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**UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES
INSTITUTE OF HUMAN NUTRITION**

**THE IMPACT OF POST OPERATIVE OEDEMA ON CLINICAL
RECOVERY AND ITS POTENTIAL CAUSES**

by

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Submitted for the degree of

Doctor of Medicine

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UNIVERSITY OF SOUTHAMPTON
ABSTRACT
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**THE IMPACT OF POST OPERATIVE OEDEMA ON CLINICAL RECOVERY
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Emmanuel Onome Itobi

The postoperative period is characterized by massive shifts of fluid between body compartments and accumulation of fluid in the extracellular space, which may manifest clinically as central and or peripheral oedema. The incidence of oedema in patients undergoing routine major abdominal surgery (MAS) is unknown and there are no objective means of quantifying or monitoring its presence. Furthermore, the aetiological factors responsible for post-surgical oedema formation in patients with no overt signs of cardiovascular disturbance are poorly understood and the relationship between the development of oedema and clinical outcomes such as the recovery of gastrointestinal function, postoperative complications and duration of hospital stay is unclear.

Observational studies were therefore conducted on patients undergoing MAS. The presence of oedema was related to changes whole-body impedance (Z), obtained at four frequencies (5, 50, 100 and 200 kilohertz (kHz)) using bioelectrical impedance analysis (BIA) and to clinical outcomes. The fluid intake and output and changes in plasma concentration of albumin, total protein, C-reactive protein (CRP) and reduced glutathione in whole blood (GSH) were compared before and after surgery in patients who subsequently developed oedema (OD group) and patients who consistently remained free of oedema (NOD group)

Oedema occurred in 40 per cent of the patients observed prospectively and was significantly related to age (odds ratio 1.087 (95 per cent confidence interval (c.i), 1.016 - 1.163; $P=0.016$). The preoperative ratio of Z at 200 kHz to 5 kHz (Z_{200}/Z_5) was higher in patients who subsequently developed oedema than those who did not (0.809 v 0.799; $P = 0.015$), suggesting that it may be possible to identify patients who are prone to abnormal fluid shifts preoperatively. The change in (Z) was greater in the oedematous than non-oedematous groups (at all frequencies ($P < 0.001$)), and more so at lower frequencies (5kHz) than higher frequencies (100 kHz) ($P < 0.001$). The impedance quotient (ht^2/Z) in the whole group changed in a similar direction at each frequency but to a greater extent in the OD compared to NOD groups.

The total volumes of administered fluids in both groups of patients were similar but the average urine output per kg body weight was significantly lower in the OD compared to NOD patients (29.4(2.3) *versus* 40.5(3.7) mls/kg, $P = 0.023$). There were no significant differences before and after surgery in the concentrations of albumin, total proteins and GSH in both patient groups. Preoperative CRP concentration in the OD and NOD patients were similar but the mean (s.d) CRP concentration over duration of observation in the OD compared to the NOD patients was significantly greater (148 (54.1) *versus* 89.6 (43.8) mg/L, $P = 0.006$). Oedema was associated with a significant delay in the recovery of gut function (median (range) (6(3-17) *versus* 5(1-13) days, $P = 0.020$) and prolonged hospital stay (17(8-59) *versus* 9(4-27) days, $P = 0.001$) and increased incidence of postoperative complications (65 *versus* 22%, $P = 0.011$).

This study shows that the incidence of early postoperative oedema is high and preoperative identification and monitoring of surgical patients vulnerable to abnormal fluid shifts may be possible with non-invasive techniques. Age, impaired ability to excrete administered fluid load and an exaggerated inflammatory response to surgical trauma rather than hypoalbuminaemia and hypoproteinaemia were significant factors for oedema formation. Postoperative oedema was associated with a significant increase in postoperative morbidity.

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List of Publications

Peer reviewed abstracts

Itobi E, Stroud M, Elia M. Oedema and gastrointestinal function following abdominal surgery. *Clinical Nutrition* 2004; 23: 867-868.

Itobi E, Elia M, Stroud M. Generalized oedema following abdominal surgery: Fluid overload or poor output? *Clinical Nutrition* 2004; 23: 850-851

Itobi E, Stroud M, Elia M. Value of multifrequency bioelectrical impedance analysis in assessing fluid disturbances in patients undergoing major abdominal surgery. *Clinical Nutrition* 2004; 23: 849-850.

E. Itobi, M. Stroud, M. Elia. Can pre-operative bioelectric impedance predict oedema development after major abdominal surgery? *International Journal Body Composition Research* 2005; 3(3): 122.

Original paper

Itobi E, Stroud M, Elia M. Recovery after major abdominal surgery in oedematous and non-oedematous patients and the potential value of multifrequency bioimpedance measurements. *British Journal of Surgery* 2006; 93(3): 354-361

Author's Letter

M. Elia, M. Stroud, E. Itobi. Authors' reply: Impact of oedema on recovery after major abdominal surgery and potential value of multifrequency bioimpedance measurements (*Br J Surg* 2006; 93(3): 354-361) *British Journal of Surgery* 2006; 93(6): 770.

Declaration of authorship

I, Mr Emmanuel Onome Itobi hereby declare that the thesis entitled

THE IMPACT OF POST OPERATIVE OEDEMA ON CLINICAL RECOVERY AND ITS POTENTIAL CAUSES

and the work presented in the thesis are both my own and have been generated by me as a result of my own original research. I confirm that this work was done wholly while in candidature for a research degree at this University.

I was responsible for obtaining ethical approval, the recruitment and assessment of patients, obtaining and preparation of blood samples prior to analysis, the collection of clinical data and the presentation of the findings of this research project.

The statistical analysis of all data obtained during this project was done with the help of Professor M. Elia.

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Abbreviations

ANOVA	Analysis of variance
AAP	Acute phase proteins
APR	Acute phase reactants
ARDS	Adult respiratory distress syndrome
ASA	American Society of Anaesthesiologists
BIA	Bioelectrical impedance analysis
c-AMP	3' 5' Cyclic adenosine monophosphate
ECW	Extracellular water
DVT	Deep vein thrombosis
GSHPX	Glutathione peroxidase
GSH	Glutathione (reduced state)
GSSG	Oxidised glutathione
ICW	Intracellular water
IL	Interleukins
KHz	Kilohertz
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
MAS	Major abdominal surgery
MODS	Multiple organ dysfunction syndrome
OH[·]	Hydroxyl radical
SPANOVA	Split plot analysis of variance
SOD	Superoxide dismutase
TBW	Total body water
TNF-α	Tumour necrosis factor-alpha

Chapter 1

Introduction and outline of thesis

Generalised oedema occurring in central or peripheral regions of the body is a commonly observed clinical finding in patients who have undergone major abdominal surgery.

Oedema is also often reported in patients who are malnourished (Shizgal 1981b) and in those with septic complications following major surgery (Lobo et al. 1999). However, in the absence of obvious cardiac and renal impairment the incidence of this finding is unknown and its impact on clinical outcomes such as the recovery of gastrointestinal function, duration of hospital stay and perioperative morbidity in surgical patients is not certain.

In order to examine the relationship between generalised peripheral oedema in the early postoperative period and clinical outcomes, it is important to establish the frequency of this finding in patients undergoing routine abdominal surgical procedures. Oedema can often be easily demonstrated on clinical examination when pitting is present, usually around the ankles, but objective quantification is difficult and may be impracticable as it also occurs in areas of the body that are not easily assessed. Bioelectrical impedance analysis (BIA) is a simple non-invasive technique that utilizes the ability of human tissue to conduct small amounts of electric current. BIA has been employed in the evaluation of extracellular and total body water in post-surgical patients (Hoffer et al. 1969; Scheltinga et al. 1992). The estimation of body fluid compartments by this method has been correlated with other more complex techniques utilised for the estimation of body composition (Hannan et al. 1994). Examining the changes in whole body impedance in patients before and after surgery and comparing these in patients who develop oedema

with those who do not should enable evaluation of changes in cell membrane properties that are related to surgery.

The aetiological factors and the pathophysiologic mechanisms underlying oedema formation in various disease states remain poorly understood. Hypoalbuminaemia and hypoproteinaemia are common findings in oedematous states and it has been traditionally believed that these are the primary determinants of oedema formation in the post-surgical patients (Moss 1967). However it is also known that low albumin *per se* does not lead to oedema formation as evidenced by the absence of oedema in the rare individuals with congenital deficiency of albumin (Bearn & Letwin 1978). Hypoalbuminaemia and oedema are also common features of some forms of severe protein-energy malnutrition (Cohen & Hansen 1962; Picou & Waterlow 1962). It has been argued that when oedema complicates malnutrition, it represents a failure of the adaptive mechanisms to nutrient deprivation rather than a consequence of low albumin *per se* (Jackson 1990; Gopalan 1968). Some studies suggest that the occurrence of oedema in malnourished states is often precipitated by a stressful event such as infection (Frood et al. 1971), surgical complications or injury (Lobo et al. 1999).

Golden and Ramdath (1987) suggested from their studies on patients with oedematous malnutrition, that nutrient deprivation impairs the ability to maintain normal levels of critical antioxidants, which renders the individual susceptible to free radical induced cell membrane damage. They highlighted this as the potential mechanism responsible for oedema formation observed in malnourished states and were able to show significantly lower levels of reduced glutathione (GSH) in the red cells of individuals with oedematous malnutrition compared to malnourished individuals without oedema (Golden & Ramdath 1987). Furthermore, advancements in the field of molecular biology have allowed more light to be shed on role of mediators such as oxygen derived free radicals whose interactions with the vascular endothelial surface lead to increase permeability

allowing the loss of fluid from the vascular compartment to the interstitial space (Korthuis et al. 1985; Perry & Fantini 1987). It is therefore, possible that the stress of major abdominal surgery in vulnerable individuals may lead to free radical induced disruption of the normal regulatory mechanisms that control the movement of water and electrolytes resulting in a similar picture to that observed in protein-energy deficiency states.

Some aspects of the current methods of caring for postoperative patients, especially the tendency to administer large volumes of fluids during and after major abdominal surgery may also be a potential contributor to early postoperative oedema formation. Large volumes of crystalloids are often administered in the perioperative period in an attempt to stabilise blood pressure and maintain good urine output. These end points are often attained at the expense of tissue oedema. Some studies have drawn attention to the potential negative consequences of the resulting oedema following aggressive fluid administration on the function of major organs such as the myocardium and gastrointestinal tract (Weinstein & Doerfler 1992; Lobo et al. 1999).

Most importantly the relationship between postoperative oedema and clinical outcomes has not been established. Although oedema has been observed in surgical patients with septic complications the effect of oedema on clinical outcomes does not appear to have been studied.

The purpose of the studies reported in this thesis was to examine the inter relationship between the presence of oedema in post surgical patients and clinical outcomes and the potential causes of oedema such hypoalbuminaemia, antioxidant status, perioperative fluid administration and inflammatory response to surgical trauma. Within this framework, the specific value of BIA was explored as a marker of fluid shifts within the body compartments. An outline of this thesis is shown in *Figure 1.1*.

Outline of thesis

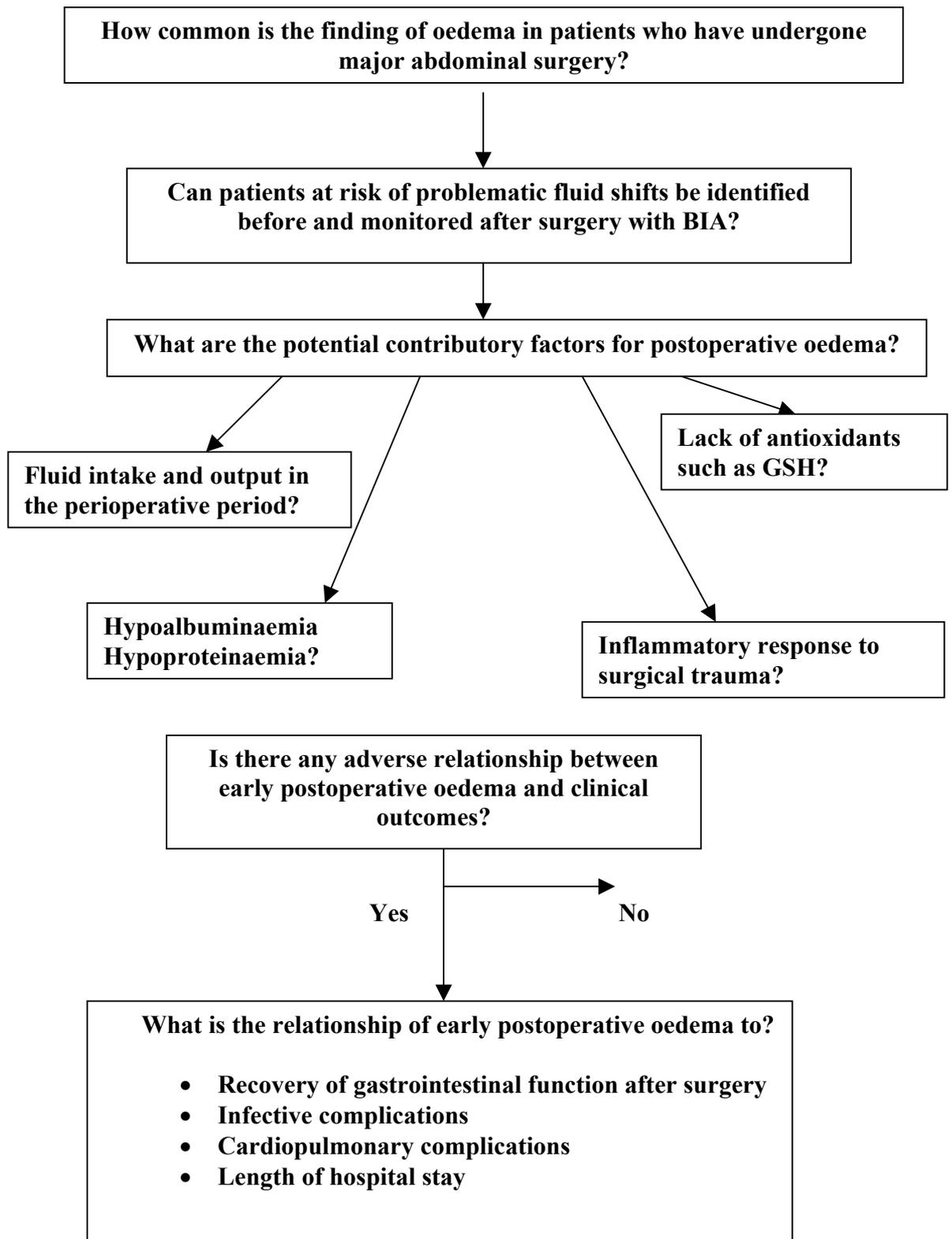


Figure 1.1

Chapter 2

Literature review

This review of literature will discuss what is currently known about the pathophysiology of oedema highlighting mechanisms that are of relevance to the surgical patient. It will also discuss the principles and applications of bioelectrical impedance and how these can be utilized in the assessment of oedema in surgical patients. The roles of the acute phase response and oxidative stress in surgery will be highlighted and the review will be concluded by discussing how oedema may affect clinical outcomes in the surgical patient.

Oedema is commonly associated with critically ill patients requiring significant amounts of supportive treatment. It is also observed in ward-based, post-surgical patients who are not critically ill, and who have no clinical evidence of organ failure. It is a clinical sign that has been well recognised since the earliest recordings of medical history. Celsus wrote, sometime in 25-50 AD about a chronic malady that may develop in patients who collect water under their skin that the Greeks called “hydrops” (Spencer WG 1938). Despite the benefit of time and advances in physiology our understanding of the causes of oedema is far from complete. In order to study the aetiology of oedema and its relationship to clinical outcomes, a review of the pathophysiologic basis of oedema formation under the following headings is valuable.

- The physiology of oedema formation
- Fluid homeostasis after surgery
- Decreased capillary oncotic pressure after surgery
- Increased vascular permeability after surgery
- Systemic effects of perioperative intravenous fluid therapy

2.1.1 The Physiology of oedema formation

Total body water is distributed between the intracellular and extracellular compartments with extracellular water making up a third of total body water. The extracellular space is comprised of the intravascular volume (25%) and interstitial space (75%) (Braunwald 1994; Guyton & Hall 2000).

Starling described the physiological forces thought to be involved in the production of oedema more than a century ago (Starling 1896). These factors include the differential gradient in hydrostatic pressure between the capillaries and the interstitial space, the difference in oncotic pressures of the capillary blood and interstitial space and the capillary permeability a property that varies from organ to organ (Webb 2000). The flow of fluid across the capillary wall and interstitial space is related by the equation given below (Michel 1997):

$$F_m = K_f ((P_c - P_i) - \sigma (\pi_c - \pi_i))$$

Where F_m = flow of fluid across capillary wall, K_f = capillary filtration coefficient, P_c = capillary hydrostatic pressure, P_i = interstitial hydrostatic pressure, σ = reflection coefficient, π_c = capillary colloid oncotic pressure, π_i interstitial colloid oncotic pressure. The difference in hydrostatic pressures at the arteriolar and venous ends of the capillary allows a net loss of fluid at the arteriolar end and a net gain at the venous end. However, not all filtered fluid is returned to the capillary. The lymphatic system performs the essential task of returning excess filtered fluid and protein molecules from the interstitial space to the vascular compartment via the thoracic duct. Any perturbation of this delicate homeostatic mechanism will lead to oedema formation

The interstitial space has the ability to accommodate a vast amount of fluid and it has been estimated that a quantity of fluid amounting to 10% of the patients body weight may be accumulated before pitting oedema is evident (Cho & Atwood 2002). Diskin and others have suggested that up to 4.5kg of excess water may be accumulated without

oedema being physically obvious by pitting (Diskin et al. 1999). In post-surgical patients this implies that a significant amount of fluid may be accumulated prior to clinical detection.

2.1.2 Fluid homeostasis after surgery

It has long been evident that the period following major abdominal surgery is characterized by haemodynamic and metabolic instability initiated by the body's physiological response to trauma. The loss of circulating blood volume and nociceptive stimuli trigger off a neurohumoral cascade. The neurotransmitters released by this cascade cause renal vasoconstriction and reduce glomerular filtration. There is also an increase in the activity of endocrine organs such as pituitary and adrenal glands leading to an increase in the circulating levels of hormones such as catecholamines, cortisol, aldosterone and antidiuretic hormone (ADH) (Moore & Brennan 1975). These hormones increase the reabsorption of sodium and water by the nephrons. The reabsorption of sodium in the proximal tubules of the nephron is mediated by angiotensin II and norepinephrine. Sodium and water reabsorption also increases in the collecting tubules where it is mediated by aldosterone and antidiuretic hormone (Rose 1994).

More recently it has also been shown that endothelium derived factors such as nitric oxide and prostaglandins also play an important role in this complex regulatory mechanism by limiting sodium and water excretion. (Townsend et al. 1995; Martin & Schrier 1997). The net effect of the interactions of these neurotransmitters is an increase in the tissue content of water, sodium and chloride accompanied by a reduction in potassium following surgical trauma (Moore 1959; Bergstrom et al. 1981).

2.1.3 Decreased capillary oncotic pressure after surgery

A significant amount of protein loss occurs during major abdominal surgery. This loss occurs mainly as plasma volume and its constituent proteins leading to a reduction in the effective extracellular fluid volume and the oncotic pressure of the extracellular space. Hoye and colleagues estimated that an average loss of 24% plasma volume and 33% of the initial total circulating protein occur during a major abdominal surgery such as radical hysterectomy. The volume of plasma lost during major abdominal surgery is often undervalued when estimates are calculated from the volume of blood loss (Hoye et al. 1972).

The loss of proteins lead to a reduction of osmotically active particles such as albumin which in health can provide up to 80% of colloid osmotic pressure (Blunt et al. 1998). The loss of albumin that occurs after major surgery is dependent on the degree of blood loss and severity of surgery and is estimated to range from 27-29% in metabolically stable patients (Hoye et al. 1970).

In healthy individuals, there is a constant trans-capillary membrane escape of albumin that has been estimated to be approximately 6 gram per hour or 4-5% of the intravascular albumin mass. This trans-capillary loss has been shown to increase as early as 6 hours following minor trauma (Myers et al. 1984), while it may rise by up to 100% in patients undergoing cardiac surgery and 300% in patients with septic shock (Fleck et al. 1985). This increased membrane permeability allows further loss of albumin as it escapes into the interstitial space, the gastrointestinal tract, areas surrounding traumatised tissue, and body fluids such as urine. The sequestration of albumin in the gastrointestinal tract, traumatised tissues and the body's excretory fluids can be likened to sequestration of albumin into a non-circulating or non-exchangeable pool (Hoye et al. 1972).

A significant fall in albumin and total concentration also occur during periods of anaesthesia alone and this has been suggested to due effect of surgical stress (Carli & Elia 1991). Further loss of albumin can occur from changes in the synthesis and catabolism of

albumin during illness although this may not happen acutely (Blunt et al. 1998). In animal studies synthesis of albumin has been shown to decrease during injury and inflammation (Ballantyne et al. 1973; Milland et al. 1990) an action mediated by TNF-alpha and interleukins 6 (IL-6) both of which depress albumin gene transcription. However, in human studies the situation is more complicated. In patients with septic shock the synthesis of albumin can vary enormously, from very decreased to significantly increased (Fleck et al. 1984). Albumin catabolism is increased during the stress response augmented by raised levels of corticosteroids, which also increases the catabolism of other proteins. However, the absolute fractional degradation rate of albumin may be so reduced in the presence of low albumin concentrations to a rate that is seen during normal conditions (Whicher et al. 1987)

Albumin is the most abundant plasma protein representing about 60 – 80% of all circulating protein. However only 40% of albumin is in circulating volume. In the healthy state 60% of albumin is in reserve in the interstitial space from where it can translocate to the circulating volume to correct an acute drop in circulating total protein (Kaminski & Hasse 1992). The amount of albumin that can be mobilized from this space is however limited. Secondly the combination of a fall in capillary hydrostatic pressure and an increase in the interstitial colloid osmotic pressure attenuates the increase of the interstitial fluid flow (Fadnes 1975; Kramer et al. 1982)

The net effect of the massive loss of protein via the various channels mentioned above is a reduction in colloid oncotic pressure, the capillary oncotic gradient and an increase in the potential for accumulation of fluid in the interstitial space. It remains unknown however what levels of protein loss eventually culminate in oedema formation and the significance of protein loss and its interaction with other potential mechanisms is unclear.

The levels of circulating plasma proteins are often used as markers of underlying malnutrition (Seltzer et al. 1979) and as indicators of severity of illness either by using the concentration of plasma proteins alone or in combination with other indexes. The latter use of albumin suggests that low levels of albumin may reflect severity of illness rather than oncotic pressure (Chwals et al. 1992; Buzby et al. 1980 and Gunel et al. 1998). This view is supported by absence of oedema in the rare individuals with congenital deficiency of albumin (Bearn & Letwin 1978) and the finding that administering large amounts of albumin in the early post operative period does not prevent the occurrence of peripheral oedema (Zetterstrom & Hedstrand 1981)

2.1.4 Increased vascular permeability after surgery

The vascular endothelium normally serves as a barrier that minimizes the movement of fluid and proteins from the capillary to the interstitial space. Loss of the function of this barrier from any insult allows the escape of fluid and protein to the interstitial space and consequent oedema formation (Michel & Curry 1999). Our understanding of the alteration to the structure and function of the vascular endothelium triggered by inflammatory response to cellular injury has significantly improved in the last two decades. It would appear that the interaction between activated neutrophils and vascular endothelium is particularly significant in the changes that occur in membrane function following injury.

Neutrophils can be activated by a variety of stimuli that include tissue injury, hypoxia, sepsis or ischaemia (Fujishima & Aikawa 1995). The activation of neutrophils is also mediated by the following inflammatory agents - activated complements (C3a, C5a), cytokines such as interleukins (IL1, IL6 and IL8) and tumour necrosis factor (TNF). Other known potent mediators of neutrophil activation are bacterial endotoxin, leukotriene B4 and platelet activating factor (Dahlen et al. 1981). Cell surface receptors and glycoproteins

are expressed by the activated neutrophils that promote their adhesion to other cells especially vascular endothelium (Cioffi et al. 1993). These glycoproteins expressed by activated neutrophils, endothelial cells and platelets are believed to govern the “slow rolling” phase of leukocyte- endothelial adhesion (Harlam & Winn 2002).

Following activation, neutrophils release proteases and oxygen derived free radicals, which are toxic to surrounding cells and cause further cellular damage through peroxidation of the lipid constituents of cell membranes (Bulkley 1983). Evidence for this mechanism is suggested by the increased serum concentrations of lipofuscin and malondialdehyde that have been observed in patients suffering from trauma both of which are by-products of free radical mediated lipid peroxidation (Roumen et al. 1994; Girotti et al. 1991). Alterations in the structural matrix of the capillary membrane result in an increased distance between endothelial cells and the provision of a route for protein leak (Ley & Arfors 1982). Furthermore neutrophils can also migrate into the interstitial space through such openings where, depending on their gene expression, they may die, accumulate or release further inflammatory mediators (Aird 2002). The combination of these events, cellular damage, neutrophil and endothelial cell activation and interaction following major trauma may lead to the initiation of a systemic immune response, which propagates further cellular destruction. This process is thought to be fundamental to post-trauma complications such as adult respiratory distress syndrome (ARDS), which has as its hallmark loss of membrane function and increased permeability of the respiratory membrane (Petty 1982).

Man is particularly susceptible to the deleterious effects of this inflammatory response as human neutrophils are capable of generating far higher concentrations of free-radicals in response to inflammatory stimuli compared to other mammals (Young & Beswick 1986). Although the production of free radicals by neutrophils following activation is a host defence mechanism that facilitates the destruction of potentially

harmful organisms, cellular damage may occur at sites remote from the injury. Over the course of evolution cells have adapted by developing free radical scavenging pathways that provide cellular protection from such free radical induced injury. These pathways involve enzymes and antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, tocopherols, ascorbic acid and reduced glutathione (GSH). The protection provided by such antioxidants to free radical mediated tissue damage is the theoretical basis for experimental treatment of patients with exaggerated inflammatory response patterns following major trauma or burns, with agents that enhance antioxidant defences. So far, these experimental treatments have not resulted in successful clinical therapy, but it remains an exciting area of research (Fujishima & Aikawa 1995; Marzi et al. 1993).

2.1.5 Systemic effects of perioperative intravenous fluid therapy

Fluid administration and resuscitation are an integral part of the management of surgical patients during the perioperative period. It is not uncommon to administer large amounts of intravenous fluids during such periods. Although the choice between crystalloids and colloids continues to be a topic for debate, the complications of this intervention have received little attention. The skin has been reported to be the most susceptible tissue to developing oedema in patients receiving large volumes of crystalloids (Pappova et al. 1977). This oedema was reversed in experimental animals by administration of colloids giving rise to the “push/pull” theories of oedema formation within soft tissues.

Intravenous administration of saline has also been shown to lead to a persistent rise in the flow of lymph from the soft tissues long after restoration of osmotic gradient to normal. It has been suggested that a change occurs in the underlying matrix of the soft tissue that allows fluid movement in only one direction. The persistence of this flux has been used to explain why clinically apparent oedema requires prolonged periods to resolve (Harms et al. 1981; Gump et al. 1970).

It has been well known for several decades that the body's ability to excrete excess salt and water is impaired during surgical illness (Wilkinson et al. 1949). This is mainly due to adrenocortical hormone activation as part of the neurohumeral response to trauma. The role of cytokines has also been implicated in this process (Moore et al. 1994; Rem et al. 1980). The net effect of all the above processes is a general tendency for post surgical patients to accumulate salt and water. The administration of excessive volumes of fluids to such patients increases the risk of developing oedema and hypoalbuminaemia (Gil et al. 1997).

Tindall and his colleagues examined the effect of administering saline containing fluids to patient recovering from abdominal surgery and they were able to demonstrate that saline containing fluids further delayed the onset of diuresis during the postoperative recovery period. Marked salt and water retention were observed in the saline-receiving patients compared to patients administered dextrose infusions (Tindall & Clark 1981). Such patients successfully lose weight and develop a negative fluid balance when their sodium and water intake are restricted (Lobo et al. 1999).

Although the deleterious effects of fluid overload in critically ill post-surgical patients has been documented in several studies, this relationship in routine ward-based patients does not appear to have been fully explored (Alsous et al. 2000; Lowell et al. 1990). The oedema resulting from crystalloid administration may lessen or negate the benefits of increased oxygen delivery. Care needs to be taken in interpreting any alteration in organ function with respect to the fluid type and volume being administered. Abnormalities of fluid and electrolyte balance may adversely affect organ function and surgical outcome when perioperative fluid therapy is not targeted at the needs of the patient (Walsh et al. 2008). It is possible that oedema formation is one of the mechanisms through which the deleterious effect of fluid overload is produced.

2.2 Bioelectrical Impedance Analysis (BIA) in the evaluation of oedema

Although inspection and palpation are sufficient to identify the presence of oedema, the objective quantification of it is not precise. Traditionally, the severity of oedema has been graded on a four-point scale, which starts from slight to very marked (Bate 1991).

However, in clinical practice, oedema may occur in areas of the body that are often inaccessible to the clinician. There is strong evidence from recent studies that exaggerated increase in the extracellular water compartment following surgical trauma is associated with a trend towards increased complication rates (Lowell et al. 1990; Starker et al. 1983). The monitoring of body composition during illness would therefore be a valuable tool that would potentially identify patients at risk of complications, and help in assessing their response to intervention.

2.2.1 Clinical and historical background

A plethora of techniques have been utilised in the analysis of body composition during health and various disease states. They vary from simple methods that employ anthropometry to methods that use very complex instruments such as in vivo neutron activation analysis. In the clinical situation, the use of anthropometric parameters such as skin fold thickness and mid arm muscle circumference to estimate body fat and protein reserve lack precision and in the presence of oedema and critical illness are prone to misclassification (Collins 1982). The accurate estimation of body cell mass, fat and water content requires sophisticated and expensive laboratory techniques that are often impracticable in a hospital setting.

The electrical properties of tissues were recognised more than a century and half ago (Herman 1871) but it was not until four decades ago that studies were undertaken that related bioelectrical impedance measurements to total body water (TBW) (Thomasset

1963). BIA allows a simple, non-invasive method of assessing total body water and its distribution between the fluid compartments in the body. In healthy individuals it can also be used to estimate the lean body and fat mass (Lukaski et al. 1985).

2.2.2 Principles of bioelectrical impedance analysis

BIA is based on the ability of the fat-free mass to conduct small amounts of electrical current. Since it is the water containing electrolytes that conducts electrical current, it follows that the resistance of tissues to an applied current is directly related to their fluid content. Well-hydrated fat-free mass is a good conductor whilst poorly hydrated adipose tissue is a good insulator. The ability of tissues to oppose current is called impedance and it is this electrical property that is measured by BIA. Whole-body bioelectrical impedance is the impedance obtained to the flow of a small amount of electrical current applied to the whole body. Impedance is written as Z (Ohms, Ω) and its components are resistance (R , Ω), the largest contributor to impedance (approximately 98%) and reactance or the capacitive resistance (X_c , Ω) (contributing approximately 2%) (Scheltinga et al. 1992). Resistance (R) arises from the extra- and intracellular fluid and capacitance is derived from the anatomical arrangement of cell membranes. The resistance (R) of a homogenous conductor of uniform cross-sectional area is proportional to its length (L) and inversely proportional to its cross-sectional area (A). The human body is however not a uniform conductor. Conductivity and the degree of tissue hydration are variable across segments of the body. Despite this limitation, an empirical relationship can be derived between impedance quotient (Length^2/Z) and the volume of water containing electrolytes in the body that conducts electrical current otherwise known as lean mass. In clinical practice it is far easier to measure height than measuring the conductive length from the wrist to the ankle. The impedance index (height^2/Z) can be related to lean mass, which contains about 73% water (Hoffer et al. 1969).

The lack of field homogeneity in the body implies that the term height^2/Z refers to a theoretical cylinder, which must be matched in real geometry by appropriate coefficients. These coefficients depend on various factors such as weight, anthropometry and sex and arise from the variability in the body segments and the non-linearity in conduction arising from mixture effects (i.e. conductor to non-conductor interactions and variations in the height to conductive length) and constitute potential sources of errors.

Several electrical models have been used to characterize the behaviour of biological circuits in vivo. Some of them are simple whilst others are quite complex. One of the models commonly used is one in which R due to extracellular fluid is arranged in parallel to a second arm containing capacitance and R of the intracellular fluid in series. Another model takes into account the effect of “mixing”. This model is based on a theory that the R of conductive fluids increases as the amount of the non-conducting material increases. Explained in a different way, the conductive path taken by the current as it curves around the non-conducting particles increases. In vivo, the non-conducting particles are represented by cells. Hanai devised a formula based on this model by studying the electrical property of emulsions (the mixture theory) and his formula has been extrapolated for use in humans (Hanai 1968).

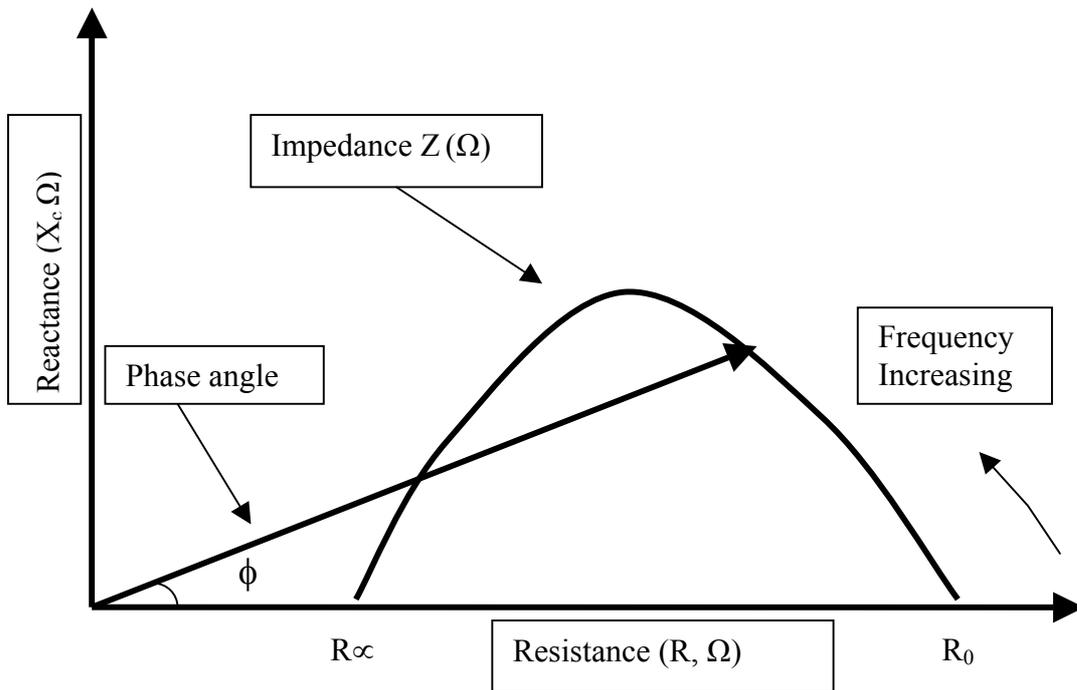
Multifrequency BIA (MF- BIA) uses linear regression model of impedance at several frequencies. Since 1969, the conventional method used for predicting water volume with impedance has used equations formed by regressing $\text{height}^2/\text{impedance}$ against a known dilution volume, where height is an index of conductive length (Hoffer et al. 1969). Apart from the geometrical assumptions such equations also assume uniform conductivity in the body hence the variability in several equations. Furthermore the early BIA equations were validated in inadequate populations (usually healthy people), which, led to large variations in the results obtained when applied to patients with diseases and the many formulae published in medical literature. This made the clinical interpretation

often very difficult. To overcome this, it has been recommended that the use of general predictive equations across populations with different age and ethnicity should be avoided (Kushner 1992).

At a frequency of zero or frequencies close to zero, current is unable to penetrate the cell membrane, which acts as an insulator. The current therefore only passes through the extracellular water (ECW), which is responsible for the measured value of R (or R_0). At a frequency of infinity or very high frequencies the cell membrane is penetrated and the total R (or R_∞) is provided by both intracellular water (ICW) and extracellular water (ECW), which make up TBW. In reality, multiple dispersions prevent the use of direct current (zero frequency) or very high frequency alternating current, so it is impracticable to obtain reliable impedance measurement at frequencies below 5KHz and frequencies above 1MHz. However, the value of R at the ideal frequencies can be obtained by plotting the reactance versus R at various frequencies, which gives a curve fitting trace (Cole-Cole plot) (Cole 1941). With the aid of this plot, R_0 and R_∞ , which represent the ideal resistances for calculating ECW and TBW respectively, can be obtained *Figure 2.1*. The use of this method is called bio-impedance spectroscopy. There are several commercially available BIA analysers capable of measuring R and capacitance over a wide range of frequencies.

Whole-body impedance is measured classically by means of four surface electrodes. The emitting electrodes are typically placed on the dorsum of the right hand and right foot just proximal to the metacarpophalangeal and metatarsophalangeal joints whilst the detecting electrodes are placed on the right wrist (between the radius and ulnar) and right ankle (between the medial and lateral malleoli) on the right side of the body. BIA instruments are portable and can be easily used at the bedside. The results are rapidly obtained and are reproducible.

Fig 2.1 The Cole-Cole plot



Cole-Cole plot and graphical derivation of the phase angle; its relationship to resistance (R (Ω)) on x axis, reactance (X_c (Ω)) plotted on y-axis as $-X_c$ (Ω), impedance Z and the frequency of the applied current.

Several commercial devices are available that measure bioelectrical impedance.

They range from devices that measure impedance at a single frequency to multi-frequency analysers. It is also possible to measure the contribution to whole body impedance from different segments of the body such as the arms, trunk and lower limbs. It is argued by some that segmental impedance overcomes some of the errors that arise as a result of the variation in the shape of the body segments and underestimation of the contribution to whole body impedance from the trunk (Tatara & Tsuzaki 1998).

2.2.3 Clinical applications of Bioelectrical Impedance Analysis

The electrical properties of tissues are affected in various ways by disease, nutritional state and hydration and such changes are reflected in the changes of the relationship between

capacitance (X_c) and resistance (R). A variety of indices have been derived to assess the changes in the electrical properties of tissue membranes. Examples of such indices include the phase angle (ϕ) and the ratio R_0/R_∞ *Figure 2.1*. It has been suggested that these indices have the potential to predict outcomes in certain disease states (Schwenk et al. 1998; Schwenk et al. 2000). Furthermore, it has been shown that when X_c and R are plotted graphically (after standardising for height) different diseases tend to form distinct clusters (Piccoli et al. 2000). Scheltinga and colleagues also showed how BIA might be utilised to monitor changes in cell membrane properties during critical illness. They demonstrated that there was a significant difference in cell membrane capacitance (X_c) in critically ill patients compared with controls. The change in X_c was also strongly correlated with extracellular water volume (Scheltinga et al. 1990). These findings suggest that different diseases may produce recognisable changes in cell membrane properties that can be identified with BIA and it is possible that monitoring such changes is potentially valuable with respect to diagnosis and prognosis.

The assessment of oedema following surgical procedures is not routinely undertaken in clinical practice but studies have shown that when BIA was employed in the assessment of lower limb oedema following vascular surgery, the change in impedance was more specific of oedema related to tissue injury compared to simple measurement of changes in limb volume obtained by other non invasive techniques (Belanger et al. 1998).

Patients with trauma and sepsis retain fluid in response to therapeutic interventions such as nutritional support and gain weight as a result of the expansion of the extracellular space. Such weight gain may be misinterpreted as an improvement in protein stores (Hill et al. 1991). Post trauma patients who exhibit an increase in extracellular water following nutritional intervention tend to have increased postoperative complication rates compared to patients who lose water (Starker et al. 1983; Starker et al. 1986). The monitoring of the response of surgical patients to therapeutic intervention such as intravenous fluids and

nutritional support remains difficult to achieve in clinical practice. The observation of changes in weight in the postoperative period provides limited information on fluid distribution, which can vary significantly depending on the type of surgery. Such changes are not reflected by postoperative fluid balance measurements but can be monitored in surgical patients by BIA (Carlson et al. 1994). BIA eliminates the need for the use of relatively complex investigations and provides this information at the bedside. It might therefore identify patients who retain fluids in the extracellular space following surgery.

The ratio of the total exchangeable sodium (Na_e) to the total exchangeable potassium (K_e) is a measure of the extracellular mass expressed as a function of the body cell mass (Shizgal 1981b). Forse and Shizgal have shown that this ratio is a sensitive index of identifying dangerous levels of expansion of the extracellular mass in post surgical patients with septic complications (Forse & Shizgal 1980). This ratio was also shown to be the best method of predicting the risk of dying compared with anthropometric, biochemical and immunological indexes when applied to a group of septic and malnourished surgical patients (Tellado et al. 1989). It has been suggested that similar information may be obtained from the ratio of extracellular water to total body water ECW/TBW (Hannan et al. 1994). Shizgal studied a group of malnourished and critically ill patients and was able to demonstrate that the loss of body cell mass is reflected by a decrease in exchangeable potassium per total body weight and a decrease in the intracellular water per total body weight. This is regarded as a crude measure of the body cell mass. The expansion of the extracellular mass was also reflected by an increase in both the exchangeable sodium per total body weight and extracellular water per total body weight (Shizgal 1981a).

2.3 The acute phase response and surgery

The trauma of surgery induces a variety of host defence responses, which include a change in the concentration of a group of plasma proteins collectively known as the acute phase reactants (APRs) or acute phase proteins (APPs). The changes in the concentration of the APPs occur as part of a common inflammatory response to physical or chemical injury, the presence of endotoxins (Buttenschoen et al. 2001), bacterial, viral or parasitic infection, or neoplastic growth (Kushner 1982). The bulk of the APPs are hepatic in origin but production at extrahepatic sites has been identified. The stimulus for the transcription of acute-phase protein (APP) genes in the liver is brought about by the complex interaction of cytokines, growth factors and glucocorticoid hormones that are released during the systemic defence reaction in response to trauma. The cytokines that have been implicated in this process are multiple. Potent cytokines whose roles have been extensively studied include TNF-alpha, interleukin-6 (IL-6) and interleukin-10 (IL10). APP synthesis may be regarded as a non-specific response of the liver, in so much as different types of trauma elicit the production of the same proteins (Pullicino et al. 1990).

The APPs that increase their concentration during the acute phase response are known as positive APPs. Some proteins like complement C3 and ceruloplasmin are increased by two to threefold, others like fibrinogen and hepatoglobulin rise up tenfold, whilst spectacular APPs like serum amyloid A (SAA), rat α_2 macroglobulin (α_2 M) and CRP can be elevated by several hundredfold within 1-3 days of the acute phase response (Fey & Fuller 1987). Proteins with transiently decreased plasma concentrations are known as negative APPs. Well known examples are serum albumin, α_{2u} -globulin, rat α_1 -inhibitor III (α_1 I3), transthyretin (TTR) and vitamin D-binding globulin (Lonberg-Holm et al. 1987; Northemann et al. 1989). APPs return to their original levels at the end of the acute phase response, typically after 3 to 5 days. Their levels may remain persistently high in the presence of chronic inflammation or continued tissue irritation (Fischer et al. 1976;

Colley et al. 1983).

The changes in the serum concentrations of these proteins have been shown to be useful in monitoring complications such as infection or sepsis after surgery or trauma, and predicting the clinical course of malignant and other diseases.

CRP is a prototype human APR and was discovered more than six decades ago (Macleod & Avery 1941). CRP has been demonstrated to be a useful clinical marker to monitor inflammation and the recurrence of neoplastic growth (Fujita et al. 1999). That CRP is remarkably well conserved in different animal species suggests its role is critical during inflammatory response to injury. Reports from recent studies have shown that CRP can opsonize bacteria, parasites, foreign particles and immune complexes and facilitate their clearance by phagocytic cells. CRP has also been shown to be capable of binding to chromatin and rendering it soluble (Robey et al. 1984; Volanakis 1982). This role has led to suggestions that CRP has critical a role as a modulator of the immune response which may be mediated by CRP acting as a scavenger and facilitating the clearance factor of chromatin fragments released from damaged cells during inflammation stress (Robey et al. 1985). It is thus possible that during inflammation that APPs typified by CRP may represent a non-specific first line of defence that is effective against a variety of pathological processes and has to its advantage the ability of rapid inducibility to enable it provide increased protection immediately after the insult or injury (Yother et al. 1982). The broad spectrum of the activities of this heterogeneous group of proteins assists the injured organism in restoring homeostasis by assuming a protective role. APPs accomplish this by inactivating vasoactive, proteolytic and cytotoxic molecules liberated from damaged tissues and accumulating phagocytic cells, and by participating in a feedback control mechanism that prevents overload of the organisms' immune response.

2.4 Oxidative stress and surgery

The potential role of oxidative stress in surgical patients was highlighted by Del Maestro more than two decades ago when he attempted to associate disease patterns and site of production of free radicals (Del Maestro 1980). Free radicals are chemical species that have a single electron in their outer orbit. They are extremely reactive and unstable and are capable of reacting with organic and inorganic molecules. Such organic molecules can be protein, carbohydrate and lipid components of cell membranes. Free radicals can produce widespread derangement in cellular metabolism by raising the intracellular concentration of Ca^+ , direct attack on nucleic acids of DNA leading to eventual fragmentation and direct attack on cell membranes and their intrinsic ion transporters (Halliwell, Gutteridge, & Cross 1992).

An inability to dispose free radicals has been suggested to be a significant aetiological factor for oedema formation when oedema complicates protein-energy deficient states (Golden & Ramdath, 1987). More recently the interaction of oxygen derived free radicals and vascular endothelium has also been shown to increase vascular permeability.

2.4.1 Origin of free radicals

In biologic systems oxygen derived free radicals are generated from the tetravalent reduction of molecular oxygen to form water. This process occurs in the cytochrome oxidase complex. This efficient process is called aerobic glycolysis and is utilised by most cells to generate energy in the form of adenosine triphosphate (Gregory & Bulkley 1983). However, the univalent reduction of molecular oxygen that may occur along this pathway generates reactive species such as the superoxide ($\bar{\text{O}}_2^{\cdot}$) radical, hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^{\cdot}), *Figure 2.2*. The rate of leak of free radicals from this system has been estimated to be about 1%-2% (McCord 1983).

Free radicals are also generated from the activities of phagocytic cells especially during an inflammatory process. The phagocytic cells depend on a “burst” of free radical generation within their lysosomes to produce lysis of bacterial cells. The subsequent release of lysosomal enzymes further promotes cellular damage as it leads to the release of more free radicals and potent hydrolytic enzymes *Figure 2.3*.

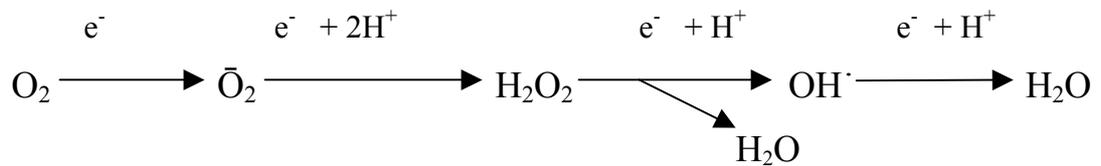
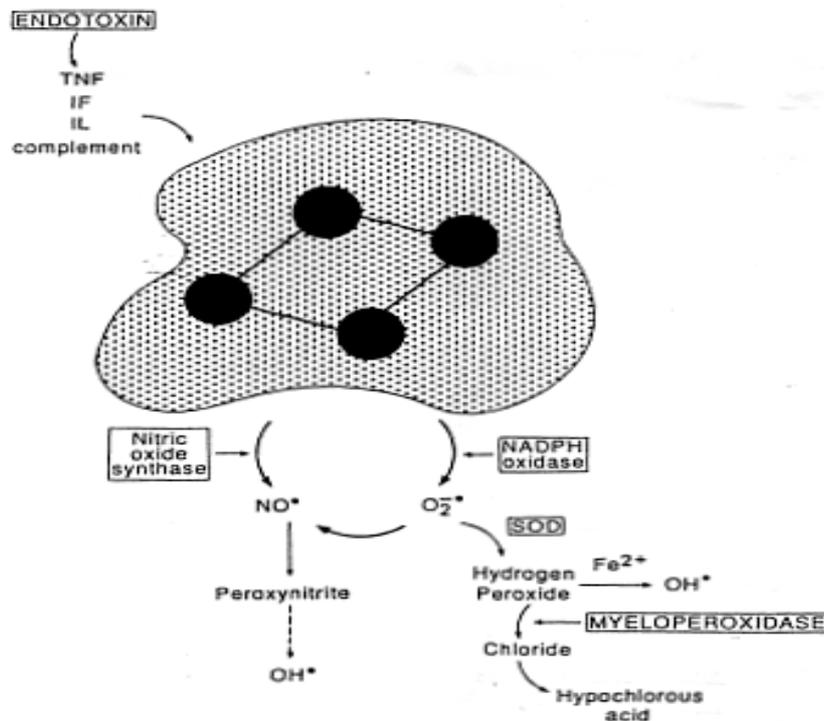


Fig 2.2 Univalent pathway for the reduction of molecular oxygen.

By a series of single-electron transfers, molecular oxygen is first reduced first to the superoxide free radical and from superoxide (\bar{O}_2^\cdot), with the addition of two electrons to, hydrogen peroxide (H_2O_2). Hydrogen peroxide is then univalently reduced, with the addition of another proton, to water and the hydroxyl radical (OH^\cdot). A final univalent reduction and the addition of another proton convert the hydroxyl radical to water. (Modified from Del Maestro RF: An approach to free radicals in medicine and biology)

Fig 2.3 Production of oxygen derived free radicals by activated neutrophils



TNF- α , tumour necrosis factor- α , IF γ , interferon γ ; IL, interleukins; NO^\cdot , nitric oxide; SOD, superoxide dismutase; \bar{O}_2^\cdot , superoxide radical; OH^\cdot , hydroxyl radical; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form), Fe^{2+} , ferrous iron. Modified from Goode HF and Webster NR: Free radicals and antioxidants in sepsis.

2.4.2 Antioxidant defence mechanisms in humans

In evolutionary terms aerobic organisms have had to develop intracellular mechanisms for the detoxification of oxygen molecule and its by products. This cellular protection is provided by antioxidants that inactivate or prevent the formation of free radicals (Gregory & Bulkley 1983). Free radicals may also decay spontaneously when they are not removed by antioxidants. Antioxidants can be divided into two broad groups based on their method of action. They are either enzymatic or non-enzymatic. The enzymatic group include Superoxide dismutase (SOD), Catalase and Glutathione peroxidase *Figure 2.4*. The non-enzymatic group includes vitamin C, tocopherol, sulfhydryl-containing compounds like cysteine and glutathione. Glutathione is a potent endogenous mainly intracellular antioxidant that can neutralize the destructive potential of reactive oxygen metabolites and is discussed in further detail later in this chapter (Deneke & Fanburg 1989). Serum proteins such as albumin, ceruloplasmin and transferrin also belong to this group, *Table 2.1*. Most of the antioxidant protective pathways require micronutrients such as selenium, manganese, copper and zinc and deficiency of any one of these nutrients might lead to diminished free radical protection in some cellular compartment.

Table 2.1 Endogenous antioxidants

-
1. Low molecular weight compounds, e.g., vitamins C, E, β -carotenes
 2. Sulfhydryl group donors, e.g., glutathione
 3. Proteins with sulfhydryl groups
 4. Enzymes, e.g., superoxide dismutase, catalase, glutathione peroxidase.
-

Modified from an introduction to free radical biochemistry. Cheeseman KH and Slater TF

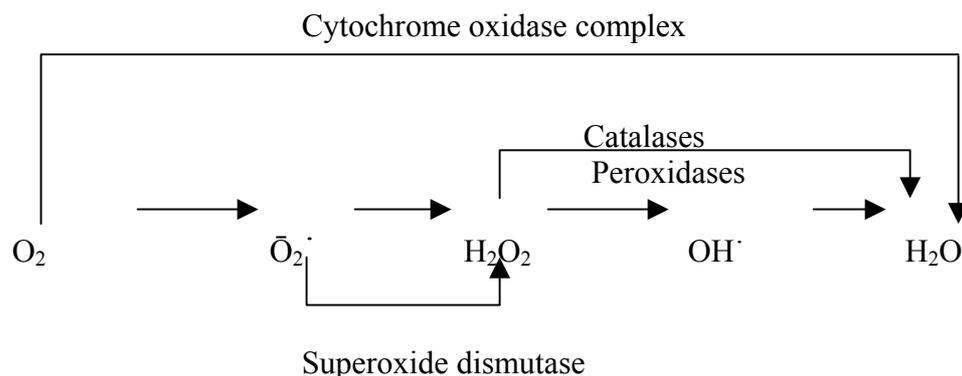


Fig 2.4 Endogenous antioxidants

Endogenous mechanisms for the detoxification of oxygen free radicals generated by the univalent reduction of molecular oxygen. SOD catalyses the dismutation of superoxide to hydrogen peroxide and oxygen without the oxidation of other molecules in the intracellular environment. Similarly, the catalases and peroxidases catalyse the reduction of hydrogen peroxide directly to water, without the production of the toxic hydroxyl radical. These enzymes thus serve to detoxify or prevent the production of all three of the highly reactive and hence toxic species produced from the univalent reduction of molecular oxygen. (Modified from Del Maestro RF: An approach to free radicals in medicine and biology.

2.4.3 Free radicals and membrane permeability

The damaging effects of free radicals on the cell membrane and intercellular matrix have been implicated in a wide range of disease processes. Some of the diseases of surgical importance in which free radicals have been implicated include reperfusion syndrome following limb ischaemia, intestinal ischaemia, circulatory shock, radiation injury and peripheral oedema (Del Maestro 1980).

Oxygen derived free radicals damage cell membranes by directly attacking structurally important polyunsaturated fatty acids within the phospholipid structure of the cell membrane. This process is called lipid peroxidation and is directly attributable to the action of the superoxide and hydroxyl radicals. The additional effects of secondarily generated lipid peroxides, which are further produced in a self-perpetuating chain-

reaction, further amplify the destruction of cell membranes *Figure 2.5*. Using the cheek pouch experimental model it has been demonstrated that the intravascular generation of the superoxide radical significantly increases the macromolecular permeability in post capillary venules. This increase in permeability is prevented by the administration of superoxide dismutase, an enzymatic scavenger of free radicals (Ley & Arfors 1982).

Whilst there is abundant evidence to show that the activities of free radicals significantly alter membrane permeability, the exact contribution of this factor to the aetiology of post-surgical oedema is unknown. It is possible that, the alteration in membrane function produced by unchecked free radical attack of cell membranes is one of the significant contributors to postoperative oedema formation. Individuals with pre-existing impaired antioxidant capacity before surgery or who develop impairment of their antioxidant defences after surgery will be more prone to developing post-surgical oedema. If this is the case, the degree of post-surgical water retention should correlate with antioxidant status. It is also possible that a sub-clinical degree of water retention may be present prior to surgery in those with impaired pre-operative antioxidant status. Furthermore, if the above are true, monitoring the changes in total body water and its distribution may correlate with levels of circulating antioxidants in blood and may therefore act as a proxy for oxidative damage.

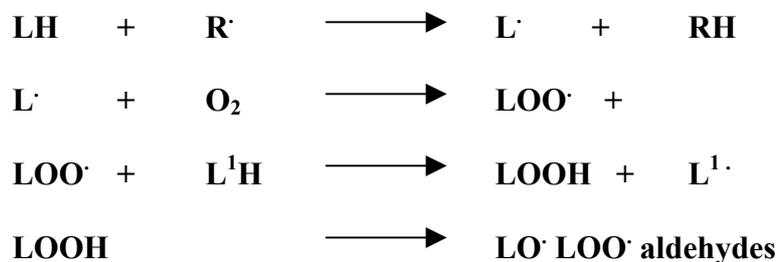


Fig 2.5 Reactions of free radicals and secondary generation of free radicals

LH = Target polyunsaturated fatty acid (PUFA) molecule in cell membrane

R[·] = Initiating oxidising radical, **L[·]** = Fatty acid radical

LOO[·] = Fatty acid peroxy radical, **L¹H** = New PUFA molecule

LOOH = Lipid hydroperoxide, **LO[·]**

From an introduction to free radical biochemistry. Cheeseman KH and Slater TF

2.4.4 Glutathione

Glutathione is an endogenous intracellular antioxidant that plays a central role in cellular protection from oxidative stress. Several clinical and experimental studies indicate that the level of this tripeptide is depleted in severe surgical illness (Reichard et al. 1981; Shi et al. 1982 and Sugino et al. 1989). It has been suggested that impairment of the antioxidant protection provided by glutathione during critical illness could lead to unopposed free radical mediated damage to major organs and organ failure (Robinson et al. 1992).

2.4.4.1 The synthesis of glutathione

Glutathione is a tripeptide of L-glutamate, L-cysteine and glycine. The molecular structure is shown below *Figure 2.6*. The first step in glutathione synthesis is the combination of glutamate and cysteine. This reaction is the rate-limiting step in glutathione synthesis and is catalysed by gamma-glutamylcysteine synthetase. Glutathione limits the activity of this enzyme through a biofeedback mechanism (Meister & Anderson 1983). Hormones may also induce the activity of this enzyme. In the second step glycine is added to gamma-glutamylcysteine in a reaction catalysed by glutathione synthetase, which is not subject to negative feedback, by glutathione *Figure 2.7*. The gene coding for gamma glutamylcysteine synthetase has been identified on human chromosome 6 (Sierra-Rivera et al. 1995). There are several varieties of gamma-glutamylcysteine synthetase and the type expressed is tissue dependent (Shi et al. 1996).

The reduced form of glutathione (GSH) is synthesized in virtually all cells from its amino acid precursors. The liver is the main source of plasma glutathione and under physiological conditions 90% of circulating glutathione is synthesized in the liver (Kaplowitz et al. 1985; Meister 1982 and DeLeve & Kaplowitz 1990). Most of the glutathione synthesized in the liver is exported to the plasma and the remaining is excreted in the bile (Lauterburg et al. 1984). The synthesis of glutathione in the liver is controlled

by both substrate availability and hormones. It is known that fasting decreases liver GSH content, whereas refeeding replenishes it without affecting the activity of GSH synthesizing enzymes. It would appear that the concentrations of cysteine and methionine are critical for restoring hepatic GSH synthesis during refeeding (Tateishi et al. 1974). Insulin and glucocorticoids stimulate hepatic GSH synthesis through induction of gamma-glutamylcysteine synthetase (Lu et al. 1992). Conversely glucagon and c-AMP stimulating agents down regulate hepatic GSH synthesis by inhibiting gamma-glutamylcysteine synthetase a process brought about by phosphorylating the enzyme (Lu et al. 1991).

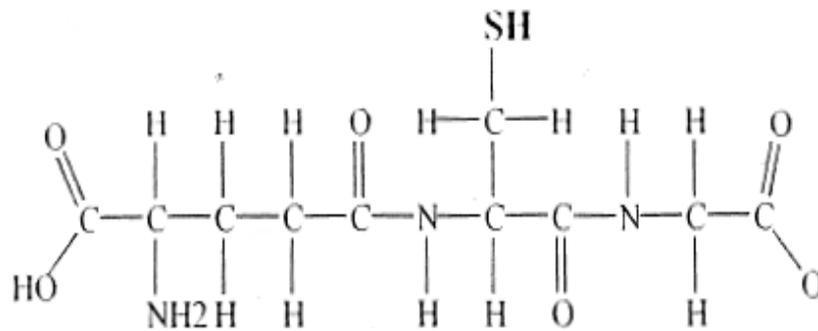
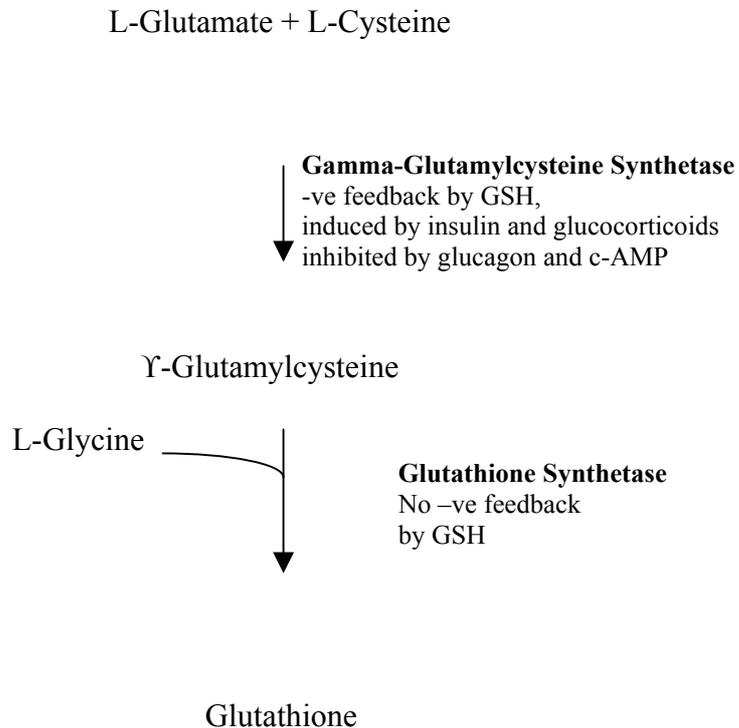


Fig 2.6 The structure of glutathione

Structure of gamma-L-glutamyl-L-cysteinyl-glycine (Glutathione)

From glutathione metabolism of human skeletal muscle in surgical trauma (Luo et al. 1996).

Fig 2.7 The steps in the synthesis of glutathione



The synthesis of glutathione (GSH). (Modified from Lomaestro and Malone, 1995)

2.4.4.2 The metabolism of glutathione

Glutathione is the most prevalent and most important intracellular non-protein thiol and sulfhydryl compound in mammalian cells (Meister 1988). In humans reduced glutathione occurs in millimolar concentrations intracellularly but only in small amounts in plasma and other body fluids *Table 2.2*. The role played by plasma GSH in maintaining cellular concentration of GSH in tissues is not clear. Plasma contains less than 2% of the GSH present in whole blood. Ninety eight percent of the GSH in whole blood is carried in the red blood cells (Dass et al. 1992). Certain organs such as lung and intestinal epithelial cells are able to utilize plasma GSH. Most cells lack the capacity for the direct uptake of exogenous GSH but epithelial cells such as proximal tubular cells, endothelial cells,

retinal and pigmented epithelial cells are capable of exogenous GSH uptake. This supports GSH dependent detoxification systems (Hagen et al. 1990a). This process allows for rapid maintenance of GSH availability by routes other than can be provided by synthesis alone. Although it is known that plasma concentration of GSH can be increased by oral administration of GSH, GSH is synthesized from amino acids transported into the cells and from the activity of transpeptidases at the cell surface. These transpeptidases also play a role in salvaging amino acids from circulating GSH, which are subsequently utilised in the intracellular synthesis of GSH.

Little is known about the significance of dietary intake of GSH in health or disease. The amount of GSH in various food sources also remains unknown. Estimated daily intake in humans is believed to be about 150mg of GSH per day. It is possible to achieve a two- to five-fold increase in plasma GSH concentration by giving as much as 15g/Kg body weight of GSH (Hagen et al. 1990b). It is believed that GSH is absorbed from the intestinal lumen into the enterocytes. It is then exported from the enterocytes into the blood from where uptake from plasma by cells occurs. Gastrointestinal transport of GSH appears to occur via a non-energy requiring, sodium dependent, carrier mediated diffusion (Aw et al. 1991; Hunjan & Evered 1985).

The breakdown of GSH occurs extracellularly. This process is catalysed by transpeptidases and dipeptidases bound to the external surface of cell membranes. Under physiological conditions GSH is exported out of the cell to membrane bound enzymes where its degradation provides a source of amino acids. It is possible that a small amount of oxidised glutathione (GSSG) is exported as well. However during periods of oxidative stress GSSG excretion from the cell increases (Toborek & Hennig 1994). Transpeptidation of glutathione requires the presence of certain amino acids which act as acceptors. Such amino acid acceptors include cystine, methionine and glutamine. Transpeptidation of GSH leads to the formation of gamma-glutamyl amino acids and cysteinylglycine.

Cysteinylglycine is then split by dipeptidases to glycine and cysteine. The gamma-glutamyl amino acid provides a substrate for gamma-glutamyl cyclotransferase and is converted to 5-oxoproline and the corresponding amino acid. 5-oxoproline is converted to glutamate by a reaction catalysed by 5-oxoprolinase (Meister 1988) *Figure 2.8*.

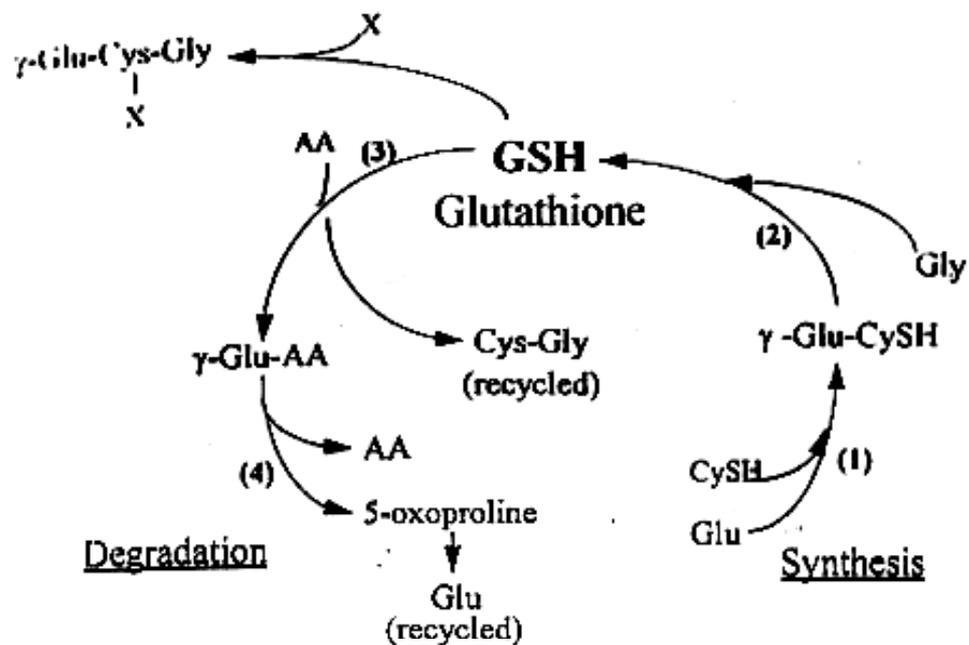
There is a rapid turnover of GSH in plasma. The kidneys remove more than 80% of plasma GSH and the half-life of intravenously administered GSH is less than two minutes (Amano et al. 1994). The half-life of GSH in epithelial fluid is however much longer and this raises the question of the possible existence of a different control mechanism (Holroyd et al. 1993). The concentration of glutathione in plasma thus depends on hepatic synthesis, the degree of oxidative stress, extrahepatic uptake and degradation, and glutathione absorption.

Table 2.2 Glutathione concentration in various tissues

Eye lens	~10mM
Liver	5-7mM
Lung/kidney/heart	~ 2-3mM
Red blood cell	~ 2mM
Plasma	< 0.05mM

The concentration of Glutathione in various tissues (Luo et al. 1996).

Fig 2.8 An outline of the metabolism of Glutathione



Enzymes: (1) γ -glutamylcysteine synthetase; (2) glutathione synthetase; (3) γ -glutamyltranspeptidase; (4) γ -glutamyl-cyclotransferase; AA = amino acid e.g. methionine; X=foreign compounds. Modified from glutathione metabolism of human skeletal muscle in surgical trauma (Luo, Hammarqvist, Andersson, & Wernerman 1996).

2.4.4.3 The functions of glutathione

Glutathione plays a central role in the protective repertoire against oxidative stress. It acts as an antioxidant by directly scavenging free radicals as well as the detoxification of endogenous and exogenous substances (Deneke & Fanburg 1989). It is also involved in the regulation of protein synthesis and degradation, transmembrane transport of amino acids and the regulation of intracellular redox state. There is evidence to suggest that it plays a role in gene expression (Wernerman et al. 1999). A summary of the metabolic functions of GSH is listed below *Table 2.3*

GSH exerts most of its properties as an antioxidant by serving as a substrate for the selenium-dependent antioxidant enzyme glutathione peroxidase (GSHPX). GSH is utilised by glutathione peroxidase as a hydrogen donor to reduce hydrogen peroxide and other organic peroxides to water and alcohol respectively. Reduction of the oxidised glutathione (GSSG) is catalysed by glutathione reductase, a flavin-containing enzyme, which uses reduced nicotinamide-adenine dinucleotide phosphate NADPH as the reducing power (Toborek & Hennig 1994). NADPH is regenerated from NADP through the pentose-phosphate pathway and the enzyme glucose-6-phosphate dehydrogenase is the first enzyme in this pathway. The enzymes involved in the oxidation-reduction reactions of GSH maintain cellular level of glutathione predominantly in the reduced state, which accounts for more than 90% of total intracellular glutathione *Figure 2.9*. During exposure to oxidative stress GSSG accumulates within the cytosol. The GSSG/GSH ratio can thus be used as an index of oxidative stress. GSSG diffuses more easily out of the cell than GSH and its continuous formation and efflux eventually leads to the depletion of the intracellular pool of glutathione (Akerboom & Sies 1994).

The lipid bilayer of cell membranes is enriched with GSH. Within the cell membrane GSH prevents the peroxidation of the lipid structures and its accompanying deleterious effects on cellular function by maintaining a reduced state in other membrane

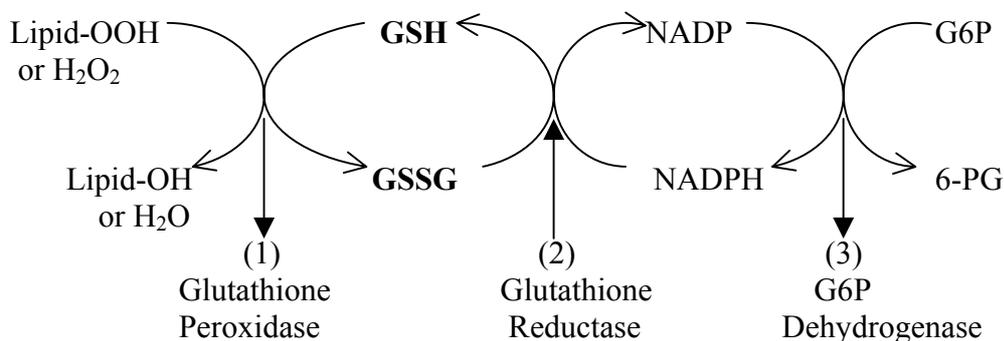
bound thiols and antioxidants such as α -tocopherol (Wu et al. 1994). GSH in the lipid bilayers therefore plays a critical role in maintaining cell membrane integrity and function and it is possible that this function can be overwhelmed during periods of oxidative stress (Toborek & Hennig 1994).

Table 2.3 Functions of Glutathione

1. Serve as a substrate for GSH peroxidase in the reduction of hydrogen peroxides and peroxides to water and alcohol respectively.
2. Conjugate exogenous and endogenous toxic compounds, rendering them more water-soluble.
3. Maintain the sulfhydryl residues of certain proteins and enzymes in the reduced state
4. Store cysteine in a non-toxic form and provide a vehicle for inter organ transport of cysteine
5. Synthesis and repair of DNA
6. Maintain α -tocopherol (vitamin E) and ascorbic acid (vitamin C) in the reduced states
7. Direct scavenger of free radicals.

Modified from the Metabolic Functions of Glutathione (Modified from Lomaestro and Malone, 1995)

Fig 2.9 Oxidation and reduction of Glutathione



The oxidation and reduction of glutathione. Enzymes: (1) glutathione peroxidase; (2) glutathione reductase; (3) glucose-6-phosphate dehydrogenase

2.4.4.4 The role of glutathione in inflammation and surgery

Surgical trauma and critical illness are associated with accelerated production of free radicals. During such periods, a variety of antioxidant systems protect tissues from free radical induced cellular damage (Bulkley 1983; Del Maestro et al. 1980). Glutathione plays a key role in the body's overall antioxidant defence system (Deneke & Fanburg 1989). By acting as a scavenger of free radicals GSH protects protein thiol groups from oxidant injury (McCord 1983). A more oxidized state of glutathione is associated with increased rate of protein degradation (Khairallah et al. 1985). In experimental animals and humans, tissue concentration of GSH falls following major trauma or elective abdominal surgery (Reichard et al. 1981; Luo et al. 1996). Depleted tissue GSH has been shown to increase mortality rates after haemorrhagic shock in animals (Robinson et al. 1992). The supplementations with precursors of glutathione are used to preserve GSH concentration in the liver and reduced cellular toxicity induced by the administration of acetaminophen (Puri & Meister 1983). In experimental models depletion of hepatic glutathione stores was associated with liver oedema following ischaemic-reperfusion injury (Suzuki et al.

1994). GSH provides cellular protection over a variety of cellular metabolism as listed in *Table 2.3*. The diminished ability to maintain adequate levels of circulating GSH during periods of inflammatory stress has been suggested to be partly due to irreversible consumption of GSH as a reducing agent, availability of substrates and the impaired re-utilization of oxidized glutathione (Jahoor et al. 1995; Reid et al. 2000 and Hammarqvist et al. 1997).

2.4.4.5 Glutathione and oedema

Impaired synthesis of erythrocyte GSH was identified as a vital factor in the development of oedematous states in children with protein-energy malnutrition (PEM). The imbalance in antioxidant defence created by the deficiency of glutathione renders the cell membranes susceptible to free radical induced damage (Golden & Ramdath 1987). This view is supported by other recent studies that have found elevated levels of the biomarkers of lipid peroxidation in patients with oedematous malnutrition (Fechner et al. 2001; Lenhartz et al. 1998 and Reid et al. 2000). The unchecked activities of free radicals produce global disruption of cell membrane that is typically seen in some disease states such as ischaemia-reperfusion injury. Such clinical states are characterized by increased permeability of endothelial cell membrane and generalised oedema (Del Maestro 1980; Fishel et al. 2003). In severe cases vital subcellular organelles such as mitochondria are involved and cell death is the end result. In experimental models such terminal event has been prevented by the administration of glutathione monoesters (Martensson & Meister 1989).

2.5 The complications of post-surgical oedema

Although the occurrence of generalised oedema per se in the early postoperative period does not appear to have been directly related to clinical outcome parameters, several studies have shown that hypoalbuminaemia and fluid overload, which are both common features of oedematous states, are associated with prolonged recovery of gut function (Lobo et al. 2002; Moss 1967). Oedema is also observed in patients with acute septic complications following major surgery (Lobo et al. 1999) and furthermore, a positive fluid balance (another feature of oedematous patients) has also been positively correlated with mortality in septic post-surgical patients (Alsous et al. 2000).

The presence of oedema increases the distance between tissue cells and the vascular space and consequently the distance for oxygen delivery from the blood supply leading to a decrease in the oxygen tension in such affected tissues (Heughan et al. 1972). The low oxygen tension of oedematous tissues impairs collagen synthesis and epithelialization. The combination of these processes ultimately delays wound healing (Hunt & Pai 1972; Pai & Hunt 1972). Furthermore the low oxygen tension impairs cellular immunity in oedematous tissue as some of the bacteriocidal functions of white cells depend on the presence of oxygen in adequate concentration. For example, leukocyte activity against *Staphylococcus aureus* is impaired in the setting of tissue hypoxemia (Hohn et al. 1976). These factors may provide an explanation for why oedematous tissues are more likely to breakdown and become infected.

The tendency to retain salt and water following injury or surgery is well reported as part of the post-trauma metabolic response (Cuthbertson 1945; Moore & Brennan 1975). These changes typically lead to an expansion of the extracellular compartment which may be further compounded by use of intravenous fluids (Bergstrom et al. 1981). The expansion of the extracellular compartment or mass as it is sometimes referred to, is accompanied by depletion of protein stores achieved by the accelerated breakdown of

body protein (Shizgal 1981b). As most of the body proteins consist of either structural or functional cellular elements, the end result is a reduction in cell mass - the metabolically active component of the body (Moore & Boyden 1963). In critically ill surgical patients, the over-expansion of the extracellular fluid compartment is associated with increased morbidity and mortality (Lowell et al. 1990). It has also been shown that patients who following surgery exhibit an exaggerated increase in extracellular water tend to have increased postoperative complication rates compared to patients who lose water (Starker et al. 1983).

Attention has been drawn to the potentially negative side effects of oedema that occurs following fluid resuscitation. Oedema of the gastrointestinal tract may have very complicated effects on albumin kinetics, fluid flux, and ion flux. It may lead to development of ileus, increased nasogastric tube output, which may be incorrectly construed as unremitting obstruction rather than a consequence excessive use of crystalloids (Marx 2003). Intestinal oedema may also reduce intestinal absorptive function and lead to diarrhoea. Myocardial oedema may have implications in patients who demonstrate decreased ventricular function during sepsis or other disorders in which aggressive fluid administration is routine (Weinstein & Doerfler 1992). Despite the potential for post-surgical oedema to produce adverse clinical outcomes, there appears to be no known study that has established a direct causal relationship between oedema and clinical outcomes such as recovery of gut function and length of hospital stay.

2.6 Summary of literature review

Generalised oedema is not an uncommon clinical finding in patients who have undergone major abdominal surgery. Although generalised oedema is often assumed to be benign, there is evidence to suggest that it may be linked to poor clinical outcomes or significant morbidity even if such patients show no sign of critical illness. The presence of generalised oedema in patients following major abdominal surgery indicates the loss of capillary membrane function and may also be indicative of the existence of significant systemic perturbations elsewhere.

Examination of the previous literature reveals several key issues that need to be resolved:

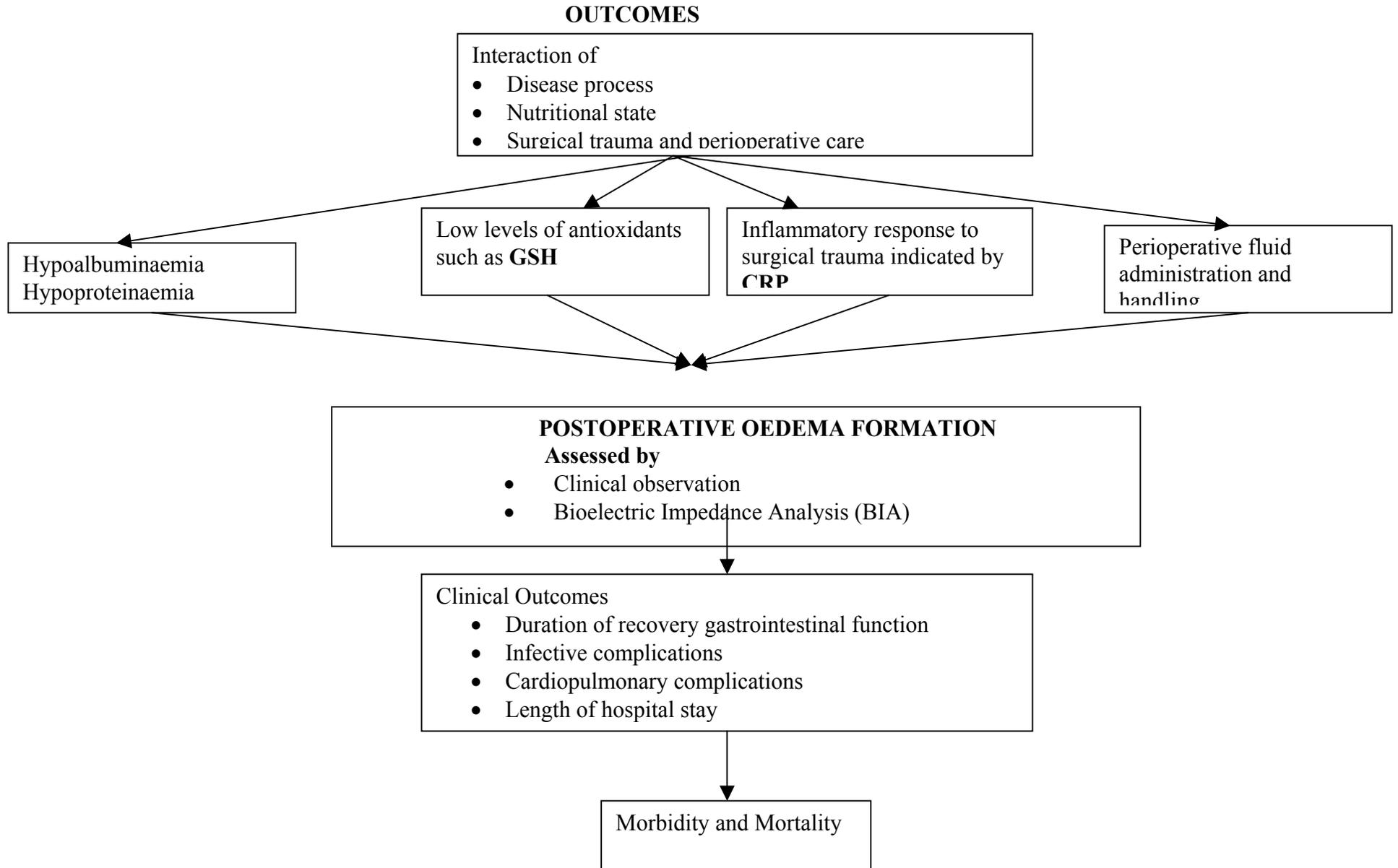
1. The incidence of oedema in patients undergoing routine surgical procedures is unknown.
2. Hypoalbuminaemia and hypoproteinaemia occur universally after major or minor surgical procedures. This finding in the early postoperative period is not sufficient enough to explain the appearance of oedema in some individuals. The aetiological factors responsible for oedema formation are multifactorial and the interactions of these factors remain complex. It would appear that the importance of any one of these factors varies according to the state of health or disease. There is a need to explore the interactions between surgical intervention and perioperative management - in particular, fluid management, oxidative stress and the response to surgical trauma.
3. Bioelectrical impedance is a non-invasive tool that can be used to examine the changes in body water distribution that reflect changes in cell membrane function following major abdominal surgery. The value of this technique in quantifying and monitoring oedema in surgical patients is unknown. There is a need to determine whether the assessment of body composition in post-surgical patients using BIA would be of value in identifying patients who have the propensity to expand their extracellular mass and who may be at increased risk of complications.

4. Most importantly there is a need to better understand the association, and underlying mechanisms, that relate the presence of oedema with recovery of gastrointestinal function and clinical outcome.

These issues have been captured in the form of a putative causal chain that highlights the inter relations of the causes of post surgical oedema and its consequences on clinical outcomes (*Fig 2.10*). The work described in this thesis was an attempt to address these issues.

Fig 2.10

CAUSAL CHAIN OF THE CAUSES OF POSTOPERATIVE OEDEMA AND ITS EFFECTS ON CLINICAL



Chapter 3

Hypotheses and objectives

3.1 Hypotheses underlying this study

Generalised oedema is often seen early during postoperative recovery in patients who have undergone surgery. The incidence of generalised oedema following routine major abdominal surgery and its relationship to clinical outcomes is not known. The factors that contribute to any propensity to develop oedema are poorly understood.

The over-arching hypothesis of this work is that the presence of early postoperative oedema impacts negatively on clinical outcomes. To explore this central hypothesis the following four interrelated and testable hypotheses were established:

- a. That generalised peripheral oedema is common in early postoperative period after major surgery.
- b. The use of bioelectrical impedance analysis can objectively quantify the changes in body fluid compartments and identify abnormal fluid shifts in surgical patients
- c. The presence of oedema in the early postoperative period is unrelated to hypoalbuminaemia and or hypoproteinaemia but strongly related to other risk factors such as perioperative fluid administration, the inflammatory response to trauma and antioxidant capacity
- d. The presence of oedema in the early postoperative period is significantly associated with poor clinical outcomes in patients who have undergone major abdominal surgery.

3.2 Objectives of study

The inter-related hypotheses stated above will be examined tested within a single longitudinal study.

Hypothesis 1.

This will be tested by determining the incidence of postoperative generalised oedema in a consecutive series of hospital admissions of patients who have undergone major abdominal surgery. This part of the study is described in Section 5.2

Hypothesis 2.

This will be tested by examining the changes in the distribution of total body water (as measured by Ht2/Z50 and other variables derived by BIA) in a prospective series of observations in clinically oedematous and non-oedematous patients following major abdominal surgery. This part of the study is described in Section 5.3.

Hypothesis 3.

The role of the following potential aetiological factors in the development of postoperative oedema would be examined by serial measurements of these factors over a period of time and comparing the data obtained from patients who developed oedematous and those consistently non-oedematous after surgery. This part of the study is described in Section 5.4.

- Fluid administration and excretion in the perioperative and postoperative period. (Section 5.4.1).
- The changes in total protein and albumin concentration after major abdominal surgery (Section 5.4.2).
- The inflammatory response to trauma after major abdominal surgery measured as concentration of C-reactive protein (Section 5.4.3).
- Antioxidant status measured as the concentration of reduced glutathione (GSH) in whole blood (section 5.4.4).

Hypothesis 4

The impact of postoperative generalised oedema on clinical outcomes in patients who have undergone major abdominal surgery will tested by comparing the time interval for the recovery of gastrointestinal function, data of postoperative complications and length of clinical recovery in oedematous and non-oedematous patients from a cross-section population of patients. This part of the study is described in Section 5.5.

Chapter 4

Materials and methods

This chapter describes the design and methods of data collection of the two main studies used in examining the hypothesis. Where appropriate some analyses involving combined data from the two studies were made. When combined analysis is undertaken the eligibility criteria for patient selection will be clearly stated.

4.1 Study design

Two observational studies were undertaken to examine the research hypotheses.

- A pilot study
- A longitudinal study

However for the evaluation of the relationship between postoperative oedema and clinical outcomes it was necessary to combined data from both studies.

4.1.1 Study population

Subjects for both studies were recruited from patients admitted to the Surgical Directorate, Southampton University Teaching Hospital for abdominal surgery. The types of surgical procedures patients underwent included small bowel resection, colectomy, anterior resection, abdomino-perineal resection, oesophago-gastectomy, cysto-prostatectomy, radical prostatectomy and nephrectomy. Patients were excluded if they underwent major abdominal surgery for vascular pathologies such as aneurysms, if no procedure was undertaken because the disease was considered to be inoperable at surgery and if patients or their relatives were unable to give informed consent. A copy of the consent form is shown in appendix A1.

4.1.2 Reference population

The reference population used in both studies was made of twenty-one subjects. These were generally healthy volunteers selected from patients attending the day case unit of the Surgical Department at the Royal South Hants Hospital for minor surgical procedures. They received written information detailing their role in this study and an informed consent was obtained. Reference values for reduced glutathione and body composition measured by bioelectrical impedance analysis were obtained from this population.

4.1.3 Pilot study

The pilot study was a cross sectional observational investigation to examine the difference in GSH in patients with and without oedema at a single time point after major abdominal surgery. The aims of this pilot study were as follows:

- To explore the differences in the concentration of whole blood GSH in patients who developed oedema compared to those who did not after major abdominal surgery
- To allow the principal researcher to develop laboratory skills for the preparation and storage of blood samples especially for glutathione analysis.
- To provide information on the other possible variables of interest for further exploration in the longitudinal study.

Patients who had undergone major abdominal surgical procedures were recruited and divided into two groups - Oedematous patients, (OD) group, and non-oedematous patients, (NOD) group, on the basis of whether or not oedema was present at the time of assessment, and whether they or their relatives were able to provide consent. Recruitment was not therefore consecutive.

The caring medical team had undertaken pre-operative assessment of all the patients in the pilot study. After surgery, single point assessment was done for the purpose of this

study by the principal researcher. During this assessment, a nutritional history exploring recent weight loss or reduced dietary intake, anthropometric measurements and blood samples were obtained. Whole-body bioimpedance was also measured at four frequencies (5, 50, 100 and 200 kilohertz). In order to eliminate bias, an independent assessor not involved in the study verified the presence and degree of oedema and there was a one hundred per cent agreement between observations made.

4.1.4 Longitudinal study

The longitudinal study was a cross-sectional prospective observation in which the parameters detailed below were measured in consecutive eligible patients undergoing routine admission before and after major abdominal surgery. The changes in these parameters were compared between patients who became oedematous post-operatively and those who remained consistently oedema free. There was insufficient information available from the previous literature or pilot study to conduct a formal power analysis to determine the number of subjects required to test the primary hypotheses. A pragmatic approach was adopted whereby the maximum number of subjects was studied within the timeframe of the investigation.

Prior to surgery a clinical examination was undertaken when the patients came for their pre-anaesthetic evaluation. A brief nutritional history exploring recent weight loss, anthropometric data and blood samples were obtained. The patients were clinically examined for the presence of oedema as described in section 4.5. Whole-body impedance was measured at frequencies of 5, 50, 100 and 200 kilohertz. Details of data collection are described in subsequent sections. Clinical examination, whole body bioimpedance and blood samples were repeated on days 1, 3 and 5 after surgery. The total daily fluid intake and output for the initial five days following surgery was collected retrospectively as described in section 4.7.

Patients were divided into two groups – Oedematous group (OD) and non-oedematous group (NOD) on the basis of whether or not they developed oedema during the study period. Patients were placed in the NOD group only if they had no oedema present at each of the three time points they were examined after surgery. In order to eliminate bias, an independent assessor not involved in the study verified the presence of oedema with a one hundred per cent agreement between observations made.

4.1.5 Combined pilot and longitudinal analyses

To overcome the limitations of small population size some of the analyses were undertaken on a combined population of patients from the pilot and longitudinal studies. Eligibility criteria for inclusion in the combined population are listed below.

- Patients were only placed in the NOD group if they had repeated examinations (a minimum of three) to exclude the presence of oedema.
- Patients were excluded from this analysis if an insufficient number of examinations was undertaken to be certain that oedema did not occur at any point within 7 days post surgery.
- Patients were also excluded from this analysis if assessment was done more than 7 days post surgery.

4.2 Ethics

This research was undertaken in accordance with the principles of the Declaration of Helsinki. Ethical approval for this research was obtained from the local office of the Southwest Hampshire Research Ethics Committee REC 325/01.

4.3 Anthropometry

Materials

- Tape Measure
- Electronic Stadiometer, CMS Weighing Equipment Ltd, Camden, London, U.K.
- Electronic weighing machine, CMS Weighing Equipment Ltd, Camden, London, U.K.
- Skinfold calliper, Holtan Ltd, Crymych, U.K.
- Competency training was provided within the Institute of Human Nutrition for the various equipments used in the study.

4.3.1 Weight and height

At the preoperative assessment patients weight was measured using an Electronic scale (CMS Weighing Equipment Ltd, Camden, London, U.K). The patient stands upright on the instrument scale and weight is read to the nearest 0.1kilogram (kg). Height was measured with an Electronic Stadiometer (CMS Weighing Equipment Ltd, Camden, London, U.K) to the nearest 0.1centimeter (cm). Three readings of each parameter were taken and the average determined. The body mass index (BMI) was determined from the average weight and height.

Waist and hip circumferences were measured with a simple tape measure. The anatomical waist was taken to be a line running through the highest points of the iliac crests. The hip circumference was measured at a line running across the tuberosities of the greater trochanters of the right and left femur. Waist and hip circumferences were measured to the nearest 0.1 cm. The average of three readings was taken for each.

4.3.2 Mid upper arm circumference, waist circumference, hip circumference and triceps skin fold thickness

Mid upper arm circumference (MUAC) was obtained with a simple tape measure. The MUAC was measured at the mid point of the distance from the Acromium process to the Olecranon process of the elbow of the upper arm. Readings were made to the nearest 0.1centimetres. The waist circumference was measured across the apex of both iliac crests and the hip circumference across the prominences of the greater trochanter on both sides. The triceps skin fold thickness (TSFT) was measured using skin fold callipers (Holtan Ltd, Crymych, U.K). TSFT was measured by pinching the skin fold overlying the triceps muscle midway between the Acromium and the Olecranon of the elbow joint. TSFT was measured in millimetres and result expressed to one decimal place. The average of three readings was for both MUAC and TSFT.

4.4 Body Composition

Materials

- Quadscan 4000[®] (Bodystat Limited, Isle of Man, UK).
- Disposable, self-adhering Gelled Electrodes (Bodystat Limited, Isle of Man, UK).
- 50 - Ohm resistor (Bodystat Limited, Isle of Man, UK).
- Software CD (Bodystat Limited, Isle of Man, UK).
- A pair of colour coded leads

4.4.1 Obtaining a reading with the Quadscan 4000®

The Quadscan 4000® (Bodystat Limited, Isle of Man, UK) is a portable device designed for measuring whole-body bioelectrical impedance *Fig 4.1*. The Quadscan 4000 is supplied with a CD containing the software to enable downloads of data on to computer.



Figure 4.1 Quadscan 4000® (Bodystat Limited, Isle of Man, UK)

Before using the Quadscan 4000® to measure whole-body impedance (Z) height, weight, waist and hip circumferences are obtained as described in section 4.3.2. Whole-body impedance measures were obtained with the subject lying comfortably in the supine position. The arms and legs are placed slightly apart. It must be ensured before readings are obtained that the patient's thighs do not touch. In obese subjects the limbs are spread further apart to prevent this from occurring. It is also recommended that readings be obtained after subjects have been lying for at least five minutes. Self-adhering disposable electrodes (Bodystat Limited, Isle of Man, UK) are placed on the back of the right hand (just proximal to the knuckles of the index and middle fingers) and right wrist (between the heads of the radius and ulnar). Similar electrodes are placed on the dorsum of the right foot (proximal to first and second toes) and on the anterior surface of the right ankle (at the level between the medial and lateral malleoli). The electrodes behind the fingers and

toes called the “injecting” or “source” electrodes, whilst the electrodes placed on the wrist and ankle are the “sensing” electrodes. The alligator clips of the leads are clipped to the gel-free foil tabs of the electrodes. The red leads are connected to the injecting electrodes on the back of the fingers and toes whilst the black leads are connected to sensing electrodes on the wrist and ankle *Figure 4.2a and b*.

Figs 4.2 a and b Sites for the application of proximal and distal electrodes

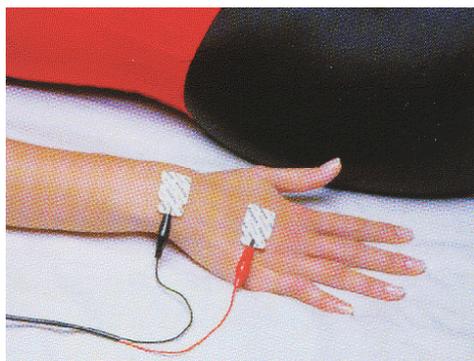


Fig 4.2 a



Fig 4.2b

Fig 4.3a Sites on the wrist for the application of proximal electrodes; Fig 4.3b Sites on the foot for the application of distal electrodes.

Whole-body bioelectric impedance was measured at four frequencies (5, 50, 100 and 200 kilohertz (KHz)). All readings were taken in the mornings to minimize variations that may occur over during daily activity. Whole-body bioelectric impedance readings were also obtained with patients in a relatively stable state. The Quadscan 4000[®] is able to store up to 99 readings at a time and after this any additional record is stored by deleting the first record of stored readings. The stored data in the Quadscan 4000[®] can be downloaded directly onto a computer as a spreadsheet. The Quadscan 4000[®] provides estimates of the volumes of TBW, ECW and ICW using the manufacturer’s equation programmed into the machine. The manufacturer’s equation was not revealed. Estimates of third space water, fat mass and the lean body mass can also be obtained from the Quadscan 4000[®] machine but these parameters were not the subject of this study.

4.5 The assessment of oedema

The presence of oedema following major abdominal surgery was the primary clinical outcome measure. The presence of oedema on visual inspection was confirmed by the presence of pitting. Pressure was applied to the area of the body (usually the ankles) with the tip to the index finger. The pressure may also be applied to any part of the lower or upper limbs or the sacral region depending on the distribution of oedema. The depression of the skin should remain for at least 2 seconds after removing the pressure. The principal researcher was solely carried out the physical examination for the presence of oedema but in order to eliminate bias an independent clinician not involved with the study verified the presence of oedema in all patients who had oedema.

4.5.1 The grading of oedema

The degree of oedema was quantified using a four-point scale (Bate 1991) -

1. Absent – Oedema absent on clinical examination
2. Mild – Mild degree of pitting confined to the ankles or upper limbs
3. Moderate – Moderate degree of pitting that is present at the ankles but not extending beyond the knee.
4. Severe - Severe degree of pitting present at the ankles or involving lower limbs, trunk and upper limbs.

4.6 Measures of clinical outcome

The following measures of clinical outcomes were collected retrospectively from the medical records of the patients following discharge from hospital.

- Duration of ileus
- Length of hospital stay
- Infective complications
- Cardiopulmonary complications
- Admission to intensive care unit (ICU) or high dependency unit (HDU)
- 30 day mortality

4.6.1 Duration of ileus

The duration of ileus was used as a crude measure of the recovery of gut function following surgery. The duration was estimated in two ways:

1. The number of days to the first bowel movement or passage of flatus and
2. The number of days to tolerate solid hospital food following surgery.

4.6.2 Length of hospital stay

Length of hospital stay was measured as the number of days spent in hospital after surgery until medically fit for discharge. If the patient remained in hospital after being deemed fit for medical discharge, the extra number of days on the ward was not counted.

4.6.3 Infective complications

Infective complications were diagnosed clinically and confirmed by microbiological or radiological tests. Although the clinical suspicion of infective complications was recorded during the study the results of microbiological and radiological tests were analysed

retrospectively. Confirmed infective complications were further subdivided into minor and major infective complications depending on clinical severity.

4.6.4 Cardiopulmonary complications

These were defined as cardiopulmonary problems thought to have been precipitated by surgery and which were not present preoperatively. They include postoperative myocardial infarction, arrhythmias and left ventricular failure.

4.6.5 Admission to ICU or HDU

All admissions to an intensive care or high dependency unit following surgery were analysed retrospectively. The number of days spent in ICU/HDU was also noted.

4.6.6 Mortality

Data on deaths occurring within 30 days following surgical procedures were also collected. Such data was also recorded whether patient had been discharged or not from hospital as long as it occurred within 30 days following surgical procedure.

4.7 Fluid intake and output

Fluid administration in this study did not follow a set protocol. The anaesthetist was responsible for the administration of intraoperative fluids whilst postoperatively the caring surgical team was responsible for this. The amount and type of fluid administered reflected typical clinical practice, and was influenced by the perception of need judged from blood and fluid losses, urine output and cardiovascular signs. The daily total fluid intake was the sum of all fluids administered by any route. This included oral, intravenous and fluids administered through routes such as epidural infusions, blood or blood products.

The data on fluid intake and output were collected retrospectively and only in the longitudinal group of patients. Fluid output including estimates of intraoperative blood loss, urine, losses from the gastrointestinal tract and drains were documented.

The daily cumulative fluid balance for the initial five days following surgery was calculated from the difference between the daily fluid intake and output after allowing for insensible water loss of one litre/day (the value routinely used at Southampton University Hospital at the time of this study). No further adjustment was made for insensible water loss as none of the patients in this study had prolonged periods of pyrexia or ventilation (Cox 1987).

The process of data collection was dependent on records made by individuals not involved in the study. Although fluid volumes appeared to be reasonably well recorded, the exact type of fluids (e.g. normal saline infusion (0.9% sodium chloride solution) or 5% dextrose infusion) was not always well documented. This made it impossible to calculate sodium intakes. It was also impossible to find full data for all patients where such data was missing and precluded the use of some subjects in fluid analysis.

4.8 Laboratory assessment

4.8.1 Glutathione analysis

The analysis of whole blood GSH was undertaken in the laboratory of the Institute of Human Nutrition. The preparation of the samples for storage and for analysis for GSH were done solely by the principal researcher who prior to starting the study underwent training in this laboratory in pipetting techniques and determination of coefficient of variation, the handling and storage of blood samples.

Materials

- Monobromobimane (Fluka, Cat no. 69898 MW 271)
- Acetonitrile (HPLC grade)
- Dulbeccos PBS (Sigma Dulbeccos PBS 0.137M NaCl, 1mMKH₂PO₄, 3mMKCl, 8mM NaH₂PO₄, pH 7.5)
- Serine (Sigma # S4500, MW105.1)
- Boric acid (Sigma #B0252, 100g)
- 70% Perchloric Acid
- Methanol
- Acetic acid glacial

4.8.1.1 Sample workup

Blood for GSH was obtained in a 10ml EDTA bottle and was processed promptly. Where samples had to be transported, they were placed on ice and transported in the dark and processed within two hours of collection. 10 µL of well-mixed venous blood in an EDTA bottle was pipetted using a Microman's pipette and added to 300µL of a phosphate buffered derivatising reagent. This contained serine, boric acid, monobromobimane and penicillamine (137 mmol/L NaCl, 1mmol/L KH₂PO₄, Na₂HPO₄, 50mmol/L boric acid, 1.25mmol/L monobromobimane, 30µmol/L penicillamine, pH 7.4). Samples were

prepared in duplicate and left to stand in the dark for 20 minutes before freezing for later analysis. The reaction of GSH with monobromobimane in the derivatising reagent forms light sensitive derivative, which was stable on storage at -20°C.

The remaining blood in the EDTA bottle was centrifuged at 2500 rpm at a temp of 4°C for 10minutes. Plasma was decanted and stored in duplicate for future analysis.

4.8.1.2 GSH measurement

Samples derivitised for GSH as mentioned above, were thawed slowly in the dark and deproteinized by addition of 300µL of 0.75M perchloric acid. After mixing and centrifugation at 10000g, at 4°C for 10 minutes, the supernatant was analysed by high-pressure liquid chromatography (HPLC) with fluorometric detection. Analysis was carried out on a C₁₈ reverse phase column with a mobile phase of 20% methanol in 0.1mol/L acetate buffer pH 3.8. Quantification was by comparison with standard GSH solutions treated in an identical manner. Precision of the measure was determined prior to commencing the study and further determined from the replicates throughout the analysis with a co-efficient of variation of 3.24%.

4.8.2 Plasma albumin, total protein and C-reactive protein (CRP)

Samples were collected into 10ml Vacutainer bottles containing lithium heparin and processed and analysed using Advia 2400 Multi analyser[®] (Bayer) within routine clinical chemistry (SUHT) in batches soon after collection. Plasma proteins were analysed routinely by spectrophotometry using Bromocresol Purple. Results were available later in the day but were collated retrospectively at the end of the study.

4.8.3 Plasma electrolytes, urea and creatinine

Blood samples for plasma electrolytes were analysed routinely using by the ion exchange technique. Batch analysis of blood samples for subjects was undertaken on the same day.

Although the results were available on the same day as the tests the results were collated retrospectively at the end of the study.

4.9 Statistical analysis

Results are presented as mean and standard error for all measured variables except for the following: number of days to solid food, bowel movement and length of hospital stay.

This set of data is expressed as median and range. Comparisons between the non-oedematous and oedematous groups were made using the Student t test or the Mann-Whitney U test for continuous outcome variables that were not normally distributed (number of days to solid food, bowel movement and discharge). Rank analysis of covariance was used to adjust for differences in age between groups (Huitema 1980).

Logistic regression was used to assess differences in binary outcome variables with and without age as a covariate. Fisher exact test was used in 2x2 contingency tables with low expected frequencies. One-way repeated measures analysis of variance was used to test for changes in repeated measurements of one variable (impedance or impedance ratios) in the same group of patients. Two-way repeated measures analysis of variance (2-way ANOVA) was used to test for differences between two sets of repeated measurements carried out in the same group of subjects. Split-plot ANOVA (SPANOVA) was used to test for differences in repeated measurements between oedematous and non-oedematous groups. Analysis of covariance (ANCOVA) was used to establish intra-individual relationships between changes in fluid balance (dependent variable) and ht^2/Z (covariate)(with subject as the fixed factor, after testing for homogeneity of regression). A value of $P < 0.05$ was taken to indicate a significant result. Statistical analyses were performed with SPSS for Windows, version 10.0 (SPSS Chicago, Illinois).

Chapter 5 Results

5.1 The pilot study

The primary aim of the pilot study was to examine the difference in the concentration of reduced glutathione (GSH) in whole blood in oedematous (OD) and non-oedematous (NOD) patients who had undergone major abdominal surgery. Patients were divided into 2 groups - (OD) and (NOD) on the basis of whether or not oedema was clinically present at the time of assessment. Findings in each group were compared to each other and with the reference population. Physical and biochemical measurements obtained from the reference population are shown in *Table 5.1.1*

Table 5.1.1 Physical characteristics and laboratory findings in the reference population

Number (n): (Male/Female)	14/7
Age (years):	26.8 – 82.4 (56.4)
BMI (kgm^{-2}):	22.8 – 37.4 (28.2)
Albumin (g/L):	36 – 48 (41.1)
Total Protein (g/L):	65 – 78 (72.8)
C-reactive protein (mg/L):	1.9 – 9.9 (3.0)
GSH ((mmol/L):	0.94 – 1.61 (1.17)

Variables are expressed range (mean)

5.1.1 Pilot study - Subjects and surgical procedures

24 patients (aged 41.9 – 89.5 years; Male-Female ratio 14:10) who had undergone major abdominal surgery were recruited. There were 14 patients in the OD and 10 in the NOD

groups respectively. The various surgical procedures undertaken are shown in *Table 5.1.2*. There was no significant difference in types of procedures undertaken in both groups of patients.

5.1.2 Pilot study - Age and physical characteristics

The OD group of patients were significantly older than the NOD group – mean (s.d) 73.0 (8.4) *versus* 61.6 (14.7) years, $P = 0.045$. There was however no significant difference in the BMI or gender distribution and ASA scores in both groups *Table 5.1.3*.

5.1.3 Pilot study - The number of days post surgery and assessment

The number of days following surgery that assessment was undertaken ranged from 1 – 12 days. There was no difference in OD and NOD groups in the number of days following surgery that clinical assessment was undertaken; median (range) 7.0 (1-12) *versus* 5.0 (2-8) days respectively, $P = 0.146$ *Table 5.1.3*.

Table 5.1.2 Surgical Procedures in Pilot Study

Cross-sectional Group	Non-Oedematous (N = 10)	Oedematous (N = 14)	Total (N = 24)
Resection of the colon (Malignant)	4	4	8
Resection of the colon (Benign)	3	4	7
Other cancers			
Oesophagogastrectomy	0	1	1
Nephrectomy	0	1	1
Gastrojejunostomy (Ca pancreas)	1	0	1
Other diagnoses			
Small bowel resection	1	2	3
Repair of perforated duodenum	0	1	1
Mesenteric Ischaemia	0	1	1
Open Cholecystectomy	1	0	1

Table 5.1.3 Physical characteristics and laboratory findings in the non-oedematous and oedematous patients (Pilot Study)

	Non-Oedematous	Oedematous	p Value
All patients			
Number (n): (Male/Female)	10 (7/3)	14 (7/7)	0.421 ^a
Age (years): (mean (s.d.))	61.6 (14.7)	73.0 (8.4)	0.045 ^b
BMI (kgm ⁻²): mean (s.d.)	29.3 (6.8)	27.5 (4.3)	0.463 ^b
Number of Days post surgery when assessed: median (range)	5.0 (2-8)	7.0 (1-12)	0.146 ^c
ASA Score (n for scores 1, 2, 3)	6, 9, 3	2, 13, 5	0.209 ^d

a = Chi square; b = Student's t test; c = Man-Whitney U Test; d = Fisher's test

5.1.4 Pilot study - GSH and haemoglobin concentration

The concentrations of haemoglobin and GSH in whole blood were significantly lower in the OD compared to NOD group 10.3 (1.6) *versus* 12.0 (1.3) g/dL; $P = 0.005$ and 0.767 (0.17) *versus* 0.995 (0.18) mmol/L; $P = 0.004$ respectively. There was however no difference in the concentration of GSH when the difference in haemoglobin concentration was taken in account by expressing the concentration of GSH relative to the concentration of haemoglobin: 7.45 (1.19) *versus* 8.3 (1.44) $\mu\text{mol/gHb}$; $P = 0.128$, for OD and NOD groups respectively *Table 5.1.4*.

The haemoglobin and GSH concentrations were significantly lower in the NOD patients compared to the volunteers. Haemoglobin concentration was (12.00 (1.27) *versus* 14.33 (1.04) g/dL, $P < 0.001$) and GSH concentration was (1.0 (0.18) *versus* 1.19 (0.16) mmol/L, $P = 0.013$) in the NOD and volunteers respectively.

Similarly the haemoglobin and GSH concentrations of the study population as a whole were significantly lower when compared to the reference population. The concentration of haemoglobin was 11.0 (1.6) *versus* 14.3 (1.0) g/dL; $P < 0.001$ and GSH was 0.862 (0.203) *versus* 1.187 (0.158) mmol/L; $P < 0.001$ for the study and reference populations respectively. Again when the difference in red cell concentration is considered there was no significant difference in the GSH/Hb ratio 7.8 (1.34) *versus* 8.3 (1.03) $\mu\text{mol/gHb}$, $P = 0.178$, in the study and reference populations respectively.

5.1.5 Pilot study - Albumin, total protein and C-reactive protein

The concentration of protein in the oedematous and non-oedematous patients, the concentrations of albumin and total protein at the time of assessment were significantly lower in OD compared to NOD; 18.86 (6.21) *versus* 27.50 (5.38) g/dL, $P < 0.001$ for albumin and 50.21 (8.22) *versus* 58.40 (5.34) g/dL, $P = 0.005$ for total protein. CRP

concentration on the other hand was significantly raised in the OD compared to NOD at the point of assessment 104 (35.6) *versus* 60.7 (37.1) mg/L, $P = 0.010$.

Table 5.1.4 Haematological and biochemistry measurements in pilot study

	Volunteers	Non-Oedematous	Oedematous	p value*	p value**
Patient group	(n=21)	(n=10)	(n=14)		
Neutrophil	4.38(2.99)	7.68(1.95)	10.84(4.38)	0.082	<0.001
WBC	6.76(3.13)	9.92(2.15)	12.89(4.39)	0.125	<0.001
Haemoglobin g/dL	14.33(1.05)	11.99(1.27)	10.31(1.55)	0.008	<0.001
GSH (mmol/L)	1.19(0.16)	1.00(0.18)	0.77(0.17)	0.004	<0.001
GSH/Haemoglobin μmol/gHb.)	8.30(1.03)	8.30(1.44)	7.45(1.19)	0.128	0.178
Albumin (g/L)	41.15(2.27)	27.50(5.38)	18.86(6.21)	<0.001	<0.001
Total Protein (g/L)	72.63(3.64)	58.40(5.34)	50.21(8.22)	0.0005	<0.001
C-reactive protein (mg/L)	2.92(1.93)	104.4(35.55)	60.70(37.11)	0.010	<0.001

Values are expressed as means (s.d.) TSFT = Triceps skin fold thickness, * = (Student's t test Non-oedematous *versus* Oedematous), ** = (Student's t test Post surgical patients vs volunteers)

5.1.6 Pilot study – Preliminary discussion

It has been suggested that an impaired ability to mount an adequate response to free radical induced cellular injury is a critical factor in the development of oedematous states in protein-energy deficiency and that this impaired antioxidant defence is reflected in a low concentration of red blood cell reduced glutathione (Golden & Ramdath 1987). The concentration of GSH within tissues such as muscles has also been demonstrated to fall in critically ill post-surgical patients although the fluid status of such patients was not described (Hammarqvist et al. 1997). The main aim of this pilot study, therefore, was to explore the differences in the concentration of whole blood GSH in patients who developed oedema compared to those who did not after major abdominal surgery.

The concentration of GSH was significantly lower in the OD compared to NOD patients, and in all post-surgical patients when compared to controls. However, the concentration of GSH was largely affected by the differences in the concentrations of haemoglobin between the OD and NOD patients and all post-surgical patients and volunteers. The observed statistically significant differences in GSH concentration in the OD *versus* NOD patients and all post-surgical patients compared to the reference population was lost when the concentration of GSH per gram of haemoglobin was compared in these respective groups.

Surgery was associated with blood loss that appeared to be greater in the OD patients compared to the NOD patients. As this was an observational study there was no control over blood loss or replacement during or after surgery. It is also possible that haemodilution contributed to the lower concentrations of haemoglobin seen in the oedematous patients. However, although the effect of haemodilution was not specifically quantified, it is assumed that that the effect of this was similar in the OD and NOD patients as the concentrations of haemoglobin and GSH in the NOD compared to controls

were significantly lower. Haemoglobin (12.00 (1.27) *versus* 14.33 (1.04) g/dL, $P < 0.001$) and GSH (1.0 (0.18) *versus* 1.19 (0.16) mmol/L, $P = 0.013$).

Other factors that could have contributed to the observed differences in the concentration of GSH in the oedematous and non-oedematous patients include age, type of surgery (and hence degree of trauma) and the intensity of the inflammatory response to injury. There did not appear to be a significant difference in the type of surgical procedures undertaken in the oedematous and non-oedematous patients *Table 5.1.2*.

The pilot study showed that the trauma of major surgery was associated with elevation in the concentration of positive markers of inflammatory response such as C-reactive protein and a decrease in the concentration of albumin in the post-surgical patients when compared to controls. This response pattern to surgical trauma is well known and has been demonstrated in previous studies and (Brewster et al. 1994; Buttenschoen et al. 2001 and Shakespeare et al. 1989). CRP concentration was significantly greater and albumin concentration significantly lower in OD compared to NOD patients, *Table 5.1.4*. Although the data collection on the concentration of C-reactive protein was incomplete, CRP values when used in combination with albumin concentration, suggest that the inflammatory response to trauma was greater in the OD compared to the NOD patients (Jakeways et al. 1994).

5.2 Results of longitudinal study

The results presented in this section are from data obtained from consecutive patient admissions that also had repeated observations. Analysis of the data obtained was used to determine the incidence of oedema, the role of BIA in monitoring oedema and the aetiological contributors to postoperative oedema formation in the early post operative period.

5.2.1 Longitudinal study - The analysis of the incidence of oedema

The incidence of oedema was analysed in the patients recruited in the longitudinal study. For accurate analysis the patients from the pilot were not eligible.

5.2.2 Longitudinal study - Subjects

33 consecutive admissions to the surgical wards for major abdominal surgery were recruited and 30 (91%) completed the study. Following assessment preoperatively, one patient had a myocardial infarction prior to surgery, one patient had disseminated malignant disease on laparotomy and another patient's operation was postponed. These three patients were therefore eliminated from the study. None of the patients had clinical evidence of oedema when assessed preoperatively. The physical characteristics, pre-operative biochemistry and surgical procedure undertaken in the patients analysed in the longitudinal study are shown in *Tables 5.2.1* and *5.2.2*.

5.2.3 Longitudinal study - Age, BMI, ASA score and preoperative biochemistry

12 (40%) of the patients developed oedema following major abdominal surgery (OD group) and 18 (NOD group) remained consistently non-oedematous. The OD group were

significantly older than the NOD group; mean (standard deviation (s.d)) 74 (9) *versus* 63 (14) years, $p = 0.020$ Student's t test) the risk of oedema was related to age (odds ratio 1.090 (95% confidence interval, 1.007 -1.181; $P = 0.034$)

The pre-operative BMI was similar in OD and NOD patients; mean (s.d.) 26.0 (2.7) *versus* 26.7 (6.1). All the patients in this study were all within American Society of Anaesthesiologists (ASA) score (1-3). Six patients in the NOD group had ASA score 1, whilst there was none with this score in the OD group. The distribution of the ASA score overall was however not significantly different between OD and NOD groups.

The preoperative concentrations of albumin, total protein and CRP were not significantly different in the OD and NOD patients.

The use or non-use of an epidural for anaesthesia was documented in 27 (90%) of the patients. An epidural was used in 15 (83%) of the NOD and 5 (56%) of the OD patients and there was no difference in the use epidural in either group. The distribution of surgical procedures undertaken in both groups of patients was identical *Table 5.2.2*.

Table 5.2.1 Physical characteristics and laboratory findings in the non-oedematous and oedematous groups used in the analysis of the incidence of oedema (Longitudinal study)

	Non-Oedematous	Oedematous	P Value
Number (n): (n (Male/Female))	18 (11/7)	12 (6/6)	0.547 ^a
Age (years):	63 (14)	74 (9)	0.020 ^b
BMI (kgm ⁻²):	26.7 (6.1)	26.0(2.7)	0.705 ^b
Pre-op Albumin (g/L):	38.3 (5.1)	36.5(3.5)	0.301 ^b
Pre-op Total Protein (g/L):	73.9(5.6)	73.2(5.8)	0.716 ^b
Pre-op CRP (mg/L): median (range)	3.9 (2.0 – 61.4)	8.1 (2.0 -72.0)	0.185 ^c
ASA Score (n for scores 1, 2, 3)	6, 9, 3	0, 9, 3	0.082 ^d
Use of epidural (per cent)	15(83)	6(50)	0.102 ^d

Results are expressed as mean (s.d.) except as indicated. a = Chi squared; b = Student's t test; c = Man-Whitney U Test; d = Fischer

Table 5.2.2 Surgical Procedures in the Longitudinal Study

Longitudinal Group	Non-oedematous (N=18)	Oedematous (N=12)	Total (N=30)
Cancer of the colon	12	7	19
Other cancers			
Cancer of the liver	1	0	1
Cancer of the stomach	0	1	1
Cancer bladder	0	1	1
Cancer of the prostate	2	0	2
Other diagnoses			
Small bowel resection	1	2	3
Colitis	1	0	1
Calcific pancreatitis	1	1	2

5.3 Longitudinal study - Oedema, total body water (TBW) and whole-body bioelectric impedance analysis (BIA)

This analysis compares the serial changes in whole-body bioelectrical impedance (Z) obtained at 4 frequencies (5, 50, 100 and 200kHz) before and after abdominal surgery in patients who developed oedema with those patients who remained consistently free of oedema after abdominal surgery, in order to gain further understanding of the changes in the various compartments that make up TBW and cell membrane electrical properties that occur with oedema formation.

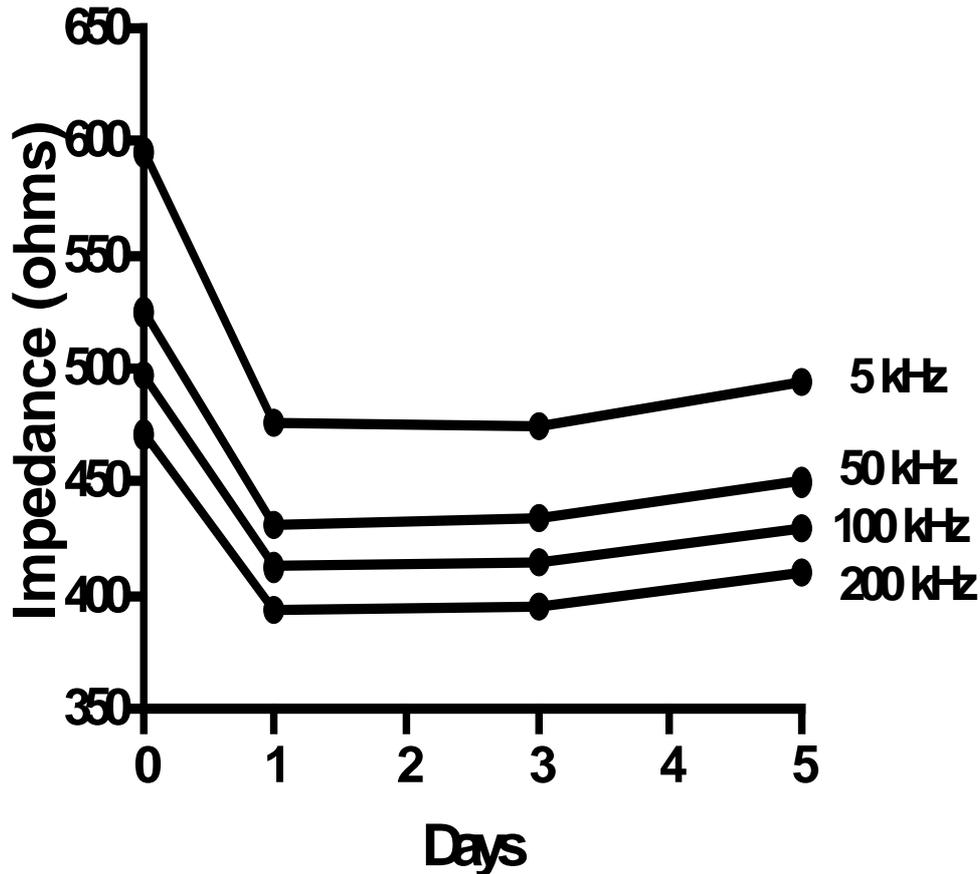
5.3.1 Longitudinal study - Physical characteristics of subjects analysed for the changes in BIA and relationship to oedema and TBW

The data analysed in this section were obtained from patients observed in the longitudinal study. Fluid data were collated as described in section 4.7. Only patients with matching fluid and complete bioelectrical impedance analysis data (pre-op and days 1, 3 and 5 after surgery) were selected for analysis. Complete data sets were successfully retrieved in 22 (73 per cent) patients (Male 11, Female 11) aged from 28 – 85 years. Preoperative BMI in the OD and NOD groups were similar (25.8 ± 3.1 versus 27.2 ± 6.5 kg/m², $P = 0.560$). Postoperatively there were 9 patients who developed oedema (OD group) and 13 patients (NOD group)

5.3.2 Longitudinal study - Changes in Z in OD and NOD patients

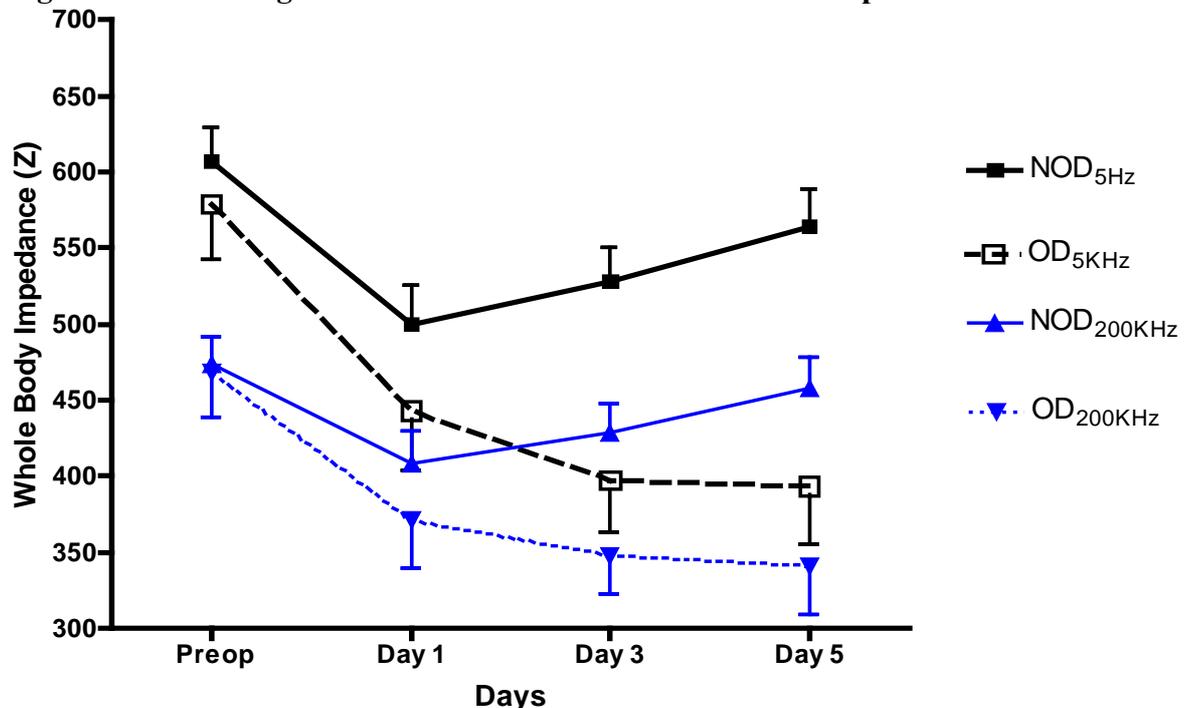
Whole body bioimpedance (Z) decreased significantly after surgery at all measured frequencies (5 kHz, 50 kHz, 100 kHz, and 200 kHz) in the group as a whole ($P = 0.001$, SPANOVA) and in the oedematous and non-oedematous groups separately, *Figure 5.3.1*. The change in impedance was greater in the oedematous than non-oedematous groups at all frequencies, and more so at lower than at higher frequencies. The average percentage decrease relative to the preoperative value was greater at 5kHz compared to 200kHz in both oedematous and non-oedematous groups (28.4 % at 5kHz versus 24.1% at 200kHz, ($P = 0.003$; oedematous group) and (12.6% at 5kHz versus 8.7% at 200kHz ($P < 0.001$; non-oedematous) *Figure 5.3.2*.

Figure 5.3.1 Changes in Z at frequencies of 5, 50, 100 and 200 KHz after surgery



Change in impedance at 5kHz, 50kHz, 100 kHz, and 200kHz following surgery (mean values shown). Impedance measure preoperatively and on days 1, 3 and 5. The reduction was significant at each frequency ($P < 0.001$) and at all time points.

Fig 5.3.2 Changes in Z at 5 and 200KHz in OD and NOD patients



Change in impedance at 5kHz (thin lines) and 200kHz (thick lines) following surgery (mean \pm (s.e.m.) values shown). Whole body impedance was measured preoperatively and on days 1, 3 and 5. The reduction was significant at each of these two frequencies but was greater in the OD (broken lines) than NOD (solid lines) patients and more so at 5KHz than at 200KHz ($P < 0.001$).

5.3.3 Longitudinal study - Changes in impedance quotient (ht^2/Z) and fluid retention in OD and NOD patients

The relationship between intra-individual change in ht^2/Z (the difference in the value of ht^2/Z on different days compared to pre-operative values) and measured fluid balance was assessed using a General Linear model, with fluid balance as dependent variable and subject as the fixed factor (*Figures 5.3.3 and 5.3.4*). The impedance quotient (ht^2/Z) in the whole group changed in a similar direction at each frequency but to a greater extent in the OD compared to NOD groups ($P < 0.001$ to 0.003 depending on the frequency) (see *Figure 5.3.3* for changes in ht^2/Z at frequency of 50kHz, $ht^2/Z_{(50kHz)}$). Prior analysis had established no significant gender effect, and therefore males and females were analysed together. Although the overall r^2 was high at all frequencies ($r^2 = 0.925$ - 0.949), there was significant variation in the gradient between subjects at 5, 50 and 100 kHz ($P < 0.050$), and a borderline significant variation at 200 kHz ($P = 0.054$). More consistent results were obtained when OD and NOD patients were analysed separately (there was homogeneity of regression (no significant differences in gradient between subjects) at all individual frequencies, except at 50 kHz in the oedematous group). The common intra-individual gradient ($(\Delta \text{fluid balance}) / (\Delta ht^2/Z)$) was steeper in the OD compared to NOD patients (e.g. at 5kHz the mean (s.e.m.) was 0.291 (0.068) *versus* 0.059 (0.053) litre ohms cm^{-2} , $P < 0.05$), whilst at 100 kHz it was (0.265 (0.061) *versus* 0.094 (0.071) litre ohms cm^{-2} , $P = 0.08$). In all cases, the overall r^2 value obtained by the ANCOVA model was significant in both the NOD ($r^2 = 0.667$ to 0.677 , depending on the frequency) and OD patients ($r^2 = 0.811$ to 0.817). Although the model describes a common intra-individual gradient within the OD or NOD groups, the absolute values of ht^2/Z at given values of fluid balance (i.e. the position of the parallel lines on the graph) differed significantly between subjects ($P < 0.001$ e.g. at 5kHz the standard error of the intercept was 1.15 litres in the NOD and 1.45 litres in the OD group).

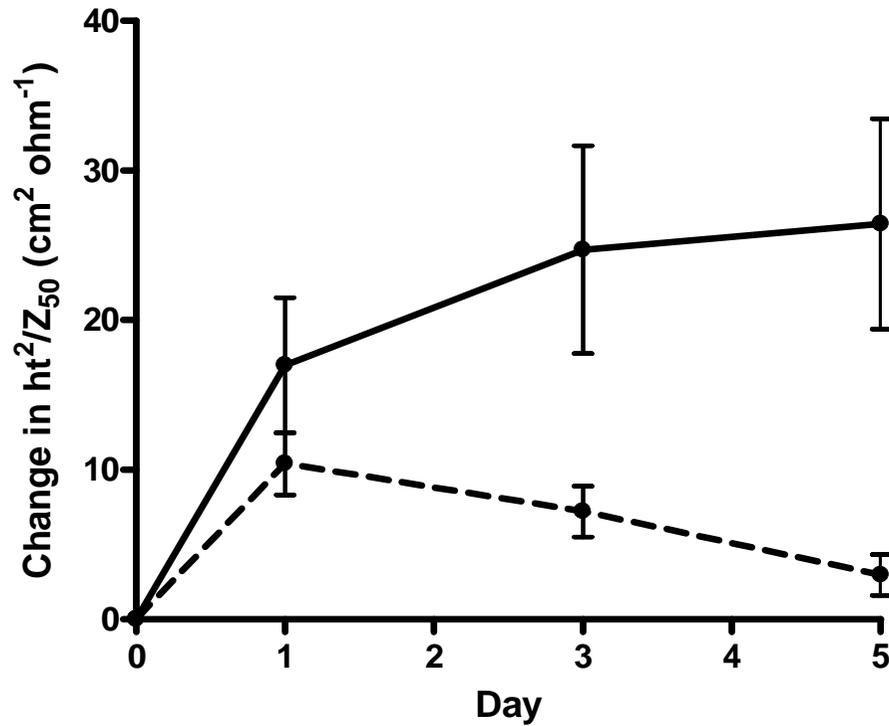


Figure 5.3.3 Change in the impedance quotient height²/Z₅₀ following surgery in OD and NOD patients
 Change in the impedance quotient height²/Z₅₀ (mean ± (s.e.m.) (cm²ohm⁻¹)) following surgery. The increments in the oedematous group (solid line) were significantly greater than in the non-oedematous group (dashed line) (SPANOVA, *P* < 0.001)

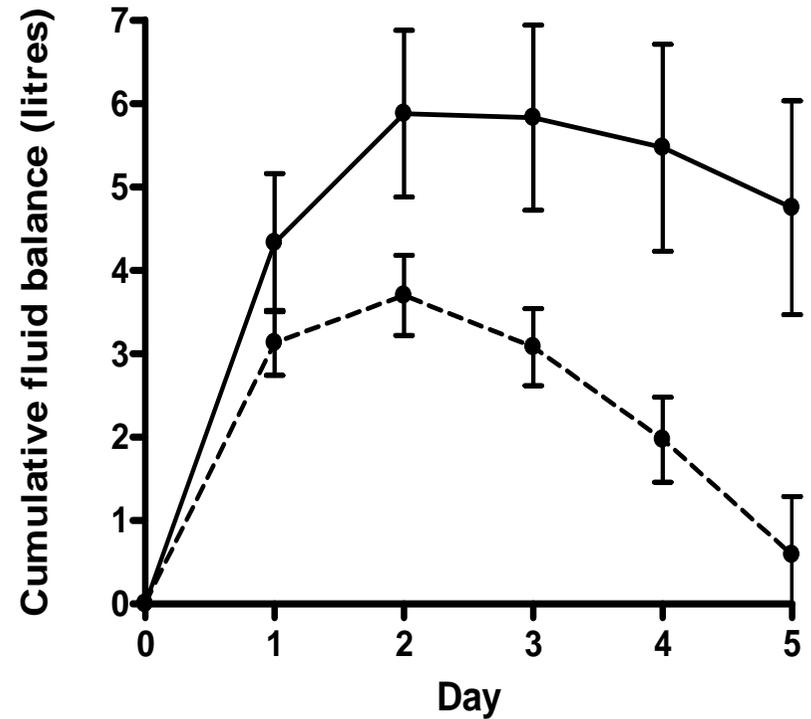


Figure 5.3.4 Daily cumulative fluid balance following surgery in OD and NOD patients
 Daily cumulative fluid balance following surgery (mean ± (s.e.m.) (Litres)). The overall fluid balance in oedematous (solid line) was significantly more positive than in the non-oedematous patients (dashed line) (SPANOVA; *P* = 0.009).

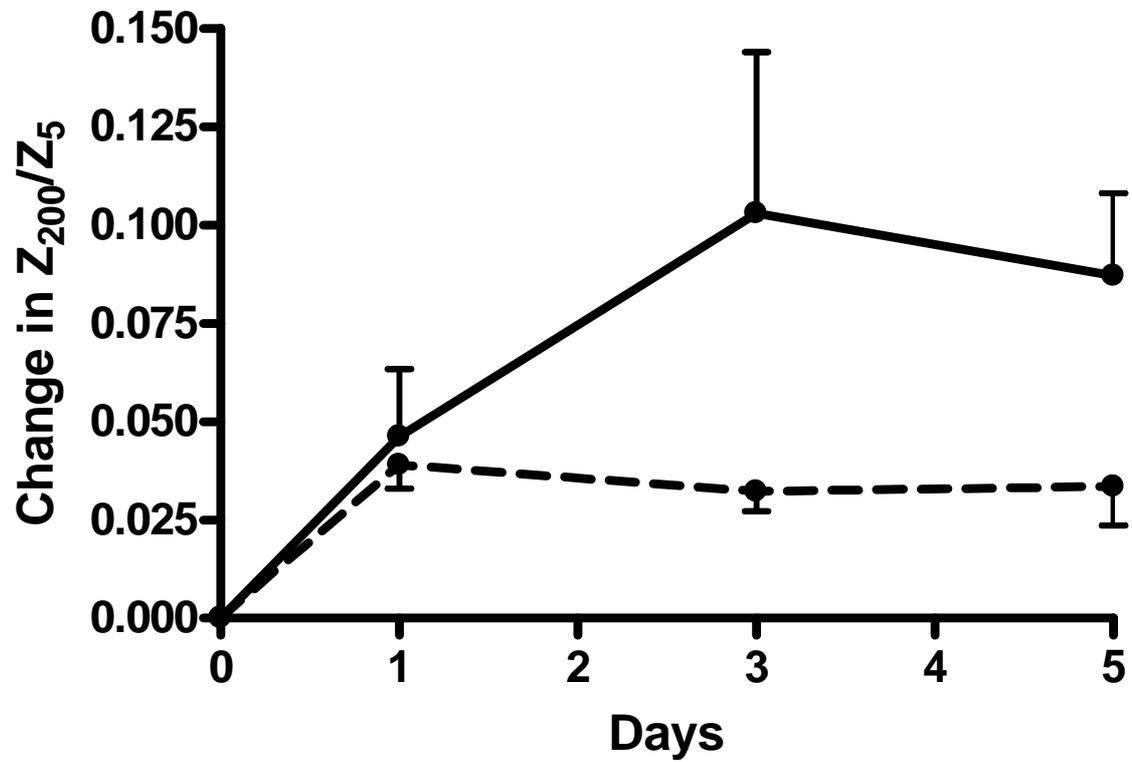
5.3.4 The ratio Z_{200}/Z_5 in OD and NOD patients

The Z_{200}/Z_5 (the ratio of impedance at 200kHz, influenced by extra and intracellular water at 5kHz, influenced mainly by extracellular water, to impedance. Measuring this ratio gives an idea of the changes in ECW in relation to TBW. The preoperative ratio of Z obtained at 200 kHz to 5 kHz (Z_{200}/Z_5) was significantly higher in those that went on to develop oedema than those who did not (0.809 v 0.799; $P = 0.015$), and the increment after surgery was also greater but not significant in those that developed oedema ($P = 0.090$) *Figure 5.3.5*.

A predisposition to developing post-operative oedema is suggested by measurements of a bio-electrical impedance ratio (Z_{200}/Z_5) obtained pre-operatively. The ratio Z_{200}/Z_5 became exaggerated post-operatively in those that developed oedema.

The Quadscan 4000[®] provided values for total body water using manufacturer's equations, which were not released. The changes in directly measured fluid balances were compared with those predicted by bioelectrical impedance using 2-way repeated measures ANOVA in the OD and NOD patients. The changes in fluid balance (days 1, 3 and 5) were similar by the two methods for the non-oedematous group (2.31 v 2.62 litres respectively; with a mean (s.e.m.) difference of 0.30 (0.75). The discrepancy was greater for the OD compared to the NOD group 8.65 *versus* 5.00 litres; difference 3.6 (1.82) litres. The overall difference between the two methods in the study population as a whole was strongly related to the magnitude of the fluid balance (mean of two methods) ($r = 0.88$), with the bioimpedance method (BIA) progressively overestimating the BIA-derived fluid balance as the volume of fluid balance measured from the charts became more positive.

Figure 5.3.5 Change in the impedance ratio Z_{200}/Z_5 (impedance at 200kHz to impedance at 5kHz)



Change in the impedance ratio Z_{200}/Z_5 (impedance at 200kHz to impedance at 5kHz) following surgery (mean \pm standard error) ($P < 0.001$). The increase in the oedematous group (solid line) was greater than in the non-oedematous group (SPANOVA, $P = 0.07$). Within each group there were significant increments at each time point relative to the pre-operative value (day 0).

5.4 Longitudinal study - Potential aetiological factors in early postoperative oedema formation

One of the main objectives of longitudinal study was to explore factors that may be contributory to the risk of developing early post-operative oedema. The following potential aetiological factors were explored.

- Fluid administration and output.
- Plasma and total protein concentration.
- The inflammatory response to the trauma of major abdominal surgery.
- Antioxidant status measured as the concentration of reduced glutathione (GSH) in whole blood.

5.4.1 Longitudinal study - Oedema and fluid administration after major abdominal surgery.

The data analysed in this section were obtained from patients observed in the longitudinal study in whom it was possible to obtain a complete fluid data set - 26 (87 per cent) patients. The 26 (15 M, 11 F) patients ages ranged from 48-85 years. The patients who developed oedema (OD, n = 11) were older (74.3 ± 10.5 versus 64.0 ± 12.0 years; $P = 0.030$) but had similar BMI ($25.8.0 \pm 2.8$ versus 24.9 ± 3.5 kg/m²; $P = 0.455$) to patients who remained persistently non-oedematous during the study period (NOD, n = 15). There was no difference in the frequency of use of epidural or the distribution of ASA scores in both patient groups *Table 5.4.1*. Similarly there was no significant difference in the gender distribution, type of surgical procedures undertaken in both groups of patients *Table 5.4.2*.

Table 5.4.1 The physical characteristics of patients analysed for the role fluid administration in post-surgical oedema (Longitudinal study)

	Non-Oedematous	Oedematous	p Value
Number (n): (n (Male/Female))	15 (9/6)	11(5/6)	0.692 ^a
Age (years):	64 (12)	74.3 (10.5)	0.029 ^b
BMI (kgm ⁻²):	24.9 (3.5)	25.8(2.8)	0.471 ^b
Pre-op Albumin (g/L):	38.9 (4.4)	36.0(3.2)	0.062 ^b
Pre-op Total Protein (g/L):	73.9(5.1)	58.2(6.5)	<0.001 ^b
Pre-op CRP (mg/L): median (range)	3.6 (2.0 – 61.4)	7.1 (2.0 -72.0)	0.247 ^c
ASA Score			0.177
1	4	0	
2	9	9	
3	2	2	
Use of epidural (per cent)	12/15(80)	5/9(55.5)	0.356 ^d

Variables are expressed as means (s.d.) unless where indicated. a = Chi square; b = Student's t test; c = Man-Whitney U Test; d = Fisher's test

Table 5.4.2 Surgical Procedures in the patients used for fluid analysis (Longitudinal study)

	Non-oedematous (N=15)	Oedematous (N=11)	Total (N=26)
Cancer of the colon	10	6	19
Other cancers			
Cancer of the liver	1	0	1
Cancer of the stomach	0	1	1
Cancer bladder	0	1	1
Cancer of the prostate	1	0	1
Other diagnoses			
Small bowel resection	1	2	3
Colitis	1	0	1
Pancreatic resection	1	1	2

5.4.1.1 Longitudinal study - Daily fluid intake in OD and NOD patients

Fluid volumes are expressed as mean (standard error of mean (s.e.m.)). The average total daily fluid intake over the initial five days following surgery did not differ significantly between OD and NOD patients *Figure 5.4.1* (upper graph). The average total daily fluid intake was (4.32 (0.25) *versus* 4.11(0.15) litres respectively, $P = 0.455$). The total volume of crystalloids (4.15 (0.48) *versus* 3.40 (0.29) litres, $P = 0.322$) and colloids (1.00(0.33) *versus* 0.63(0.33) litres, $P = 0.167$) administered intra-operatively were similar in the OD and NOD patients.

5.4.1.2 Longitudinal study - Daily fluid output in OD and NOD patients

In contrast to the above, the average daily urine output over the first five post-operative days was significantly lower in the OD compared to the NOD patients 2.15 (0.16) *versus* 2.69 (0.14) litres, $P = 0.020$. This pattern was repeated in the average daily total fluid output over the same period 3.37 (0.14) *versus* 4.0 (0.18) litres, $P = 0.017$ *Figure 5.4.1* (lower graph).

The average urine output per kg body weight over the course of five days was also significantly lower in the OD compared to NOD patients 29.4 (2.3) *versus* 40.5 (3.7) mls/kg, $P = 0.023$ (SPANOVA) *Figure 5.4.2*. This statistical difference did not exist after adjustment for age difference with Rank ANCOVA. However, the urine output per kg body weight over the initial three days was also significantly lower in OD compared to NOD patients 24.5 (2.4) *versus* 35.2 (3.3) mls/kg, $P = 0.018$ (SPANOVA) and remained so after adjustment for age, $P = 0.032$, *Table 5.4.3*

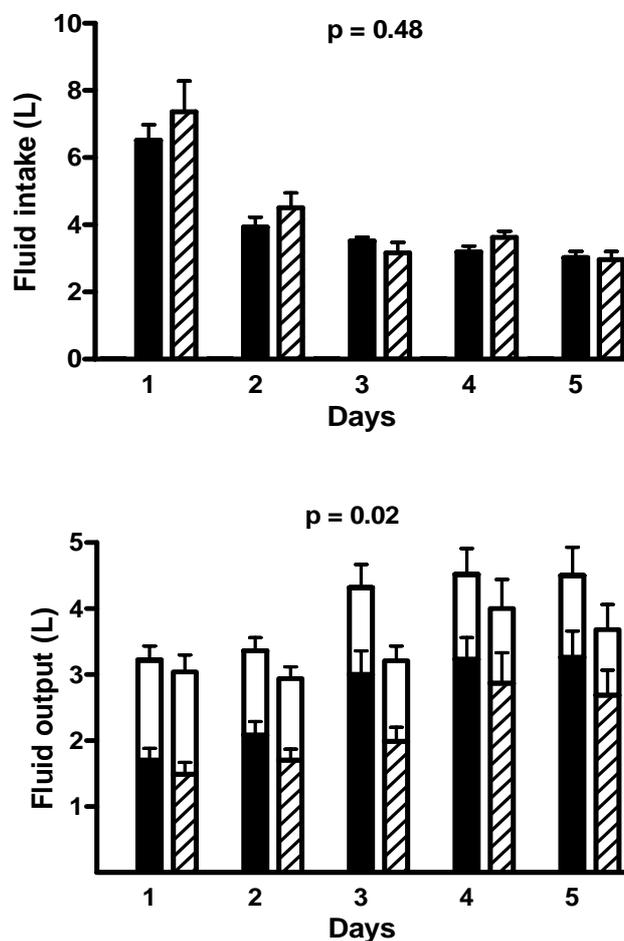
5.4.1.3 Longitudinal study – Fluid Balance in OD and NOD patients

Overall the net fluid balance was therefore significantly more positive in the oedematous group and maximum levels of fluid retention achieved by the second postoperative day. In

the OD group this was approximately 5.9 (1.0) with a range of -0.80 - 12.0 litres compared to 3.7 (0.48) with a range of 0 - 9.3 litres in the NOD group *Figure 5.4.3*.

From the combined intake and output data it is clear that oedema is related to inability to excrete similar fluid load administered in the postoperative period.

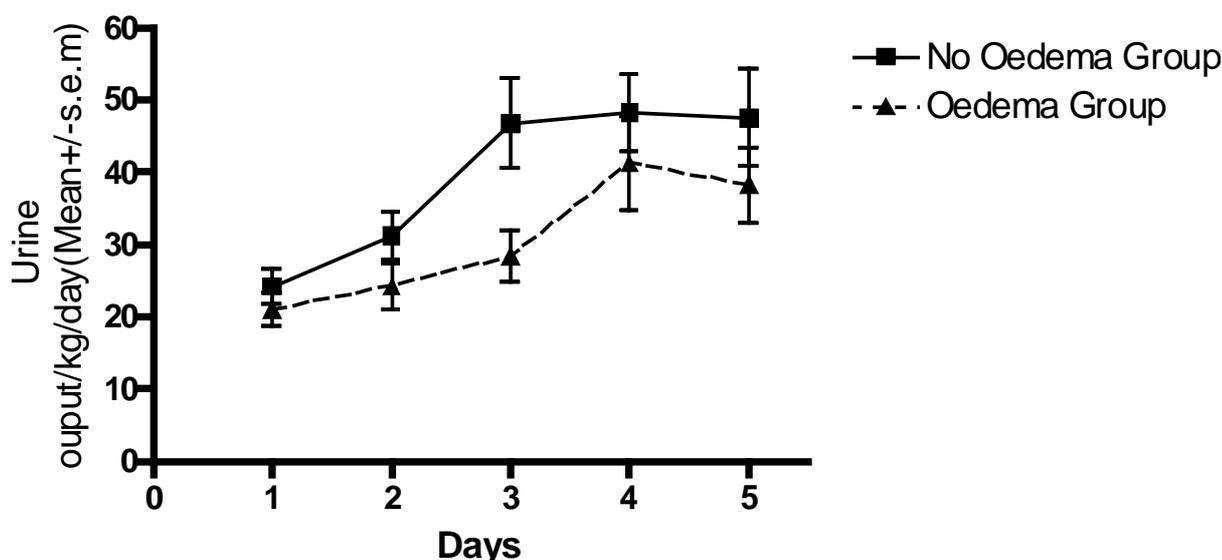
Figure 5.4.1 Daily fluid intake and output over five days post surgery OD and NOD patients



Upper graph: Daily volume of fluid administered ((mean \pm s.e.m.); Litres (L)) to NOD (black bars) and OD (shaded bars). The overall difference between the groups was not significant (SPANOVA, $p = 0.48$).

Lower graph: Daily urine and total fluid output ((mean \pm standard error of mean); Litres (L)) in OD and NOD patients. The black (NOD) and shaded parts of the bars (OD) represent urine output, and the white part of the bars above these correspond to the non-urine output (insensible loss, blood loss and losses from gastrointestinal tract and drains). Both urine output (SPANOVA, $P = 0.02$) and total fluid output ($P = 0.027$) were significantly lower in the oedematous patients.

Figure 5.4.2 Daily average of urine output per kilogram body weight
 $P = 0.023$



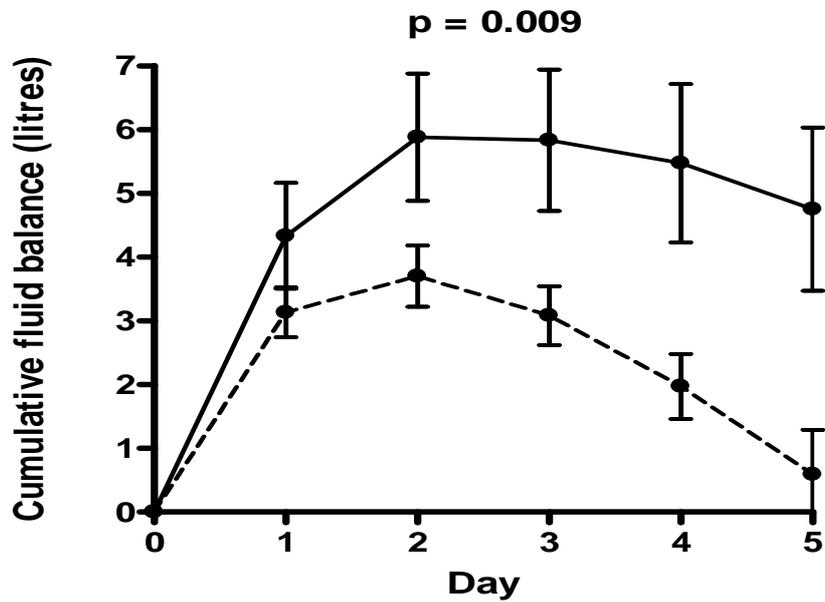
The average daily urine output/kg body weight in NOD *versus* OD patients. Average daily urine output/kg body is significantly greater in NOD (solid line) compared to OD (broken line) patients (SPANOVA; $P = 0.023$)

Table 5.4.3 Average urine output per kilogram body weight in OD *versus* NOD patients (Longitudinal study)

	Non-oedematous (N=15)	Oedematous (N=11)	p Value (Unadjusted for age)	p Value (Adjusted for age)
Age (years): (mean (s.d.))	64 (12)	74.3 (10.5)	0.029 ^a	
Average output/kg body weight over 5 days (mls/kg): mean (s.e.m.)	40.5(3.7)	29.4(2.3)	0.023 ^b	0.116 ^c
Average output/kg body weight over 3 days (mls/kg): mean (s.e.m.)	35.2(3.3)	24.5(2.4)	0.018 ^b	0.032 ^c

a= Man-Whitney U Test; b = SPANOVA; c = Rank ANCOVA

Figure 5.4.3 Cumulative fluid balance in patients with/without oedema over five days post surgery



Daily cumulative fluid balance following surgery (mean \pm s.e.m. (Litres)). The overall fluid balance in oedematous (solid line) was significantly more positive than in the non-oedematous patients (dashed line) (SPANOVA; $P = 0.009$).

5.4.2 Longitudinal study - Oedema, plasma albumin and total protein concentrations

Data from all 30 patients involved in the longitudinal study were analysed with comparisons made with comparisons made with the control population in an attempt to gain insights into the influence of surgery on plasma albumin and protein concentrations.

5.4.2.1 Longitudinal study - Changes in plasma albumin and total protein concentrations in OD and NOD patients

There was no difference in the preoperative concentration of plasma albumin in the OD compared to NOD patients 36.5 (3.5) *versus* 38.3 (5.1), $P = 0.301$ but when compared to plasma albumin concentration in the reference population, the preoperative plasma albumin in the OD group was significantly lower 36.5 (3.5) *versus* 41.2 (2.3), $P = 0.004$. There was no difference in the plasma albumin concentration between reference population and NOD group *Table 5.4.4*.

The data for intraoperative blood loss and the use of colloids and blood perioperatively was incomplete. The intraoperative blood loss was (mean (s.e.m.)) 1.09 (0.46) *versus* 0.94 (0.17) Litres, $P = 0.71$ and volume of colloid used perioperatively was 1.00 (0.39) *versus* 0.63 (0.16) Litres, $P = 0.32$ in the OD compared to NOD patients respectively.

Marked significant changes occurred in the concentrations of plasma albumin and total plasma protein with time in all the post surgical patients as a group. These changes were evident within twenty-four hours following surgery *Figures 5.4.4a and b*. There was a fall in the plasma albumin and total protein concentrations in NOD and OD patients which was more marked on the first day and showed a tendency towards the pre-operative levels by the fifth day post-surgery. The fall in plasma albumin concentration from

preoperative levels in the NOD and OD patients was 29 *versus* 34 percent on day 1; and 22 *versus* 28 percent on day 5; and for total protein concentration it was 25 *versus* 30 percent on day 1 and 15 *versus* 20 percent on day 5 *Figures 5.4.4a and b*. Although significant changes in the concentrations of albumin and total protein occurred on all the days patients were evaluated after surgery the pattern of changes observed in the NOD and OD patients were similar, $P = 0.094$ and 0.376 (SPANOVA) for plasma albumin and total protein respectively.

The analysis of the above data did not examine other components of total plasma protein such as fibrinogen and macroglobulins. The data shows that there is a universal fall in plasma albumin levels after surgical trauma. The pattern of this fall is similar in patients who develop oedema after major surgery compared to those who don't. This finding in combination with findings from other studies where albumin substitution did not prevent the occurrence of peripheral oedema after surgical trauma (Zetterstrom & Hedstrand 1981) or reduce microvascular hyper-permeability would suggest that the changes in plasma albumin concentration alone are not sufficient to explain the occurrence of oedema after surgery.

Table 5.4.4 Age, haematological and biochemistry measurements in volunteers and NOD and OD patients (Longitudinal study)

	Volunteers	Non-Oedematous	Oedematous	p value*	p value**
Pilot group	(n=21)	(n=18)	(n=12)		
Age (years)	56.4(15.0)	62.6(14.5)	74.3(9.4)	0.012 ^a	
Neutrophil	4.38(2.99)	4.38(1.3)	3.98(1.9)	0.515 ^a	
WBC	6.76(3.13)	6.56(1.5)	6.20(2.6)	0.635 ^a	
Haemoglobin g/dL	14.3(1.05)	13.5(1.8)	12.9(1.7)	0.381 ^a	0.030 ^b
GSH (mmol/L)	1.19(0.16)	1.19(0.18)	1.14(0.16)	0.492 ^a	
GSH/Haemoglobin (µmol/gHb)	8.30(1.03)	8.85(1.42)	9.02(1.89)	0.791 ^a	
Albumin (g/L)	41.2(2.27)	38.3(5.1)	36.5(3.5)	0.301 ^a	0.004 ^b
Total Protein (g/L)	72.63(3.64)	73.9(5.6)	73.2(5.8)	0.716 ^a	
C-reactive protein (mg/L): median(range)	2.0(1.9-9.9)	4.3(2.0-61.4)	11.4(2.0-72.0)	0.115 ^c	0.005 ^d

Values are expressed as mean (s.d.) except where indicated. * = (^a = t- test, ^c = Mann Whitney U NOD vs OD patients), ** = (^b =ANOVA-test Oedema patients vs volunteers, ^d = Kruskal-Wallis test)

Changes in plasma albumin and total protein concentrations in patients with/without oedema

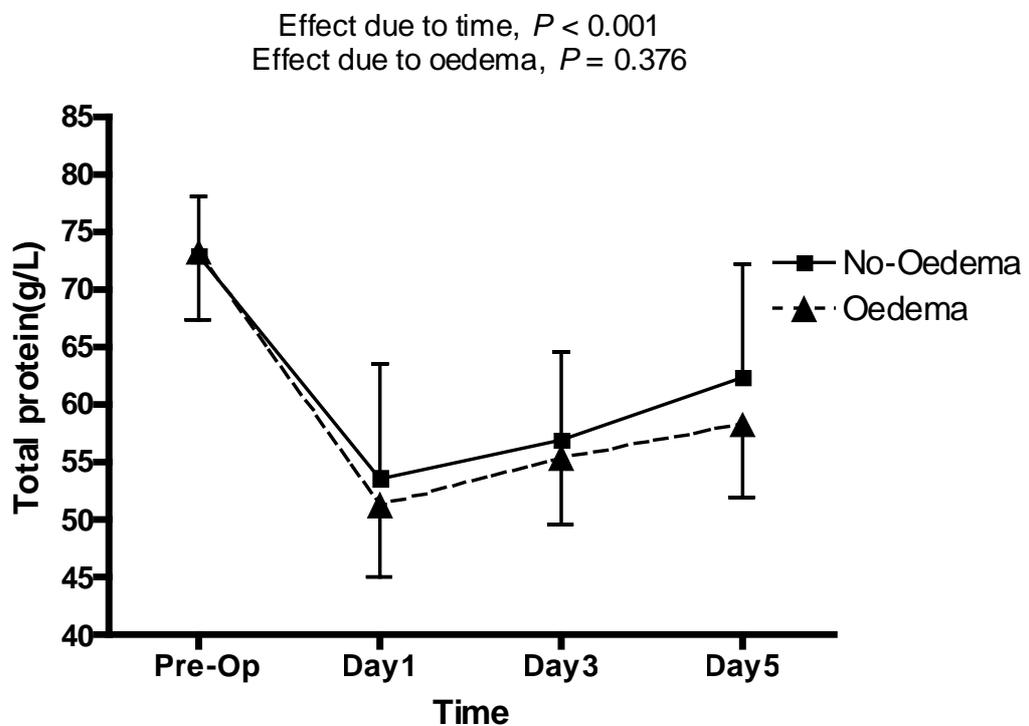
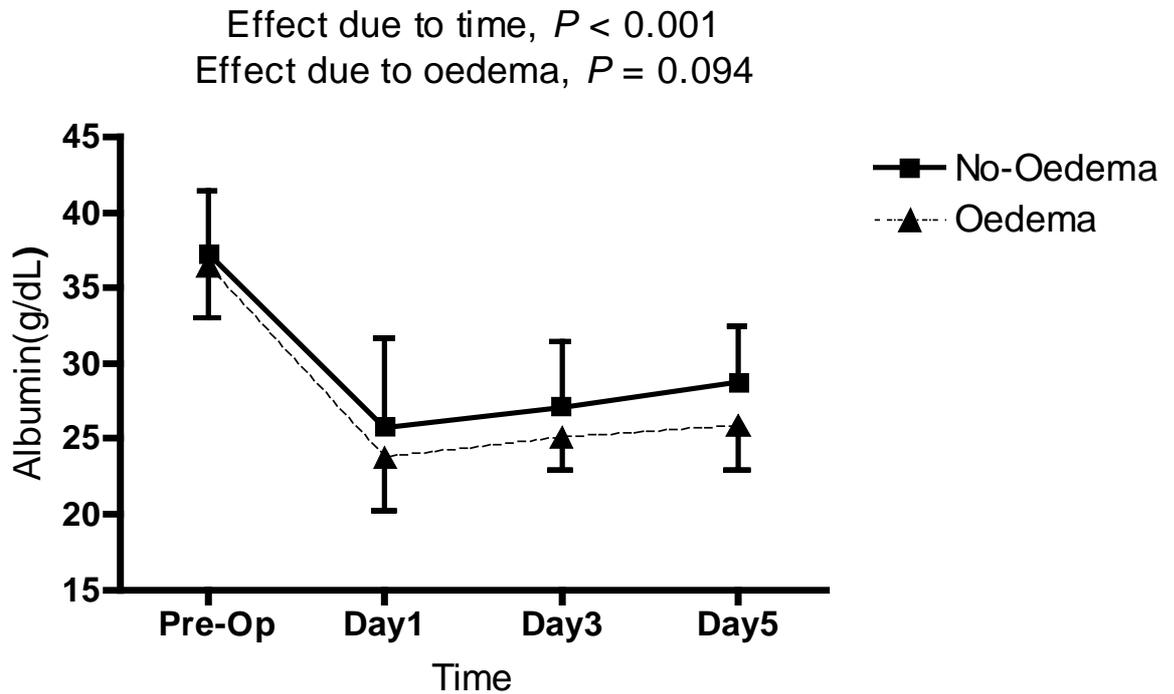


Figure 5.4.4(a) Upper chart – The changes in plasma albumin concentration in NOD (unbroken line) and OD (broken line) patients over the initial five days post surgery. (b) – The changes in plasma total protein concentration in NOD and OD patients over the initial five days post surgery.

5.4.3 Longitudinal study - Oedema and inflammatory responses as measured by C-reactive protein (CRP) in OD and NOD patients

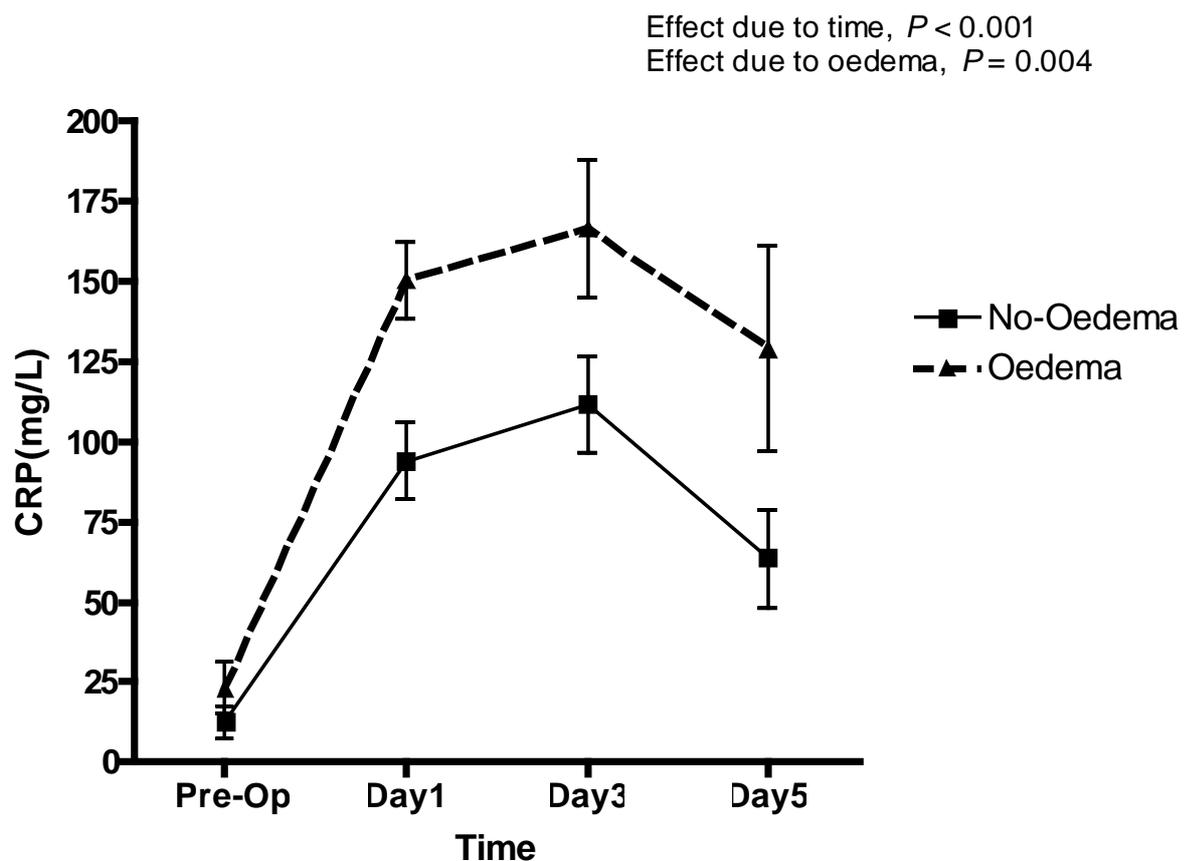
Data for the CRP analysis was obtained from all patients in the longitudinal study already described in 5.2.2. CRP was measured as a marker of the inflammatory response to surgical trauma. The use of CRP for this purpose is strongly supported by medical literature (Fischer et al. 1976, Goransson et al. 1998)

Prior to undergoing major abdominal surgery the median (range) of levels of CRP in plasma in NOD compared to OD patients was 4.3 (2.0-61.4) *versus* 11.4 (2.0-72.0) mg/L, $P = 0.115$ (Mann Whitney U) *Table 5.4.4*. After surgery, there was an increase in the levels of CRP and this peaked by 72 hours in both groups of patients. Comparing the preoperative plasma levels of CRP in the NOD and OD patients with the reference population revealed that the preoperative CRP concentration in the OD patients was significantly greater than the CRP concentration of the reference population, 11.4 (2.0 – 72.0) *versus* 2.0 (1.9 – 9.9) mg/L, $P = 0.005$, whilst there was no observed difference in the preoperative CRP concentration of the NOD compared to the reference population

Although the patterns of response of CRP following surgery were similar, there was a main effect due to time in CRP levels over the initial five days post-surgery, $P < 0.001$ and the magnitude of the rise in CRP levels was greater in the patients with oedema compared to the non-oedema patients, $P = 0.004$ *Figure 5.4.5* The average concentration of CRP over the postoperative period (Days 1-5) was significantly greater in the OD compared to the NOD group of patients mean (s.d) 148 (54.1) *versus* 89.6 (43.8) mg/L, $P = 0.006$ (Student t test). Age adjustment was made with Rank ANCOVA and the difference in concentration of CRP remained significant; $P = 0.004$.

Whilst there is no data on the duration of surgical procedures, there was no difference in the distribution of surgical procedures in both groups and an assumption is made that the degree of surgical trauma in both groups is similar. These results above show that although a similar pattern of inflammatory response to surgical trauma was observed in both groups of patients the intensity of this response was greater in the OD compared to the NOD group of patients. Secondly, comparison with the controls showed that patients in the OD group demonstrated raised inflammatory markers suggestive of an inflammatory response to their disease before surgery.

Figure 5.4.5 Changes in the concentration of C-reactive protein (CRP) in patients with/without oedema



Concentration in OD (broken lines) compared to NOD (straight line) patients preoperatively and on days 1, 3 and 5 after surgery.

5.4.4 Longitudinal study - Oedema and changes in antioxidant capacity (Whole blood GSH)

To examine the effect of antioxidant capacity as a potential aetiological factor for postoperative formation the concentration of GSH in whole blood was analysed before and after surgery. Complete data set over the duration of study was obtained in 26 (87%) of the longitudinal study patients.

The preoperative haemoglobin concentration in patients who subsequently developed oedema (OD group) following surgery was lower compared to controls 12.9 (1.7) *versus* 14.3 (1.0) g/dL, $P = 0.030$ *Table 5.4.4*, but this however, did not cause any significant difference in preoperative GSH concentration of the OD patients compared to the controls 1.14 (0.16) *versus* 1.19 (0.16), $P = \text{NS}$.

There was a significant variation in the concentration of whole blood reduced glutathione (GSH) with time. This was characterised by significant fall in GSH concentration within the first 24 hours following major surgery and a trend towards restitution to preoperative values by 72hours in the NOD patients *Figure 5.4.6*. However in the OD patients the fall in GSH concentration continued until day 3 with signs of recovery of GSH concentration appearing by day 5. The delay in the recovery of GSH to preoperative levels in the OD group of patients however did not produce significant effect over time.

Since plasma contains less than two percent of the GSH content of whole blood we expressed GSH concentration per gram of haemoglobin to allow adjustments for the effects of blood loss and haemodilution following major surgery. The overall pattern of the change in the concentration of GSH in the OD and NOD was identical after such adjustments were made. The concentration of GSH per gram of haemoglobin remained constant in both patient groups and showed no variation with time *Figure 5.4.7*.

The identical pattern of change in GSH levels in oedematous and non-oedematous patients in this study suggests that GSH as a marker of antioxidant capacity is not an aetiological factor for oedema formation in the postoperative period. A limitation of this study is that the other components of glutathione metabolism were not measured. These include oxidised GSH (GSSG), plasma and red blood cell glutathione and the enzymes that regulate synthesis and catabolism of glutathione.

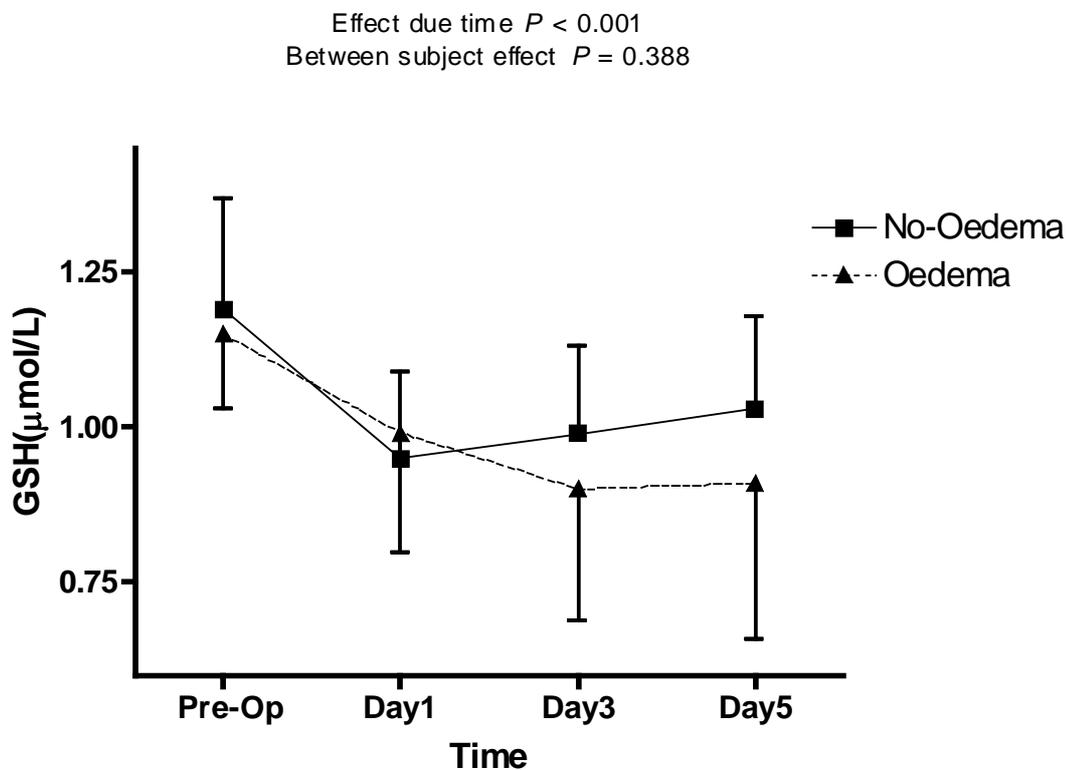


Figure 5.4.6 Changes in the concentration of GSH in OD and NOD patients

The changes in the concentration of GSH in NOD (straight line) compared to OD (broken line) over days 1, 3, and 5 post-surgery.

Effect due to time, *P* NS
Between subject effect, *P* NS

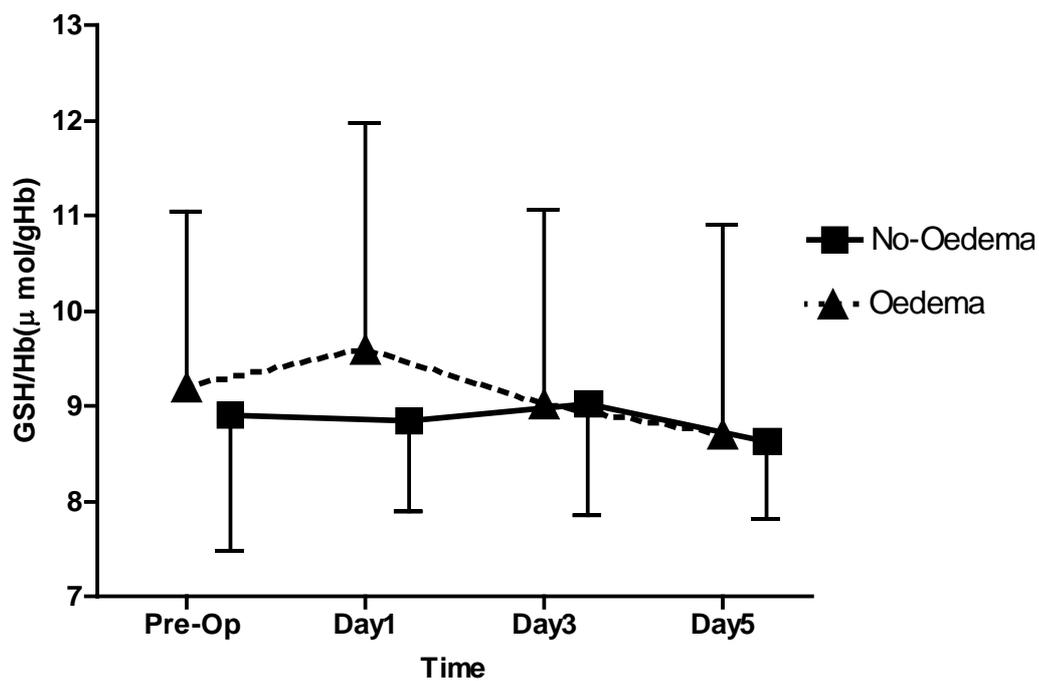


Figure 5.4.7 Changes in the concentration of GSH/Hb in OD and NOD patients

The changes in the levels of GSH/Hb in NOD (solid line) compared to OD (broken line) pre-op and on days 1, 3 and 5 post-surgery

5.5 Combined population - Oedema and postoperative clinical outcomes

This section compared clinical outcomes in OD and NOD groups of patients in order to ascertain the impact of post-surgical oedema on clinical outcomes after major surgery. The analyses in this section were undertaken on the combined population of patients from the pilot and longitudinal studies

The markers of clinical outcomes evaluated are listed below

- Recovery of gut function – number of days to tolerate solid hospital food and the number of days taken to achieve first bowel movement.
- Length of stay (LOS) in hospital.
- The incidence of complications
 - Cardiopulmonary complications
 - Infective complications

5.5.1 Combined population – Subjects and surgical procedures

38 patients were eligible for inclusion in this analysis. There were 18 NOD (Male:Female 11:7) and 20 OD (Male:Female 10:10) patients. The types of surgical procedures undertaken in both groups are shown in *Table 5.5.1*. The physical characteristics of patients in each group are shown in *Tables 5.5.2*. The type of surgical procedures undertaken, pre-operative BMI, ASA, albumin and total protein concentrations in both groups showed no statistical difference. The patients in the OD group were significantly older than the NOD patients 73.5 (8.7) *versus* 62.6 (14.5) years, $P = 0.010$, and age was related to the subsequent development of oedema (odds ratio (OR) 1.087 (95 per cent confidence interval (c.i.) 1.016 -1.163; $P = 0.016$) *Table 5.5.2*.

Table 5.5.1 Combined population – Surgical procedures in OD and NOD patients

	Non-Oedematous (N= 18)	Oedematous (N=20)	Total (N=38)
Resection of the colon (Malignant)	12	12	24
Resection of the colon (Benign)	1	1	2
Other cancers			
Cancer of the liver	1	0	1
Cancer of the stomach	0	2	2
Cancer of the bladder	0	1	1
Cancer of the prostate	2	0	2
Other diagnoses			
Small bowel resection	1	3	4
Pancreatic resection	1	1	2

Table 5.5.2 Combined population - Physical characteristics and laboratory findings in the non-oedematous and oedematous groups

	Non-Oedematous	Oedematous	P Value
All patients			
Number (n): (Male/Female)	18 (11/7)	20 (10/10)	0.547 ^a
Age (years): (mean (s.d.))	62.6 (14.5)	73.5 (8.7)	0.010 ^b
BMI (kgm ⁻²): mean (s.d)	26.7 (6.1)	26.0 (3.3)	0.656 ^b
ASA Score (n for scores 1, 2, 3)	6, 9, 3	2, 13, 5	0.209 ^a
1	6	2	
2	9	13	
3	3	5	0.209 ^a
Pre-op Albumin (g/L): mean (sd)	38.3 (5.1)	34.4(6.7)	0.059 ^b
Pre-op Total Protein (g/L): mean (s.d)	73.9(5.6)	59.7(7.3)	<0.001 ^b
Pre-op CRP (mg/L): median (range)	3.9 (2.0 – 61.4)	8.1 (2.0 -72.0)	0.099 ^c

a = Chi squared; b = Student's t test; c = Man-Whitney U Test

5.5.2 Combined population - The recovery of gut function in OD *versus* NOD patients

The times taken to tolerate solid food and achieve first bowel movement were significantly prolonged in the OD compared to the NOD patients. Time to tolerate solid food remained significantly prolonged in the OD group after statistical adjustments for the difference in age. This however was not the case for the time taken to achieve first bowel movement. The median (range) number of days to tolerate solid and achieve first bowel movement following surgery, with age adjusted *P* values in parenthesis for patients with OD compared to the NOD patients are: (6 (5-25) *versus* 5 (1-8) days, *P* = 0.001 (0.030)) and (6 (3-17) *versus* 5 (1-13) days, *P* = 0.020 (0.124)) respectively *Table 5.5.3*.

5.5.3 Combined population - The length of stay (LOS) in hospital in OD *versus* NOD patients

Oedema formation was associated with prolonged LOS in hospital when a comparison is made with the NOD patients with a median (range) of 9 (4-27) *versus* 17 (8-59) days, *P* = 0.001 (with correction for age difference *P* = 0.030) *Table 5.5.3*.

Table 5.5.3 Combined population - Clinical outcomes in NOD and OD groups

Outcomes	Non-Oedematous NOD	Oedematous OD	P value (Unadjusted for age)	P value (Adjusted for age)
	(N=18)	(N=20)		
No of days to tolerate solid food: median (range)	5(1-8)	6(5-25)	0.001 ^a	0.030 ^b
No of days to bowel movement: median (range)	5(1-13)	6(3-17)	0.020 ^a	0.124 ^b
No of days in Hospital: median (range)	9(4-27)	17(8-59)	0.001 ^a	0.03 ^b
Admission to High Dependency Unit: n/total (%)	4/18(22.2)	7/20(35)	0.389 ^c	0.433 ^c
Subjects with any complications: n/total (%)	4/18(22.2)	13/20(65)	0.011 ^c	0.018 ^c
Subjects with infective complications: n/total (%)	3/18(17)	9/20(45)	0.069 ^c	0.060 ^c
Subjects with cardiopulmonary complications: n/total (%)	1/18(6)	4/20(20)	0.217 ^c	0.359 ^c

a = Mann-Whitney U Test; b = Rank ANCOVA; c = binary logistic regression

5.5.4 Combined population - Infective complications in OD versus NOD patients

2 (11%) patients in the NOD group developed anastomotic leak and 1 (5.6%) had superficial wound infection after surgery. In the OD group, 2 (10%) patients developed intra abdominal collections. This was associated with and anastomotic leak in one patient. Another 2 (10%) of OD patients had superficial wound infection. The following major infective complications were observed in one patient each (5.0%), *C. difficile* diarrhoea infection, empyema thoracis, fungal oesophagitis, pneumonia and wound dehiscence *Table 5.5.4*. Although the total incidence of infective complications was higher in the oedematous patients compared to the non-oedematous patients but this was not statistically significant (45% vs. 17%, $P = 0.069$) *Table 5.5.3*.

Table 5.5.4 Combined population- Infective complications in OD and NOD patients

	Non-Oedematous (N= 18)	Oedematous (N=20)	Total (N=38)
No infective complication	15	11	26
Major Infective Complication			
Clostridium defficile diarrhoea		1	1
Empyema thoracis		1	1
Fungal Oesophagitis		1	1
Intra Abd Abscess + Anastomotic Disruption	1	1	2
Intra abdominal collection	1	1	2
Pneumonia		1	1
Wound Dehiscence		1	1
Minor Infective Complication			
Urinary tract infection		1	1
Wound infection	1	1	2
Subtotal (Infective complication)	3	9	12

5.5.5 Combined population - Cardiopulmonary complications in OD versus NOD patients

Cardiopulmonary complications were recorded in 4 of 20 (20%) oedematous patients. These were mainly acute cardiac related events. Only one patient in the non-oedematous group developed cardiopulmonary complication in the form of a deep venous thrombosis

Table 5.5.5.

Table 5.5.5 Combined population - Cardiopulmonary complications in OD and NOD patients

	Non-Oedematous (N= 18)	Oedematous (N=20)	Total (N=38)
No cardiopulmonary complication	17	16	33
Cardiopulmonary complications			
DVT	1	0	1
Left Ventricular Failure	0	1	1
Myocardial Infarction	0	2	2
Arrhythmias	0	1	1
Subtotal	1	4	5

5.5.6 Combined population - Oedema and post-surgical complications in OD and NOD patients

The overall combined incidence of infective and cardiopulmonary complications was significantly higher in the oedematous compared to the non-oedematous patients (65% vs. 22.2%, $P = 0.011$) and this significance was maintained with correction for age difference ($P = 0.018$)

When patients with cardiovascular complications were excluded the times to tolerate solid food, achieve first bowel movement and LOS were significantly prolonged in patients who developed oedema after abdominal surgery compared to patients in whom oedema was not observed. The observed difference in these parameters was maintained after adjusting for the difference in age in the two groups of patients. When patients with cardiovascular or major infective complications are excluded the time to tolerate solid food and LOS were significantly prolonged in oedematous patients compared to the non-oedematous patients and remained so after adjusting for age *Table 5.5.6*.

Table 5.5.6 Combined population - Clinical outcomes in OD versus NOD groups after exclusion of patients with major infective and/or cardiopulmonary complications

	Non-Oedematous	Oedematous	P value ^a	P value ^b
	NOD	OD		(adjusted for age)
Major infective complications are excluded	N = 16	N = 13		
No of days to tolerate solid food: median (range)	5(1-7)	6(5-25)	0.015	0.103 ^b
No of days to bowel movement: median (range)	4.5(1-13)	6(3-17)	0.1	0.479 ^b
No of days in Hospital: median (range)	8.5(4-27)	17(8-50)	0.001	0.02 ^b
Cardiopulmonary complications are excluded	N = 17	N = 16		
No of days to tolerate solid food: median (range)	5(1-8)	7(5-25)	0.0005	0.0005
No of days to bowel movement: median (range)	5(1-13)	7(3-17)	0.012	0.045
No of days in Hospital: median (range)	9(4-27)	17.5(12-47)	0.001	0.001
Major infective/cardiopulmonary complications excluded	N = 16	N = 8		
No of days to tolerate solid food: median (range)	5(1-7)	7(5-9)	0.006	0.030
No of days to bowel movement: median (range)	4.5(1-13)	6(3-17)	0.061	0.235
No of days in Hospital: median (range)	8.5(4-27)	17.5(12-22)	0.001	0.006

a = Man-Whitney U Test; b = Rank ANCOVA

Chapter 6

General discussion

In this chapter, the findings of the experimental work will be discussed in relation to the to the stated hypotheses of these studies. The chapter will be concluded by discussing the clinical implication of the findings from this project in the care of surgical patients and suggestion for the future.

6.1 The incidence of early post operative oedema in patients undergoing major abdominal surgery

Oedema is often seen in patients recovering from abdominal surgery on the surgical wards. Its incidence and impact on clinical recovery are uncertain. This study shows that development of oedema in patients undergoing major abdominal surgery is common, affecting 40 per cent of consecutive admissions for major abdominal surgery. Lowell and colleagues reported a similar incidence of fluid overload in post-surgical patients who were managed in an intensive care unit, although they did not indicate whether their patients were oedematous or not (Lowell et al. 1990). It is possible that the incidence of oedema may vary between centres, and may depend on the type of patients studied and surgery undertaken, the perioperative nutritional status of the patients, presence of complications, and attitude to fluid administration in the perioperative period.

The patients who developed oedema following major abdominal surgery were significantly older than those who did not. Age as an independent factor was significantly

associated with the risk of development of oedema (odds ratio (OR) 1.087 (95 per cent confidence interval (c.i.) 1.016 -1.163; $P=0.016$). The effect of age on tissue hydration and the relationship of total body water are at best debatable. Whilst some studies have reported no age related changes to the degree of hydration of fat free mass (Ritz 2000), other studies have reported that aging is associated with expansion of ECW in healthy individuals (Aloia et al. 1998; Silva et al. 2005). However, in disease states such as sepsis the prolonged over expansion of extracellular water in the elderly has been described (Cheng, Plank, & Hill 1998). The degree to which this factor alone contributed to oedema formation is not certain but this study did show it was not the only factor responsible for the finding of oedema in surgical patients and on its own did not explain the significant increase in poor clinical outcomes observed in this study. Furthermore it well known that age blunts the reserves with which the elderly can mount a response to critical illness (Watters & Bessey 1994) It is possible that these factors contributed to oedema formation in patients observed in this study

This study shows that the incidence of oedema in patients undergoing routine surgical procedures is high. It would appear that the relevance of this finding in surgical patients is not given the proper attention it deserves. The occurrence of oedema suggests faltering of the homeostatic mechanisms that regulate the movement of salt and water. It may reflect some limitation of the patients' reserve to cope with stress induced by surgical trauma and may be an early pointer to the patient with the potential to develop organ dysfunction and postoperative complications (Redl et al. 1993).

A limitation of this study is the small sample size, which was mainly due to limited time and manpower available for recruitment of patients. This made it impossible to match patients for age. It is possible that a bigger sample size may have demonstrated a difference in other preoperative factors such as the ASA score, BMI and the presence of raised inflammatory markers that could indicate a propensity to become oedematous.

6.2 Oedema, distribution of TBW and bioelectric impedance analysis

Measuring the changes in body weight during the early post-operative period can accurately indicate changes in fluid balance, but such measurements can be difficult or impossible to undertake as patients are often too ill to move and specialised weighing equipments are not routinely available. Furthermore, such measurements do not reflect the distribution of fluid within the body (Hannan et al. 1994). A further aim of this study was to assess the potential role of bioelectrical impedance in identifying fluid overload and monitoring distribution of TBW in oedematous and non-oedematous patients in the perioperative period.

The present study provides several insights into postoperative fluid disturbances. Firstly, the postoperative decrease in whole body bioelectrical impedance (Z) and the increase in the impedance quotient h^2/Z , which are explained by an increase in the water space (positive fluid balance), were well established before the clinical appearance of clinical oedema are more objective than clinical inspection of the patient for the presence of oedema. Furthermore, similar changes in the whole body impedance were observed postoperatively in the non-oedematous patients although they were less marked compared to the patients who became oedematous.

Secondly, both the absolute and fractional increase in ht^2/Z was greater at 5 kHz than at higher frequencies, with the result that the ratio Z_{200}/Z_5 also increased to near significant levels. At low frequencies the current does not penetrate through the cell membrane and therefore the measured resistance reflects that of extra-cellular fluid. As the frequency increases, there is increasing penetration of the current through the cell membrane so that the measured resistance reflects total body water. The increased ratio Z_{200}/Z_5 obtained from the analysed data in this study demonstrates that the extracellular

fluid compartment increased more than the intracellular fluid compartment. The significant preoperative value of this ratio suggests it may be possible to identify patients at risk of post-surgical oedema prior to surgery.

Thirdly, the oedematous group not only showed a greater increase in ht^2/Z than the non-oedematous group, but also demonstrated that the increase was greater for each litre of fluid accumulating within the body. The reason for this is not clear, but a possible explanation concerns differences in tissue impedance and differences in the distribution of retained fluid within the segments of the body. The trunk contributes only about ~10 % of the whole body resistance but contains a much greater proportion of body water (Bracco et al. 1996). Accumulation of fluid here makes a much smaller difference to whole body impedance than accumulation of the same amount of fluid in other thinner body segments, which offer greater resistance to the flow of current. Thus accumulation of fluid in the trunk, for example from sequestration of fluid into the gastrointestinal tract, would be expected to have little effect on whole body impedance (Cha et al. 1995). In contrast, accumulation of fluid within the limbs, which is known to have occurred in the oedematous patients, would be expected to have a greater effect on Z and ht^2/Z . This hypothesis would be tested by undertaking segmental measurements of impedance, which were not done in this study. Tatara and Tsuzaki compared estimates of ECW from segmental BIA and whole body BIA in patients undergoing gastrointestinal surgery. They were able to show a significant underestimation of ECW with whole body BIA. ECW prediction with segmental BIA was similar with estimate from net fluid balance and this was attributed to marked changes in impedance contributed by trunk with segmental BIA (Tatara & Tsuzaki 1998).

Finally, this study demonstrated a discrepancy between measured fluid balance and that predicted by the bioelectric impedance analysis in the oedematous and non-oedematous patients, which progressively increased as the fluid balance became more

positive. The study draws attention to the potential problems of extrapolations that use equations derived in healthy subjects in patients with disease states associated with fluid disturbance.

6.3 Potential aetiological factors for early postoperative oedema formation

6.3.1 Oedema after major abdominal surgery: fluid intake or fluid output?

Patients frequently develop oedema after surgery but it is uncertain whether oedematous patients receive more fluid or are less able to excrete a fluid load compared to patients without oedema. One of the aims of the study was to provide some answers to this question.

Although this study was observational, analysis of the data obtained strongly suggests that patients who develop oedema after major surgery have an impaired ability to excrete administered fluid load. This impaired ability to excrete fluid is reflected in the low daily average urine output per kilogram body weight observed over the initial five days post-surgery in the oedema patients compared to the patients who remained oedema free, 29.4 (2.3) *versus* 40.5 (3.7) mls/kg, $P = 0.023$. Over the initial five days after surgery, this impaired ability to excrete administered water was related to age. Further analysis of the data however showed that over the initial three days after surgery the impaired urine output was independent of age *Table 5.4.3*. There was no attempt to control the amount of fluid administered in this study and the administered fluid volume reflected typical practice. This study highlights that certain patients may be vulnerable to oedema formation as a consequence of the volume of administered fluid in the perioperative

period. The critical volume of intake at which oedema becomes obvious may vary from one individual to another and although the determination of such volume has not been the objective of this study, the need to individualize perioperative fluid administration to optimize outcome is further supported by the findings from this study (Rosenthal & Rosenthal 1999).

The reduced ability to excrete administered load of fluid seen in the oedematous patients in this study may be partly due to a combination of age related blunting of functional reserves and the over expansion of ECW that has been reported in septic elderly patients (Watters & Bessey 1994; Cheng et al. 1998). It has also been shown that the magnitude of the inflammatory response to surgical injury may have a direct relationship to the degree of organ dysfunction seen in surgical patients (Bruce et al. 1999). Furthermore, certain genotypic expressions of interleukin-10 (IL-10) has been associated with increased multiple organ dysfunction syndrome (MODS) in multiple trauma patients (Schroder et al. 2004).

Fluid accumulation following surgical trauma is a recognised physiological response but pathological fluid accumulation is seen in critically ill and septic surgical patients with detrimental effects on organ function (Bergstrom et al. 1981; Plank et al. 1998). Although the type of crystalloids administered was not reported in this study, several studies have reported an association between fluid overload especially with salt containing fluids and oedematous post-surgical patients (Gil et al. 1997; Tindall & Clark 1981).

This study shows that significant fluid accumulation occurs in the ward based surgical patients some of whom may not reveal any signs of fluid overload. Such fluid accumulation may cause major organ dysfunction such as impaired gastrointestinal absorption, hepatocellular derangement and reduced myocardial contractility. It may also delay wound healing and increase the chances of wound infection (Marx 2003).

6.3.2 Oedema and plasma albumin and total protein

In the pilot study, the OD patients demonstrated significantly lower concentrations of plasma albumin and total protein compared to the NOD patients. The assessment of the patients in both groups had occurred on different days after surgery making it difficult to make definite interpretations of this finding, which was further examined, in the longitudinal study. Marked changes occurred in the concentrations of plasma albumin and total plasma protein with time in the longitudinal study population as a group and these changes were evident within twenty-four hours following surgery *Figures 5.4.4a and b*. There was however no difference in the pattern of changes observed in the concentrations of plasma albumin and total protein in the patients who remained consistently oedema free and patients who developed oedema after undergoing major abdominal surgery.

Assuming no problem with hepatic synthesis, the early fall in plasma albumin and total protein concentrations observed in this study is most likely to be attributable to either haemodilution secondary to the effects of resuscitation fluids in the perioperative period, or an increase in capillary permeability and trans-membrane capillary loss that is known to occur as early as six hours following uncomplicated surgery. The normal trans-capillary loss has been estimated to be approximately 6 gram per hour or 4-5% of the intravascular albumin mass but in critically ill patients, this transcapillary escape rate of albumin may increase to up 20% per hour (Sun et al. 1993; Fleck et al. 1985). In previous studies, the fall in the concentration of the plasma albumin within the initial 24 hours of trauma has been shown in previous studies to be due to a prompt increase in microvascular permeability (Myers et al. 1984). The trans-capillary escape of albumin and increase in membrane permeability are not altered by exogenous albumin administration and there is evidence to suggest that it is cytokine mediated as part of the acute-phase response (Margaron & Soni 2002; Fleck 1989).

Loss of albumin could also have occurred from haemorrhage and exudation from large wounds (Robarts 1979). Such loss occurs mainly as loss of plasma volume during abdominal surgery and it is often underestimated from blood losses alone (Hoye et al. 1970)

The loss of albumin from changes in the synthesis and catabolism of albumin during illness in humans is more complex. In animal studies, a reduction in the synthesis of albumin occurs during injury and inflammation, an action mediated by TNF-alpha and interleukins 6 (IL-6) both of which depress albumin gene transcription (Ballantyne et al. 1973; Milland et al 1990). In humans however the situation is not so straightforward. In patients with septic shock the synthesis of albumin can vary enormously, from very decreased to significantly increased (Fleck et al. 1984; Blunt et al. 1998). Albumin catabolism is increased during the stress response augmented by raised levels of corticosteroids, which also increases the catabolism of other proteins. The absolute fractional degradation rate of albumin may be reduced in the presence of low albumin concentrations to a rate that is equivalent to normal or may be so reduced (Whicher et al. 1987)

Although this study did not explore the mechanisms of albumin and protein loss in surgical patients, the finding of a similar pattern of change in the concentrations of plasma albumin and total protein in oedematous and non-oedematous patients suggests that the changes in the circulating concentrations of plasma proteins is not significant aetiologically for oedema formation in the early postoperative period.

6.3.3 Oedema, CRP and inflammatory response to surgery

The changes in the serum concentrations of acute phase proteins (APPs) such as CRP have been demonstrated to be useful in monitoring complications such as infection or sepsis after surgery or trauma, and predicting the clinical outcomes after surgical treatment of malignant and other diseases (Fischer et al. 1976; Fujita et al. 1999). Observation of the time course of the changes in CRP concentration after surgery showed that peak concentration is achieved in 48-72 hours and levels then fall back to preoperative levels by the fifth day (Colley et al. 1983). The changes in CRP concentration in this study are in keeping with this. Peak levels of CRP were recorded on the third day and a downward trend in CRP levels on day five in both patient groups.

The finding in this study of significant increase the magnitude of CRP levels in the oedematous compared to the non-oedematous patients suggest either amplification of a normal response to surgical trauma or an increase in the stress inducing stimuli experienced by this group of patients. Both patient groups however showed a downward trend in CRP levels after the third day. There may be several explanations for the observation in this study.

The OD group of patients were older than the patients who remained oedema free but the increase in CRP production did not show a relationship to age. The stress response to surgery has been shown not to be age dependent although the recovery of serum protein levels may be delayed in the elderly (Puskarich-May et al. 1996).

Elevation in the levels of CRP is dependent on type of surgery undertaken and the degree of trauma. It is not affected by use of opioids, type of anaesthesia or the use of epidurals (Brewster et al. 1994; Brix-Christensen et al. 1998 and Moore et al. 1994). The type of surgical procedures and arguably the degree of trauma in both groups of patients used in this analysis was similar and so was unlikely to account for the pattern observed.

Although the concentration of APRs such as CRP may vary as a non-specific metabolic response to surgical stress, monitoring their levels has value during critical illness as markers of acute metabolic stress (Dickson et al. 1987). The magnitude of the CRP response in the OD group may reflect the significantly high incidence of infective and cardiopulmonary complications observed in the group that could have combined to act as a continued source of stimulus for continued reprioritization of hepatic acute phase plasma protein release (Sganga et al. 1985)

It is also possible that the increased inflammatory response observed in the oedematous patients in this study may reflect triggering of the inflammatory stimulus before surgery. This preoperative inflammatory response is suggested by the low preoperative albumin levels in the patients who subsequently developed oedema after surgery compared to the reference population 36.5 (3.5) *versus* 41.2 (2.3), $P = 0.004$. This finding is in keeping with findings from other studies that have identified low albumin and raised levels of inflammatory markers including CRP as evidence of preoperative inflammatory response to the surgical disease (Bocsi et al. 2002; Gibbs et al. 1999). The raised inflammatory markers were also used as indicators of postoperative complications (Goransson et al. 1998; Haupt et al. 1995).

Furthermore, it is now known that certain genomic polymorphisms in the genes coding for some cytokines are associated with adverse outcomes in multiple trauma patients. Examples of such phenotypic expressions have been identified within the TNF-alpha locus and interleukins-10 (IL10). These expressions are associated with increased production of proinflammatory cytokines when such individuals are exposed to trauma and this finding is positively correlated with high incidence of multiple organ dysfunction syndrome (MODS), sepsis and mortality (Schroder et al. 2004; Sherry et al. 1996)

6.3.4 Oedema and antioxidant capacity (Whole blood GSH)

An accelerated production of free radicals from damaged tissues following surgical trauma and critical illness has been well documented (Bulkley 1983; Del Maestro et al. 1980).

Glutathione plays a key role in the body's overall antioxidant defence system. The liver is the central organ in glutathione metabolism and plasma and erythrocyte concentrations are thought to reflect the synthetic capacity of the liver. It has been suggested that whole blood GSH provided a different and possibly more accurate picture of the glutathione metabolic status than plasma GSH (Dass et al. 1992).

A fall in the concentration of GSH was observed in this study in patients with and without oedema within the initial 24 hours after surgery with a trend to return to the preoperative levels by the third day in patients without oedema. Although there was a delay in GSH returning to preoperative levels in the patients with oedema after surgery but this was not significant. A 40% fall in skeletal muscle GSH, a 20% fall in plasma GSH and no change in whole blood GSH has been reported in a small series of patients undergoing elective surgery (Luo et al. 1996). It was suggested that a decreased liver output and or increased organ uptake of GSH might be responsible for this observations although study of the amino acid pattern in skeletal muscles after major trauma showed that there was enough substrates available intracellularly to maintain the production of GSH. The observed changes in the concentration of GSH in the early postoperative period in this study can be explained to be due to the effects of blood loss and haemodilution (Robarts et al. 1979; Robarts et al. 1979).

The finding of a steady concentration of GSH after the effects of blood loss and haemodilution were taking into consideration gives supportive evidence to previous suggestions that whole blood GSH is tightly regulated at the expense of tissue GSH. It would appear that maintaining a steady level of circulating GSH may have implications for the inter organ transport of GSH. Secondly all the patients in this study remained

essentially metabolically stable during the duration of their hospital stay and were not exposed to significant oxidative stress to deplete liver stores of GSH (Robinson et al. 1992).

The finding in this study of similar changes in GSH concentration of oedematous and non-oedematous patients raises the question of what the role of GSH in whole blood is as an aetiological factor for postoperative oedema? The oedematous patients in this study demonstrated an increased inflammatory response to surgical stress that may have been accompanied by increased free radical generation but was not reflected in the GSH levels. The role of oxidative injury in altering membrane permeability has been extensively demonstrated in other studies (Del Maestro et al. 1981; Korthuis et al. 1985 and Ley & Arfors 1982). Most of these studies explored GSH in surgical patients who were septic or critically ill and demonstrated significant decline in tissue concentration of GSH as opposed to the fairly metabolically patients observed in the studies undertaken for this project (Lyons et al. 2001; Hammarqvist et al. 1997).

This study has some limitations. The absence of tissue (such as muscle) GSH, plasma GSH and oxidised glutathione (GSSG) did not allow for a full picture of GSH metabolism in these patients to be appreciated. It is possible that the small size of the population studied may be an additional limiting factor for not observing a difference in GSH after surgery in the oedematous and non-oedematous patients.

6.4 The impact of early postoperative oedema on clinical outcomes

Having established the incidence of oedema and the potential causes in ward-based patients undergoing surgical procedures the next step in the outline of this study was to examine the relationship between post-surgical oedema and clinical outcomes.

A difficulty encountered during this study was the inability to weigh patients on a regular basis in the immediate postoperative period that made it impossible to correlate oedema with weight gain. Nevertheless, the presence or absence of oedema was clear enough to permit conclusions on the relationship between oedema and clinical outcomes.

The most important finding of this study is that the presence of oedema was associated with slower post-operative recovery of gastrointestinal function (delays in opening bowels and toleration of solid food), significantly increased the combined cardiopulmonary and infective complications and longer length of hospital stay. These observations raise three important questions –

- Firstly, is the presence of oedema per se, the cause of the slower post-operative recovery observed in the oedematous patients?
- Secondly, does the presence of cardiopulmonary and infective complications predispose to oedema formation and/or delay in clinical recovery?
- Thirdly, is the older age of the oedematous patients responsible for the prolonged recovery of gut function and length of hospital stay?

To answer these questions, the data on clinical outcomes were further analysed after excluding patients with evident infective and cardiovascular complications. These showed that recovery remained slower in the oedematous compared to non-oedematous group. Similarly, the apparent effect of oedema per se remained after controlling for the age difference between the OD and NOD groups, and when patients with any complications were included or excluded in the analysis.

Although this study was not specifically designed to establish causality, the data therefore suggest that oedema could be involved in a causal chain leading to delayed clinical recovery, especially since plausible mechanisms exist to explain the findings. For example, fluid overload in experimental animals and in humans delayed gastrointestinal motility and gastric emptying (Barden et al. 1938; Lobo et al. 1999). The function of vital organs such as the hepatic and respiratory system, are disturbed by the presence of oedema (Hill 1988; Arieff 1999). Wound healing in oedematous tissues is also delayed and the tissues are more susceptible to bacterial infection as the immune response in such tissues is altered ((Pai & Hunt 1972; Hohn et al. 1976). Furthermore, a recent randomised controlled trial demonstrated that restricting fluid and salt intake after colorectal surgery to no more than 2 litres and 77 mmol of sodium per day (the intake in the control group was based on routine practice of 3 litres or more per day as observed in this study) significantly reduced postoperative gastric emptying time, time to tolerate food, infective complications, and length of hospital stay (Lobo et al. 2002). Unfortunately the presence of oedema was not reported in that study and therefore it is not clear if oedema was more common feature in the control group, and if so, whether more complications occurred in those who developed oedema.

This study supports the findings of other studies where oedema was reported in surgical patients with septic complications (Lobo et al. 1999), extreme haemodilution delayed gastrointestinal recovery (Mecray et al. 1937), and the treatment of fluid overloaded surgical patients included water and salt restriction (Frost et al. 2001; Gil et al. 1997). It draws a strong inference to the possibility of a direct relationship between postoperative oedema and adverse outcomes and calls for further large-scale studies.

6.5 Conclusions and implications of findings

- The first part of the hypothesis of this study was to demonstrate that the incidence of postoperative oedema in patients undergoing routine major abdominal surgery was high. This study shows that oedema occurs in forty percent of patients admitted for routine abdominal surgical procedures.
- A valuable finding of this study is that the use of simple, non-invasive bedside bioimpedance analysis before surgery has the potential to identify patients with the propensity to develop oedema after major abdominal surgical procedures and can be used to monitor such patients. The use of BIA was more sensitive than clinical observation alone in monitoring changes in total body water body water and detecting abnormal fluid accumulation, which it did before clinical signs became apparent.
- The third part of the hypothesis of this study was to demonstrate that factors other than the circulating levels of albumin and total protein were more significant contributors to postoperative oedema formation. The factors examined were, fluid intake and output, plasma protein levels, the intensity of the inflammatory response to surgical trauma and levels of reduced glutathione (GSH) as an antioxidant marker.
- Age was a single independent factor for oedema formation and it is possible that age related changes in the connective tissues and blunting of functional reserves may account for this finding.
- Another significant factor identified by this study is the impaired ability to excrete administered fluid volume. This is important as none of these patients showed any overt sign of organ failure and suggests that our present methods of assessing and monitoring patients after surgery may not be sufficient to identify patients at risk. Fluid administration in the perioperative operative period remains a complex area of surgical care and should be based on a monitored physiologic application of the

Starling principles of cardiac function to individualize therapy and optimise and tissue perfusion. Emphasis needs to be given to intraoperative fluid shifts resulting in hidden fluid loss (Rosenthal & Rosenthal 1999).

- The magnitude of the inflammatory response to surgical trauma was greater in the oedematous compared non-oedematous patients. This difference in inflammatory response to trauma was independent of age. There was evidence of an inflammatory response and abnormal fluid distribution prior to surgery in the patients who developed oedema. Such patterns have been shown in previous studies.
- The changes in circulating albumin, total protein and GSH levels did not appear to be contributory to postoperative oedema formation. Early postoperative changes in albumin concentration are mainly due to redistribution from increased membrane permeability, haemodilution and a complex interaction of synthesis and catabolism.
- Changes in GSH levels could be accounted for by blood loss in the early postoperative period. Corrected GSH levels were fairly constant in both patient groups. Whilst GSH plays a key in the body's repertoire of antioxidant defence mechanisms it is by no means representative of the body's entire antioxidant defence. Several protective pathways exist that also play vital roles against oxidative stress. This include vitamin C and E (Richard et al. 1990), xanthine oxidase which is known to reduce leukocyte endothelial interaction and superoxide dismutase which has been shown to reduce MODS, length of stay in ICU and reduction in of CRP levels when administered to multiple trauma patients (Redl, et al. 1993; Marzi et al. 1993).
- Early postoperative oedema is an independent factor for delayed clinical recovery in surgical patients by mechanisms that require further studies.

The aetiological factors for postoperative formation are multifactorial and their interaction is complex. The metabolic response to injury is modulated by a variety of neuro-humeral mechanisms that are not fully understood but which have as their goal, providing the individual the best chance of survival. They include neurotransmitters, hormones and potent inflammatory stimulators cytokines that alter leukocyte function leading to free radical generation. The potential factors examined in this study represent only a fraction of the vast array of possible mechanisms for postoperative oedema formation. The secretion of vasoactive substances such catecholamines, pituitary and adrenocortical hormones alter the vascular tone and increase salt and water retention. The increased secretion of hormones such as cortisol and growth hormone enhance insulin resistance and promote the catabolism of proteins.

The observations in this study show a strong link between oedema and delayed recovery after major surgical procedures and highlight the gaps in our understanding of the causes and its clinical significance. The initial findings of this study offer a platform for the designing and conduct a future study that would more directly test the primary hypotheses outlined herein. This would in essence take the form of a prospective study within a well-characterised surgical population where ht^2/Z at a single or multiple frequencies would be determined alongside concurrent precise measures of fluid balance. By relating the change in ht^2/Z to clinical outcomes it would be possible to address the following questions:

1. Can ht^2/Z predict clinical outcome, earlier or more confidently than simple clinical observations of oedema?
2. Whether a care pathway that includes BIA measures would be associated with better clinical outcomes than routine conventional care without BIA?

3. There is particular merit in exploring preoperative evaluation of inflammatory, antioxidant status and distribution of total body water. Such studies would provide valuable information for the care of surgical patients.

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