications. There are several studies in adults. This is the first randomised blinded controlled trial addressing the outcome after laparoscopic or open surgery in children.

8.3.3.1   Early postoperative outcome was not altered by laparoscopy

The meta-analysis of randomised controlled trial comparing open and laparoscopic Nissen fundoplication in adults revealed that the laparoscopic approach favoured a quicker recovery, quicker discharge and generally fewer complications compared to open surgery. There are few studies in children that compare the outcome after open and laparoscopic surgery. Most of these are comparative, historical and non-randomised studies. The data presented in this chapter shows that in children randomised to laparoscopic and open Nissen fundoplication, there was no significant difference in length of stay and time to full feed between groups. Nurses in charge of feeding were blinded to patient allocation by an occlusive dressing, and there was a standardised feeding regimen in use. Patients were objectively assessed and fed according to a standardised protocol. Most patients were fully fed at 48 hours postoperatively, with no

significant difference between groups. Most patients were discharged after establishing feed, this therefore translated into similar hospital stay in both groups.

Although the study was not powered for the clinical outcome, this still represents the first objective data on the clinical outcome of children randomised to laparoscopic *versus* open surgery.

8.3.3.2 Laparoscopy did not reduce postoperative pain

One of the major initiating stimuli to the metabolic response to surgery is pain. Indeed improved intra- and postoperative pain management causes less postoperative stress responses and improves postoperative course (Anand & Carr 1989, Kehlet 2000, Rutberg et al 1984). The less invasive nature of laparoscopy leads to the assumption that it is less painful and therefore less stressful than open surgery.

During this study all patients received equivalent doses of paracetamol and NSAIDs in the postoperative period. As well, all nurses and pain control team members were blinded to patient allocation. There was no significant difference in morphine requirements and pain scoring between open and laparoscopic groups. Huang *et al* (Huang et al 2001) found that there was significantly less pethidine requirements in patients randomised (non-blinded) to laparoscopic and needlescopic compared to open appendicectomy. In agreement with this, Till *et al* (Till et al 1996) described reduced analgesia requirement in a non-randomized paediatric population undergoing laparoscopic compared to open appendicectomy. However, Beanes *et al* (Beanes et al 1995) found no significant differences in morphine requirements after open or laparoscopic splenectomy in children. However these latter 2 studies were not randomised. In randomised single-blind studies of children undergoing appendicectomy,

Lejus *et al* found that there was no difference in PCA requirements between open and laparoscopic groups (Lejus et al 1996); whereas Lintula *et al* found reduced requirement in the laparoscopic group (Lintula et al 2001). Therefore the data is inconsistent. Overall, the data from controlled trials suggest that there may not be a huge difference in postoperative pain between approaches.

There is little data on the pain outcome with respect to Nissen fundoplication in children. In one comparative study there was no significant difference in the total morphine requirements and NSAID doses between children undergoing open or laparoscopic Nissen (Dick et al 1998), although the requirements for morphine on day 1 were higher in the laparoscopic group. The findings in this trial concur with their findings. There are no previous randomised studies that have evaluated the pain response to Nissen fundoplication in children.

8.3.3.3 No difference in outcome in the late postoperative period

Follow up was 2 years in this group of patients at time of assessment. There was no significant difference in postoperative complications and recurrence rates between groups. There was also no significant difference in the incidence of dysphagia; this contrasts to findings of the meta-analysis in adults.

However, there was significantly more postoperative retching in the open group. The reporting of retching was a subjective assessment made by the parents or carers and is open to bias. At time of this reporting parents were no longer blinded. Therefore this may be one possible flaw in this outcome measure. However, this is a large difference to be due solely to parental bias.

The reason for the increased incidence of retching in the open group is difficult to speculate on. It was unlikely to be due to difference in postoperative gastric motility, as both groups established feeds in comparable times. There was also no significant difference in the incidence of gastric paresis or ileus between groups in the early postoperative period. However the long term effect on gastric motility is not known. There may be a difference in long term gastric motility to explain the differences. This can be the subject of another prospective study. In addition further follow up of this randomised cohort may provide further clarification of any differences between groups and provide important comparative information about long term outcome after open *versus* laparoscopic Nissen fundoplication.

8.4   Conclusions

Meta-analysis of randomised Nissen fundoplication in adults revealed quicker postoperative recovery, less postoperative complications and quicker return to normal activities after open Nissen fundoplication. Open Nissen was however associated with a higher incidence of postoperative dysphagia.

The results of this randomised controlled trial in children are different to the findings of the meta-analysis in adults. There was no difference in the postoperative recovery in this cohort of children. There were similar pain scores and analgesia requirements in both groups, and no difference in the incidence of postoperative dysphagia in children.

The differences in the findings between adults and children further highlight the need for studies in children to assess the influence of laparoscopy on outcome in children, which may differ to that reported in adults.

	Open	Laparoscopy	p value
Weight	12.80 (7.40, 18.25)	14.50 (9.80, 23.00)	0.71
Age	47.60 (7.88, 100.88)	66.87 (20.87, 126.23)	0.33
Neurological impairment	15 (75%)	15 (79%)	0.77
Congenital anomaly	0	1 (11%)	0.87

Table 8.1 *Demographics for patients in randomised controlled trial*

(Median, interquartile range; number and percentages for categorical data).

	Open	Laparoscopy	p value
Operation length	80 (75, 95)	160 (135, 195)	<0.001
Time to full feed	2 (2, 4)	2 (2, 4)	0.85
Hospital stay	4.5 (3, 7)	5 (4, 7)	0.57
Infection (%)	5	16	0.60
Ileus (%)	16	11	0.63

Table 8.2 *Early postoperative outcome for patients in randomised controlled trial (Median, interquartile range).*

	Open	Laparoscopy	p value
Dysphagia	0/16 (0%)	1/16 (6.3%)	1.0
Recurrence	3/18 (16.7%)	1/14 (7.1%)	0.61
Re-do fundoplication	1/15 (6.7%)	0/14 (0%)	1.0
Retching	10/18 (55.6%)	1/16 (6.3%)	0.003

Table 8.3 *Late postoperative outcome for patients in randomised controlled trial.*

Post-operative Dysphagia

Study	Open Surgery n/N	Laparoscopy n/N	RR (fixed) 95% CI	RR (fixed) 95% CI
Bais	0/46	7/57		0.08 [0.00, 1.40]
Chrysos	11/50	13/56		0.95 [0.47, 1.92]
Heikkinen	2/19	3/19		0.67 [0.13, 3.55]
Laine	3/55	5/55		0.60 [0.15, 2.39]
Luostarinen	6/15	4/13		1.30 [0.47, 3.62]
Nilsson	0/30	5/25		0.08 [0.00, 1.32]
Total (95% CI)	22/215	37/225		0.62 [0.39, 1.00]
Test for overall effect: P = 0.05				

0.01 0.1 1 10 100

Favours Open Surgery   Favours Laparoscopy
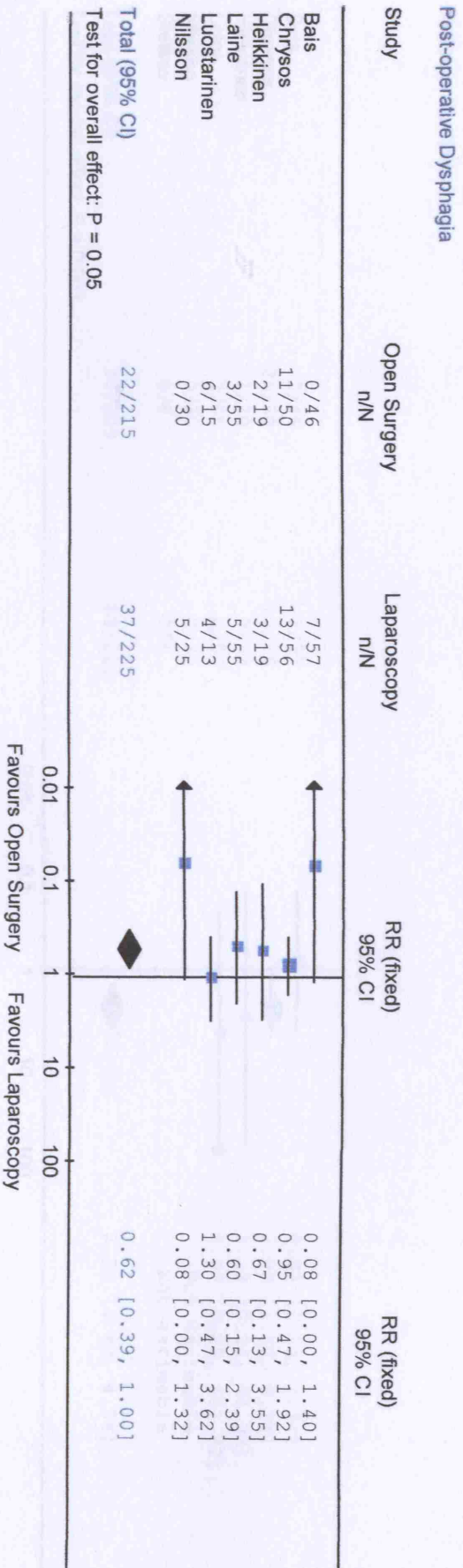
Figure 8.1 *Forest plots of relative risks (RR) for postoperative dysphagia in the meta-analysis of randomised controlled trials on open versus laparoscopic Nissen fundoplication.*

Systemic Complications

Study	Open Surgery n/N	Laparoscopy n/N	RR (fixed) 95% CI	RR (fixed) 95% CI
Bais	2/46	3/57		0.83 [0.14, 4.74]
Chrysos	25/50	10/56		2.80 [1.50, 5.24]
Heikkinen	1/20	0/22		3.29 [0.14, 76.33]
Laine	2/55	0/55		5.00 [0.25, 101.82]
Nilsson	0/30	0/25		Not estimable
Sietses	0/8	0/8		Not estimable
Total (95% CI)	30/209	13/223		2.50 [1.43, 4.38]

Test for overall effect: P = 0.001

Favours Open Surgery   Favours Laparoscopy
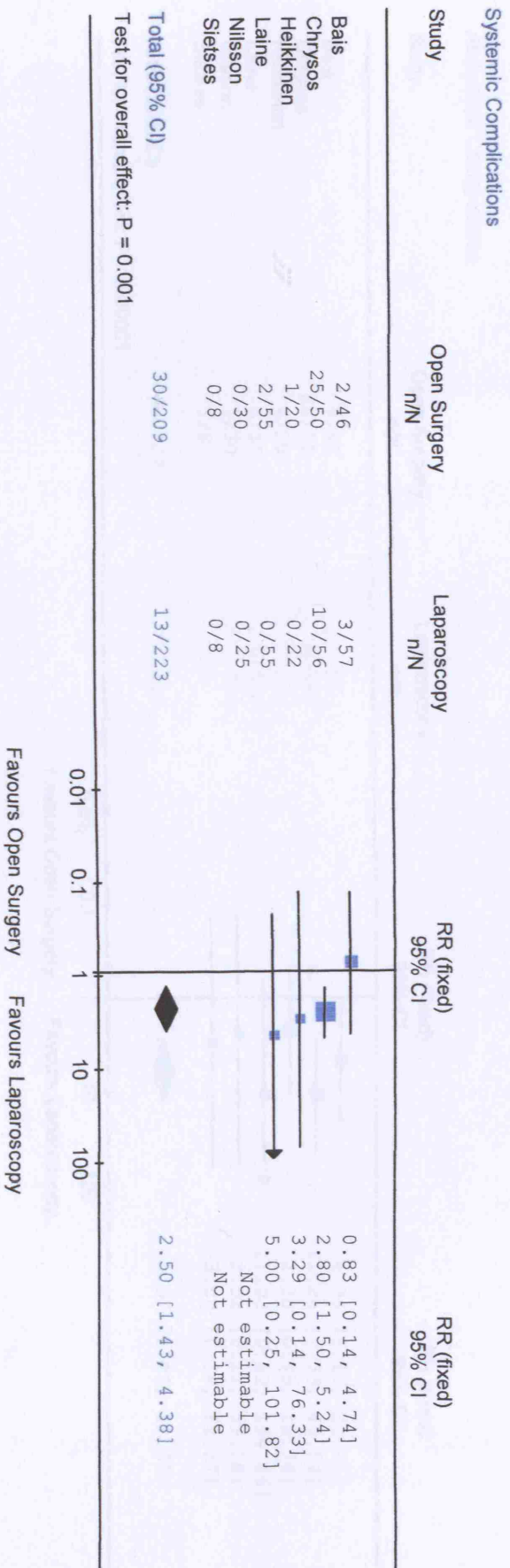
Figure 8.2 *Forest plots of relative risks (RR) for systemic complications in the meta-analysis of randomised controlled trials on open versus laparoscopic Nissen fundoplication.*

Abdominal Complications

Study	Open Surgery n/N	Laparoscopy n/N	RR (fixed) 95% CI	RR (fixed) 95% CI
Bais	8/46	2/57		4.96 [1.11, 22.21]
Chrysos	20/50	2/56		11.20 [2.75, 45.54]
Heikkinen	4/20	2/22		2.20 [0.45, 10.74]
Laine	5/55	0/55		11.00 [0.62, 194.26]
Nilsson	1/30	0/25		2.52 [0.11, 59.18]
Sietses	1/8	0/8		3.00 [0.14, 64.27]
Total (95% CI)	39/209	6/223		5.97 [2.82, 12.67]

Test for overall effect: P < 0.00001

0.01   0.1   1   10   100

Favours Open Surgery   Favours Laparoscopy
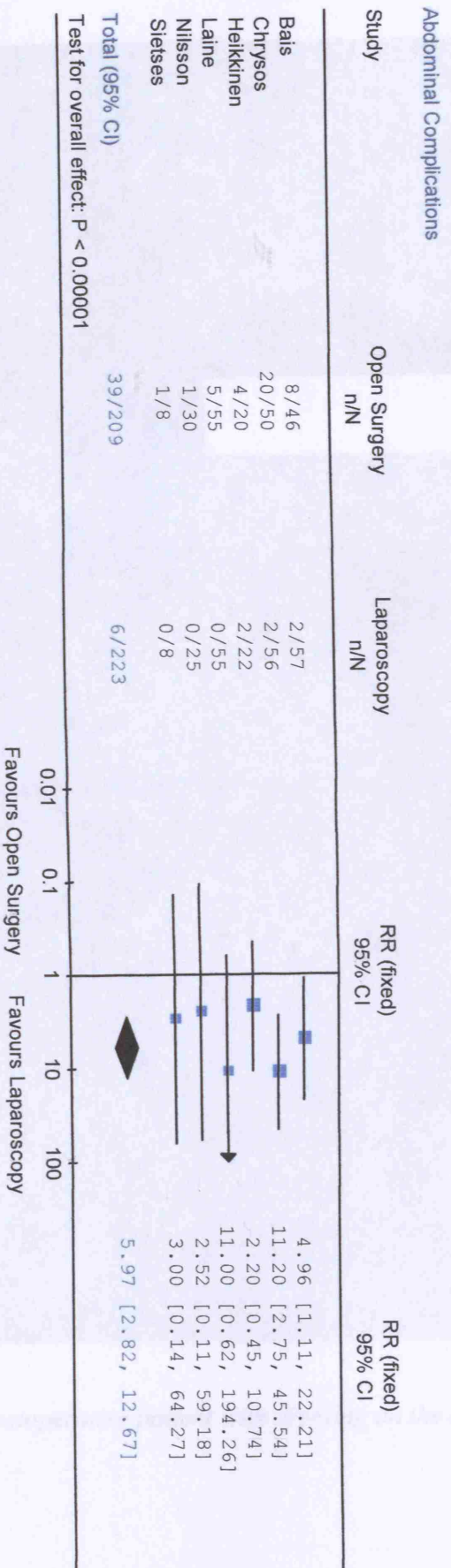
Figure 8.3 *Forest plots of relative risks (RR) for postoperative abdominal complications in the meta-analysis of randomised controlled trials on open versus laparoscopic Nissen fundoplication.*
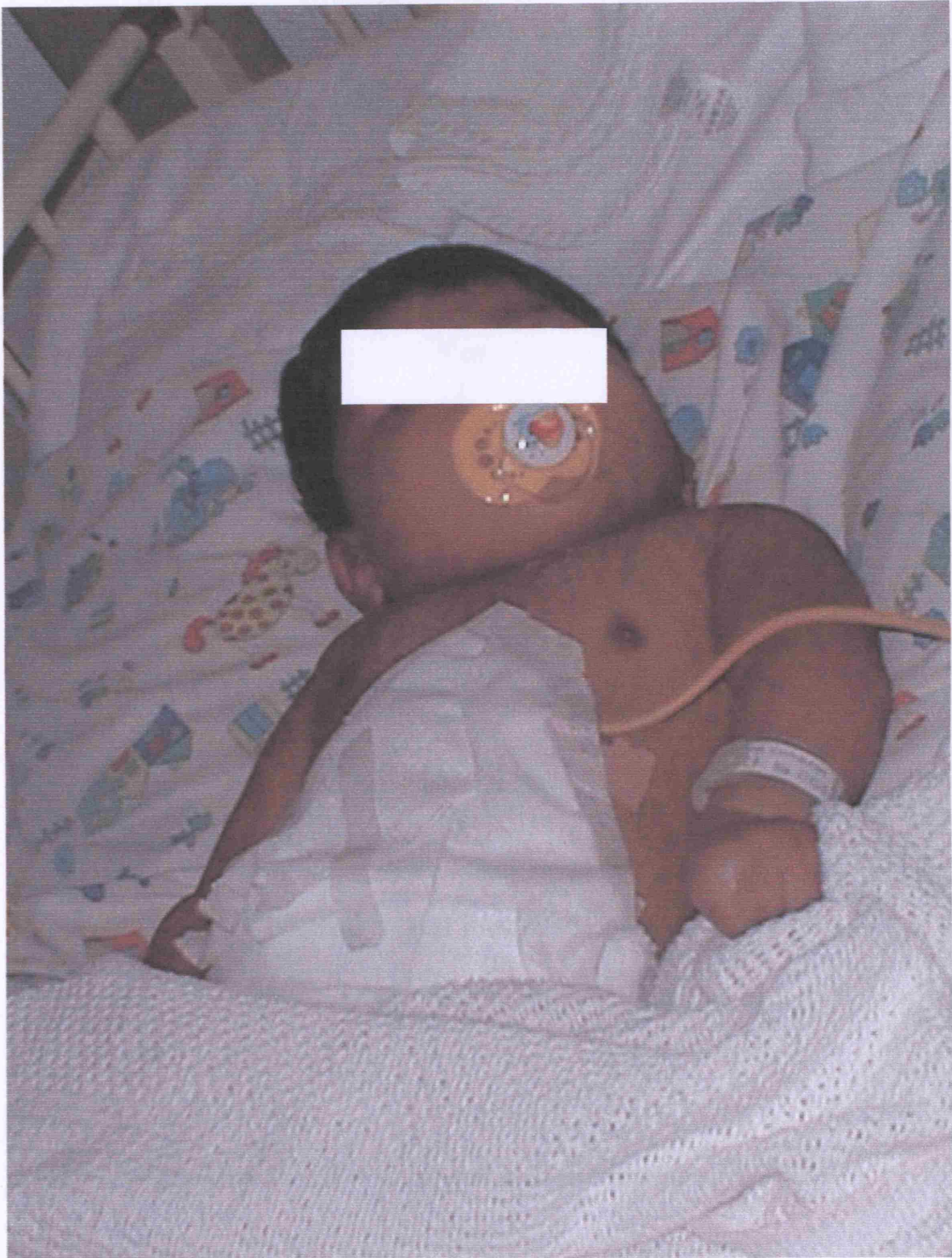
Figure 8.4 *Postoperative patient with dressing on the abdominal wall used for blinding.*

Day 1 (24 hrs) post op
↓
Bilious stomach aspirates? → Yes→ review 8-12 hrs
↓
No
↓
Infants: aspirates < 27 mls/hr
Child: aspirates < 5 mls/kg/hr? → No → review 8hrs
↓
Yes
↓
Introduce Milk 2mls/kg/2hrs
Increase 2mls/kg/4hrs
↓
Tolerated? → No → withhold 12 hrs
↓
Yes
↓
Introduce Feeds or
Soft diet after 8-12 hrs
↓
Tolerated? → No → Milk/Fluids
↓
Yes
↓
Graduate to full feed as tolerated.
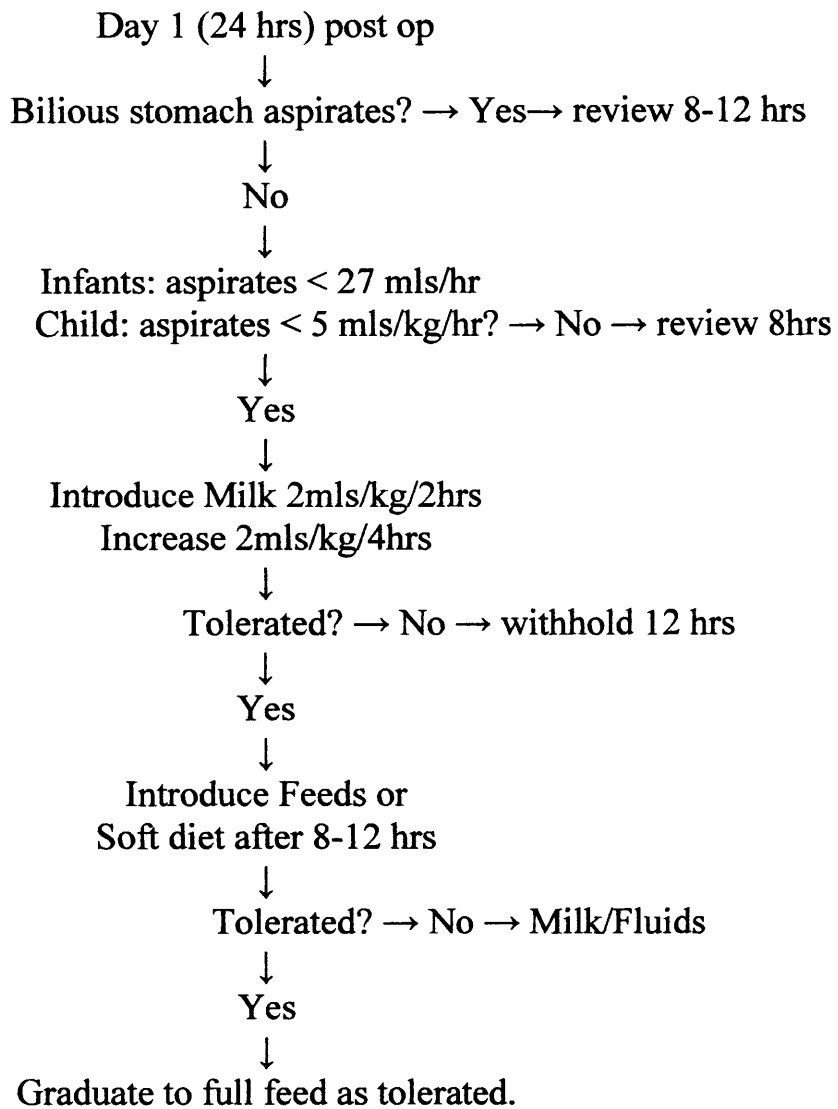
Figure 8.5 *Feed flow chart used in the postoperative period.*
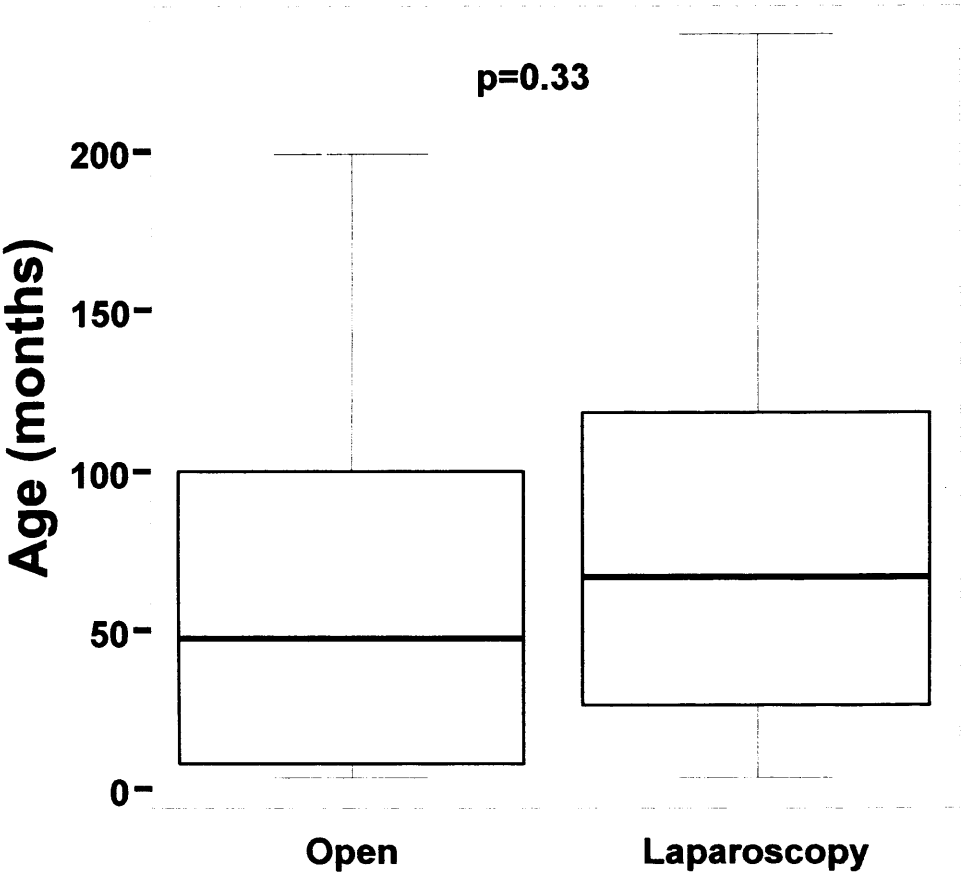
Figure 8.6 *Patient age in open and laparoscopic groups*

(Median, interquartile range and 95th centiles)
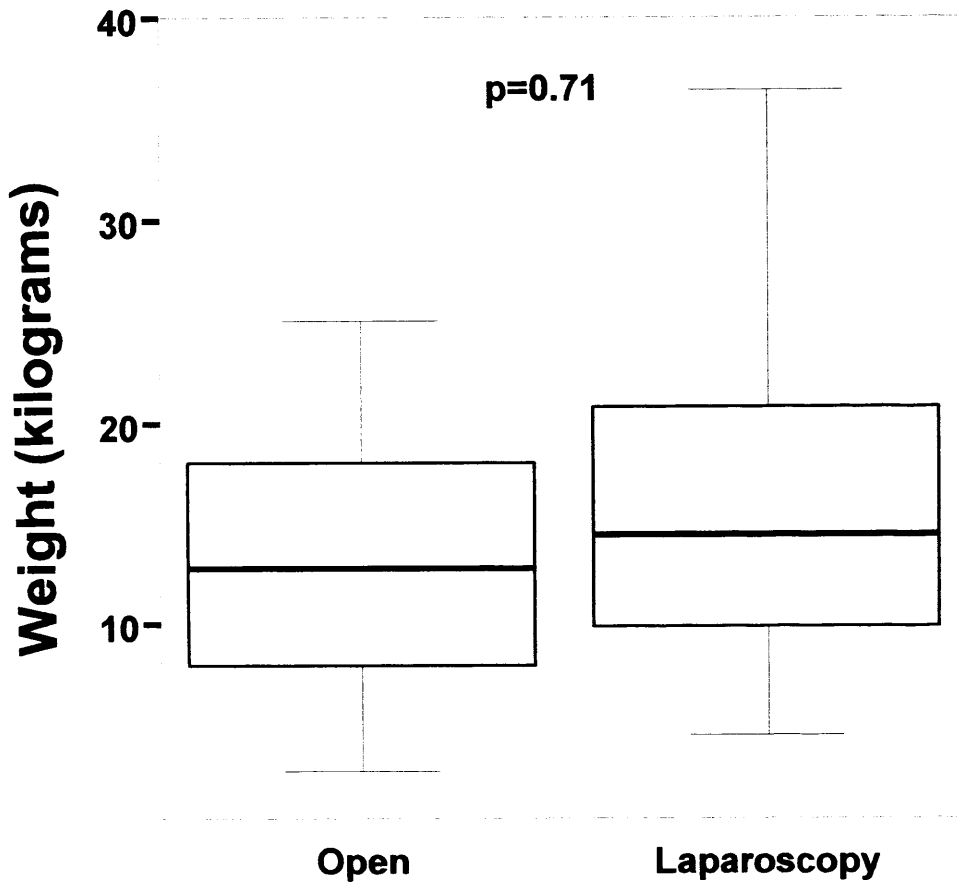
Figure 8.7 *Patient weight in open and laparoscopic groups*

(Median, interquartile range, and 95th centiles)
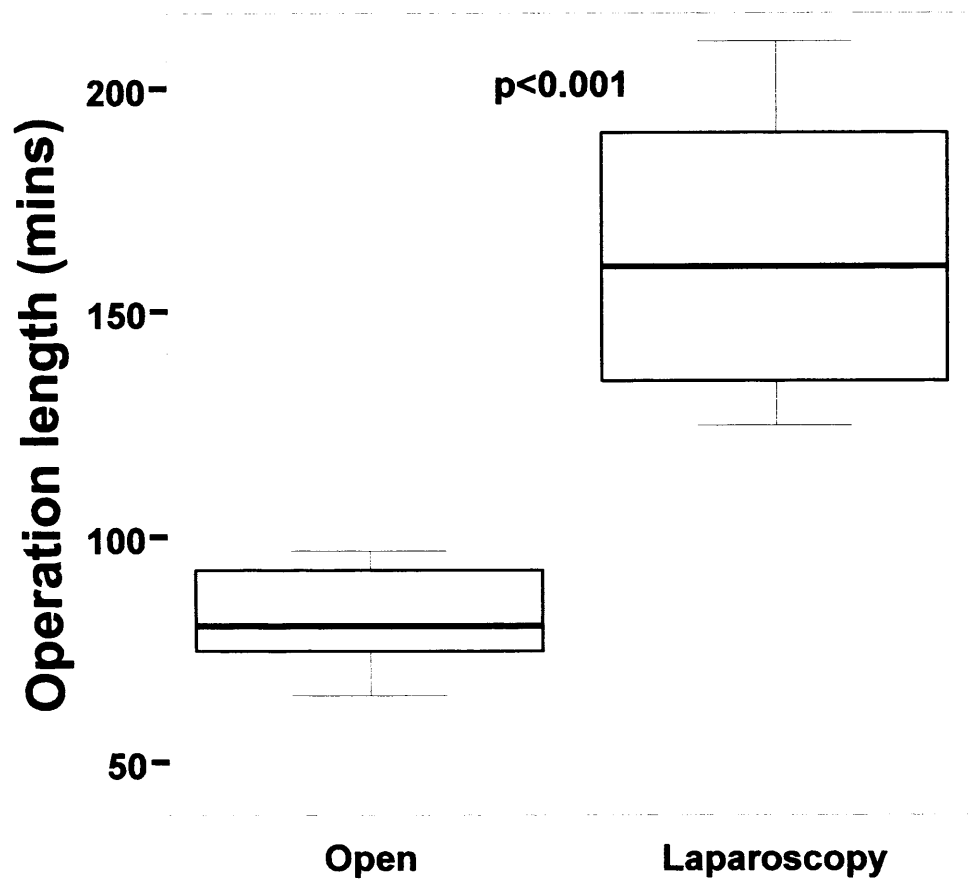
Figure 8.8 *Length of operation in open and laparoscopic groups*

(Median, interquartile range and 95th centiles)
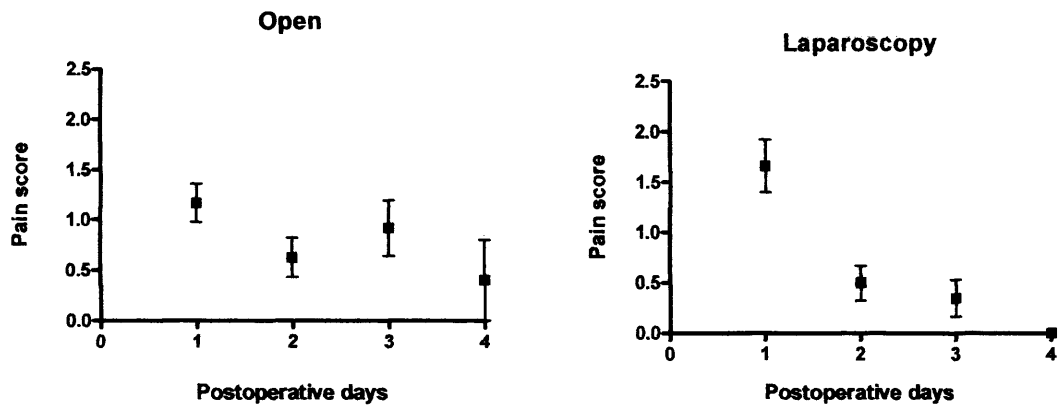
Figure 8.9 *Daily pain scores in patients in the postoperative period.*
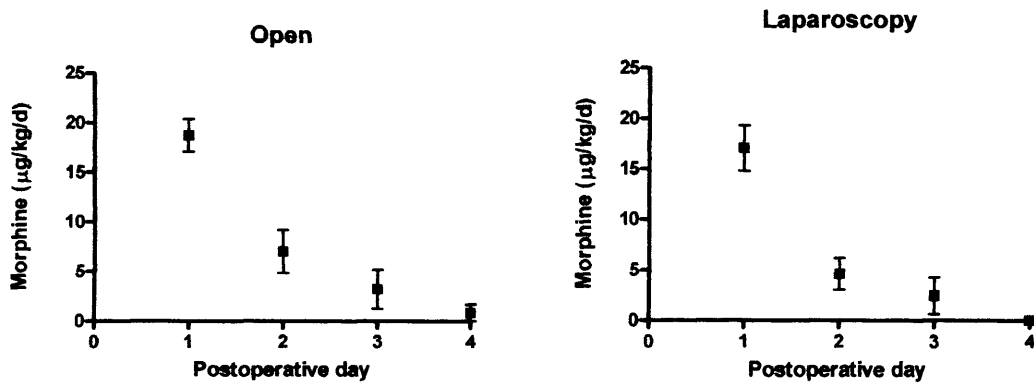
Figure 8.10 *Morphine requirements in the postoperative period*
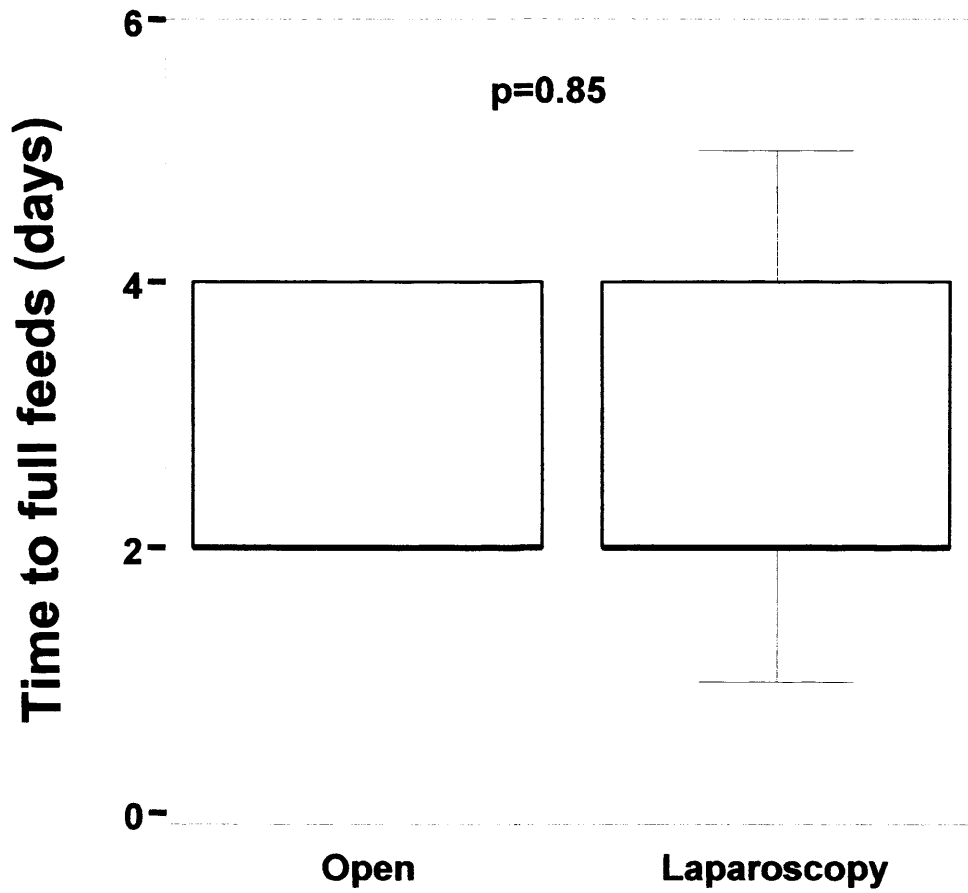
Figure 8.11 *Time to full feeds in patients in open and laparoscopic groups*
(Median, interquartile range, and 95th centiles)
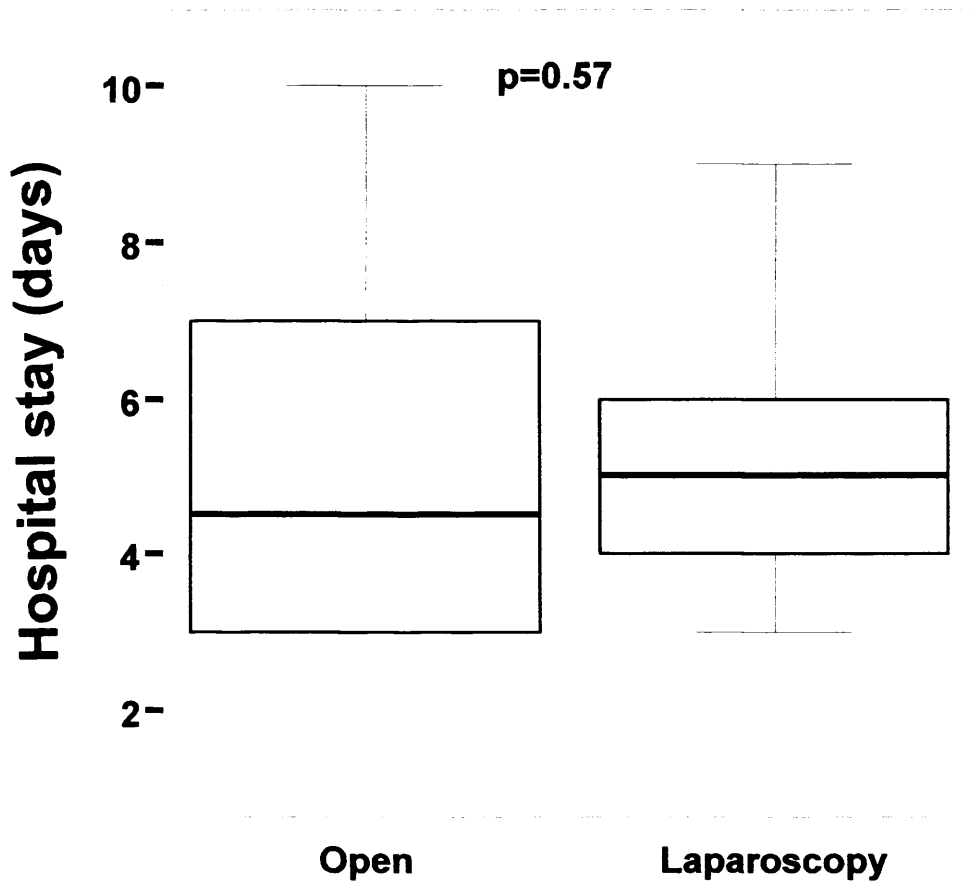
Figure 8.12 *Hospital stay in patients after open and laparoscopic surgery*

(Median, interquartile range, and 95th centiles)

CHAPTER 9

Final Discussion

9.1   Introduction

This chapter summarises the findings and conclusions presented throughout this thesis. The investigations performed addressed the impact of laparoscopy on the metabolic, inflammatory response, and clinical outcome after surgery in children. The results of each chapter are discussed in relation to each other.

Few studies have been performed to evaluate the effects of laparoscopy in children. The benefits cannot be assumed. Laparoscopy has most convincingly been shown to be of clinical benefit to adults undergoing cholecystectomy. The impact of laparoscopy on the inflammatory and metabolic response in adults has revealed mixed results. There seems to be a dampening of the inflammatory and immune response (Gupta & Watson 2001), but little, if any, effect on the metabolic response in adults (Kehlet 1999). Most of this data has been established in patients undergoing laparoscopic cholecystectomy. The evidence in adults undergoing larger operation is mixed. The findings in adults does not necessary translate into the paediatric population.

The findings in this thesis are summarised.

9.2   Intraoperative handling of CO_2 in children differs form that in adults

In **Chapter 3** the effect of intraperitoneal CO_2 insufflation on CO_2 elimination in the intraoperative period was described. There was an increase in CO_2 elimination throughout the period of CO_2 insufflation in children. This differs to the early steady state of CO_2 elimination achieved after approximately 15 minutes in adults (Mullett et al 1993). The increase in CO_2 elimination was more marked in younger and smaller children, suggesting that age modifies the intraoperative handling of CO_2; with smaller and younger patients absorbing relatively more CO_2. End tidal CO_2 levels increased in

line with the increase in CO_2 elimination, and were controlled by an increase in minute ventilation. This continuous increase in CO_2 elimination in children resembles the pattern seen with extraperitoneal insufflation in adults.

This pattern of continuous increase in CO_2 elimination in children differs from that described in adults. Two possible explanations of this continuous increase in CO_2 elimination are hypothesised. Firstly, there may be a difference in the peritoneal handling of CO_2 in children compared to adults. The relatively larger peritoneal surface area compared to body mass may alter the metabolic handling of CO_2 in children. Alternatively, there may be an increase in whole body metabolism during laparoscopy compared to open surgery as suggested in **Chapter 4** and described below. The increased CO_2 absorption may give rise to local acidosis. Local acid-base balance at the peritoneal level and systemic acid-base balance may alter the overall metabolic response in children undergoing laparoscopy. The local peritoneal absorption of CO_2 insufflated and acid-base balance in children undergoing laparoscopy needs further investigation.

9.3   Intraoperative period is associated with an increase in core temperature and O_2 consumption in children undergoing laparoscopy

The effect on laparoscopy on whole body energy metabolism and thermoregulation in the intraoperative period was described in **Chapter 4**. There was a continuous intraoperative increase in core temperature and O_2 consumption in children undergoing laparoscopy. This was not observed in children undergoing open surgery. Although these were not randomised groups, the two groups were comparable in respect to age and weight. Multilevel model analysis revealed that independent of any differences in groups, there was a significant increase in core temperature and O_2 consumption in the laparoscopy group compared to open surgery. The increase in core temperature and

oxygen consumption was more marked in younger and smaller children. Results in **Chapter 5** confirmed the increase in O_2 consumption and core temperature intraoperatively in children randomised to laparoscopy but not those randomised to open surgery. These observations have not been previously described in children, and differ to that described in adults.

This intraoperative increase in intraoperative core temperature and metabolism in the laparoscopic group may be related to intraoperative heating form laparoscopic instrumentation. However this is unlikely to significantly increase oxygen consumption, as temperatures were in the thermoneutral range. Alteration in intraoperative thermoregulation is likely to be one of the mechanisms for the increase in core temperature and oxygen consumption in the laparoscopic group. Children are particularly sensitive to changes in their thermoregulatory environment intraoperatively (Plattner et al 1997). Anaesthesia inhibits thermoregulation and results in hypothermia (Dicker et al 1995). This and can be reversed by stimulating metabolic processes, for example, by amino acid infusion (Sellden et al 1994, Zamparelli et al 2000). The increase in metabolism in the laparoscopic group represents an alteration in intraoperative thermoregulation and metabolism in children undergoing laparoscopy.

The finding of an intraoperative hyperthermia has clinical significance. There has been a decrease in patients requiring intraoperative heating to maintain core temperature during laparoscopy, and use of the cooling function of the temperature regulating devices in some instances. This clinical observation supports the explanation that laparoscopy altered intraoperative thermoregulation in children.

9.4   Laparoscopy alters protein and energy metabolism

The altered intraoperative metabolism and thermoregulation had effects on the postoperative metabolism. This was investigated in a randomised trial in **Chapter 5.** Resting energy expenditure was decreased 24 hours after open surgery, but showed no significant change after laparoscopy. There was a consistent increase in resting energy expenditure 4 hours after laparoscopy, suggesting a continuation of the intraoperative response. Thus laparoscopy altered the postoperative whole body energy metabolism compared to open surgery.

The anaesthesia induced decrease in the metabolic rate and thermogenesis intraoperatively (Dicker et al 1995, Ohlson et al 1994) may be responsible for the decrease in energy and protein metabolism in the early postoperative period in the open group. Studies in the 40's demonstrated an early decrease in metabolic rate after trauma and surgery, which could be partially reversed by maintaining thermoregulation (Cuthbertson 1932, Cuthbertson 1942). The findings in this thesis of a decrease in thermoregulation and metabolism intraoperatively, followed by a diminished metabolic rate in the postoperative period mirrors those findings connecting thermoregulation and postoperative metabolic response.

Laparoscopy in children may alter this metabolic pattern by altering intraoperative thermoregulation and metabolism. Laparoscopy was shown to cause a relative preservation or increased in metabolism both intraoperatively (**Chapter 4**) and postoperatively (**Chapter 5**). The increase in core temperature and oxygen consumption intraoperatively maintained the postoperative energy metabolism.

Results in **Chapter 5** also demonstrated a difference in the postoperative protein metabolism between open and laparoscopic groups. There was a decrease in protein catabolism in the early (4 hours) postoperative period after open surgery. This confers with the findings in the early postoperative period in adults (Carli et al 1990). There was no significant change in protein catabolism after laparoscopy. **Chapter 6** investigated the link between the metabolic response and the endocrine response. Although insulin levels correlated with protein catabolism in the postoperative period in the open group, this correlation did not exist in the laparoscopic group. Thus, laparoscopy dissociated hormonal control of protein metabolism. Previous studies have linked metabolic acidosis and increased protein catabolism in children. Children with increasing acidosis had higher rates of protein breakdown (Boirie et al 2000). Children undergoing laparoscopy were shown to have increased end tidal CO_2 (**Chapter 3**) and may have concomitant acidosis. It is theoretically possible that alterations in acid base balance, either locally in the peritoneal cavity, or systemically, may alter postoperative protein metabolism. This specific link between protein metabolism and acid base balance after laparoscopy has not been investigated.

Several studies have linked intraoperative thermoregulation and amino acid metabolism. Amino acid infusion increases thermogenesis and metabolism, both in tissue culture and in the intraoperative period, by counteracting the decrease in thermogenesis associated with anaesthesia (Sellden & Lindahl 1999, Zamparelli et al 2000). This increase in thermogenesis associated with intraoperative amino acid administration has been shown to result from the increased metabolic rate, possibly due to increased protein turnover (Sellden & Lindahl 1998). Amino acid administration resulted in an increase in oxygen consumption. This increased metabolism translates into a decrease in intraoperative hypothermia. This increase in metabolism associated with protein metabolism supports

the link between intraoperative core temperature, maintained protein metabolism and energy metabolism described throughout this thesis. The maintained protein turnover and thermoregulation during laparoscopy may be responsible for the increase in the intraoperative metabolism seen. This increased response intraoperatively then continued into the early postoperative period and resulted in the maintained metabolic processes. There was a significant correlation between the change in protein metabolism and the change in energy metabolism in the laparoscopic group.

Whatever the mechanism, laparoscopy and open surgery affect the metabolic responses to operative stress in children differently.

Cytokines are well established as initiators and markers of the inflammatory response to operative stress. Results in **Chapter 7** showed that there was no significant difference in circulating IL-6, IL-10, TNF-α or systemic markers of oxidative stress. IL-6 and energy metabolism are related in the postoperative period in some studies (Kotani et al 1996). There was no correlation between IL-6 and energy metabolism in either group in this thesis. The difference in the metabolic responses is therefore not the result of differences in the inflammatory response between groups.

9.5   Postoperative monocyte function after laparoscopy and laparotomy

The postoperative decrease in monocyte class II MHC expression was described in **Chapter 7**. Monocyte MHC expression is crucial for monocyte stimulation of T-cell specific response. The decrease in monocyte MHC expression was noted after both open and laparoscopic surgery. However, laparoscopy was associated with relatively preserved monocyte class II MHC expression compared to open group. This suggested that operation type may be an independent determinant of monocyte function. There

have been mixed reports on the effect of laparoscopy on monocyte function in adults, the balance suggest a moderate benefit in maintaining postoperative immune function (Gupta & Watson 2001).

The mechanism behind the difference in monocyte function is unclear. There was no significant increase in circulating IL-10. Increased circulating IL-10 has been shown to depress monocyte class II expression in the postoperative period, but circulation IL-10 did not increase in this study. However IL-1ra levels significantly correlated with monocyte class II expression. IL-1ra is not known to affect monocyte class II expression. However the question arises as to whether the decrease may be related to this anti-inflammatory cytokine, by possible effects on intracellular gene expression.

One other possible mechanism for the decrease in monocyte class II MHC expression in the postoperative period is the decreased availability of glutamine. Studies have shown, both *in vivo* and *in vitro*, that the decrease in monocyte MHC expression postoperatively is reversed by administration of glutamine (Spittler et al 2001). Decreased protein catabolism in the postoperative period, as described in **Chapter 5,** may decrease a major source of glutamine in the fasting, post-absorptive, postoperative state. The preservation of protein catabolism in the laparoscopic group may contribute to the maintenance of monocyte function by maintaining postoperative glutamine availability.

9.6   Clinical outcome in children randomised to open and laparoscopic Nissen fundoplication

Meta-analysis of randomised control studies of Nissen fundoplication in adults revealed a difference in the incidence of infective complications and abdominal complications

that favoured laparoscopic surgery. On the contrary, there was a higher incidence of postoperative dysphagia after laparoscopic Nissen. This was not found in this randomised trial in children.

The clinical outcome (Chapter 8) in children randomised in a blinded, randomised trial demonstrated no difference in the early postoperative outcome between groups. There were similar postoperative recovery, pain scores and morphine requirements in the 2 groups. There was also no difference in the time to full feed or the time to discharge between open and laparoscopic Nissen fundoplication in children. Postoperative dysphagia, infection and recurrence were also not different between groups.

9.7   Overall conclusions

A few theories can be put forward to explain the differences in energy metabolism noted throughout this thesis. During laparoscopy in children there is an increase in the metabolic rate and O_2 consumption in the intraoperative period, which results in increased core temperature. Altered thermoregulation intraoperatively may be the initiating factor. This increase in energy metabolism in the laparoscopic group translated into a maintained protein metabolism and resting energy metabolism in the postoperative period, compared to open surgery. Overall these changes lead to maintenance of the metabolic processes relative to open surgery in the early postoperative period.

Alternatively, this increased metabolism may be partially driven by the CO_2 used during laparoscopy, being more readily absorbed in younger patients. Local acidosis may occur because of the increased CO_2 absorption during laparoscopy in children. This acidosis may give rise to the increased protein catabolism seen in the laparoscopic group

compared to open surgery. This catabolism may then maintain intra- and postoperative energy metabolism as protein turnover is an energy dependent process.

There was also a decrease in monocyte class II MHC expression in the open group that was relatively preserved by laparoscopy. The surface expression of monocyte class II MHC expression is dependent on glutamine levels. It can be postulated that the preserved class II expression may be related to improved availability of glutamine due to maintained protein catabolism in the postoperative period in the laparoscopic group.

Proposed physiological response to laparoscopy in children

Laparoscopy

? Intraoperative acid load   **Intraoperative hyperthermia**

Maintained protein catabolism ⇄ **Maintained metabolic rate**

? Improved glutamine availability   **Increased resting energy metabolism**

Preserved monocyte HLA-DR

9.8 Further studies

9.8.1 CO_2 metabolism

Results suggest that children have a different CO_2 absorption profile compared to adults. However the CO_2 elimination observed in this thesis can be the result of both increased absorption and metabolism. Isotope labelled CO_2 insufflation with estimation of labelled CO_2 in exhaled gases can differentiate between CO_2 absorbed and metabolically produced.

9.8.2 Intraoperative metabolism

The increase in intraoperative metabolism can be further validated and investigated using operations of greater magnitude. One important question that remains is the cause and effect relationship between the metabolic response and core temperature. It is envisaged that an animal model is the first step in addressing this question. Controlling intra-abdominal and core temperature while measuring oxygen consumption can be performed in an animal study. The impact of laparoscopy on the intraoperative temperature regulation in children can then be further investigated.

Another explanation that has been proposed for some of the differences between laparoscopy and open surgery on the postoperative response is the effect of CO_2 absorption and acidosis on the metabolic response. Blood gas analysis of pH levels with concomitant measurement of protein turnover may confirm or refute this link.

9.8.3 Immune response in the postoperative period
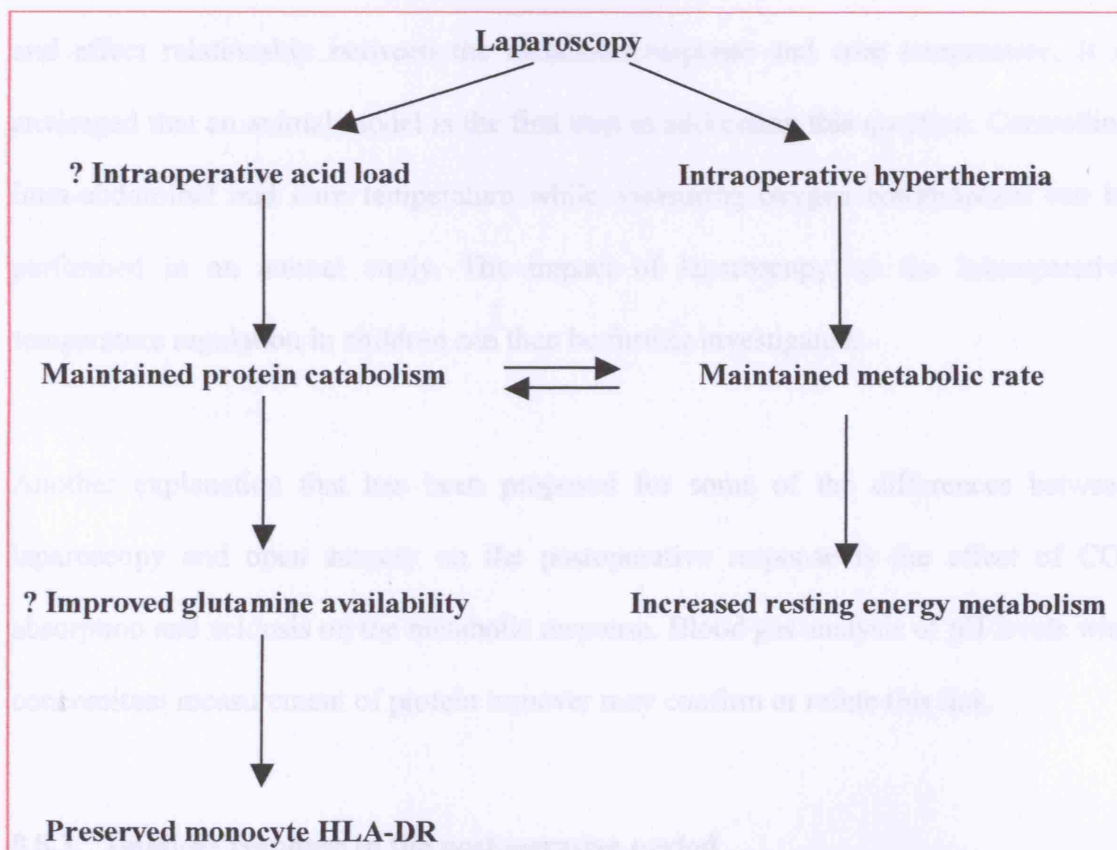
Further studies should be aimed at further elucidating the mechanism for the decrease in monocyte class II MHC expression in the postoperative period and the mechanism for the relative preservation afforded by laparoscopic surgery. Postoperative levels of

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glutamine may be one of the factors leading to decreased monocyte class II expression. Studies of *in vivo* glutamine flux in the postoperative period, along with measurements of monocyte class II expression, can be performed to test this hypothesis. *In vivo* studies of protein catabolism and glutamine turnover after laparoscopy in relation to monocyte class II expression may confirm the link. Administration of glutamine in the perioperative period may be one mechanism of decreasing the rate of postoperative infections. This has not yet been performed in children to my knowledge.

9.8.4 Postoperative energy metabolism

Postoperative metabolism was investigated in the early postoperative period. However we did not study the changes in energy and protein metabolism in the late postoperative period. Changes in the late catabolic postoperative period may determine postoperative outcome. Also, the response to operations of greater magnitude can be investigated. Nissen fundoplication was not associated with a significant pro-inflammatory response. Operations associated with a pronounced pro-inflammatory response may be studied to gain a better insight into the possible beneficial effects of laparoscopy in the metabolic response in children.

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APPENDIX I: PUBLICATIONS ARISING FROM THIS WORK

1.   McHoney M, Corizia L, Eaton S, Kiely EM, Drake DP, Tan HL et al.

CARBON DIOXIDE ELIMINATION DURING LAPAROSCOPY IN CHILDREN IS

AGE DEPENDENT.

J.Pediatr.Surg. 2003;38(1):105-10.

2.   McHoney M, Eaton S, Wade A, Klein NJ, Stefanutti G, Booth C et al.

INFLAMMATORY RESPONSE IN CHILDREN AFTER LAPAROSCOPIC VS

OPEN NISSEN FUNDOPLICATION: RANDOMIZED CONTROLLED TRIAL.

J.Pediatr.Surg. 2005;40(6):908-13.

3.   McHoney M, Corizia L, Eaton S, Wade A, Spitz L, Drake D et al.

LAPAROSCOPIC SURGERY IN CHILDREN IS ASSOCIATED WITH AN

INTRAOPERATIVE HYPERMETABOLIC RESPONSE.

Surgical Endoscopy 2006;20(3):452-7.

4.   McHoney M, Klein NJ, Eaton S, Pierro A.

DECREASED MONOCYTE CLASS II MHC EXPRESSION FOLLOWING MAJOR

ABDOMINAL SURGERY IN CHILDREN IS RELATED TO OPERATIVE STRESS.

Pediatric Surgery International 2006;22(4):330-4

PRESENTATIONS AND PRIZES

1. INTRAOPERATIVE THERMOREGULATION ALTERS POSTOPERATIVE METABOLISM AFTER LAPAROSCOPIC SURGERY IN CHILDREN

Best Presentation

British Association of Paediatric Endoscopic Surgeons   Sept 2005

2. HORMONAL RESPONSE AFTER OPEN AND LAPAROSCOPIC SURGERY IN CHILDREN; RANDOMIZED CONTROLLED TRIAL

Peter Paul Rickman Prize session   July 2005

British Association of Paediatric Surgery Annual Conference

3. LAPAROSCOPIC AND OPEN SURGERY HAVE DIFFERENT EFFECTS ON POSTOPERATIVE PROTEIN METABOLISM

Peter Paul Rickman Prize session   Aug 2004

British Association of Paediatric Surgery Annual Conference

4. POSTOPERATIVE METABOLIC RESPONSE AFTER LAPAROSCOPIC NISSEN FUNDOPLICATION

Best oral presentation

Nutrition society; British Association for Parenteral and Enteral Nutrition Nov 2003

5. LAPAROSCOPY BLUNTS THE POST-OPERATIVE METABOLIC RESPONSE TO SURGERY IN CHILDREN

Journal of Pediatric surgery; best clinical presentation prize

CAPS Conference   Sept 2003

6. MONOCYTE HLA-DR EXPRESSION IS DEPRESSED IN CHILDREN

FOLLOWING MAJOR SURGERY

CAPS Conference   Sept 2003

7. CARBON DIOXIDE ELIMINATION DURING LAPAROSCOPY IN CHILDREN

IS AGE DEPENDENT

BAPS Conference   July 2002

8. INTRAOPERATIVE METABOLISM IN CHILDREN UNDERGOING

LAPAROSCOPIC SURGERY

Clinical Nutrition and Metabolic Group (BAPEN)   Nov 2001

9. LAPAROSCOPIC SURGERY IN CHILDREN INDUCES AN INTRAOPERATIVE

HYPERMETABOLIC RESPONSE

BAPS Conference   July 2001

APPENDIX II: Multi-Level Modelling Analysis

Mixed (or random) effects models are sometimes known as multilevel or hierarchical models. Each individual trajectory is modelled, and some commonality or correspondence between the trajectory parameters for each individual is assumed. This communality or correspondence is necessary to make the model interpretable; otherwise it would be hard to identify trends.

For example, we could fit lines/curves to each child's measurements. This is a lot of separate curves and/or single points (for the children with only one measurement) to comment on and combine in some useful way. It is also a lot of parameters. If we assume some common form for the patterns of measurement change between children and allow the model parameters to follow a defined distribution, then this removes a large number of parameters. For example, if we assume that each child's measurements follow a linear trend, there are 2 parameters to be estimated for each child: the slope and intercept of their fitted line. Fitting lines independently for each child will require twice the number of parameters than there are children in the sample. By assuming that the slope and intercept are each normally distributed between children, then we need only estimate 4 parameters: the mean and standard deviation of the normal distributions of the intercepts and slopes. Hence we borrow strength across subjects to make inferences about individual subjects by assuming the parameters are from some specified distribution.

The parameters which define the distribution of child specific values (ie. the mean and standard deviations of the normal distributions for the intercept and slopes in the above example) show the extent to which children vary around the overall averages. If there is

much variation in individual child levels (intercepts) and changes over time (slopes) then the standard deviations of these will be large.

A second source of variation is the extent to which a child's observations vary around their predicted trajectory. In the line example above, each child has an estimated fitted line (with intercept and slope from the estimated distributions) and their actual measurements may not lie exactly on this line – there will be some variation around the predicted line.

Multilevel models allow quantification of the sources of variability. In the simplest case of children assessed over time, there are 2 sources (or levels) of variation:

- Between child (variation in trajectory parameters)
- Within child (around their fitted trajectory)

The between individual variability might be further partitioned into several levels. For example, some hospitals may tend to make higher measurements on average and these hospitals are within wards (which may vary in outcome because of social or other ward-related features). In this example, there are 4 levels: repeat measurements within individuals, within hospitals, within wards.

Summary of multi-level model analysis in the randomised trial

Five time points were measured

    1- Pre-op

    2 – Immediately post-op

    3 – 4 hours post

    4 – 24 hours post

    5 – 48 hours post

REE measured at times 1,3,4; protein catabolism measured at times 1 and 3; all others measured at all 5 time points

Models fitted with age, length and operation length; then time; then open or laparoscopic (open/lap) parameter; then open/lap-time interaction terms.

Time represented by series of dummy variables: 2 for REE, 1 for protein catabolism, 4 for other variables. Similarly, 2,1 and 4 interaction terms between open/lap and time.

All variables apart from HLA% show some degree of upward skewing. HLA% is distinctly downwardly skew. Upwardly skew variables were logged (natural logs taken), adding 0.9 to Il1ra and insulin for whom there were zeros. This resulted in near normal distributions for all of these variables. Squaring HLA% made the distribution less skew, but cubing was even more effective.

Change in -2 log-likelihoods for models based on suitably transformed data (log to base e for all except HLA which was cubed) when time, open/lap and interaction terms are added to a basic model incorporating adjustment for age, weight and operation length.