

**Application of near infrared spectroscopy for evaluation of liver
oxygenation and function**

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This thesis is dedicated to my parents for their great help throughout my life and to my wife for her support during this period of my studies and to my sons Amir and Ahmed with my love to all of them.

Abstract

There is no readily available technique for measurement of liver oxygenation and function. Near infrared spectroscopy (NIRS) is a non-invasive technique originally developed for measuring cerebral oxygenation. This thesis has investigated its application for measurement of changes in hepatic tissue oxygenation and function. A new algorithm was developed for the hepatic application of NIRS. It was validated by comparing tissue oxygenation changes measured by NIRS with hepatic vein oxygenation in pigs. A significant correlation was found between hepatic vein oxygen partial pressure and hepatic oxyhaemoglobin (HbO_2), deoxyhaemoglobin (Hb), and cytochrome oxidase (Cyt Ox) ($r = 0.87$, -0.86 , and 0.91 , respectively). NIRS measurement of Cyt Ox changes as an indicator of the intracellular tissue oxygenation correlated ($r = 0.91$) with cellular adenosine triphosphate levels measured by magnetic resonance spectroscopy.

A possible role for NIRS in monitoring hepatic blood flow was investigated in pigs by correlating liver tissue oxygenation and blood volume measured by NIRS with hepatic artery and portal vein blood flow. Changes to liver blood inflow were immediately reflected by alterations in liver tissue oxygenation and blood volume. With hepatic artery occlusion there was a significant decrease in HbO_2 and total haemoglobin (HbT). Portal vein occlusion caused a greater reduction in HbO_2 and HbT with a significant decrease in Cyt Ox. Total occlusion caused a further a decrease in HbO_2 and HbT with a significant decrease in Cyt Ox.

To investigate the possible role of NIRS in evaluating liver dysfunction, a rabbit model of ischaemia reperfusion (I/R) injury was used. Changes in hepatic HbO₂ correlated with reduced flow in the hepatic microcirculation (HM) ($r = 0.94$). HbO₂ changes correlated with the hepatocellular injury as shown by serum ALT, AST, and LDH ($r = 0.89, 0.81$ and 0.77 , respectively). Also, HbO₂ changes correlated with the reduction in the bile volume ($r = -0.91$). Intracellular oxygenation as reflected by changes of Cyt Ox correlated with HM changes ($r = 0.93$). A significant correlation was found between the Cyt Ox and serum ALT, AST, and LDH ($r = 0.97, 0.90$, and 0.85 , respectively). Also, Cyt Ox correlated significantly with bile volume ($r = -0.93$).

NIRS can measure hepatic indocyanine green (ICG) and this was studied in different animals models. In a rabbit model of acute hepatic dysfunction, ICG uptake rate correlated significantly with total hepatic blood flow (THBF) and HM ($r = 0.79$ and 0.59 , respectively). In a rabbit I/R model, ICG excretion rate correlated with serum ALT, AST, and LDH ($r = -0.73, -0.61$, and -0.56 , respectively) and bile volume ($r = 0.83$). In a rabbit model of fatty liver ICG rate of uptake correlated with THBF ($r = 0.94$) and HM ($r = 0.96$) and its excretion rate correlated with serum bilirubin ($r = -0.89$), ALT ($r = -0.87$), AST ($r = -0.90$), and albumin ($r = 0.95$).

In conclusion NIRS can be used to monitor changes in hepatic tissue oxygenation and function. This technique has potential for clinical application in liver surgery and transplantation for evaluating hepatic tissue viability.

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List of abbreviations

ADP	Adenosine diphosphate
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BC	Bile canaliculus
BD	Bile duct
BDL	Bile duct ligation
BW	Body weight
CAB	Chromotrope aniline blue
CO	Carbon monoxide
CV	Central venule
Cyt Ox	Cytochrome oxidase
DPF	Differential pathlength factor
E	Endothelial cell
ET	Endothelin
FADH ₂	Reduced Flavin adenine dinucleotide
FID	Free induction decay
FiO ₂	Fraction of inspired oxygen
FSC	Fat storing cell
H and E	Haematoxylin and eosin

HA	Hepatic artery
HABF	Hepatic artery blood flow
HAO	Hepatic artery occlusion
HAPO ₂	Hepatic artery oxygen partial pressure
Hb	Deoxyhaemoglobin
HbD	Difference between HbO ₂ and Hb
HbO ₂	Oxyhaemoglobin
HbT	Total haemoglobin volume
HC	Hepatic cell
HM	Hepatic microcirculation
HO	Heme oxygenase
HR	Heart rate
HSO ₂	Hepatic tissue oxygen saturation
HVPO ₂	Hepatic vein oxygen partial pressure
I/R	Ischaemia/reperfusion
ICAM-1	Intercellular adhesion molecule-1
ICG	Indocyanine green
IL-1	Interleukin-1
K ⁺	Potassium
KC	Kupffer cell
L	Lymphatic
LD	Laser diode
LDF	Laser Doppler flowmetry

LDH	Lactate dehydrogenase
LW	Liver weight
LW/BW	Liver weight/Body weight
MABP	Mean arterial blood pressure
MEGX	Monoethylglycinexylidide
N	Nerve
Na ⁺	Sodium
NAD ⁺	Nicotine adenine dinucleotide
NADH	Reduced nicotine adenine dinucleotide
NADP ⁺	Nicotine adenine dinucleotide phosphate
NADPH	Reduced nicotine adenine dinucleotide phosphate
NIR	Near infrared
NIRS	Near infrared spectroscopy
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NTP	Nucleoside triphosphate
pHi	Intracellular pH
Pi	Inorganic phosphate
PME	Phosphomonoesters
PMT	Photomultiplier tube
PNF	Primary non-function
Ppm	Part per million
PV	Portal vein

PVBF	Portal vein blood flow
PVO	Portal vein occlusion
PVP	Portal venous pressure
PVPO	Portal vein partial occlusion
PVPO ₂	Portal vein oxygen partial pressure
Redox	Reduction/oxidation
RF	Radio frequency
SaO ₂	Arterial oxygen saturation
SD	Space of Disse
SLV	Sub lobular venule
SOD	Superoxide dismutase
SP	Sieve plate of endothelial fenestrae
THBF	Total hepatic blood flow
TNF α	Tumour necrosis factor alpha
TO	Total occlusion
VO ₂	Oxygen consumption rate
XO	Xanthine oxidase

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Statement of originality

I have carried out the work in this thesis over the last two years. Contributions of other colleagues to this work have been mentioned in the text and the acknowledgments.

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Chapter 1

Introduction

1.1 Review of liver transplantation and the clinical problem

Starzl and his group carried out the first successful human liver transplant in 1967 (Starzl et al. 1968). Since then the results of transplantation have improved dramatically making liver transplantation the treatment of choice for most patients with irreversible liver disease (Starzl et al. 1989). Current 1-year survival rate of up to 90% are reported (Gordon et al. 1991, Gilbert et al. 1999).

The most important factors to the improved outcome of liver transplantation are better patient selection, refinement and standardisation of liver harvesting techniques (Gubernatis, 1989), and better organ preservation using University of Wisconsin solution (Todo et al. 1989b). In addition, there have been improvements of the recipient implantation procedure (Makowka et al. 1988), postoperative care (Shaw et al. 1989), and better immunosuppressive drugs. The latter has been partially influenced by the introduction of cyclosporine and more recently FK506 therapy (Wonigeit, 1989).

Liver transplantation may be considered in any patient with an estimated survival (due to liver disease) of less than 1 year. The main indications for liver transplantation are chronic liver disease, usually with established cirrhosis (Webberley and Neuberger, 1994). In childhood biliary atresia and metabolic diseases are common indications (Esquivel et al. 1987, Cohen et al. 1989). For some disorders such as primary biliary cirrhosis, sclerosing cholangitis, and chronic active hepatitis with cirrhosis, the role of transplantation in patient management is obvious (Williams et al. 1992, Benhamou, 1994). For other

hepatic diseases such as fulminant hepatic failure, it may be difficult to select those patients who require liver replacement early enough in their illness so that a suitable organ can be found and the procedure carried out before the onset of irreversible complications (Peters et al. 1993, Williams and Wendon, 1994). Despite the remarkable progress in liver transplantation, graft and patient loss still occurs. The main posttransplant complications are hepatic artery occlusion, portal vein thrombosis, primary non-function (PNF), and acute graft rejection. These complications results in primary failure of the graft in 10-20% of patients which is fatal without re-transplantation (Quiroga et al. 1991, Yoong et al. 1998). The reported incidence of hepatic artery thrombosis is about 3% of adult transplants and occurs most frequently within the first 2 weeks after transplantation (Sanchez-Bueno et al. 1994, Scheiner et al. 1997). Risk factors are paediatric patients where the incidence is up to 7.5% (Mazariegos et al 1999a), patients with complex vascular reconstruction (Drazan et al. 1996), and elevated haematocrit > 44% (Buckels et al. 1989). Early hepatic artery thrombosis is associated with considerable morbidity and mortality. It can produce a spectrum of clinical illness including fulminant hepatic necrosis, delayed bile leakage, and relapsing bacteraemia (Bell et al. 1990, Sanchez-Bueno et al. 1994).

The incidence of post-transplant portal vein thrombosis is up to 10-15% of patients (Langnas et al. 1991, Davidson et al. 1994, Chardot 1997). Risk factors include pre-existing portal vein abnormalities (thrombosed, sclerosed portal vein) or portosystemic shunts and children, particularly with a hypoplastic portal vein

(Langnas et al. 1991, Davidson et al. 1994, Chardot 1997). The clinical presentation is variable, ranging from fulminant liver failure to an asymptomatic diagnosis by portal ultrasound (Recordare et al. 1993, Chardot et al. 1997). Acute graft rejection remains an important problem following liver transplantation (Farges et al. 1996, Wiesner et al. 1998). About 60% of liver transplant patients show some signs of acute rejection of their new organ. A lower incidence of rejection may be achieved by using potent regimens of prophylactic immunosuppression (Farges et al. 1996, Wiesner et al. 1998). Diagnosis of acute rejection on clinical grounds alone is unreliable. Acute rejection causes fever, malaise, and reduction in bile output which becomes pale and watery (Farges et al. 1996, Wiesner et al. 1998). The earliest biochemical evidence is a sharp rise in bilirubin, transaminases, and alkaline phosphatase (Bao et al. 1994, Akamatsu et al. 1997). Confirmation of the diagnosis is achieved by biopsy which shows mixed inflammatory infiltrate of the portal tracts, disruption of the biliary epithelium, and endothelialitis (Ray et al. 1988).

PNF is characterised by severe impairment of graft function associated with hepatocellular failure and massive necrosis (Cisneros et al. 1991, Mazariegos et al 1999a). Incidence of PNF varies widely depending on the criteria used for its definition with reported incidence up to 10% (Cisneros et al. 1991, Mazariegos et al 1999a). PNF is incompatible with recipient survival and represents a major cause of early posttransplant morbidity and mortality even with early re-transplantation (Cisneros et al. 1991, Mazariegos et al 1999a). PNF is usually apparent within 24-48 hours posttransplantation. It is characterised by rapidly

rising plasma transaminases, severe coagulopathy, metabolic acidosis, reduced bile flow, hypoglycemia, hepatic encephalopathy, acute renal failure, disseminated intravascular coagulation, and death unless re-transplantation takes place (Cisneros et al. 1991, Mazariegos et al 1999a) .

Many factors are associated with a high incidence of PNF including donor liver disease such as fatty change, prolonged hypotension, elderly donors, prolonged intensive care unit stay, and long ischaemia time (Todo et al. 1989a, Gonzalez et al. 1994, Deschenes et al. 1998). The precise mechanism of PNF is not fully established. There is an increasing body of evidence which suggests that ischaemia/reperfusion (I/R) injury causes deterioration of the liver microcirculation and a significant reduction of the tissue perfusion and oxygenation resulting in liver dysfunction (Thurman et al. 1988, Vollmar et al. 1994).

One common denominator of these complications is impairment of the liver graft microcirculation and oxygenation (Kiuchi et al. 1997, Zulke et al. 1997). Early intervention with these complications can salvage the graft and could improve the graft and patient survival. Examples of optimising oxygenation and microcirculation include the use of hyperbaric oxygen therapy for hepatic artery thrombosis (Mazariegos et al. 1999b), percutaneous thrombolysis and stent placement for portal vein thrombosis (Cherukuri et al. 1998), the use of immunosuppressive drugs for acute rejection (Yoshida et al. 1996), and the use of antioxidants such as N-acetylcysteine and S-adenosylmethionine for PNF (Bzeizi et al. 1997). Thus, postoperative monitoring of the graft microcirculation

and oxygenation could be a useful tool in the transplant armamentarium for early detection and assessment of the treatment of these complications.

Organ shortage has recently become a critical issue due to the limited supply and increased demand resulting from the expansion of liver transplant indications. This problem leads to an increase in the waiting time for liver transplantation, which may lead to transplantation at more advanced and less cost-effective stage of disease with higher mortality (Harper and Rosendale, 1996, Everhart et al. 1997). As a solution for the organ shortage, many transplant centres have started to use "marginal" donor livers such as fatty liver (Chui et al. 1998). There is an increased risk of PNF of the graft after transplantation of a fatty liver and the risk increases with the severity of steatosis (Urena et al. 1998, Chui et al. 1998). Current methods of assessing the severity of steatosis have not proven to be reliable including liver function tests (Karayalcin et al. 1994), dynamic liver tests such as the MEGX (Karayalcin et al. 1994), and liver biopsy (D'Alessandro et al. 1991). At the present time decision as to whether or not to use a liver is based on gross appearance evaluated by the retrieval surgeon (Trevisani et al. 1996).

The development of clinically reliable method to assess the severity of hepatic steatosis could have an important clinical role in donor selection which could in turn help in solving the problem of donor organ shortage.

1.2 Aim and objectives of the thesis

This study investigated the potential role of near infrared spectroscopy (NIRS) in evaluating liver tissue oxygenation and function. The aim was investigated through the following objectives:

1. Development of NIRS algorithm for measuring hepatic tissue oxygenation and indocyanine green (ICG).
2. Validation of hepatic tissue oxygenation measurement with NIRS during graded hypoxia by comparing it with hepatic vein blood oxygenation.
3. Correlation between the hepatic tissue oxygenation measured by NIRS with tissue adenosine triphosphate (ATP) measured by ^{31}P nuclear magnetic resonance (NMR) spectroscopy.
4. Application of NIRS for monitoring hepatic tissue oxygenation in correlation with liver blood flow measured by ultrasonic flowmetry.
5. Validation of NIRS for assessment of hepatic microcirculation, in comparison with laser Doppler flowmetry (LDF), and hepatic functions with acute hepatic dysfunction by measuring hepatic ICG clearance.
6. Application of NIRS for measuring hepatic oxygenation, microcirculation (in comparison with LDF), and function with I/R injury.
7. Application of the ICG clearance measurement by NIRS for assessment of hepatic microcirculation (in comparison with LDF) and function with graded fatty liver.

Chapter 2

Review of hepatic circulation, oxygenation, and energy metabolism

2.1 Liver blood Flow

The human liver is the largest organ in the body, receiving about 25% of the cardiac output even though it constitutes only 2.5% of the body weight with a total blood flow of 1 - 2.6 L/min (Doi et al. 1988, Jakab et al. 1992). The liver has a dual blood supply from the hepatic artery (HA) and the portal vein (PV). The HA supplies about 20 to 66% of the total hepatic blood flow (THBF) and the rest by the PV (Doi et al. 1988, Jakab et al. 1992). The HA supplies about 40-50% of the liver's normal oxygen requirement, as the arterial blood has a greater oxygen content than the portal blood which is postcapillary and partly deoxygenated (Tygstrup et al. 1962, Andreen and Irestedt, 1976). The high pressure, well-oxygenated arterial blood mixes with the low pressure, less oxygenated, but nutrient-rich, portal venous blood within the hepatic sinusoids. The sinusoids are drained by the hepatic venous system into the inferior vena cava.

Changes in portal vein blood flow (PVBF) produce inverse changes in hepatic artery blood flow (HABF) what is known as "hepatic arterial buffer response"(Lautt, 1985, Jakab et al. 1995) but not the reverse (Doi et al. 1988, Jakab et al. 1995). The buffer response tends to maintain the total hepatic blood flow at a constant rate for the regulation of hepatic clearance of blood nutrients and hormones (Lautt, 1985). Adenosine-induced vasodilatation has been shown to be the mediator of this buffer response (Mathie and Alexander, 1990, Browse et al. 1997). Since the adenosine-induced vasodilatation is partly mediated via

nitric oxide (NO), a role for NO has also been suggested (Mathie et al. 1991, Smits et al. 1995). While a marked effect of NO in regulating basal hepatic arterial blood flow has been shown, the buffer response remains intact after inhibition of NO synthesis which suggests that this response is largely independent of NO (Grund et al. 1997).

2.2 Hepatic microcirculation

The hepatic microvascular system includes all blood vessels involved in the delivery and removal of fluids to and from the hepatic parenchyma, namely, portal venules, hepatic arterioles, sinusoids, and central venules. The principal sites for regulating blood flow and solute exchange are in the sinusoid network, which exhibits structural and functional heterogeneity (Hase and Brim, 1966, Gumucio, 1983).

The majority of blood enters the sinusoids from portal venules. These inlets are guarded by sphincters composed of sinusoidal lining cells termed "inlet sphincters" (McCuskey, 1966, Bloch, 1970). Arterial blood enters some of the sinusoids, principally through branches of the hepatic arterioles known as arteriosinus twigs, which terminate in sinusoids near their origin from the portal venules (Bloch, 1970, Kardon and Kessel, 1980). In addition, occasional direct connections (arteriportal anastomoses) have been observed with the terminal portal venules (Bloch, 1970, Kardon and Kessel, 1980) (Figure 2.1).

Since all of these structures are independently contractile, the sinusoids receive a varying mixture of portal venous and hepatic arterial blood (McCuskey, 1966, Bloch, 1970). Blood leaves the sinusoids by passing through outlet sphincters composed of sinusoidal lining cells into the central venules (McCuskey, 1966, Bloch, 1970). Sinusoidal lining cells also may serve as sphincters within the sinusoid network and regulate the distribution of blood flow in short segments of sinusoids (McCuskey, 1966, Wisse et al. 1983) (Figure 2.1).

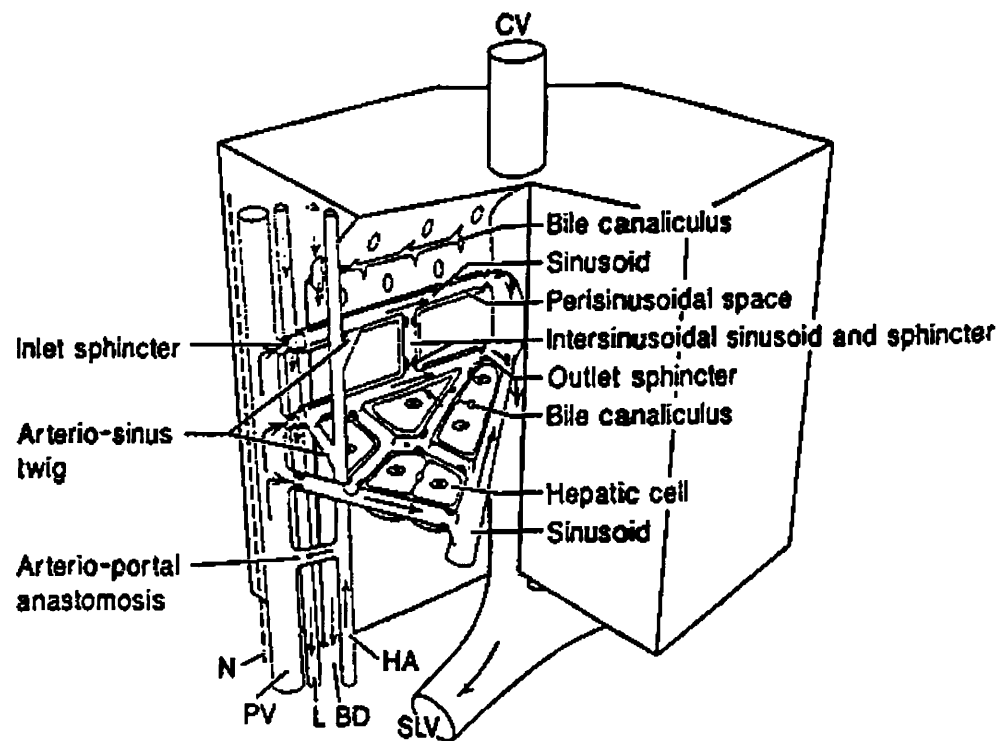


Figure 2.1 Schematic diagram of hepatic microvasculature (McCuskey and Reilly, 1993).

2.3 The hepatic sinusoid

The sinusoids are the principal sites for transvascular exchange between blood and hepatocytes (Figure 2.2). They are special capillaries, lined with endothelial cells, with an average diameter of 10 μm and an average length of 223 to 477 μm (Itoshima et al. 1974, Motta, 1977). Hepatic sinusoids differ from systemic capillaries in that the endothelial cells are fenestrated with no subendothelial basement membrane (Itoshima et al. 1974, Motta, 1977). The endothelial cell fenestrations are 100 to 150 nm in diameter and grouped in clusters called sieve plates which function as a biofilter between the sinusoidal blood flow and plasma within the space of Disse (Itoshima et al. 1974, Motta, 1975).

Kupffer cells are the resident macrophages in the liver and are attached to the luminal surface of the endothelium (Motta, 1975). These cells are phagocytic (Reske et al. 1981) and also produce both beneficial and toxic mediators that participate in non-specific host defence mechanisms and liver injury (Rogoff and Lipsky, 1981, Shiratori et al. 1984).

Perisinusoidal cells (Ito cells, fat storing cells) are stellate cells which lie in the space of Disse (Wake, 1980). They have multiple thin cytoplasmic processes which course through the perisinusoidal space and embrace the outer surfaces of the endothelium (Wake, 1980). They store excess vitamin A and other fat-soluble vitamins (Wake, 1980). Also, they play an important role in the regulation of the hepatic sinusoidal blood flow (Rockey, 1995).

Since the sinusoids have no basal lamina and collagen, the sinusoid wall is highly permeable and permits exchange between incoming blood plasma and hepatocytes through the Disse space. The perisinusoidal space is thought to function as a lymphatic space that channels plasma to the true lymphatics coursing in the portal tract (Motta, 1977, McCuskey, 1994).

The organisation of the sinusoid network exhibits anatomical and functional heterogeneity (Gumucio, 1983, Chen et al. 1993). Sinusoids in periportal areas are narrower, more tortuous and anastomotic than those adjacent to the central venules which are wider and straighter. In the periportal area, the volume of liver occupied by sinusoids is greater than that in pericentral area. Also, at the portal end, the fenestrae are larger but comprise less of the endothelial surface area than that in the pericentral region and therefore the pericentral sinusoids have higher porosity which favours the transport and uptake processes in these sinusoids (Hase and Brim, 1966, Wisse et al. 1983).

The principal site of regulation of blood flow through the sinusoids resides in the sinusoid itself (McCuskey, 1966, McCuskey, 1971). The endothelial and Kupffer cells are responsive to a wide variety of pharmacodynamic substances. By contracting or swelling they can selectively reduce the sinusoid lumen, thereby altering the rate and distribution of the blood flow (McCuskey, 1966, McCuskey, 1971). The participation of the perisinusoidal cells in regulating the sinusoid diameter has been suggested in many studies (Sakamoto, 1991, Pinznai et al. 1992).

2.4 Hepatic tissue oxygenation

The liver is supplied by blood and oxygen from two sources, namely the PV and HA. Hepatic artery oxygen partial pressure (HAPO_2) is 95 mmHg and portal vein oxygen partial pressure (PVPO_2) is about 50 mmHg (de Groot et al. 1988).

When the blood flows intermingle in the hepatic sinusoids the resulting mean hepatic tissue oxygen pressure, measured by oxygen needle electrode, is around 22 mmHg, with range of 1 - 60 mmHg (de Groot et al. 1988).

Hepatic tissue oxygenation is also heterogeneous within the liver lobule. There is a physiological oxygen gradient from the portal to the central regions of the lobules with higher oxygen concentration in the periportal than that in the pericentral region as well as inter-sinusoidal heterogeneity (Eguchi et al. 1986).

This heterogeneity is due to variations in sinusoidal blood flow (Sato et al. 1986), sinusoidal length (Itoshima et al. 1974, Motta, 1977), and different contribution of the HA to sinusoidal blood flow (McCuskey, 1966).

2.5 Cellular bioenergetics

Under aerobic conditions, the coupling of the electron transport system and ATP synthase in the mitochondrial membrane functions as an energy producing system. The mitochondrial respiratory chain is a multi-enzyme complex forming an integral part of the mitochondrial inner membrane (Nicholls and Ferguson, 1982, Erecinska and Wilson, 1982) (Figure 2.3). The respiratory chain includes five complexes, each with several subunits, some of which are encoded by nuclear genes and others by the mitochondrial genome (Wallace, 1992). The reducing equivalents nicotinic adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂), that are generated by the tricarboxylic acid cycle, are transferred to the respiratory chain as a substrate, where electrons are then transferred from NADH or FADH₂ to oxygen (Nicholls and Ferguson, 1982, Erecinska and Wilson, 1982). The electrons that are donated by NADH and FADH₂ enter the respiratory chain at complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase, an enzyme involved in the tricarboxylic acid cycle). Electrons are then sequentially transferred to complex III (cytochrome c), complex IV (cytochrome oxidase: Cyt Ox), and finally, to molecular oxygen, which then combines with protons to produce water. The passage of electrons through the respiratory chain is associated with the translocation of protons from the mitochondrial matrix into the intermembrane compartment. This generates an electrical membrane potential and proton concentration gradient (Nicholls and Ferguson, 1982, Erecinska and Wilson,

1982). The energy which is released during re-entry of protons into the matrix is then utilised by ATP synthase (complex V) to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate (P_i) (Nicholls and Ferguson, 1982, Erecinska and Wilson, 1982) (Figure 2.3). About 95% of the total cellular ATP is produced via mitochondrial oxidative phosphorylation (Nicholls and Ferguson, 1982, Erecinska and Wilson, 1982). The rate of oxidative phosphorylation is governed by the interplay of four factors: the concentration of the respiratory chain enzyme; the concentration of oxygen; the relative concentrations of the substrates including glucose, ADP, adenosine monophosphate (AMP), and P_i ; and the rate of ATP utilisation (Nicholls and Ferguson, 1982, Erecinska and Wilson, 1982).

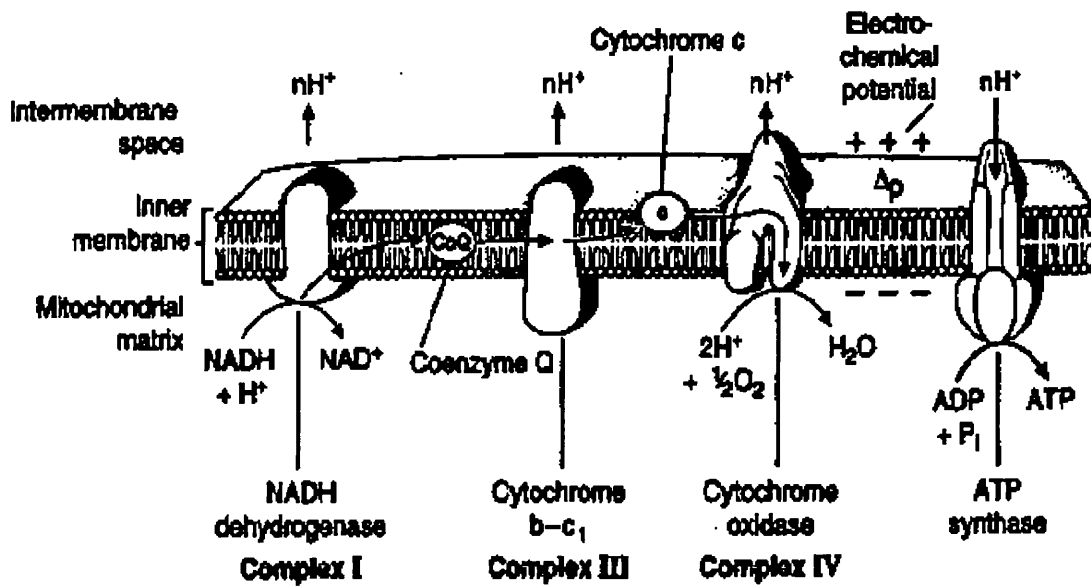


Figure 2.3 Components of oxidative phosphorylation. The major complexes of the electron transport chain, NADH dehydrogenase, cytochrome b-c complex, and cytochrome oxidase, span the inner mitochondrial membrane (Marks et al. 1996).

Chapter 3

Review of ischaemia reperfusion injury

Hepatic injury caused by I/R is an important clinical problem that can occur with circulatory shock, liver trauma (Feliciano et al. 1986), tumour resection (Belghiti et al. 1998), and liver transplantation (Chazouilleres et al. 1993). I/R injury causes hepatic dysfunction and cell injury (Chazouilleres et al. 1993) which can result in liver failure and is associated with a high morbidity and mortality (Faist et al. 1983).

3.1 Tissue changes with ischaemia

The process of cell death by ischaemia has been studied for many years and various sequences of events that can lead to cell death have been suggested (Clavien et al. 1992, Chazouilleres et al. 1993). In aerobic cells the energy necessary to maintain cell integrity is supplied by the mitochondrial system through complete reduction of oxygen to water with the concomitant production of ATP through oxidative phosphorylation (Clavien et al. 1992, Chazouilleres et al. 1993).

When oxygen supply to cells becomes insufficient by ischaemia or hypoxia, mitochondrial respiratory chain function alters because there is no final acceptor of electrons and the reduction/oxidation (redox) state of the mitochondrial enzymes becomes reduced (Gonzalez-Flecha et al. 1993). This causes inhibition of the mitochondrial ATP synthase with the subsequent reduction of oxidative phosphorylation (Lehninger, 1988, Gonzalez-Flecha et al. 1993). As a result a swift decrease of the stored energy-rich phosphates (ATP) occurs in the

tissues as metabolic processes continue while oxidative phosphorylation ceases (Kamiike et al. 1982, Hayashi et al. 1997). Reduction of cellular ATP causes disturbances of membrane ion translocation by inhibition of the ATP-dependent sodium (Na^+)/potassium (K^+) ATPase, resulting in sodium influx and intracellular sodium accumulation with cell swelling and death (Blum et al. 1991).

Intracellular calcium accumulation is strongly implicated in the development of ischaemic injury and is thought to be a crucial step in the transition to irreversible damage (Fujita et al. 1991, Dhar et al. 1996). It occurs secondary to calcium release from the intracellular stores and inhibition of the ATP-dependent calcium pumps in the plasma membrane and endoplasmic reticulum (Farber, 1981a, Hayashi et al. 1986). The increased cytosolic calcium causes activation of cell membrane phospholipases resulting in phospholipid degradation and cell membrane disruption (Farber and Young, 1981c, Farber, 1981a). Calcium also activates tissue proteases such as xanthine dehydrogenase enhancing its conversion to xanthine oxidase (XO) which play a significant role in oxygen free radical production and reperfusion injury (Ishii et al. 1990).

3.2 Tissue changes with reperfusion

Although reperfusion supplies oxygen to the ischaemic tissue, it is well established that it also triggers a complex series of events causing additional damage to the liver that is known as reperfusion injury (Thurman et al. 1988, Clavien et al. 1992). The mediators of the reperfusion injury include oxygen free radicals, cytokines, proteases, calcium, phospholipase A2, eicosanoids, platelet activating factor, endothelin, and endotoxin (Atalla et al. 1985, Clavien et al. 1992, Chazouilleres et al. 1993, Minor and Isselhard, 1993, Grace, 1994). There is large body of evidence for the role of oxygen free radicals as a mediator of the microcirculatory and parenchymal cell injury associated with I/R injury (Atalla et al. 1985, Dahm et al. 1991). The cellular origin of oxygen free radicals is probably multiple including sequestered leucocytes, stimulated Kupffer cells, sinusoidal cells, and hepatocytes (Patych et al. 1987, Dahm et al. 1991, Jaeschke and Farhood, 1991b). It has been suggested that XO might be an important source of these radicals during I/R (Hasselgren, 1987, Marotto et al. 1988). During ischaemia, the consumption of the residual amounts of ATP results in an elevated concentration of AMP, which is further catabolised to adenosine, inosine, and then hypoxanthine. Concomitantly, ischaemia causes conversion of xanthine dehydrogenase to XO secondary to elevated intracytosolic calcium and protease activation. With re-oxygenation of the ischaemic organ by restoration of the blood flow, molecular oxygen reacts with hypoxanthine and XO to produce superoxide anion, hydrogen peroxide, and hydroxyl radicals. Specific inhibitors of

XO, such as allopurinol, significantly attenuate the lesions induced by ischaemia (Nordstrom et al. 1985). Production of oxygen radicals from hepatocytes and endothelium may be dependent on this pathway (Atalla et al. 1985, Patych et al. 1987). The membrane-associated NADH is a known mechanism for the production of oxygen radicals by activated phagocytic cells such as neutrophils and Kupffer cells (Metzger et al. 1988, Dahm et al. 1991). The role of the oxygen free radicals in liver I/R is confirmed by the hepatoprotective effect of numerous antioxidants. Administration of superoxide dismutase (SOD) or catalase appears to attenuate the release of enzymatic indicators of hepatocellular injury and improve the function of the graft exposed to ischaemia (Atalla et al. 1985). Treatment with α -tocopherol (Marubayashi et al. 1986) and coenzyme Q10 (Marubayashi et al. 1984) have been demonstrated to increase survival, accelerate re-synthesis of ATP and suppress the elevation in lipid peroxide associated with hepatic I/R. Hepatic I/R is associated with a reduced level of SOD activity (Adkison et al. 1986) and depletion of endogenous coenzyme Q, α -tocopherol, and total glutathione (Marubayashi et al. 1984). A reduction in these antioxidant defences could make the cells more susceptible to the free radical reactions. Oxygen free radicals damage cell membranes by peroxidation of fatty acids within the phospholipid structure of the cell membrane (Liu et al. 1994).

The crucial role of microcirculatory impairment as a determinant of tissue viability after hepatic I/R has been investigated (Clemens et al. 1985, Vollmar et al. 1994, Vollmar et al. 1995). Study of temporal and spatial microvascular changes

with I/R using intravital microscopy showed two distinct pathophysiologic mechanisms as key components of reperfusion injury. These mechanisms are primary sinusoidal perfusion failure “no reflow” (Koo et al. 1992) and “reflow paradox” (Jaeschke et al. 1990, Vollmar et al. 1994).

In no reflow phenomenon, initially there is sinusoidal perfusion followed by progressive stasis and reduction of the sinusoidal flow (Koo et al. 1992). In reflow paradox phenomenon, reflow induces impairment of the microcirculation and tissue damage rather than improvement of the microcirculation and tissue function (Jaeschke et al. 1990, Vollmar et al. 1994).

The severity of the sinusoidal perfusion failure is dependent on the ischaemia time (Vollmar et al. 1994). This sinusoidal impairment is paralleled with the changes in hepatic tissue oxygenation, hepatocellular integrity, and parenchymal function (Vollmar et al. 1994, Chun et al. 1994). This supports the major contribution of sinusoidal impairment to the reperfusion injury by increasing the hypoxic conditions during reperfusion.

Several mechanisms contribute to the no reflow phenomena, including narrowing of the sinusoid lumens by endothelial cell swelling (Vollmar et al. 1994) secondary to ischaemia-induced ATP deficiency and the consequent failure of ion transport through the cell membrane (Lemasters et al. 1987, Carini et al. 1995). A significant reduction of leukocyte velocity with subsequent stasis and intrasinusoidal plugging has been suggested as a hindrance for blood perfusion (Vollmar et al. 1996, Vollmar et al. 1996).

Recent studies have further indicated the contribution of altered endothelin (ET)/NO balance in mediating the sinusoidal perfusion failure (Nakamura et al. 1995, Pannen et al. 1998). ET is a potent vasoconstrictor that is released from sinusoidal endothelial cells (Yanagisawa et al. 1988). It binds to ET receptors on hepatic Ito cells and regulates the microcirculation by causing sinusoidal constriction (Kawada et al. 1995, Rockey, 1995). The use of ET antagonist before I/R alleviates sinusoidal constriction and the reduction in sinusoidal perfusion (Goto et al. 1994, Nakamura et al. 1995). Also, blocking the ET-receptor improved the hepatic microcirculation and sinusoidal diameter, tissue oxygenation, neutrophil infiltration, bile production, and survival after I/R (Mitsuoka et al. 1999).

NO is potent vasodilator acting on smooth muscles via activation of the guanylate cyclase system as a second messenger and inhibits platelet aggregation and neutrophil adherence (Nathan, 1992, Marletta, 1993). It can be formed in the liver by hepatocytes, Kupffer cells, and endothelial cells (Spitzer, 1994). The role of endogenously produced NO in counteracting the increased action of ET has been demonstrated by the fact that blockade of endogenously produced NO during postischaemic reperfusion aggravates microvascular and hepatocellular injury (Wang et al. 1995, Pannen et al. 1998).

Apart from sinusoidal no reflow, reperfusion of microvessels is paradoxically associated with additional injury which is termed the "reflow paradox" (Jaeschke et al. 1990, Vollmar et al. 1994). Reflow paradox is closely linked with the inflammatory response characterised by a network of intercellular reactions

which includes leukocyte, endothelial cells, and tissue cells (Jaeschke et al. 1990, Suzuki et al. 1994b, Vollmar et al. 1995). The role of leukocyte has been confirmed by the observation that the degree of leukocyte infiltration of the reperfused tissue correlates with postoperative liver function impairment and hepatocyte injury (Jaeschke et al. 1990, Suzuki et al. 1994b). Also, in neutropenic animals there is a substantial decrease in the severity of I/R injury (Jaeschke et al. 1990).

The inflammatory response is achieved via sequential steps which include the activation of leukocytes by cytokines (Colletti et al. 1990, Suzuki and Toledo-Pereyra, 1994a), complement (Jaeschke et al. 1993), leukotrienes (Hughes et al. 1992), and increased adhesion molecules expression (Vollmar et al. 1995). The increased adhesion molecules expression results in rolling of the leukocytes on the sinusoidal lining cells via the selectins adhesion molecules (Brown et al. 1997, Palma-Vargas et al. 1997), leukocyte adhesion to the sinusoidal cell lining via intercellular adhesion molecule-1 (ICAM-1) (Vollmar et al. 1995), and leukocyte extravasation and tissue infiltration via the interaction of integrin matrix receptors and extracellular matrix molecules such as collagen and laminin (Butcher, 1991, Marubayashi et al. 1997).

Intravital microscopic studies have demonstrated leukocyte adhesion to endothelial cells of both sinusoids and postsinusoidal venules after warm (Vollmar et al. 1994) and cold ischaemia and reperfusion (Anthuber et al. 1997). It has also been shown that leukocyte adhesion in postsinusoidal venules, but not in sinusoids, is mediated by ICAM-1 and that ICAM-1- mediated leukocyte

adherence is responsible for the manifestation of excretory dysfunction and hepatocyte damage (Vollmar et al. 1995).

Pre-treatment with monoclonal antibodies to ICAM-1, lymphocyte function associated antigen-1 and CD 18 before induction of rat lobar I/R resulted in a reduced leukocyte infiltration and lipid peroxide level and enhanced the recovery of hepatic ATP with increased survival rate (Marubayashi et al. 1997).

Inflammatory cytokines including tumour necrosis factor alpha ($\text{TNF}\alpha$) and interleukin-1 (IL-1) (Colletti et al. 1990, Suzuki and Toledo-Pereyra, 1994a) act as potential mediators for the leukocyte response after hepatic I/R. $\text{TNF}\alpha$ increases the expression of ICAM-1 which facilitates leukocyte binding with endothelial cells (Colletti et al. 1998). Both $\text{TNF}\alpha$ and IL-1 can induce oxygen free radical production by the neutrophils (Shibuya et al. 1997, Shirasugi et al. 1997) which can stimulate the expression of adhesion molecules (Patel et al. 1991).

Recent studies have demonstrated the role of Kupffer cells in liver I/R injury (Jaeschke and Farhood, 1991b, Suzuki et al. 1994b, Wanner et al. 1996). Kupffer cells are activated in I/R injury (Caldwell-Kenkel et al. 1991) with the release of free oxygen radicals at the initial reperfusion period (Jaeschke and Farhood, 1991b). Kupffer cell activation causes leukocyte activation and infiltration with enhanced release of TNF-alpha and IL-1 (Suzuki et al. 1994b, Wanner et al. 1996). These released mediators may play a significant role as chemoattractants of the neutrophils and as inducers of oxygen derived free

radicals which can participate in the development of hepatic I/R injury (Colletti et al. 1990, Suzuki and Toledo-Pereyra, 1994a, Shirasugi et al. 1997).

It has been shown that there are two phases of reperfusion injury, an early phase that develops over the course of the first 1 to 3 hours of reperfusion and a later progressive phase associated with more hepatocyte necrosis and an increase of neutrophil infiltration in the liver that develops at 6 to 24 hours after reperfusion (Jaeschke et al. 1990, Jaeschke et al. 1990, Jaeschke et al. 1991a). Kupffer cells are considered the principal source of the oxidant stress during the initial reperfusion phase in the liver (Jaeschke and Farhood, 1991b, Jaeschke et al. 1991a). While the later phase mediated by the accumulated tissue leukocyte and induction of neutropenia ameliorates this late phase of injury (Jaeschke et al. 1990, Suzuki et al. 1994b).

Recently, the role of heme oxygenase (HO) in the regulation of the hepatic microcirculation and in I/R injury has been investigated. HO is the rate-limiting enzyme in the degradation of heme to biliverdin, iron, and carbon monoxide (CO) (Goda et al. 1998, Elbirt et al. 1999). The liver is the most abundant source of HO activity in the body, CO released by HO is a potent vasodilator which acts as an endogenous modulator of the basal vascular tone in the hepatic vasculature and influences sinusoidal perfusion under physiological conditions (Pannen and Bauer 1998, Goda et al. 1998). HO exists in two isoenzymes, HO-1 (inducible isoenzyme) and HO-2 (constitutive isoenzyme) (Bauer et al. 1998). With oxidative stress HO-2 is constitutively expressed in hepatocytes, Kupffer, endothelial and stellate cells (Bauer et al. 1998, Elbirt et al. 1999). HO-1

immunoreactive protein expression is restricted to Kupffer cells in the normal liver and a significant increase in HO-1 on the whole organ level occurs after provocation by oxidative stress (Bauer et al. 1998, Elbirt et al. 1999). HO-1 is induced by a variety of stimuli including reactive oxygen radicals, irradiation, endotoxins, I/R, cytokines, and hypertension (Bauer et al. 1998, Sonin et al 1999, Terajima et al. 2000). I/R produces oxygen free radicals which contribute to the hepatic injury and induce the expression of HO-1 gene in hepatocytes and nonparenchymal cells which give protection after I/R via the vasodilator effect of CO (Rensing et al.1999). The hepatoprotective role of HO in I/R has been demonstrated in studies which investigated the protective effect of doxorubicin and hyperthermic preconditioning in hepatic I/R injury (Ito et al. 2000, Terajima et al. 2000).

3.3 Assessment of ischaemia reperfusion injury

Evaluation of the graft function in the peritransplant period involves assessment of hepatocytes, endothelial cells, Kupffer cells, functional recovery of the graft, energy metabolism, and hepatic tissue microcirculation and oxygenation.

The graft's hepatocyte damage and function can be assessed by using conventional static liver function tests which include estimation of bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and prothrombin time (Rosen et al. 1998). However, a poor correlation has been shown between these tests, graft function, and survival (Burdelski et al. 1987).

The level of liver enzymes in the effluent perfusate, including lactate dehydrogenase (LDH), AST, ALT, glutamate dehydrogenase, and creatine kinase have been used as indicator of the severity of liver injury. High levels of these enzymes have been correlated with the incidence of delayed onset of function and PNF (Lange et al. 1996). The problem with static liver tests such as the liver enzymes is their relative lack of sensitivity and their inability to identify the functional reserve of the liver (Makowka et al. 1987, Burdelski et al. 1987). Markers of endothelial cell damage after I/R have been investigated. The endogenous level of Hyaluronic acid (Suehiro et al. 1997a) and its blood clearance (Wang et al. 1996) were found to correlate with graft survival and function. The graft effluent level of thrombomodulin, a surface protein on vascular endothelial cells that serves as a binding site for thrombin and plays an important role as an anticoagulant factor, showed a significant positive correlation with the ischaemia time and the incidence of PNF (Suehiro et al. 1997b). These tests are complex and not suitable for routine clinical use.

Evaluation of functional recovery of the grafted liver has been used as an index of good graft function. These included blood lactate concentrations (De et al. 1997), rate of bile flow (Bowers et al. 1987), serum and biliary bile acid analysis (Baumgartner et al. 1995), hepatic protein synthesis (Nakagohri et al. 1989), and free fatty acid accumulation (Nemoto et al. 1987).

Many studies showed the value of assessing energy metabolism. The ATP level in the donor liver was found to predict the graft metabolic capacity and synthetic function after transplantation (Wolf et al. 1997). The level of purine compounds

released into the effluent fluid has been shown to be closely related to the degree of impairment of gluconeogenesis, ureogenesis, and mitochondrial respiration (Nishida et al. 1987). The liver's ability to restore its cellular ATP content (Higashi et al. 1989, Gonzalez et al. 1994) and the beta-ATP/Pi ratio (Yang et al. 1995) after reperfusion have provided an accurate evaluation of early graft viability and function. Currently available methods for ATP measurement included enzymatic assay (Lamprechts and Trautschold, 1963), chromatography (Kamiike et al. 1982), and ^{31}P nuclear magnetic resonance (McLaughlin et al. 1979). These methods are either invasive or require a complicated procedure and time to obtain data which make them unsuitable for routine clinical use. The arterial ketone body ratio (acetoacetate/beta-hydroxybutyrate) reflects the liver mitochondrial redox potential and mitochondrial electron transfer and was used as an indicator for viability of donor liver grafts and their survival (Ozaki et al. 1991) and for assessment of posttransplant recovery of the graft's mitochondrial function (Osaki et al. 1990). The arterial ketone body measurement is complicated which makes it unsuitable for routine clinical application.

In an effort to overcome the limitations of static liver functions, more dynamic tests were used either looking at the rate of formation of metabolites by the liver, such as lignocaine metabolite or at the clearance of test substances, such as ICG or galactose.

The monoethylglycinexylidide (MEGX) test is performed in vivo in the donor to measure the metabolic rate of lidocaine conversion to MEGX by the hepatic

cytochrome P450 system. It has been proposed as a good clearance test for donor livers to predict postoperative graft function (Balderson et al. 1992, Potter et al. 1992). However, many studies have debated the test efficacy in determining the early graft function and survival (Michell et al. 1993, Woodside et al. 1998, Olinga et al. 1997).

Measurement of ICG blood clearance 24 hour posttransplantation proved to be more accurate than the conventional liver function tests as a predictor of liver-related graft outcome (Tsubono et al. 1996). Its clearance also showed significant correlation with the severity of preservation injury, the duration of intensive care unit and hospital stay, prolonged liver dysfunction, and septic complications (Tsubono et al. 1996). Moreover, ICG clearance was found to correlate significantly with free oxygen radicals and neutrophil elastase, as mediators for I/R injury, after liver transplantation (Plevris et al. 1999). Also, ICG clearance correlated with parameters of liver injury such as AST and prothrombin time at 24 hour posttransplantation (Plevris et al. 1999). Measurement of blood ICG clearance requires frequent blood sampling and gives a global idea about both hepatic circulation and function.

Other clearance tests have been used for the early detection of liver graft function including caffeine clearance and galactose elimination capacity (Nagel et al. 1990) and serum hyaluronic acid clearance (Wang et al. 1996, Suehiro et al. 1997a). Although these dynamic liver function tests are an improvement on the static tests, they are generally cumbersome and their practical usefulness is debatable (Michell et al. 1993, Jalan and Hayes, 1995).

Studies using donor liver biopsies have demonstrated that organs with PNF have higher fat, water, and amino acids content (Gruenberger et al. 1996). Also, donor biopsies with massive steatosis or hydropic degeneration are both strongly associated with PNF (Todo et al. 1989a, D'Alessandro et al. 1991). Donor liver biopsy is invasive, subjects to sampling errors, requires high quality skills in interpretation and has limitations regarding the prediction of graft function (D'Alessandro et al. 1991, Karayalcin et al. 1994).

3.4 Monitoring of the graft blood supply and tissue oxygenation

One of the most important prerequisites for primary graft function is a sufficient blood and oxygen supply to the tissue with good extra and intracellular tissue oxygenation. The hepatic blood flow has been monitored after transplantation by implanted Doppler flowmeter probes which have the potential for early diagnosis of complications in the posttransplant period such as hepatic artery occlusion (Payen et al. 1990). However, monitoring hepatic blood flow cannot assess tissue microcirculation which is the main site of impairment with I/R (Goto et al. 1992) and it is not useful in the presence of hepatic shunting where part of the hepatic blood flow does not contribute to oxygen supply (Huet et al. 1982). Furthermore, tissue oxygenation is controlled not only by blood flow, but also by oxygen supply and consumption.

Monitoring of the hepatic microcirculation (HM) using laser Doppler flowmetry (LDF) to assess the severity of I/R injury has been carried out (Chavez-Cartaya

et al. 1994, Seifalian et al. 1997a). However, the depth of penetration of LDF is very 0.6-1.3 mm (Seifalian et al. 1997b) which has the potential of sampling errors as the parenchymal damage following I/R injury is often patchy (Koo et al. 1992, Vollmar et al. 1994).

The polarographic oxygen electrode needle can be used for monitoring tissue perfusion and oxygenation (Seifalian et al. 1997b). However, this method has the same problem of sampling errors beside the inaccuracy in measurement caused by bleeding and blood collection at the tip of the electrode as the needle passes through the liver tissue (Seifalian et al. 1997b). While the new generation of oxygen electrodes may not have this problem (van Wagenveld et al. 1998). Good tissue oxygenation is vital for liver graft function and survival (Goto et al. 1992, Vollmar et al. 1996, Kiuchi et al. 1996). Measuring oxygen consumption in the liver graft after transplantation showed that an increase in the oxygen consumption was associated with good graft function (Groenland et al. 1992). Measurement of hepatic venous oxygen saturation showed that low oxygen saturation correlates with poor function of the graft and high liver enzymes (Katsuramaki et al. 1997). Direct measurement of hepatic tissue oxygenation using spectroscopy showed that tissue oxygenation is significantly correlated with the microcirculatory impairment and the liver dysfunction induced by I/R injury (Goto et al. 1992, Vollmar et al. 1996, Kiuchi et al. 1996). In addition, liver graft tissue oxygenation has also been shown to provide valuable information on early graft function and survival in both experimental animals (Sumimoto et al. 1987, Kallinowski et al. 1996) and human liver transplantation (Kitai et al. 1995).

Direct measurement of hepatic tissue oxygenation could therefore be a good indicator of liver viability and function during liver surgery and transplantation.

Chapter 4

Materials and methods

4.1 Near infrared spectroscopy

4.1.1 Tissue chromophores

In all tissues a number of colour-bearing compounds (chromophores), namely, oxyhaemoglobin (HbO_2), deoxyhaemoglobin (Hb), and cytochrome oxidase (Cyt Ox) are present in variable concentrations. They have different absorption spectra in near infrared (NIR) light (Figure 4.1) and their absorption characteristics are oxygen dependent (Jobsis, 1992).

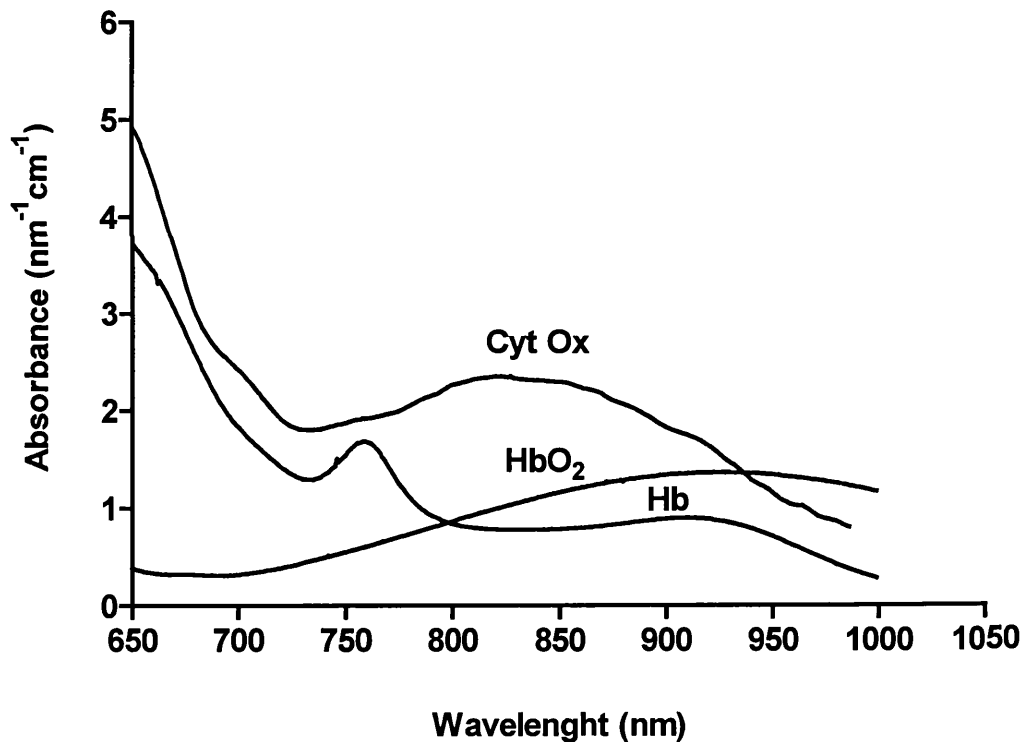


Figure 4.1 Absorption spectra of HbO_2 , Hb , and Cyt Ox in the NIR light region (Jobsis, 1992).

Haemoglobin is a protein made up of 4 subunits, each of which contains a haem moiety attached to a polypeptide chain. Each of the 4 iron atoms, with the active ferrous form (Fe^{2+}), can bind to a molecule of oxygen in a reversible physical and not a chemical manner, so that the reaction is an oxygenation and not oxidation. Each gram of haemoglobin can combine with 1.34 ml of oxygen. Oxygen releases from HbO_2 to the tissue forming Hb. Oxygen-haemoglobin reaction occurs in milliseconds and this reaction is reversible ($\text{Hb} + \text{O}_2 \leftrightarrow \text{HbO}_2$) (Little and Edwards, 1993).

Cyt Ox is the terminal enzyme complex of the cellular respiratory chain in the inner mitochondrial wall (Figure 2.3). It catalyses the reduction of O_2 to H_2O , in a four-electron reaction, with the concomitant synthesis of ATP through the oxidative phosphorylation process (Nicholls and Ferguson, 1982, Capaldi, 1990). It contains 4 redox active groups, 2 haem iron (haem a and haem a_3) and 2 copper (Cu_A and Cu_B) centres. These four metal centres accept or donate electrons during the electron transfer through the respiratory chain, changing their redox state. The oxygen-binding site of the enzyme is the binuclear unit formed of the Cu_B and haem a_3 . The donation of electrons from this unit to oxygen accounts for the majority of oxygen consumption in the tissues. The Cu_A and haem a centres donate electrons to the binuclear unit and therefore are not directly involved in oxygen reduction (Nicholls and Ferguson, 1982, Capaldi, 1990). In the absence of oxygen, electron transfer to oxygen cannot take place. Electrons accumulate on the haem and copper atoms and Cyt Ox becomes reduced. With oxygen availability the electrons are transferred rapidly from the

metal centres to oxygen and Cyt Ox becomes oxidised. Many factors can affect the Cyt Ox redox state in vivo, but the most significant factor is likely to be the oxygen concentration (Nicholls and Ferguson, 1982, Cooper et al. 1994).

All 4 centres of Cyt Ox exhibit different absorption characteristics depending on their redox state. The copper centres are optically active in the NIR light in contrast with the haem centres that absorb visible light (Jobsis, 1992, Cooper et al. 1994). However, absorption of the NIR light by Cyt Ox occurs primarily at the Cu_A centre within Cyt Ox. The oxidised Cu_A centre has a characteristic shape spectrum with a broad peak centred around 845 nm which is absent with the reduced enzyme (Jobsis, 1992, Cooper et al. 1994). The contribution of haem iron centres to absorption of NIR is less than 10% of the total signal in the reduced-oxidised spectrum (Boelens and Wever, 1980, Cooper et al. 1994).

Another synthetic chromophore that can be measured by NIRS is ICG. ICG is an anionic dye that has been used for many years to measure hepatic blood flow and as a test of liver function (Caeser et al. 1961, Grainger et al. 1983). It has a characteristic maximum absorption peak at 805 nm in the NIR light region allowing its absorption coefficient to be incorporated in the NIRS algorithm to measure directly its concentration in the hepatic tissue (Shinohara et al. 1996b).

4.1.2 Principle of near infrared spectroscopy

Light interactions with the tissue involve the combination of reflectance, scattering and absorption which depends upon many factors including the light wavelength and the illuminated tissue type (Jobsis, 1992). In the visible region of the spectrum (450-650 nm) light is strongly attenuated due to the intense absorption by haemoglobin and light scattering in tissues, which increases with decreasing the wavelength. Therefore light fails to penetrate more than 1 cm of tissue (Jobsis, 1992, Kitai et al. 1993b). However, the NIR region of the electromagnetic spectrum (700-1000 nm) represents an optical window of relative transparency and a significant amount of radiation can be effectively transmitted through biological materials over distances of up to 8 cm (Jobsis, 1992, Kitai et al. 1993b).

Although in the NIR region of the spectrum the absorption of light by Hb and HbO₂ decreases significantly, compared to that observed in the visible region of the light, their absorption spectra remain different allowing spectroscopic separation of the two compounds using only a few wavelengths (Cope, 1991).

The technique of NIRS relies upon two main physical properties: (1) the relative transparency of biological tissue to light in the NIR region of the spectrum and (2) the existence of different tissue chromophores with characteristic absorption spectra in the NIR light spectrum (Wyatt et al. 1986, Wray et al. 1988).

In tissue with homogeneous scattering the calculation of light attenuation and the relationship between the optical absorption and chromophore concentration may

be described by a modified Beer - Lambert's law. The law modifications include (a) an additive term, G, due to scattering losses and (b) a multiplier, to account for the increased optical pathlength due to scattering. This law can be used to convert the obtained optical densities to concentration changes of Hb, HbO₂, and Cyt Ox in $\mu\text{mole/L}$ per optical pathlength (Wray et al. 1988):

$A = [\alpha \cdot c \cdot (d \cdot B)] + G$. where A is the attenuation of light (optical density), α is the absorption coefficient of the chromophore ($\mu\text{mole}^{-1} \cdot \text{cm}^{-1}$), c is the concentration of the absorbing compound ($\mu\text{mole/L}$) and d is the geometrical distance between the points where light enters and leaves the tissue (cm). B the differential pathlength factor (DPF) which accounts for the increase in optical pathlength due to light scattering (which causes the optical pathlength to be greater than d) and G is a constant geometrical factor which accounts for loss of photons by scattering. As G cannot be quantified in vivo and is dependent upon the scattering coefficient of the tissue interrogated, it is not possible to measure the absolute concentration of the chromophore in the tissue from measurement of the absolute attenuation. If α , B, and d are known and G assumed to remain constant during measurement, we can measure the change in the chromophore concentration (Δc) from measuring the change in attenuation (ΔA) from the following formula: $\Delta c = \Delta A / \alpha \cdot d \cdot B$.

Since the absolute concentration of tissue chromophores are unknown and cannot be calculated due to the effect of light scattering within the tissue, all NIRS measurements are expressed as absolute concentration changes ($\mu\text{mole/L}$) from an arbitrary zero at the start of the measurement.

The absorption coefficients of HbO_2 and Hb can be obtained in cuvette studies on lysed human blood, for the oxygenated spectra these cuvettes are bubbled with different oxygen saturation (Wray et al. 1988). The absorption coefficient of Cyt Ox was obtained in vivo from the brains of experimental animals whose blood had been replaced by a blood substitute (fluorocarbon) with exposure to 100% O_2 or N_2 to obtain the oxidised and reduced Cyt Ox spectra (Wray et al. 1988, Ferrari et al. 1990).

B is dependent upon the amount of scattering in the medium which can be measured by "the time of flight " method (Delpy et al. 1988).

For simultaneous computation of the changes in concentration of a number of chromophores from changes in attenuation at a number of wavelength, a mathematical operation (algorithm) can be used which incorporates the relevant absorption coefficient for each chromophore at each wavelength (Wray et al. 1988, Cope, 1991). As there are three chromophores of interest in the tissue (HbO_2 , Hb, and Cyt Ox) it is necessary to make measurements at a minimum of three wavelengths and if more than three wavelengths are used, standard curve-fitting analysis may be used to increase the accuracy of the calculated concentration changes (Wray et al. 1988, Cope, 1991).

4.1.3 Clinical application of near infrared spectroscopy

Jobsis first applied NIRS clinically in 1977 to monitor cerebral and myocardial tissue oxygenation (Jobsis, 1977). Today it plays an important role in the diagnosis of hypoxic brain injury and in studying cerebral haemodynamics in neonates (Edwards et al. 1988, Williams et al. 1994) and intrapartum foetal cerebral circulation (Seelbach-Gobel, 1996).

It has also been used for monitoring cerebral tissue oxygenation during carotid endarterectomy (Kuroda et al. 1996) and during paediatric cardiac surgery (Daubeney et al. 1996) where it was found to be an accurate and reliable method for assessment of cerebral tissue oxygenation.

Another clinical application of NIRS is patients with head injuries for the detection of cerebral changes after closed head trauma (Kirkpatrick et al. 1995). Changes can be found on NIRS in delayed traumatic intracerebral haematoma prior to increase in intracranial pressure, a change in neurological signs or a change on CT scan (Gopinath et al. 1995).

NIRS has also been found as a reliable non-invasive method for detecting changes of cerebral haemodynamics and oxygenation with ageing and Alzheimer's disease (Hock et al. 1996).

Validation of NIRS as a tool for assessment of cerebral tissue oxygenation has been done by correlating its measurements with jugular venous oximetry measurements during labour (Seelbach-Gobel, 1996), with head injury, (Kirkpatrick et al. 1995), and in patients with acute brain disease (Tateishi et al.

1995). These studies demonstrated that the direction and the magnitude of changes in cerebral oxygenation measured by NIRS are similar to those measured by jugular oximetry which proved the sensitivity and the reliability of NIRS as a non-invasive method of evaluation of cerebral oxygenation.

Utilisation of NIRS as a non-invasive method for measurement of the cerebral blood volume has been carried out in neonates (Wyatt et al. 1990) and adults (Elwell et al. 1994). This can be done by calculating cerebral HbT from changes in the concentration of HbO₂ and Hb in response to a small change in arterial oxygen saturation (Wyatt et al. 1990).

Validation of cerebral blood flow measurement by NIRS in infants was done by comparing this technique with ¹³³xenon clearance (Skov et al. 1991). A good correlation between was found between the two techniques.

Non-cerebral applications of NIRS for monitoring tissue oxygenation are limited and most of these studies have been experimental with a limited clinical application. NIRS has been used for measurement of the tissue oxygenation in skeletal muscles and for measuring their oxygen consumption rate (VO₂) (Hampson and Piantadosi, 1988, Cheatle et al. 1991, Colier et al. 1995).

Calculating the rate of change of HbO₂ to Hb allows the rate of oxygen consumption to be calculated. This calculation has been evaluated with forearm ischaemia, venous outflow restriction and during exercise (Hampson and Piantadosi, 1988, Colier et al. 1995). The technique could be used as an indicator for the severity of peripheral vascular diseases (Cheatle et al. 1991). In

these studies NIRS was a reproducible and reliable method for the non-invasive measurement of VO_2 in human muscles.

Its use to monitor tissue perfusion and oxygenation within the microcirculation and to distinguish between the microcirculatory changes as a result of arterial, venous or total vascular occlusion has been evaluated in rabbits' limbs (Irwin et al. 1995). Subsequently it was applied to tissue flaps (Thorniley et al. 1995) where tissue oxygenation measured by NIRS correlated with flap vascularity.

NIRS can monitor changes in the extracellular and intracellular oxygenation and blood volume of myocardial tissue non-invasively and reproducibly (Parsons et al. 1990, Parsons et al. 1993, Thorniley et al. 1996). NIRS measurements and the timing of their maximum changes were significantly correlated with the duration of the occlusion periods (Thorniley et al. 1996), with the degree of the coronary vascular occlusion (Parsons et al. 1993), and with the collateral blood flow (Parsons et al. 1990).

NIRS has been used to assess mitochondrial respiratory function after ischaemia reperfusion injury in a rabbit renal transplant model. A significant correlation was found between the Cyt Ox redox changes measured by NIRS, NADH fluorescence and histological changes (Thorniley et al. 1994). It has also been applied in the same animal model to assess the effect of drug treatment on the renal tissue oxygenation after I/R injury (Vaughan et al. 1995).

4.1.4 Hepatic application of near infrared spectroscopy

The application of NIRS to liver haemodynamics is still very limited. The first study was in 1993 from Kitai and colleagues (Kitai et al. 1993b). They used a continuous absorbance spectrum in the NIR region (every 2 nm from 700 to 1000 nm) with use of a multicomponent curve fitting analysis following the Beer-Lambert law to calculate concentration changes in HbO₂, Hb, and Cyt Ox (Kitai et al. 1993b). Hepatic tissue oxygen saturation (HSO₂) in the rabbit was measured following graded hypoxia and compared with the hepatic vein oxygen saturation, as an indicator of tissue oxygenation. There was a significant correlation between the two parameters (Kitai et al. 1993b).

Tokuka and colleagues investigated the effect of hepatic vascular occlusion on the hepatic HSO₂ and the interrelationship between the HA and the PV blood flow regarding liver tissue oxygenation (Tokuka et al. 1994). NIRS measurements with vascular occlusion were consistent and reproducible with a specific pattern of tissue oxygenation associated with HA, PV and both vessel occlusion (Tokuka et al. 1994). Other studies have used NIRS for monitoring hepatic tissue oxygenation, including evaluation of triiodothyronine on tissue oxygenation recovery after Pringle's manoeuvre (Okamoto et al. 1994) and the effect of resuscitation using dextran or epinephrine after haemorrhage (Kitai et al. 1993a).

In the field of liver transplantation evaluation of the graft viability was examined using NIRS after warm I/R injury in a rat model of liver transplantation. As the

duration of warm ischaemia was increased the HSO₂ measured by NIRS was progressively reduced (Tashiro et al. 1993). The relation between HSO₂ measured by NIRS and PNF of the graft has been also studied in human liver transplantation (Kiuchi et al. 1996). They suggested two patterns of hepatic oxygenation impairment with PNF, high and homogenous, and low and heterogeneous hepatic oxygenation, due to loss of the graft oxygen consumption and reduction of tissue perfusion, respectively. It has also been shown that increased hepatic tissue oxygenation heterogeneity at the end of transplantation is associated with an increased incidence of graft rejection and poor liver function postoperatively (Kiuchi et al. 1995).

The above studies suggested an important use for NIRS for measuring hepatic tissue oxygenation in liver surgery and transplantation. Nevertheless, very little basic research has been performed to investigate the interpretation of the NIRS measurements for tissue oxygenation and its relationship with the blood flow and energy metabolism. The role of Cyt Ox as an indicator of intracellular oxygenation and its importance for measuring critical tissue hypoxia and I/R injury has not been evaluated. Previous experimental studies have been carried out in small animals (rabbits and rats) which have significant differences in hepatic haemodynamics in comparison to the human liver. Extrapolation from these studies to the human liver may not be valid.

NIRS has also been applied for assessment of liver function and blood volume using ICG. Shinohara and colleagues first reported the use of NIRS for measurement of hepatic ICG concentration in 1996 (Shinohara et al. 1996b).

They demonstrated that the elimination of ICG by the rabbit liver follows two exponential rate constants; the first reflects the dye uptake from plasma to hepatocytes and the second represents the dye removal from the liver by cytoplasmic transport and biliary excretion. The handling of ICG by the rabbit liver was studied using NIRS and rate constants established for ICG uptake and excretion using a two-compartment model. This mathematical model was validated using the effect of competitive inhibition of ICG clearance using bilirubin, microtubular toxin Colchicine, Na^+ , K^+ -ATPase inhibitor Ouabain and I/R. Good correlation was found between these different parameters affecting the hepatic ICG handling, the ICG uptake and excretion rates. These data suggest a potential role of direct ICG measurement for monitoring hepatic tissue microcirculation and function. The clinical usefulness of this technique has to be tested by applying it in the different conditions which can be encountered clinically. The ICG uptake and excretion rates also require to be validated by comparing them with standard techniques for monitoring tissue blood flow and function, respectively.

4.1.5 Near infrared spectrometer

The NIR spectrometer used in this study is the NIRO 500 (Hamamatsu Photonics K.K., Hamamatsu, Japan) (Figure 4.2 and 4.3). This spectrometer is the commercial version of an instrument developed by colleagues in the Department of Medical Physics and Bioengineering, University College London (Cope, 1991). In the NIRO 500, the light source is monochromatic light generated from semiconductor laser diodes (LD). The light is produced at four wavelengths (774, 826, 849, and 906 nm). The choice of the wavelengths is based on 765 nm, the absorption maximum for Hb; 810 nm, the isobestic wavelength at which the extinction coefficients of HbO₂ and Hb are equal which can be used to calculate haemoglobin concentration independent of oxygen saturation; 845 nm, the absorption maximum for oxidised Cyt Ox; and 900 nm, a reference wavelength (Jobsis, 1977, Wray et al. 1988).

The light is produced by laser diodes and carried to the liver via a bundle of optical fibres in sequential pulses. The optical fibres are covered by a light proof protective sheath and its distal end terminated in a very small glass prism which reflects the light through 90° to direct it into the tissue (Elwell, 1995). Photons emerging from the liver are collected by the second bundle of optical fibres and detected by a photomultiplier tube (PMT) light detector (Elwell, 1995) (Figure 4.4). The incident and transmitted light intensities are recorded and from these the changes in the concentration of tissue chromophores (μmole/L) are

calculated using an algorithm incorporating the known chromophores absorption coefficients and an experimentally measured optical pathlength (see below).

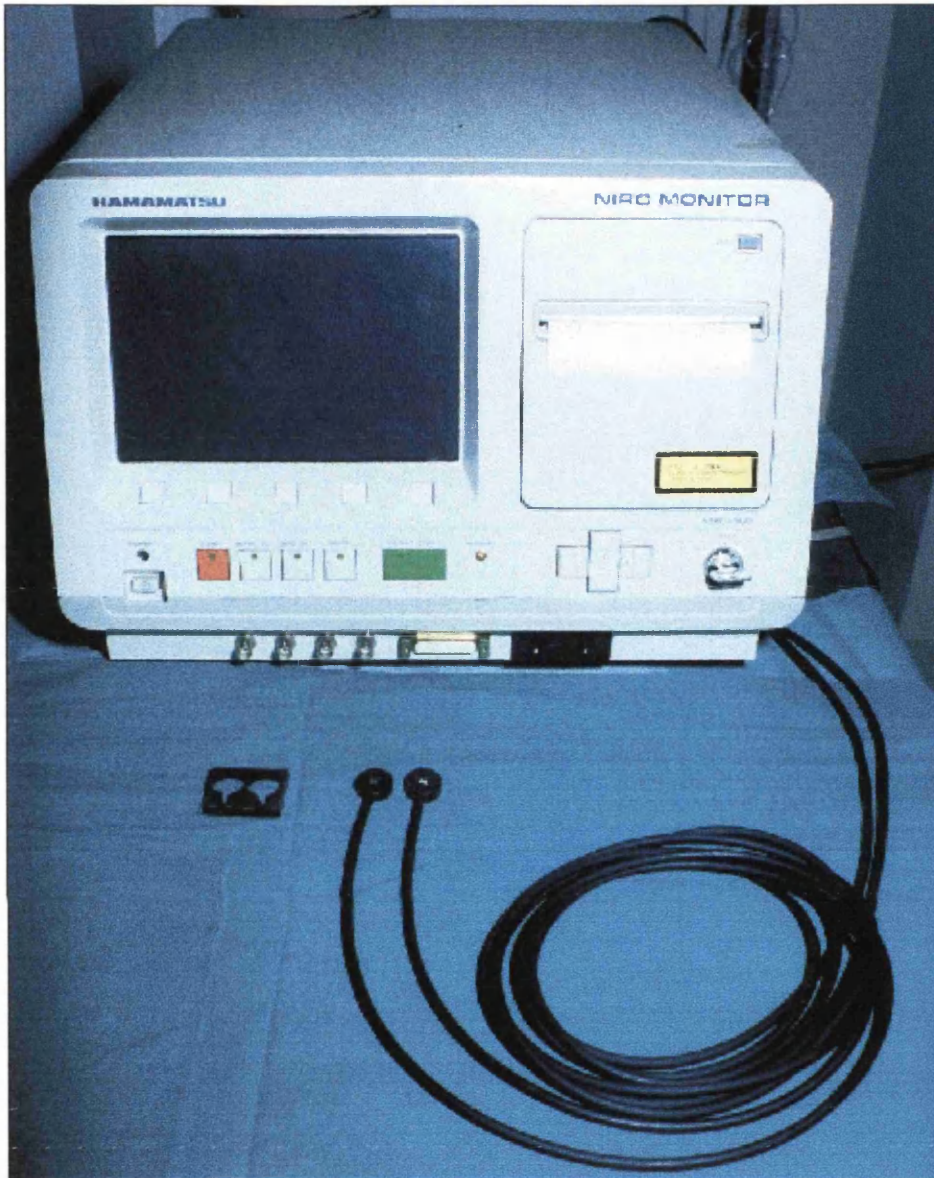


Figure 4.2 Near infrared spectrometer (NIRO 500, Hamamatsu Photonics KK, Hamamatsu, Japan).

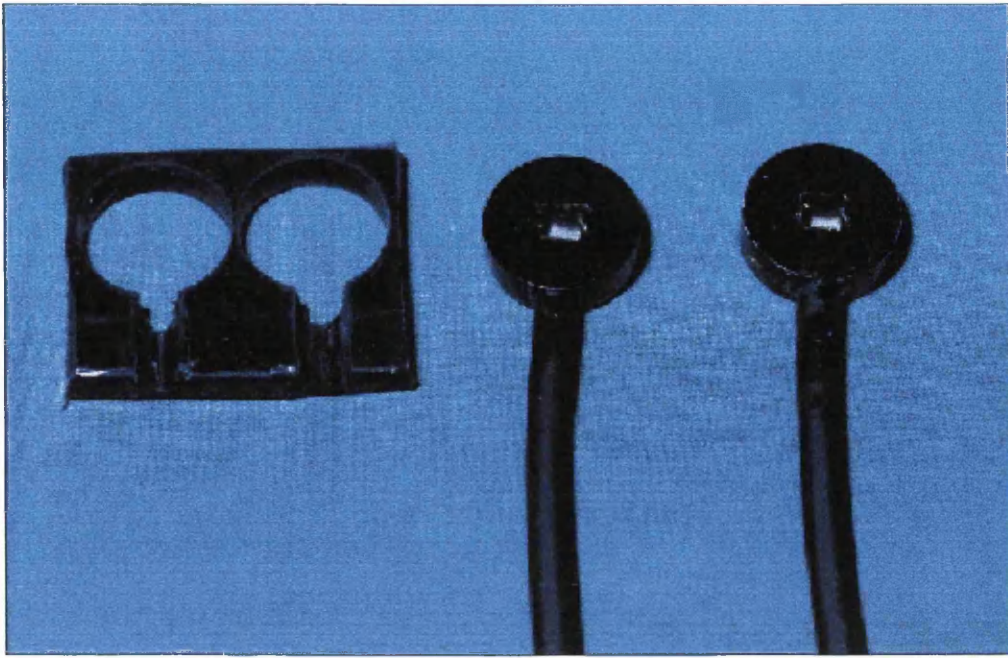


Figure 4.3 NIRO 500 probes and a rubber optode holder.

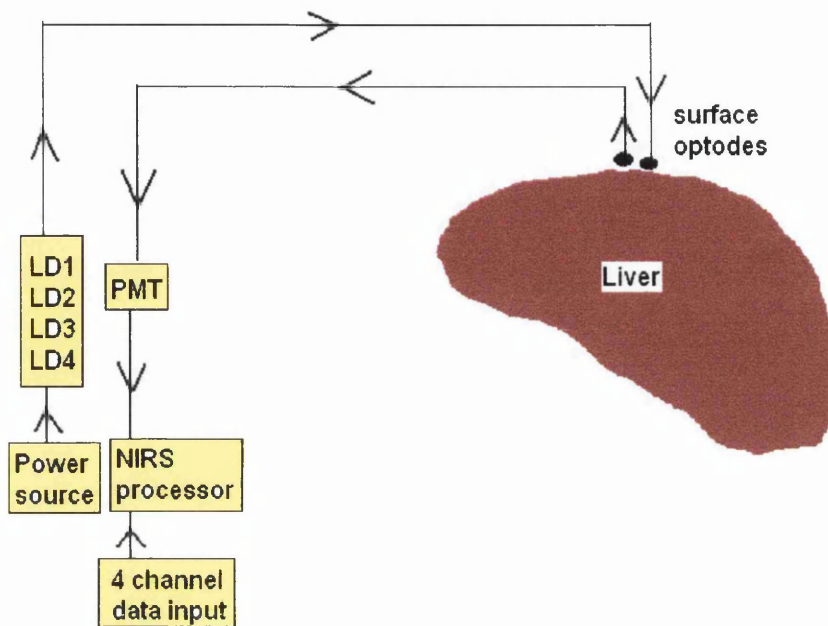


Figure 4.4 Schematic diagram of the NIRO 500 spectrometer. Four LD are used as the monochromatic light source and a PMT is used to detect transmitted light from the tissue. Data from other monitors can be recorded simultaneously via the data input channels.

The standard NIRO 500 algorithm was developed using the wavelength dependant-pathlength for the brain tissue (Essenpreis et al. 1993). As a part of this study, modification of the NIRO 500 algorithm has been carried out by colleagues in the Department of Medical Physics and Bioengineering, University College London for its hepatic application. The absorption coefficient of a freshly dissected and viable blood free pig liver was measured. The absorption coefficient was found to be 0.04 mm^{-1} at 800 nm which is some four times larger than that of normally perfused brain tissue although the transport scattering coefficient was similar near 1.0 mm^{-1} . When the absorption coefficient of blood in a normally perfused liver was added to the blood free absorption coefficient the overall absorption coefficient was near 0.1 mm^{-1} with the scattering coefficient essentially unchanged. The optical pathlength as a function of wavelength for normally perfused liver was then calculated incorporating the measured absorption coefficient and the scattering coefficient mathematically corrected for the contribution of haemoglobin absorption. The average blood content of the liver was assumed to be 12% by volume (Greenway and Stark, 1971) at an average haemoglobin saturation of 60%. The pathlength data were used to generate the final algorithm for calculating changes in chromophore concentrations (Table 4.1) where for example,

$$\Delta Hb = (1.3619\Delta A_{774nm} - 0.9331\Delta A_{826nm} - 0.7197\Delta A_{849nm} + 0.6752\Delta A_{906nm}) \frac{1}{IOS * DPF}$$

Here ΔA_{774nm} is the light attenuation at 774 nm, IOS is the interoptode spacing (2.5 cm in this study), and DPF is 2.7 measured by the time of flight technique (Delpy et al. 1988).

The time of flight method uses pumped dye laser source and synchronscan streak camera. In principle the laser beam is split and part of it is taken directly to the streak camera as a time reference. The other part of the beam is directed through the tissue sample. The temporal reference and the signal which has traversed the tissue sample are recorded simultaneously on the streak camera image. The geometrical distance between the transmitting and the detecting fibre (D) is measured. The time difference (T) between the light entering the tissue and the mean time of that which has traversed the tissue is measured from the streak image and is then used in the calculation of the DPF using the following equation: $DPF = C_v * T / D * N$

Where C_v is the speed of light in vacuum, N is the refractive index of the tissue.

The other chromophores use similar formula but with the different multipliers shown in Table 4.1.

Table 4.1 Multiplication coefficients used to calculate changes in hepatic chromophore concentrations from changes in light attenuation at the wavelengths of the NIRO 500.

Wavelength (nm)	774	826	849	906
Hb	1.3619	-0.9331	-0.7197	0.6752
HbO ₂	-0.7556	-0.5568	0.0356	1.7298
Cyt Ox	-0.1064	0.8168	0.4294	-1.0373

4.1.6 The application of NIRO 500 for measurement of hepatic tissue oxygenation

NIRO probes were mounted inside a probe holder and placed on the liver surface at a fixed site of the right lobe of the liver in all the animals in each experiment to avoid any anatomical variation which could influence in tissue oxygenation and blood volume. As a part of the modification of this instrument for use on the liver, a flexible rubber holder was made to hold the NIRS probes at a fixed spacing over the liver surface. This probe holder ensured that the sites of light entry and exit are maintained at a constant and known spacing distance which minimises the possibility of artefact due to changes in the distance between the probe ends. Also, it allows a satisfactory contact between the liver surface and the probe ends (Figure 4.3).

4.1.7 Collection and analysis of the NIRO 500 data

The NIRO includes the facility to set the attenuation and therefore chromophore concentration) changes to zero with the NIRO initial setting. Since all the measurements are changes from an arbitrary initial zero, this function is important to ensure that artefacts such as system drift, optode movement, and excessive light having a minimal effect on the data. For collection of NIRS data, a sampling time of 1 second was used. The NIRS data were continuously collected in a laptop computer connected to the NIRS. These data are the changes in light attenuation (optical densities: OD) at four wavelengths due to absorption by the tissue chromophores. A software program called ONMAIN[®] (Hamamatsu Photonics K.K., Hamamatsu, Japan) was used to convert these data into changes in concentration changes of HbO₂, Hb, and Cyt Ox (μmole/L) using the previously defined algorithm in the NIRO 500. This was then transferred to excel[®] data sheets (Microsoft Company, Seattle, USA) for analysis. The data at the relevant time points were collected as the mean of 1-minute data and calculated in regard to the baseline value at the start of the experiment.

4.1.8 Interpretation of NIRO 500 measurements

NIRS allows continuous monitoring of the changes in the following parameters:

HbO₂: the amount of oxygenated haemoglobin concentration within the blood vessels and capillaries. It increases in response to increase in blood flow or oxygen saturation and vice versa (Cope and Delpy, 1988, Irwin et al. 1995, Lane et al. 1996).

Hb: the amount of deoxygenated haemoglobin within the blood vessels and capillaries. It changes in the opposite direction to HbO₂. It also represents the state of the venous outflow and increases with its impairment (Cope and Delpy, 1988, Irwin et al. 1995, Lane et al. 1996).

HbT: the total haemoglobin is the sum HbO₂ and Hb which reflects changes in the blood volume and hence provides an indirect indication of the blood flow and tissue perfusion. It increases with increasing blood flow and with impedance of the venous outflow (Cope and Delpy, 1988, Irwin et al. 1995, Lane et al. 1996).

HbD: the difference between HbO₂ and Hb which reflects the net changes in haemoglobin oxygenation independent of any blood volume changes (Cope and Delpy, 1988, Irwin et al. 1995, Lane et al. 1996).

Cyt Ox: measurement of Cyt Ox redox state provides a good index of intracellular oxygen. Cyt Ox becomes more reduced with decreasing the blood flow or oxygen saturation and more oxidised with increasing the blood flow, or oxygen saturation (Brazy, 1991, Cooper et al. 1994). Changes in the level of glucose, as a substrate for the electrons generated in the respiratory chain, also affects the

redox state of Cyt Ox. With hypoglycaemia Cyt Ox becomes more oxidised and with glucose administration it becomes more reduced (Brazy, 1991, Cooper et al. 1994). The cell metabolic activity can affect the redox state of Cyt Ox, with increasing metabolic activity, it becomes more oxidised providing that adequate oxygen and substrate are available (Brazy, 1991, Cooper et al. 1994).

4.2 Laser Doppler flowmetry

In this study blood flow in the hepatic microcirculation was measured using laser Doppler flowmetry (LDF).

4.2.1 Principle of laser Doppler flowmetry

LDF is a relatively new technique for assessing tissue microcirculation.

Measurement is easy to perform and provides a continuous signal without interference with tissue blood flow (Shepherd et al. 1987, Almond and Wheatley, 1992, Wheatley et al. 1993a). The theory of operation of this technique has been described in detail in many studies (Shepherd et al. 1987, Almond and Wheatley, 1992, Wheatley et al. 1993a).

Briefly, a monochromatic laser light from a 2mV-helium neon laser operating at 632 nm is guided to the tissue via optical fibres. The back-scattered light from the tissue is transmitted through optical fibres to photodetectors. Only the photons which are scattered by moving red blood cells will have a Doppler frequency shift, whereas those from the static tissue matrix will not be Doppler shifted.

Mixing of these components at the photodetector surface produces an electrical signal containing all of the Doppler frequency shift information. Further processing of the signal produces an output voltage that varies linearly with the product of total number of moving red blood cells in the measured volume of a few cubic millimetres multiplied by the mean velocity of these cells.

Linearity of the LDF signal from the liver with total organ perfusion has been demonstrated and the technique has been shown to be sensitive to rapid changes in organ blood flow (Shepherd et al. 1987, Almond and Wheatley, 1992, Wheatley et al. 1993a). The LDF measurements are expressed in arbitrary perfusion units (flux). Due to the problems associated with variation in signal across the surface of the liver, it is not possible to apply a conversion factor so that the LDF signal can be expressed in absolute flow units (Almond and Wheatley, 1992, Wheatley et al. 1993a).

The application and reproducibility of LDF measurement for assessment of liver microcirculation has been validated in both experimental animals (Wheatley and Zhao, 1993b, Wheatley et al. 1993a) and human liver transplantation (Seifalian et al. 1997a).

4.2.2 Laser Doppler flowmeter

The hepatic microcirculation in this study was assessed using a commercially available dual channel surface laser Doppler flowmeter (DRT4, Moor Instruments Ltd., Devon, UK) (Figure 4.5).

The LDF was calibrated before each study against a standard reference (Brownian motion of polystyrene microspheres in water) provided by the manufacturer. To minimise any disturbance to blood flow by the LDF probe pressure on the tissue, the probe was mounted on a probe holder so that the actual probe was just in contact with the liver surface without any pressure by the

probe weight. The probe was applied to a fixed site on the liver to avoid any error due to anatomical variation in the microcirculation.

4.2.3 Laser Doppler flowmeter data collection and analysis

Data from the continuous measurement by LDF was collected via the NIRS program that can accept the input of 4 different clinical monitors. After conversion of the NIRS data to excel sheets, the LDF data at the relevant points in each experiment was calculated as a mean of 1-minute data.

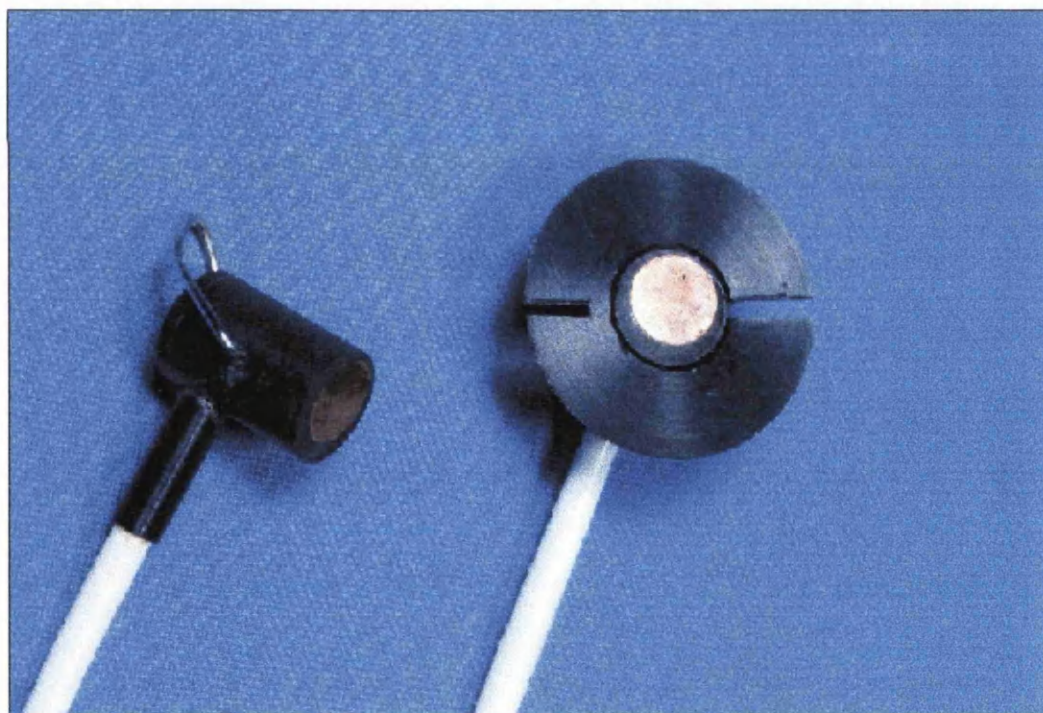
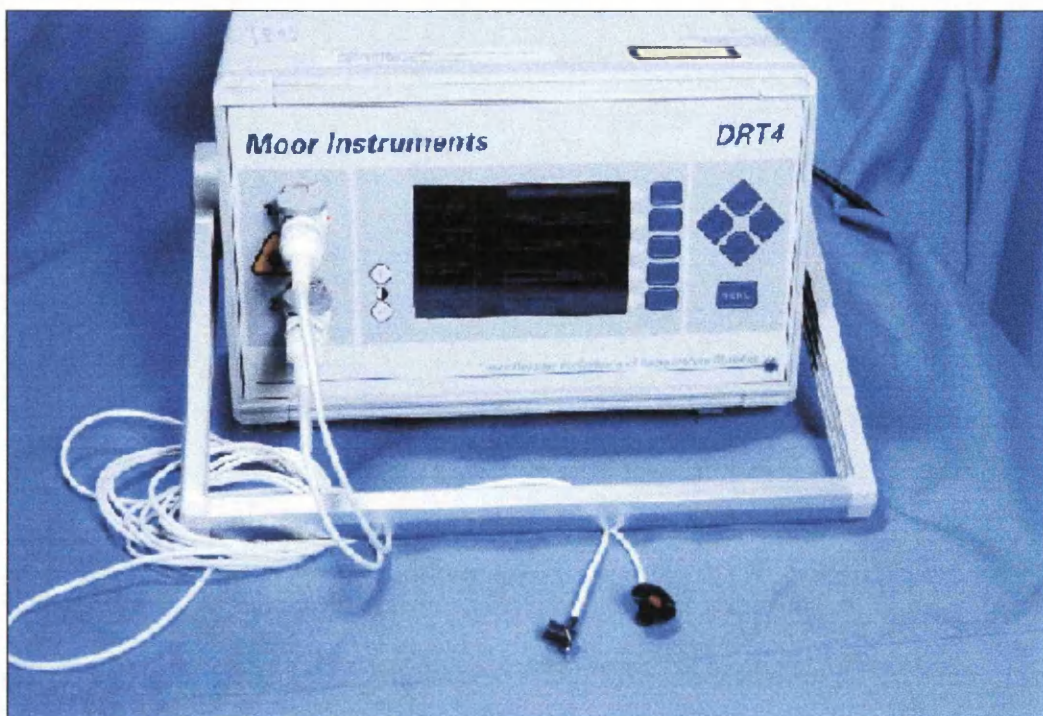


Figure 4.5 Dual channel surface laser Doppler flowmeter and its probes (DRT4, Moor Instruments Ltd., Devon, UK).

4.3 Ultrasonic transit time flowmetry

In this study blood flow through the HA and PV was measured using ultrasonic transit time flowmetry.

4.3.1 Principle of ultrasonic transit time flowmetry

Using wide-beam illumination, two transducers pass ultrasonic signals back and forth, alternately intersecting the flowing liquid in the upstream and downstream directions. The transit time of such beam is a function of the volume flow intersecting this beam, regardless vessel dimensions or cross-sectional area. In the same way that a swimmer will move quicker in the downstream direction than in the upstream direction, the speed of the ultrasound is affected by the flow of liquid passing the 'acoustic window' of the flow probe. The flowmeter derives an accurate measure of the transit time it took the wave of ultrasound to travel from one transducer to the other. The difference between the upstream and downstream-integrated transit times is a measure of volume flow rather than velocity (Takata and Robotham, 1992). The ultrasonic transit time flowmetry has been demonstrated to provide an accurate method for hepatic blood flow measurement (Doi et al. 1988, Jakab et al. 1995, Ayuse et al. 1994).

4.3.2 Ultrasonic transit time flowmeter

PVBF, HABF, and THBF (the sum of PVBF and HABF) were measured continuously using a dual ultrasonic transit time flowmeter (HT207, Transonic Systems Inc, NY, USA) (Figure 4.6). The flowmeter perivascular probes were placed around the hepatic artery and portal vein (Figure 4.6). The accuracy of this technique is dependent on careful positioning and alignment of the probe with respect to the vessel. The vessel should be positioned within the central area of uniform ultrasonic intensity of the probe window i.e. away from the probe window edges, which have lower ultrasonic beam intensity (Takata and Robotham, 1992). Also, it requires accurate selection of the probe size which was determined by the outer diameter of the blood vessel.

4.3.3 Ultrasonic transit time flowmeter data collection and analysis

Data from the continuous measurement by the ultrasonic flowmeter were collected via the NIRS program. After conversion of the NIRS data to excel sheets, the ultrasonic flowmeter data at the relevant points in each experiment was calculated as a mean of 1-minute data.

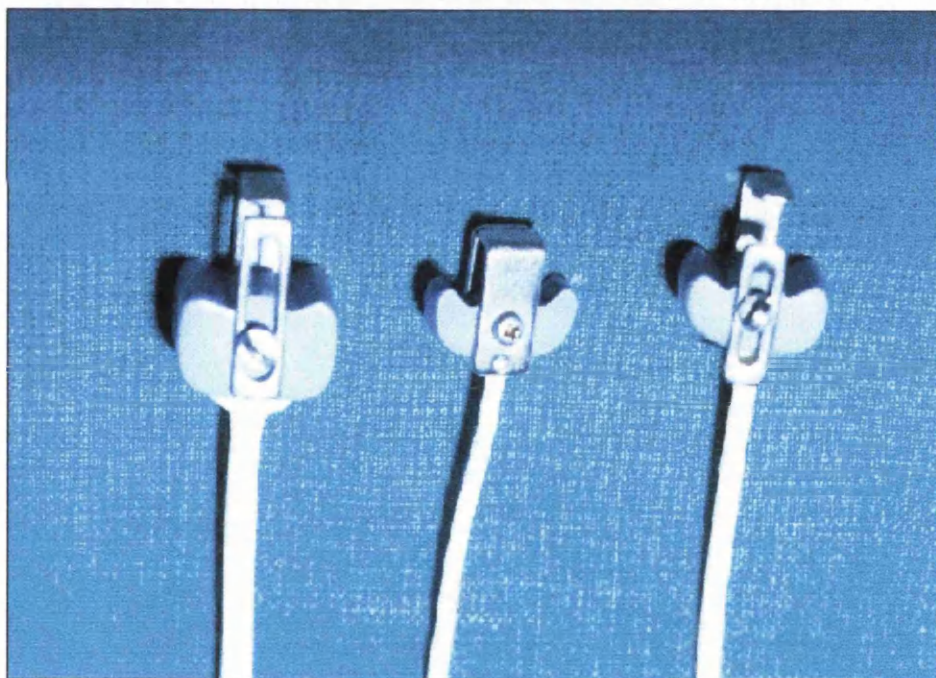
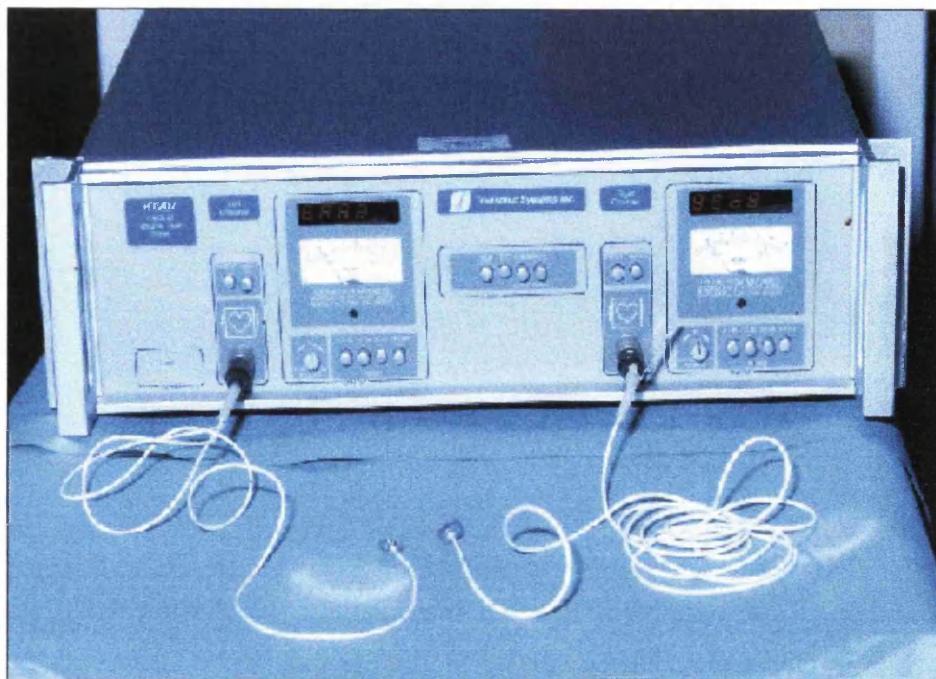


Figure 4.6 Dual ultrasonic transit time flowmeter (HT207, Transonic Systems Inc., NY, USA) and the perivascular probes.

4.4 Nuclear magnetic resonance spectroscopy

The hepatic cellular energy metabolism in this study was monitored by phosphorus nuclear magnetic resonance (^{31}P NMR) spectroscopy.

4.4.1 Principle of nuclear magnetic resonance spectroscopy

Measurement of tissue energy metabolism in this study was done using NMR spectroscopy. The principle of NMR has been described in detail in many studies (Iles et al. 1985, Williams and Gadian, 1986, Desmoulin et al. 1987). It is based on the ability of a nucleus with an unpaired number of protons such as ^{31}P to resonate upon the application of a short radio frequency (RF) pulse in the presence of a strong magnetic field. The release of RF energy after cessation of the excitation pulse (free induction decay, FID) is picked up by the spectrometer coil. The FID, which is a time signal, is then Fourier transformed into frequency signal in which the individual frequencies of the respective ^{31}P nuclei can be recognised. These frequencies are characteristic for the type of chemical bond in which the nucleus is located. A graphic display of the frequency intensities in parts per million (ppm) produces a spectrum. This spectrum gives information about the identity of a resonance peak through the position of the peak on the ppm axis relative to a reference signal, the so-called chemical shift.

4.4.2 Nuclear magnetic resonance spectrometer

In this study a 4.7 Tesla NMR spectrometer (Oxford Magnet Technology, Oxford, UK) was used. Phosphorus spectra were acquired using an acquisition time of 0.5 second, a delay time of 0.1 second, a pulse width of 70 μ second and a spectral width of 8.0 KHz with 128 averages, producing time-resolved spectra every 1 minute.

A typical ^{31}P NMR spectrum of the liver contains resonances which can be assigned to phosphomonoesters (PME), Pi, and three nucleoside triphosphate (NTP) resonances (McLaughlin et al. 1979, Iles and Griffiths, 1982). ATP contributes about 70% of the nucleoside triphosphate signal with other nucleotide triphosphates such as guanosine triphosphate contributing 30% (Chapman et al. 1981). Changes in NTP therefore imply changes in ATP. Changes in ATP were measured from the β -NTP resonance since the γ -NTP and α -NTP resonances contain contributions from the β - and α - phosphates of ADP. The α -NTP also contains contributions from nicotine adenine dinucleotide (NAD^+), reduced nicotine adenine dinucleotide (NADH), nicotine adenine dinucleotide phosphate (NADP^+), and reduced nicotine adenine dinucleotide phosphate (NADPH) (Evanochko et al. 1984). β -NTP resonance arises solely from the β phosphate of the ATP (McLaughlin et al. 1979, Iles et al. 1985).

4.4.3 Quantification of ^{31}P nuclear magnetic resonance spectra

As a consequence of using a 4.7 Tesla high field in vivo NMR system a broad co-resonance of phospholipid components of membranes, nucleic acids, and possibly slowly tumbling phosphorus containing proteins appears between 30 and -30 ppm, coinciding with the metabolites of interest (Iles and Griffiths, 1982, Williams and Gadian, 1986, Changani et al. 1997). Absolute integration of individual resonance therefore requires the removal of this broad resonance. In order to assess the changes in the groups studied, the peak heights of the five observed metabolites (PME, Pi, γ -NTP, α -NTP, and β -NTP) were measured from the spectral baseline which included a component from the phospholipid.

Individual peak heights were then divided by the sum of all the peak heights (total phosphorus peak height). The total phosphorus peak height within each experiment did not change and hence was regarded as a good standard to be employed for all experiments (Changani et al. 1997). All values are therefore expressed relative to the total phosphorus peak height and the relative peak height changes are expressed with a component of phospholipid which did not appear to change throughout the experiment (Changani et al. 1997).

Intracellular pH (pHi) was calculated from the chemical shift difference between the pH dependent Pi resonance relative to the pH independent α -NTP resonance using the NMR version of Henderson-Hasselbach equation (Malloy et al. 1986):

$$\text{pHi} = 6.75 + \log (\sigma - 10.85 / 13.25 - \sigma), \text{ where } \sigma = \text{chemical shift difference between Pi and } \alpha\text{-NTP, 6.75 is the pKa for Pi in liver tissue}$$

Chapter 5

**Validation of near infrared spectroscopy measurement of hepatic tissue
oxygenation in a porcine model: comparison with hepatic vein oxygen
partial pressure**

5.1 Introduction

The liver is generally regarded as being resistant to a reduction in oxygen supply, but hypoxia can induce profound metabolic changes which may lead to liver cell damage and impairment of function (Kahn et al. 1986, Clavien et al. 1992).

Hepatic tissue oxygenation correlates with the microcirculatory impairment and liver dysfunction induced by I/R injury (Goto et al. 1992, Vollmar et al. 1996).

Liver graft tissue oxygenation has also been shown to provide valuable information on early graft function and survival in experimental animals (Sumimoto et al. 1987) and human liver transplantation (Kitai et al. 1995). Direct measurement of hepatic tissue oxygenation might therefore be a useful method of assessing viability and function during liver surgery and transplantation. NIRS is a light based technique for measuring tissue oxygenation. It monitors changes in extracellular tissue oxygenation (HbO_2 and Hb) (Jobsis, 1992) and intracellular oxygenation by measuring redox changes of Cyt Ox (Jobsis, 1992). A new algorithm for measurement of hepatic tissue oxygenation using NIRS has been developed for measuring tissue oxygenation changes (see Chapter 4.1.5). In this experiment NIRS measurement of hepatic tissue oxygenation changes were compared with hepatic vein oxygen partial pressure (HVPO_2) in a porcine model with graded hypoxia. HVPO_2 measurement was considered as the gold standard technique for measuring hepatic tissue oxygenation (Kainuma et al. 1991, Shimizu et al. 1996)

5.2 Objectives

1. Validation of hepatic tissue oxygenations parameters (HbO₂, Hb, and Cyt Ox) measured by NIRS in comparison to HVPO₂.
2. A comparison of direct hepatic tissue oxygenation measurement by NIRS and HVPO₂ as indicators of tissue hypoxia.

5.3 Materials and methods

5.3.1 Animal preparation and surgical procedure

Large White Landrace pigs (n = 5, 24 ± 4.6 kg) were used. Following an overnight fast, the animals were pre-medicated with 0.1 ml/Kg Stresnil® (Azaperone; Janssen Pharmaceutical Ltd., Oxford, UK) intramuscular. After induction of anaesthesia using 5 mg/Kg Ketaset® (Ketamine hydrochloride; Willows Francis Veterinary, Crawley, Sussex, UK) intravenous, the animals were intubated and mechanically ventilated. Anaesthesia was maintained using Halothane (May and Baker Ltd, Dagenham, UK), nitrous oxide and oxygen via a standard anaesthetic circuit.

The animals were placed in supine position on a warming blanket (Harvard apparatus Ltd., Kent, UK) to maintain their body temperature between 37-39 °C. A pulse oximeter probe (Ohmeda Biox 3740, Ohmeda Co., Louisville, USA) was used for continuous monitoring of arterial oxygen saturation (SaO₂) and heart

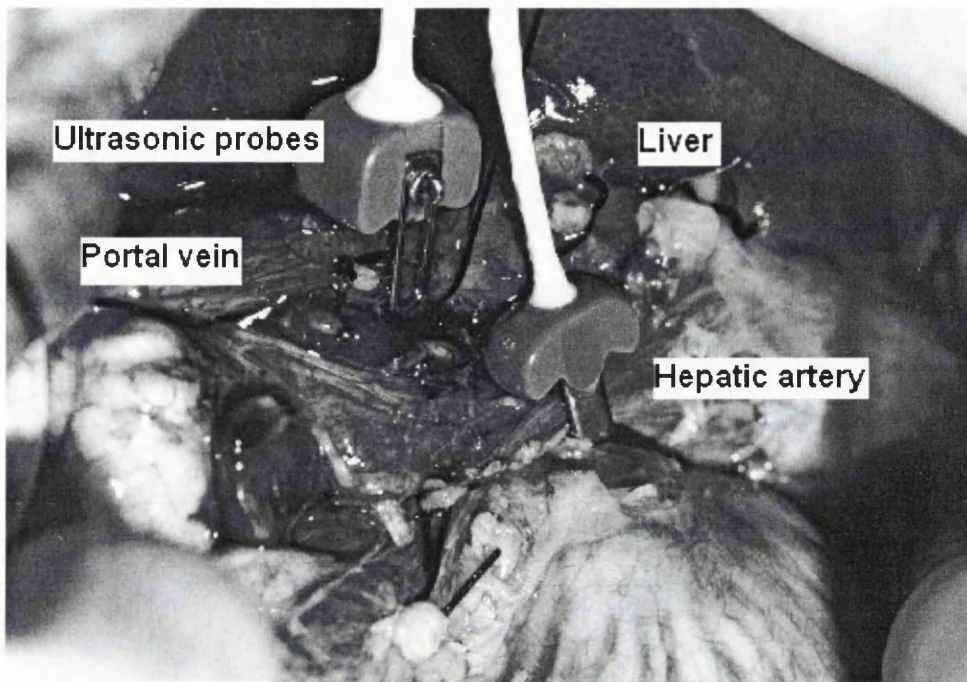
rate (HR). A catheter was inserted into the left internal jugular vein for fluid administration. Another catheter was inserted in the left carotid artery for monitoring mean arterial blood pressure (MABP) and blood sampling as an indicator of hepatic artery oxygenation.

Laparotomy was performed through a transverse subcostal incision. The ligamentous attachments from the liver to the diaphragm and the abdominal wall were divided and the liver exposed. The porta hepatis was exposed and the PV and HA were identified and carefully dissected.

For continuous monitoring of hepatic blood flow: PVBF, HABF and THBF were measured continuously using a dual transonic flowmeter. The ultrasonic flowmeter perivascular probes were placed around the HA and PV (4 and 6 mm diameter, respectively) (Figure 5.1A). The ultrasonic flowmeter perivascular probes were placed close to the hepatic hilum and therefore above the junction of non-hepatic vascular branches to measure the hepatic flow only.

The splenic vein was cannulated for blood sampling from the portal system and the hepatic vein cannulated for measuring HVPO₂. NIRS probes were placed, with a 20 mm separation, on the surface of the right lobe of the liver for continuous monitoring of the changes in hepatic tissue oxygenation and blood volume (Figure 5.1B). Blood samples (2 ml each) were obtained from the carotid artery, portal vein and hepatic vein for blood gas measurements at the end of each hypoxic period using a commercial blood gas analyser (BGElectrolytes, Instrumentation Laboratory Ltd., Cheshire, UK).

(A)



(B)

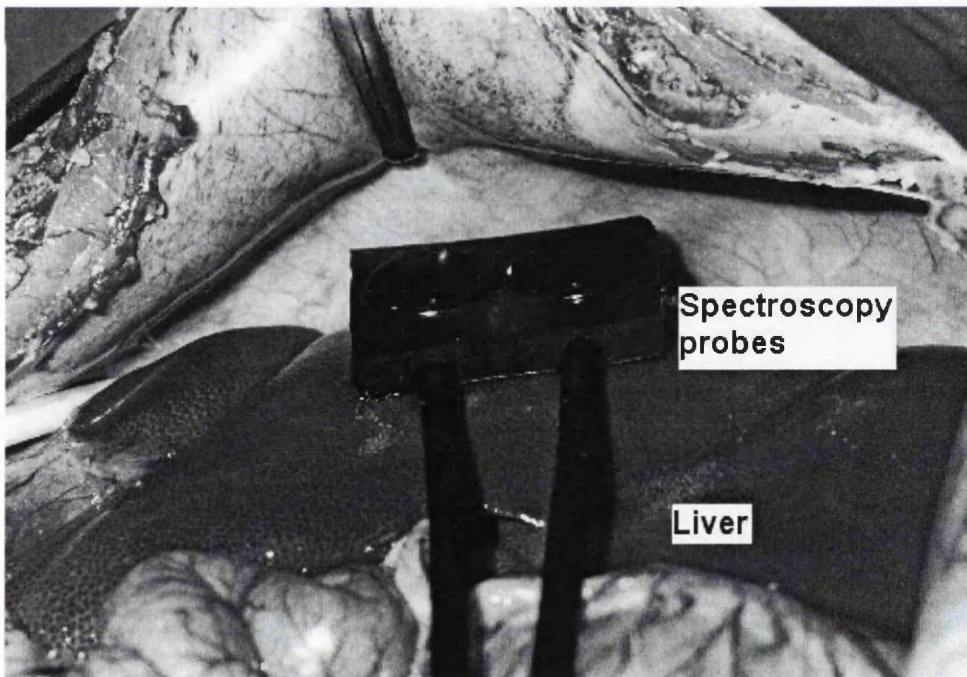


Figure 5.1 (A) Transonic flowmeter perivascular probes around HA and PV. (B) NIRO 500 probes on the surface of the right lobe of the liver.

5.3.2 Experimental protocol

The animals' parameters were allowed to stabilise for 10 minutes and then baseline measurements and blood samples were obtained. The fraction of inspired oxygen (FiO_2) was then reduced in stepwise fashion from FiO_2 of 30% (baseline) to 20, 15, 10, 8, 6, 4%. Five minutes were allowed at each hypoxia level before blood samples were taken. The selection of a 5-minute period for each FiO_2 level based on the observation that reduction and oxidation responses for Cyt Ox reached a new steady-state level within 5 minutes (Sylvia and Piantadosi, 1988). With hypoxia, hepatic vein oxygenation stabilises within a few minutes with a 50% change after 2 minutes (Winkler et al. 1986). After completion of the experiments the animals were killed by a lethal dose (20 ml, 200 mg/ml) of Euthatal[®] (pentobarbitone sodium; Rhone Merieux Ltd., Essex, UK).

5.3.3 Data collection and statistical analysis

Data from the pulse oximeter, blood pressure monitor, transonic flowmeter, and NIRS were collected continuously on a laptop computer. The data were calculated as 1-minute mean values at the beginning of the experiment before the induction of hypoxia (baseline) and at the end of each hypoxia period where the maximum changes in tissue oxygenation occurred.

The hepatic tissue oxygenation changes at the end of each hypoxic period were calculated relative to the baseline. The values are expressed as mean $\pm$ SD. For statistical analysis one way analysis of the variance (ANOVA) and Student's t-test were used with Bonferroni correction for multiple tests. $p < 0.05$ was considered statistically significant. The relationship between hepatic HbO₂, Hb, Cyt Ox, and HVPO₂ was tested using non-linear regression and Spearman's rank order correlation.

5.4 Results

5.4.1 Systemic and hepatic haemodynamics with hypoxia

All the animals tolerated all grades of hypoxia. The baseline HR was 97 ± 11 beats/min and MABP 85 ± 13 mmHg. Immediately after induction of hypoxia HR and MABP significantly increased and remained elevated for the duration of the hypoxia period (Table 5.1). No significant changes were seen in PVBF with any grade of hypoxia, whereas a significant reduction in HABF and THBF occurred with $FiO_2 \leq 10\%$ (Table 5.1).

Table 5.1 Systemic and hepatic haemodynamics at the start of the experiment
(baseline: FiO₂ 30%) and at the end of each hypoxia period.

FiO ₂ (%)	HR (bpm)	MABP (mmHg)	HABF (ml/min)	PVBF (ml/min)	THBF (ml/min)
30	97 ± 11 [‡]	85 ± 13 [‡]	178 ± 14 [‡]	917 ± 65 [•]	1095 ± 57 [‡]
20	110 ± 13 ^{‡*}	102 ± 12 ^{‡*}	163 ± 12 ^{‡*}	905 ± 111 [•]	1068 ± 105 ^{‡*}
15	131 ± 14 ^{‡*}	109 ± 14 ^{‡*}	158 ± 16 ^{‡*}	881 ± 112 [•]	1039 ± 103 ^{‡*}
10	143 ± 15 ^{‡*}	119 ± 20 ^{‡*}	125 ± 11 ^{‡*}	876 ± 111 [•]	1001 ± 102 ^{‡*}
8	146 ± 15 ^{‡*}	129 ± 20 ^{‡*}	114 ± 15 ^{‡*}	875 ± 97 [•]	989 ± 87 ^{‡*}
6	151 ± 14 ^{‡*}	138 ± 17 ^{‡*}	107 ± 17 ^{‡*}	883 ± 102 [•]	990 ± 89 ^{‡*}
4	161 ± 17 ^{‡*}	143 ± 16 ^{‡*}	98 ± 13 ^{‡*}	798 ± 89 [•]	896 ± 80 ^{‡*}

Values are mean ± SD of 5 animals. [‡]p < 0.05 and [•]p = not significant (ANOVA).

*p < 0.05 vs. baseline (Student's t test).

5.4.2 Oxygen partial pressure with hypoxia

With all grades of hypoxia there was a significant progressive reduction in HAPO₂, PVPO₂, and HVPO₂ (Table 5.2).

Table 5.2 HAPO₂, PVPO₂, and HVPO₂ (mmHg) at the start of the experiment (baseline: FiO₂ 30%) and at the end of each hypoxia period.

FiO ₂ (%)	HAPO ₂	PVPO ₂	HVPO ₂
30	106 ± 11 [‡]	51 ± 7 [‡]	40 ± 3 [‡]
20	84 ± 13 ^{‡*}	42 ± 9 ^{‡*}	33 ± 8 ^{‡*}
15	74 ± 10 ^{‡*}	38 ± 11 ^{‡*}	27 ± 5 ^{‡*}
10	53 ± 9 ^{‡*}	30 ± 8 ^{‡*}	20 ± 6 ^{‡*}
8	42 ± 10 ^{‡*}	23 ± 7 ^{‡*}	16 ± 4 ^{‡*}
6	31 ± 9 ^{‡*}	18 ± 4 ^{‡*}	9 ± 6 ^{‡*}
4	22 ± 8 ^{‡*}	13 ± 3 ^{‡*}	7 ± 2 ^{‡*}

Values are mean ± SD of 5 animals. [‡]p < 0.05 (ANOVA).

*p < 0.05 vs. baseline (Student's t test).

5.4.3 Hepatic tissue oxygenation and blood volume changes measured by NIRS with hypoxia

A typical example of NIRS measurements in one animal with graded hypoxia is shown in Figure 5.2. No significant changes occurred in the baseline tissue oxygenation parameters from the zero value at the beginning of the measurement (Table 5.3). With all hypoxic periods there was an immediate significant reduction in hepatic HbO₂ and a simultaneous increase in Hb (Table 5.3). Hepatic Cyt Ox was not significantly reduced from the baseline with FiO₂ of > 10% but was significantly reduced with further grades of hypoxia (Table 5.3). There was a significant reduction of HbT with FiO₂ ≤ 10% (Table 5.3).

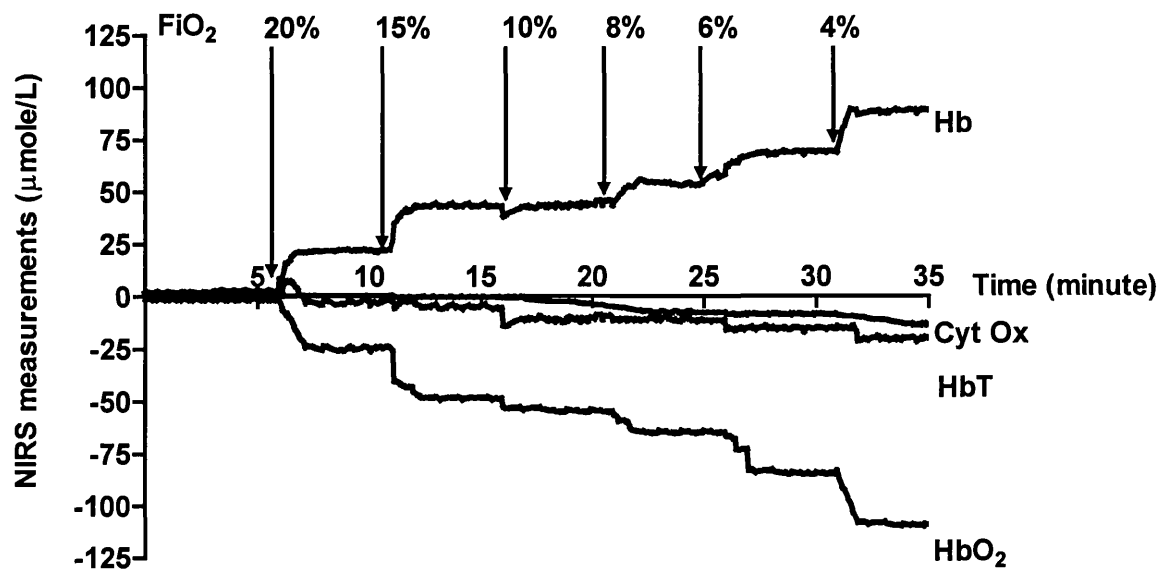


Figure 5.2 A typical example of NIRS measurements in one animal with graded hypoxia. The start of each hypoxic period is marked by arrow.

Table 5.3 Changes in hepatic tissue oxygenation ($\mu\text{mole/L}$) measured by NIRS at the start of the experiment (baseline: FiO_2 30%) and at the end of each hypoxia period.

FiO_2	HbO_2	Hb	Cyt Ox	HbT
30	$-1.54 \pm 2.9^{\dagger}$	$3.16 \pm 1.24^{\dagger}$	$-0.44 \pm 1.59^{\dagger}$	$1.62 \pm 3.4^{\dagger}$
20	$-31.01 \pm 7.98^{\dagger*}$	$26.43 \pm 7.32^{\dagger*}$	$-0.58 \pm 1.56^{\dagger*}$	$-4.58 \pm 2.74^{\dagger*}$
15	$-43.65 \pm 8.6^{\dagger*}$	$34.91 \pm 9.56^{\dagger*}$	$-0.32 \pm 1.98^{\dagger*}$	$-6.44 \pm 3.35^{\dagger*}$
10	$-60.32 \pm 6.98^{\dagger**}$	$45.02 \pm 7.99^{\dagger**}$	$-4.82 \pm 1.65^{\dagger*}$	$-13.44 \pm 5.63^{\dagger**}$
8	$-71.62 \pm 8.7^{\dagger**}$	$52.71 \pm 8.31^{\dagger**}$	$-8.5 \pm 1.72^{\dagger*}$	$-14.45 \pm 4.84^{\dagger**}$
6	$-83.40 \pm 8.9^{\dagger**}$	$64.28 \pm 9.73^{\dagger**}$	$-11.61 \pm 2.89^{\dagger*}$	$-17.25 \pm 5.11^{\dagger**}$
4	$-100.26 \pm 7.9^{\dagger**}$	$78.30 \pm 12.81^{\dagger**}$	$-15.83 \pm 4.32^{\dagger*}$	$-22.95 \pm 9.17^{\dagger**}$

Values are mean $\pm$ SD of 5 animals. Values were calculated at the end of each hypoxic period relative to baseline. $^{\dagger}p < 0.05$ (ANOVA). $^*p < 0.05$, $^{**}p < 0.01$, and $^{\circ}p$ = not significant vs. baseline (Student's t test).

A significant correlation was found between hepatic vein oxygen partial pressure and changes in hepatic tissue HbO₂ ($r = 0.87$, $p < 0.001$), Hb ($r = -0.86$, $p < 0.001$), and Cyt Ox ($r = 0.91$, $p < 0.001$) (Figure 5.3A-C).

The temporal relationship of Cyt Ox and HVPO₂ with reduction of FiO₂ is shown in Figure 5.4. At FiO₂ > 10% there was a reduction in HVPO₂ with no change in Cyt Ox. With FiO₂ ≤ 10% the reduction in HVPO₂ was associated with reduction in Cyt Ox.

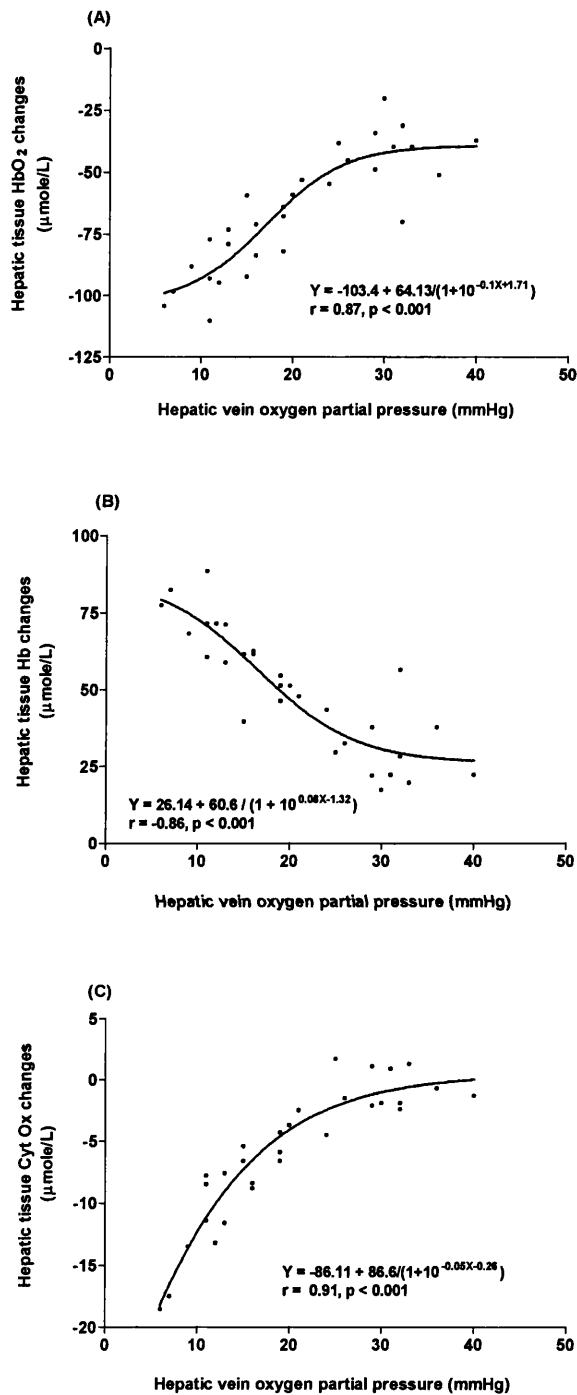


Figure 5.3 The correlation between changes in hepatic HbO₂ (A), Hb (B), and Cyt Ox (C) and HVPO₂ with graded hypoxia. Tissue oxygenation values are the mean of 1-minute measurements at the end of one hypoxic period in one animal.

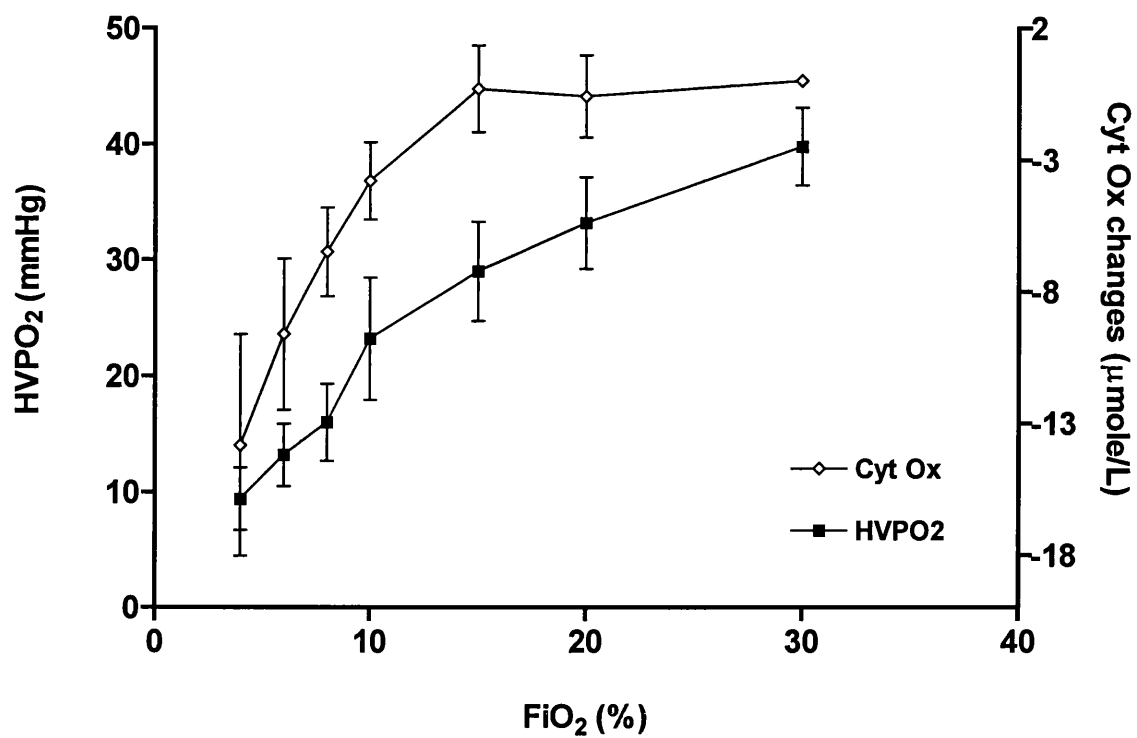


Figure 5.4 Temporal relationships of Cyt Ox and HVPO₂ with graded hypoxia.

Each point is mean $\pm$ SD of 5 animals at the end of one hypoxic period.

5.5 Discussion

This study has investigated the relationship between the changes in liver tissue oxygenation measured by NIRS with HVPO₂. As the hepatic microcirculation is predominantly venous (by volume) and the sinusoidal volume is about 35% larger in the pericentral than in the periportal areas, hepatic tissue oxygenation measured by NIRS should correlate with HVPO₂ (Miller et al. 1979, Conway et al. 1985). HVPO₂ is the sum of oxygen partial pressure in the blood at the venous end of the sinusoids and its saturation depends on oxygen supply to the liver and consumption in the tissue (Kainuma et al. 1991, Shimizu et al. 1996). HVPO₂ measurement has been used to monitor hepatic oxygenation during liver surgery (Kainuma et al. 1991) and liver transplantation (Katsuramaki et al. 1997). It has also been used as an indicator of critical hepatic hypoxia associated with irreversible hepatocellular damage and impairment of the energy metabolism (Winkler et al. 1986). The main disadvantage of HVPO₂ measurement is the requirement of direct access to the hepatic vein for blood sampling via cannulation which is an invasive procedure.

In this study a large animal model with similar anatomy and physiology to the human was used (Copper et al. 1991). All grades of hypoxia produced a significant increase in HR and MABP, which has been previously found in studies investigating the effect of hypoxia on the systemic and splanchnic circulations in pigs (Licker et al. 1998), dogs (Mathie and Blumgart, 1983), and cats (Larsen et al. 1976). These haemodynamic changes are due to sympathetic stimulation

and increased peripheral vascular resistance induced by hypoxia and mediated by chemoreceptor stimulation (Korner, 1965) and increased catecholamine secretion (Licker et al. 1998).

In this study the PVBF did not alter with hypoxia, while there was a significant decrease in both HABF and THBF with severe hypoxia ($\text{FiO}_2 \leq 10\%$). These results are in agreement with a number of previous studies of the effect of hypoxia upon the hepatic blood flow which have shown that hypoxia has no significant effect on portal blood flow (Scholtholt and Shiraishi, 1970, Hughes et al. 1979), while severe hypoxia produces a decrease in hepatic artery blood flow (Larsen et al. 1976, Hughes et al. 1979). The reduction in HABF secondary to an increase in hepatic arterial resistance is due to the sympathetic vasoconstriction response with hypoxia (Hughes et al. 1979). This response can be eliminated with hepatic artery denervation (Mathie and Blumgart, 1983). The absence of change in PVBF can be explained by the lack of sympathetic control over the PV vasomotor tone (Ito et al. 1998).

The changes in hepatic extra and intracellular tissue oxygenation (HbO_2 , Hb, Cyt Ox, respectively) and hepatic total blood volume (HbT) were measured by NIRS. At the start of the experiment, the instrument sets up at zero values for all the oxygenation parameters and all the changes were relative to this zero. It took about 5 minutes for the measurements to stabilise. No significant change in tissue oxygenation observed before the start of hypoxia. All the changes with hypoxia were calculated relative to the baseline values.

With hypoxia there was an instantaneous decrease of hepatic tissue HbO_2 with a simultaneous increase in Hb, which reflects the dissociation of oxygen from haemoglobin as oxygen is extracted by the hepatic tissue. The liver normally extracts less than 40% of oxygen presented to it at baseline blood flow. However, its oxygen extraction capacity approaches 100% during ischaemia or hypoxia (Lutz et al. 1975, Larsen et al. 1976), the main mechanism for matching hepatic oxygen supply with requirement. There was a significant reduction of the HbT with severe hypoxia (FiO_2 of $\leq 10\%$) which reflects the marked reduction in arterial blood flow.

The redox state of Cyt Ox is dependent on intracellular oxygen availability (Cooper et al. 1994). In the presence of oxygen, electron transfer takes place and the enzyme becomes oxidised. With lack of oxygen the flow of electrons from Cyt Ox decreases and it becomes reduced (Cooper et al. 1994). The reduction of the cellular oxygen supply is paralleled by decreases in the ATP/ADP and NAD^+/NADH concentration ratios and by increase in the reduction state of the mitochondrial respiratory enzymes such as Cyt Ox (Oshino et al. 1974, Chaudry et al. 1976, Wilson et al. 1979a). Thus assessment of the redox state of Cyt Ox could be used as an indicator of intracellular oxygen availability (de Groot and Noll, 1985, Cooper et al. 1994). In this study a significant reduction in Cyt Ox was found only with severe hypoxia (FiO_2 of $\leq 10\%$). This reduction occurred at a level where reduced liver blood flow (reflected by HABF, THBF, and HbT reduction) exacerbates the effect of reduced oxygen supply. The reduction of Cyt Ox only with severe hypoxia can be explained by its high affinity

to oxygen as its half-maximal oxidation (K_m) for oxygen is $< 1 \mu\text{mole}$ in isolated mitochondria (Wilson et al. 1979b), $< 3.5 \mu\text{mole}$ in hepatocytes (Jones and Kennedy, 1982), and $< 6.8 \mu\text{mole}$ in the isolated perfused liver (Nakajima et al. 1990).

This study has also compared changes in hepatic tissue oxygenation parameters using NIRS with HVPO_2 as these two measurements represent the balance between hepatic oxygen delivery and oxygen consumption in the tissue (Kainuma et al. 1991, Kitai et al. 1993b, Shimizu et al. 1996). A significant correlation ($p < 0.001$) was found between the two measurements which support the usefulness of NIRS as a non-invasive method for measuring changes in hepatic tissue oxygenation.

The temporal relationship between the changes in tissue oxygenation measured by NIRS and HVPO_2 was investigated to determine their relative value as indicators of hepatic tissue hypoxia. Mild grades of hypoxia ($\text{FiO}_2 > 10\%$) resulted in a reduction in oxygen delivery with a significant decrease in HVPO_2 . This was associated with reduction of tissue HbO_2 and increase of Hb but with no significant change in Cyt Ox. With severe hypoxia ($\text{FiO}_2 \leq 10\%$) there was reduction of hepatic Cyt Ox with a HVPO_2 of around 20 mmHg (50% of baseline). This demonstrates that HVPO_2 does not adequately reflect changes in intracellular oxygenation. This can be explained by the presence of an oxygen gradient from the sinusoidal blood to the interior of the liver cells with the steepest gradient being found across the mitochondrial membrane (Jones and Kennedy, 1982, Jones, 1986). The rate of oxygen diffusion may be a critical

factor for intracellular oxygen supply to mitochondria during hypoxia (Jones and Kennedy, 1982, Jones, 1986).

In this study the use of Cyt Ox redox changes as an index of intracellular hypoxia suggested that intracellular hypoxia starts to occur with arterial oxygen partial pressure of ≤ 53 mmHg (FiO_2 of $\leq 10\%$). This is in agreement with other studies measuring the critical arterial oxygen partial pressure level at which tissue damage from hypoxia occurs (Huckabee, 1958, Loegering and Critz, 1971, Hobler and Carey, 1973, Ukikusa et al. 1979). Reduction of the hepatic energy charge and bile flow with concomitant increase in the ratio of lactate to pyruvate in the arterial blood occurs with an arterial oxygen partial pressure of 50 mmHg (Ukikusa et al. 1979). Using blood lactate level to determine tissue hypoxia suggested that the critical arterial oxygen partial pressure is 32 mmHg (Huckabee, 1958) or 36 mmHg (Hobler and Carey, 1973). Direct assessment of tissue damage by measuring the release of glutamic oxaloacetate and creatine phosphokinase enzymes from muscles as an index of critical tissue hypoxia has similarly suggested that this occurs on breathing 8.5 - 12% oxygen (Loegering and Critz, 1971).

Clearly the data from this study would suggest that NIRS measurement of Cyt Ox is an accurate indicator of intracellular hypoxia. Whether the level of reduced Cyt Ox can be correlated with an independent indicator of altered cellular bioenergetics remained to be determined.

Chapter 6

The correlation between hepatic tissue oxygenation measured by near infrared spectroscopy and energy metabolism measured by ^{31}P nuclear magnetic resonance spectroscopy

6.1 Introduction

Liver hypoxia has a detrimental effect on hepatic cell function, metabolism, and morphology (Bradford et al. 1986). It causes depletion of high-energy phosphate moieties, such as ATP with the subsequent impairment of ATP-dependent intracellular homeostatic mechanisms (Winkler et al. 1986). The measurement of intracellular ATP has been proposed as a sensitive indicator of hypoxic liver damage (Sumimoto et al. 1988, Higashi et al. 1989). Currently available methods for ATP measurement included enzymatic assay (Lamprechts and Trautschold, 1963), chromatography (Kamiike et al. 1982), and ^{31}P NMR (McLaughlin et al. 1979). These methods are either invasive requiring tissue biopsy or require a complicated procedure and time for processing of data which make them unsuitable for routine clinical use.

NIRS can measure continuously tissue HbO_2 , Hb, as well as the redox change of Cyt Ox (Jobsis, 1992). Cyt Ox is the terminal electron carrier of the mitochondrial respiratory chain that catalyses the reduction of oxygen to H_2O which is directly coupled to the synthesis of ATP through oxidative phosphorylation (Capaldi, 1990). In aerobically respiring hepatocytes, approximately 90% of the oxygen taken up is consumed to produce ATP through Cyt Ox (de Groot and Noll, 1985) and hence the redox state of Cyt Ox may be useful for monitoring intracellular ATP levels.

Previous studies have shown the effect of hypoxia on hepatic ATP metabolism (Pass et al. 1982, Desmoulin et al. 1987, Bowers et al. 1992) but the

correlation between hepatic extracellular (HbO₂ and Hb) and intracellular (Cyt Ox) oxygenation with ATP metabolism has not been investigated.

6.2 Objectives

1. To correlate the changes in hepatic HbO₂, Hb, and Cyt Ox measured by NIRS with intracellular ATP and pH measured by ³¹P NMR spectroscopy with graded hypoxia.
2. To determine the significance of measuring Cyt Ox as an indicator of critical tissue hypoxia associated with reduction of ATP.

6.3 Materials and methods

6.3.1 Animal preparation and surgical procedure

Sprague Dawley rats (397 ± 34 g, $n = 15$), in 11 animals ³¹P NMR spectroscopy measurements alone were done and in 4 animals simultaneous ³¹P NMR spectroscopy and NIRS measurements were done. The animals were anaesthetised by 25 mg/Kg Sagatal® (pentobarbitone sodium; Rhone Merieux Ltd., Essex, UK) intraperitoneal. Laparotomy was performed through a subcostal incision. The ligamentous attachments from the liver to the diaphragm were severed and the liver was exposed. The animals were allowed to breathe

spontaneously using a mask connected to an oxygen, carbon dioxide, and nitrogen regulator.

The animals were positioned within a NMR cradle. A hot gel pad was placed under the animal with further insulation provided by cotton wool to maintain the animal's temperature.

The NIRS probes were positioned, with 20 mm spacing, on the surface of the right lobe of the liver. A single turn 2 cm diameter phosphorus tuned coil was placed directly on the liver adjacent to the NIRS probes and used to acquire blocked spectra at 26 MHz.

6.3.2 Nuclear magnetic resonance spectroscopy

The animals were positioned in the centre of the bore of a 4.7 Tesla NMR spectrometer (Oxford Magnet Technology, Oxford, UK) for acquiring the NMR spectra. The principle of the technique, data collection and analysis have been outlined in Chapter 4.4.

6.3.3 Experimental protocol

Following acquisition of baseline NMR spectra and NIRS measurements for 5 minutes the animals were exposed to 6-minute periods of consecutive graded hypoxia separated by 5-minute recovery periods. Graded hypoxia was induced by a stepwise reduction of the FiO_2 using 15, 10, 8, 6, and 4% mixtures of

oxygen balanced with nitrogen and recovery between the hypoxic periods using 30% oxygen. In this experiment recovery periods were allowed between the hypoxia periods to avoid any cumulative effect of hypoxia. At the end of the experiment the animals were killed by exsanguination.

6.3.4 Data collection and statistical analysis

Data are expressed as mean $\pm$ SD. The NIRS data were calculated as 1-minute mean values at the beginning of the experiment before the induction of hypoxia (baseline) and at the end of each hypoxia period where the maximum changes in tissue oxygenation occurred. The PME, Pi, and β -NTP, measured by NMR, were calculated at the end of each hypoxia period when these signals were maximally changed from the baseline. For statistical analysis one way analysis of the variance (ANOVA) and Student's t-test were used with Bonferroni correction for multiple tests. $p < 0.05$ was considered statistically significant. The relationships between the NIRS measurements (HbO₂, Hb, and Cyt Ox) and the energy metabolites (PME, Pi, and β -NTP) were tested using linear regression analysis.

6.4 Results

6.4.1 Hepatic tissue oxygenation and blood volume changes measured by NIRS with hypoxia

All animals tolerated and recovered from exposure to FiO_2 of 15 and 10%. At FiO_2 of 8% 4 animals died, with FiO_2 of 6% 3 animals died and with FiO_2 of 4% 4 animals died. With all hypoxic periods there was an immediate reduction in hepatic HbO_2 and a simultaneous increase in Hb (Table 6.1). Hepatic Cyt Ox was not significantly reduced from the baseline with FiO_2 of $> 10\%$ but was significantly reduced with further grades of hypoxia ($\text{FiO}_2 \leq 10\%$) (Table 6.1). There was a significant reduction of HbT with $\text{FiO}_2 \leq 10\%$ (Table 6.1). Figure 6.1 represents an example of NIRS measurements with graded hypoxia in one animal.

Table 6.1 Changes in hepatic tissue oxygenation ($\mu\text{mole/L}$) measured by NIRS at the start of the experiment (baseline: FiO_2 30%) and at the end of each hypoxia period.

FiO_2	HbO_2	Hb	Cyt Ox	HbT
30	$-2.34 \pm 1.2^{\dagger}$	$1.11 \pm 0.9^{\dagger}$	$-0.54 \pm 0.3^{\dagger}$	$-1.23 \pm 0.9^{\dagger}$
15	$-39.24 \pm 13.49^{\dagger**}$	$35.21 \pm 8.17^{\dagger**}$	$-0.15 \pm 0.24^{\dagger*}$	$-3.78 \pm 5.77^{\dagger*}$
10	$-40.16 \pm 11.19^{\dagger**}$	$31.61 \pm 5.03^{\dagger**}$	$-3.05 \pm 2.48^{\dagger*}$	$-8.55 \pm 3.28^{\dagger*}$
8	$-58.97 \pm 15.69^{\dagger**}$	$46.80 \pm 9.65^{\dagger**}$	$-5.54 \pm 2.65^{\dagger*}$	$-12.18 \pm 6.01^{\dagger*}$
6	$-67.84 \pm 18.78^{\dagger**}$	$54.73 \pm 13.65^{\dagger**}$	$-7.34 \pm 3.02^{\dagger*}$	$-13.11 \pm 7.69^{\dagger*}$
4	$-91.37 \pm 19.65^{\dagger**}$	$76.33 \pm 14.23^{\dagger**}$	$-11.13 \pm 5.48^*$	$-15.05 \pm 8.45^{\dagger**}$

Values are mean $\pm$ SD. Values were calculated at the end of each hypoxic period with regard to the baseline. $^{\dagger}p < 0.05$ (ANOVA). $**p < 0.01$, $*p < 0.05$, and $^{\circ}p =$ not significant vs. baseline (Student's t test).

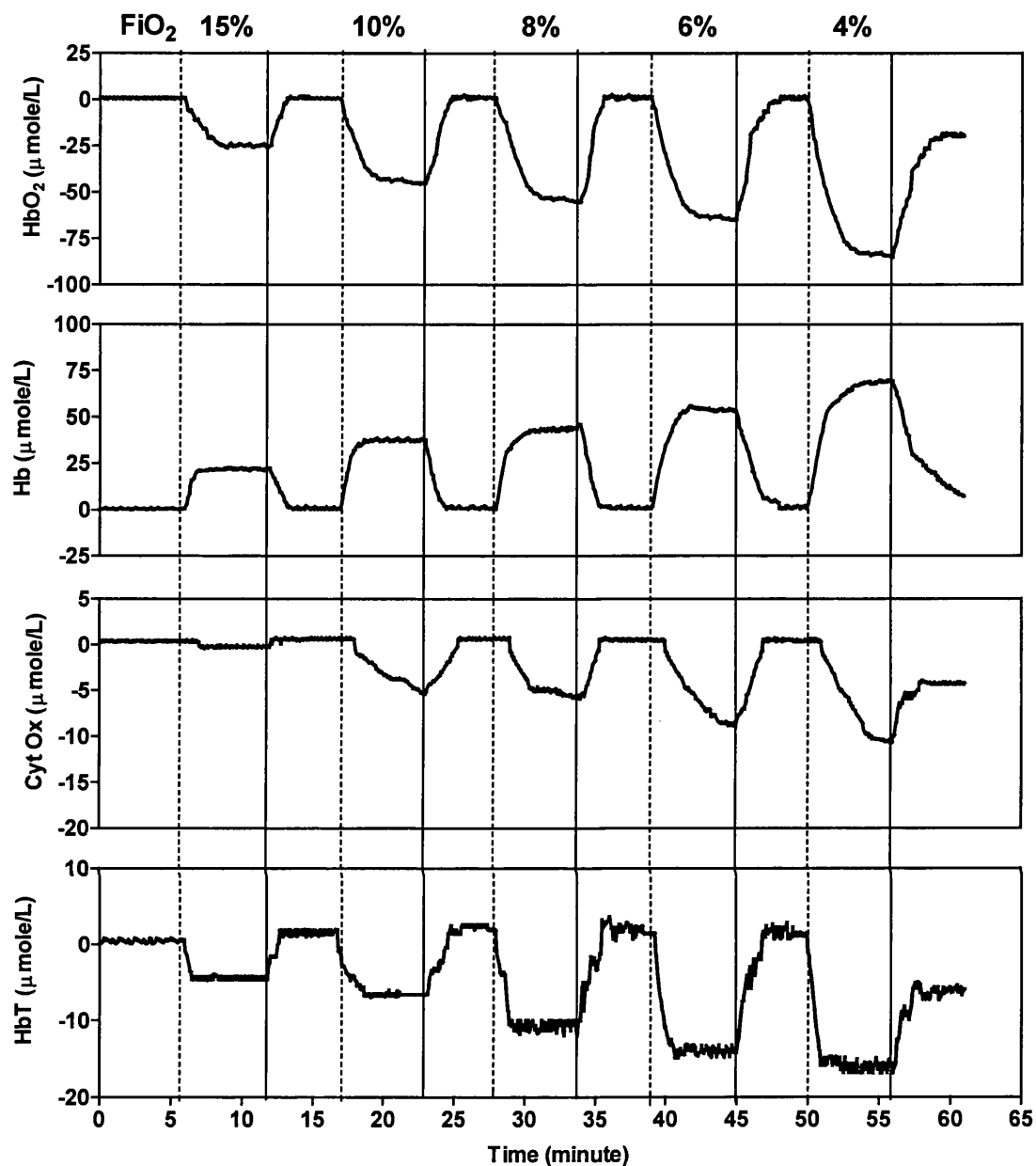


Figure 6.1 A typical example of NIRS measurements in one animal with graded hypoxia. The x-axis is time (minute). The y-axes are hepatic HbO₂, Hb, Cyt Ox, and HbT. Starts of the hypoxic periods are marked by dotted lines and recovery periods by solid lines.

6.4.2 Hepatic energy metabolism measured by ^{31}P NMR spectroscopy

There was no significant change in energy metabolites with FiO_2 of 15%. With further hypoxia ($\text{FiO}_2 \leq 10\%$), ^{31}P NMR spectra showed an increase in PME and P_i resonance with a concomitant decrease in $\beta\text{-NTP}$ resonance (Table 6.2 and Figure 6.2). Intracellular acidosis occurred only with FiO_2 of $\leq 8\%$ (Table 6.2). Hepatic PME, P_i , and $\beta\text{-NTP}$ changes through the whole experiment are summarised in Figure 6.3.

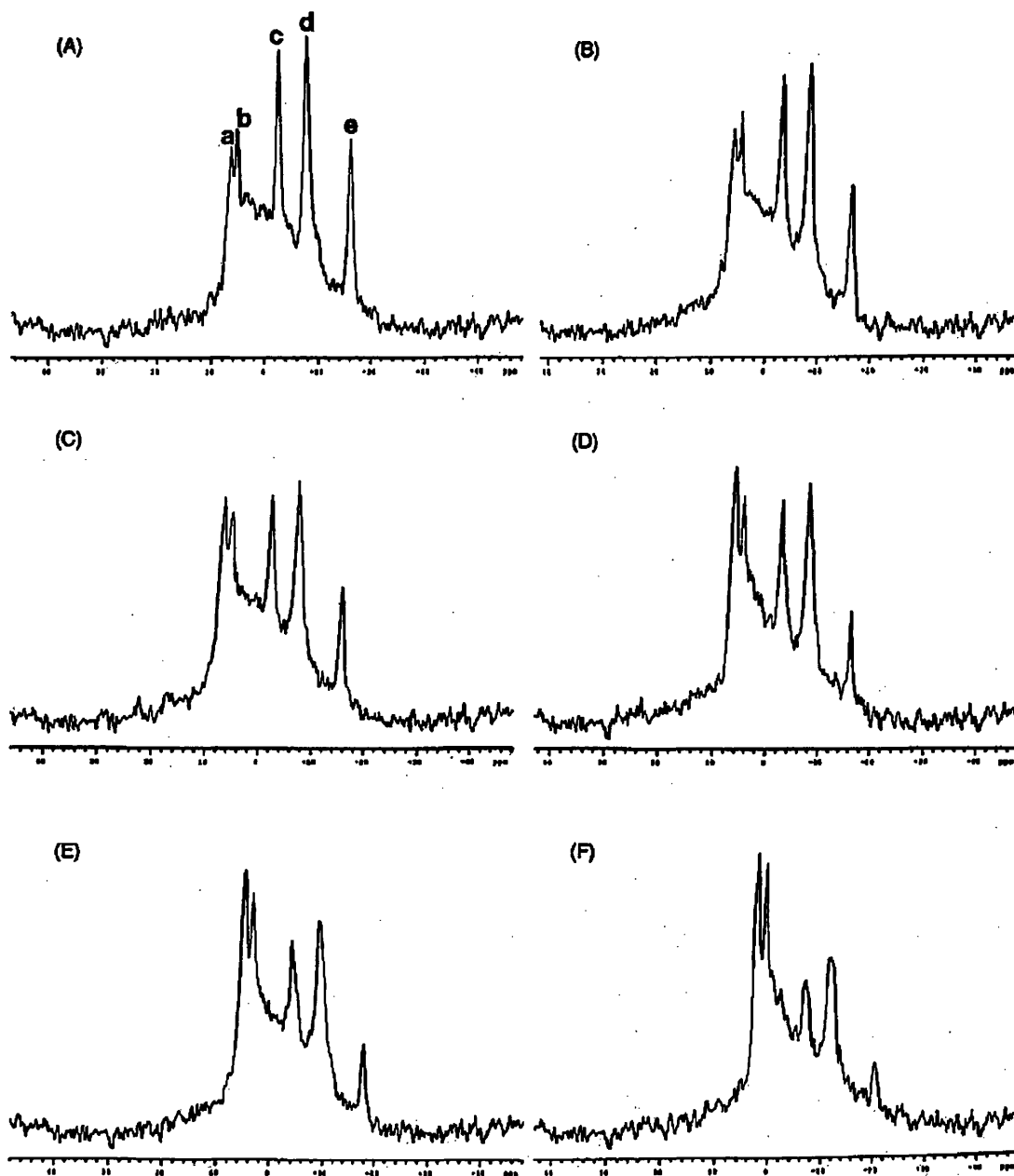


Figure 6.2 Examples of ^{31}P NMR spectroscopy spectra before hypoxia (A) and with FiO_2 of 15 (B), 10 (C), 8 (D), 6 (E), and 4% (F). Peak assignments are a: PME, b: P_i , c: $\gamma\text{-NTP}$, d: $\alpha\text{-NTP}$, and e: $\beta\text{-NTP}$.

Table 6.2 Hepatic energy metabolism and cellular pH with graded hypoxia measured by ^{31}P nuclear magnetic resonance.

FiO_2	PME	P_i	$\beta\text{-NTP}$	pHi
30%	$15.14 \pm 4.61^{\dagger}$	$15.50 \pm 3.39^{\dagger}$	$17.71 \pm 3.14^{\dagger}$	$7.23 \pm 0.06^{\dagger}$
15%	$16.50 \pm 3.60^{\dagger*}$	$16.75 \pm 2.29^{\dagger*}$	$16.13 \pm 3.51^{\dagger*}$	$7.19 \pm 0.04^{\dagger*}$
10%	$19.38 \pm 3.54^{\dagger**}$	$21.63 \pm 2.61^{\dagger**}$	$10.25 \pm 5.49^{\dagger**}$	$7.12 \pm 0.54^{\dagger*}$
8%	$21.86 \pm 4.28^{\dagger**}$	$23.75 \pm 3.54^{\dagger**}$	$9.07 \pm 3.39^{\dagger**}$	$6.97 \pm 0.11^{\dagger**}$
6%	$22.13 \pm 5.59^{\dagger**}$	$23.10 \pm 4.68^{\dagger**}$	$8.38 \pm 4.36^{\dagger**}$	$6.92 \pm 0.04^{\dagger**}$
4%	$22.83 \pm 4.47^{\dagger**}$	$25.33 \pm 6.31^{\dagger**}$	$6.51 \pm 4.29^{\dagger**}$	$6.75 \pm 0.06^{\dagger**}$

Values are mean $\pm$ SD. Values were calculated at the start of the experiment (baseline with FiO_2 of 30 %) and at the end of each hypoxic period. $^{\dagger}\text{p} < 0.01$ (ANOVA). $^{**}\text{p} < 0.01$ and $^*\text{p}$ = not significant vs. baseline (Student's t test).

PME, P_i , and $\beta\text{-NTP}$ are calculated as a percentage of the total phosphorus signal height.

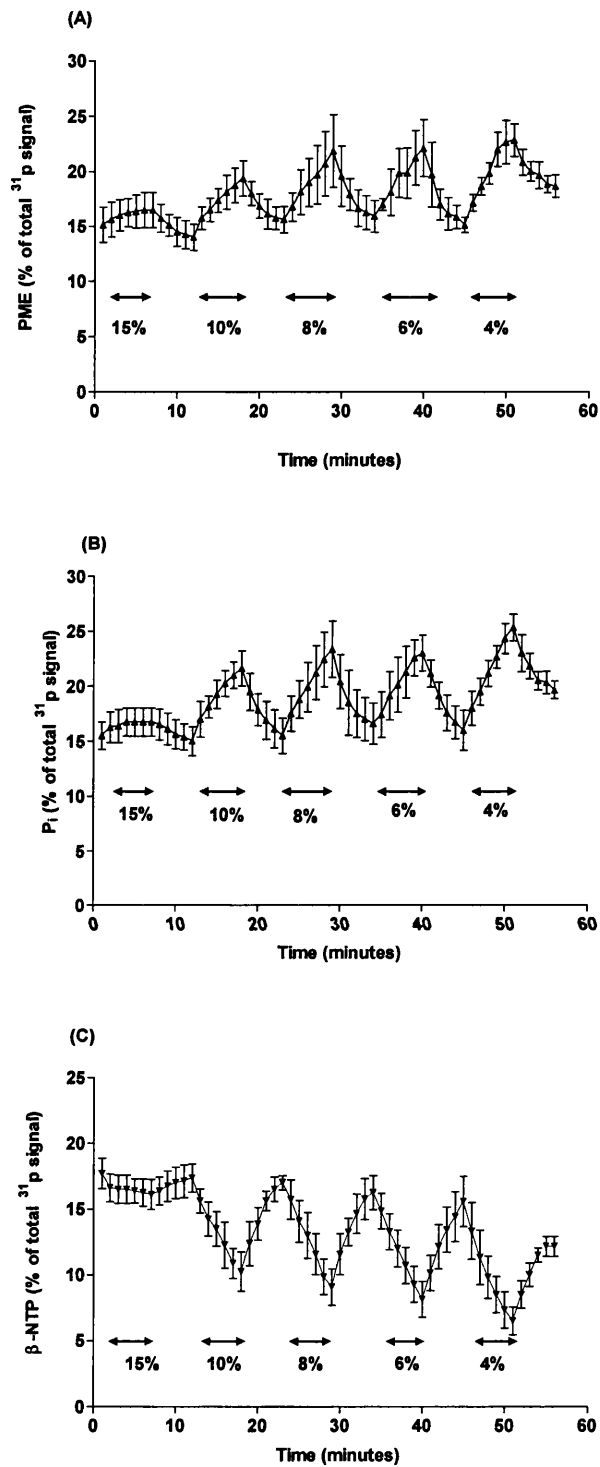


Figure 6.3 Hepatic PME (A), P_i (B), $\beta\text{-NTP}$ (C) at baseline, during hypoxia (FiO_2 of 15, 10, 8, 6, and 4%), and recovery. Values are percentage of the total phosphorus signal height. Values are mean $\pm$ SD.

6.4.3 Correlation between changes in tissue oxygenation measured by NIRS and hepatic energy metabolism measured by ^{31}P NMR spectroscopy

The correlation between the changes in hepatic tissue oxygenation parameters (HbO_2 , Hb, and Cyt Ox) and β -NTP levels are shown in Figure 6.4. β -NTP showed a positive correlation with hepatic HbO_2 and Cyt Ox ($r = 0.82$ and 0.91 , respectively, $p < 0.001$) and a negative correlation with the changes in hepatic Hb ($r = 0.79$, $p < 0.001$).

Mild hypoxia (FiO_2 of 15%) reduced HbO_2 with no significant reduction of Cyt Ox and β -NTP. Further hypoxia (FiO_2 of $\leq 10\%$) produced significant reductions in HbO_2 , Cyt Ox, and β -NTP. The reduction in Cyt Ox measured by NIRS was found to correlate temporally with the reduction of β -NTP measured by ^{31}P NMR spectroscopy (Figure 6.5).

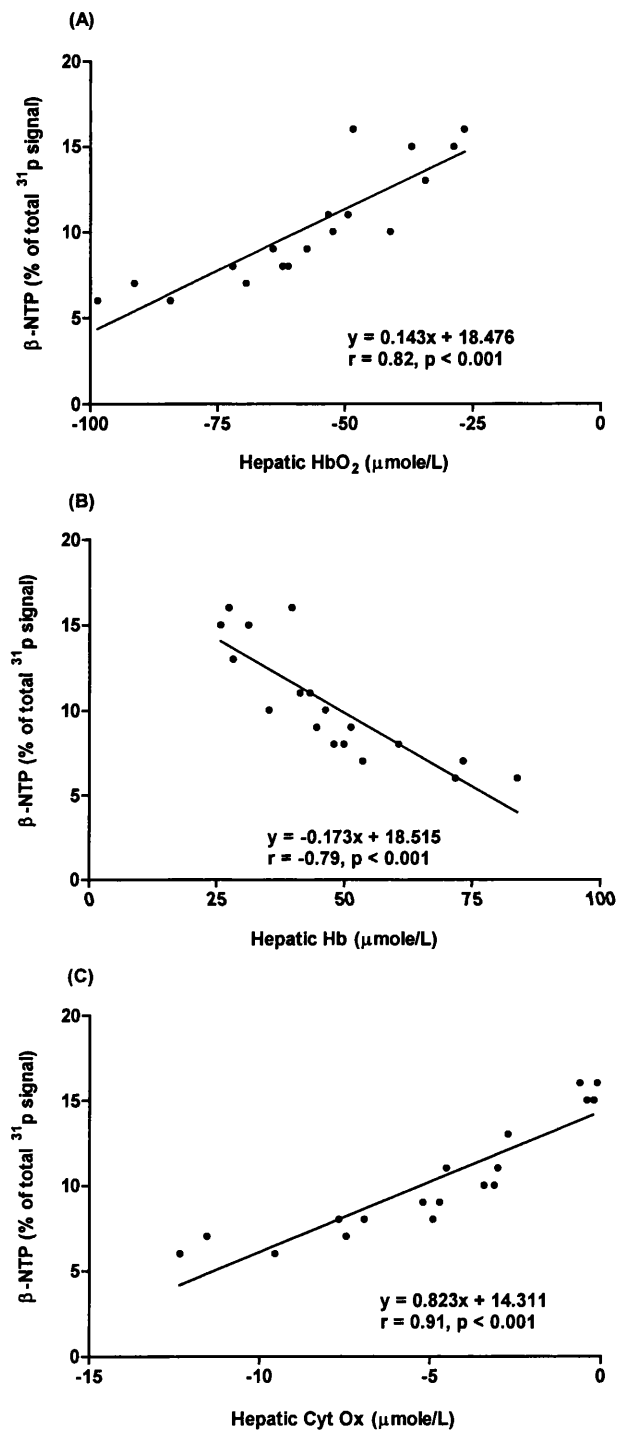


Figure 6.4 The relationship between the changes in hepatic HbO₂ (A), Hb (B), and Cyt Ox (C) and β-NTP at the end of the hypoxic periods. Each point is the change observed at the end of one hypoxic period in one animal.

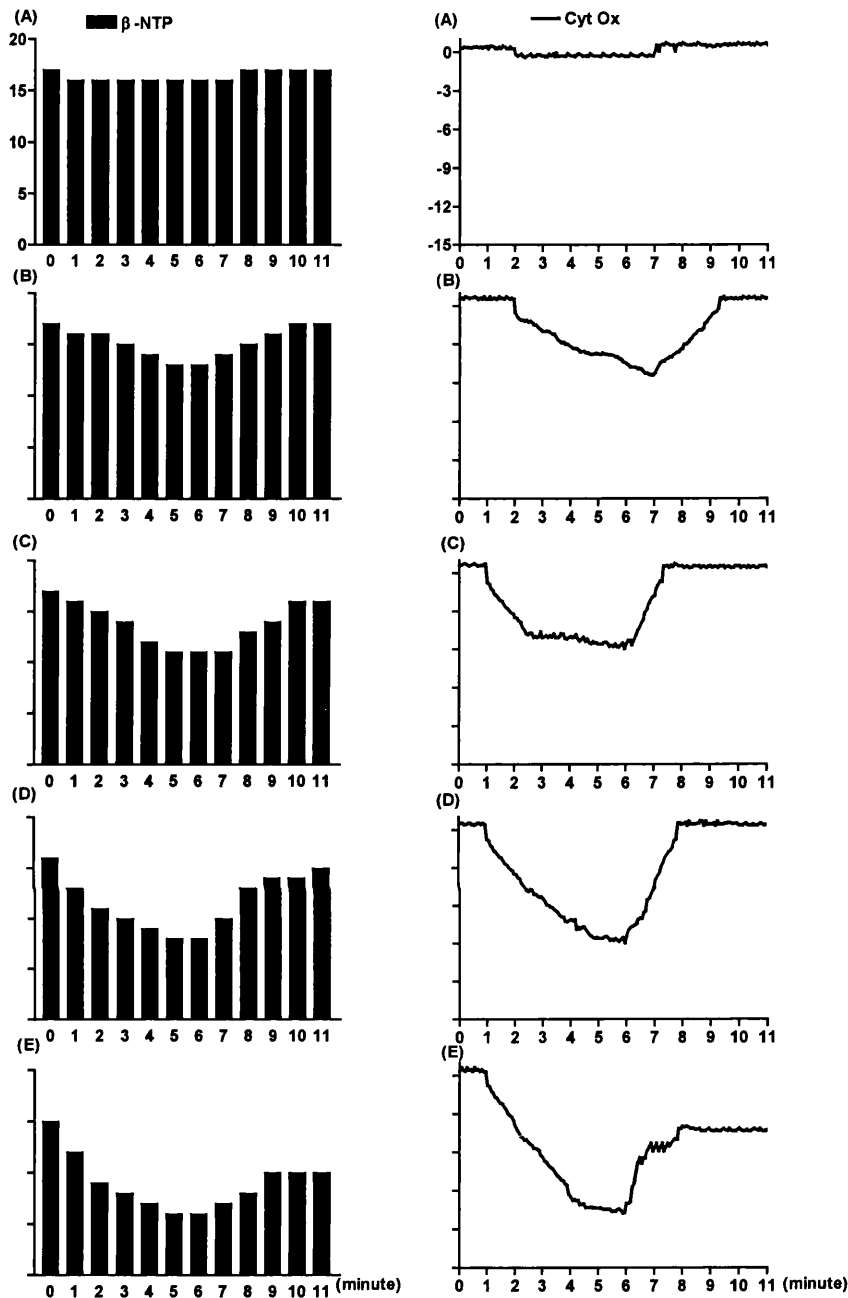


Figure 6.5 Temporal relationships of Cyt Ox and β -NTP in one animal with FiO_2 of 15 (A), 10 (B), 8 (C), 6 (D), and 4% (E). Cyt Ox measured with hypoxia (1-6 minutes) and recovery (7-11 minutes). β -NTP are one-minute values at baseline (0), hypoxia (1-6 minutes), and recovery (7-11 minutes).

6.5 Discussion

Oxygen is the energy source for living cells as it acts as the terminal electron acceptor in the oxidative phosphorylation process which leads to ATP production in the mitochondria (Capaldi, 1990). Oxygen deprivation and re-oxygenation resulting in tissue necrosis with subsequent organ failure is a major clinical problem for liver surgery and transplantation (Clavien et al. 1992).

ATP depletion is among the first alterations detectable in the hypoxic liver cell and the depression of ATP synthesis due to oxygen limitation of Cyt Ox activity has been accepted as the decisive functional lesion responsible for hypoxic cell death (Farber et al. 1981b, Hochachka, 1986). A failure of membrane ion homeostasis resulting from ATP depletion is considered to be a critical factor for cell death with hypoxia (Snowdowne et al. 1985, Carini et al. 1995). In view of the central role of ATP to cellular metabolism, the correlation between the tissue ATP level and the severity of hypoxic liver damage may be anticipated (Sumimoto et al. 1988, Higashi et al. 1989).

One limitation of this experiment is the failure to measure the blood oxygenation parameters with the different grades of hypoxia. This was impossible with the animal inside the NMR magnet because of the dead space required for extended cannula. However, the changes in blood oxygenation and pH with each grade of hypoxia have been measured in rabbits and no significant inter-animals difference was noticed at each grade of hypoxia (El-Desoky et al. 1999). In this experiment hypoxia resulted in an instantaneous decrease of hepatic tissue

HbO₂ with a simultaneous increase in Hb. Also, there was a significant reduction of the HbT with severe hypoxia (FiO₂ of $\leq 10\%$) which has been shown in the preceding chapter to be associated with a significant reduction in hepatic arterial blood flow. A significant reduction in Cyt Ox occurred only with FiO₂ of $\leq 10\%$. A mild grade of hypoxia (FiO₂ of 15%) did not induce significant changes in ATP metabolism. With more severe hypoxia (FiO₂ of $\leq 10\%$) there was significant reduction of ATP with a simultaneous increase of PME and Pi and intracellular acidosis. These results are in agreement with other in vitro and in vivo studies which have investigated hepatic energy metabolism with hypoxia (Desmoulin et al. 1987, Bowers et al. 1992, Brauer et al. 1997). In aerobic conditions, ATP degrades to ADP and Pi with the release of protons. These metabolic products are used for ATP re-synthesis through oxidative phosphorylation (Nicholls and Ferguson, 1982). Hypoxia severely limits the capability of the cell to produce ATP, so the demand for ATP exceeds supply resulting in an ADP, P_i, and protons accumulations (Wilson et al. 1977, Winkler et al. 1986). The increase of PME with hypoxia could be explained by the increase in AMP from hydrolysis of ATP and ADP, increased glycolytic activity and an increase in phosphocholine from phospholipid breakdown (Brauer et al. 1997).

In this study, the intracellular pH before hypoxia are similar to the pHi levels reported in other studies using NMR (Iles and Griffiths, 1982, Malloy et al. 1986, Desmoulin et al. 1987). The intracellular acidosis which occurred with severe hypoxia is due to proton accumulation from ATP hydrolysis, decreased H⁺ consumption through gluconeogenesis and the degradation of glycogen and

glucose to lactate (Iles and Griffiths, 1982, Desmoulin et al. 1987, Bowers et al. 1992).

This study has investigated the hepatic tissue ATP metabolic changes with hypoxia and their relationships with the changes in hepatic tissue oxygenation parameters (HbO₂, Hb, and Cyt Ox) measured by NIRS. With mild hypoxia (FiO₂ of 15%), there was significant change in HbO₂ and Hb with no significant change in Cyt Ox or ATP. With FiO₂ of $\leq 10\%$ Cyt Ox and ATP reduced significantly in addition to HbO₂ and Hb.

The reduction in ATP in this study was considered as an indicator of critical tissue hypoxia which was observed at FiO₂ of $\leq 10\%$. ATP reduction correlated temporally with the changes in Cyt Ox but not with changes in HbO₂ and Hb which supports the use of Cyt Ox as an indicator of intracellular ATP availability with critical tissue hypoxia.

Chapter 7

**Measurement of in hepatic tissue oxygenation and blood volume with
vascular inflow occlusion in a porcine model by near infrared spectroscopy**

7.1 Introduction

In chapters 5 and 6 the measurement of hepatic tissue oxygenation changes by NIRS was assessed with reduction of the oxygen supply. In this chapter NIRS was applied to measure tissue oxygenation in relation to hepatic blood flow. The importance of understanding the hepatic blood and oxygen supply has become evident due to the recognition that 1) hepatic dysfunction is a major factor contributing to morbidity and mortality in septic patients (Schwartz et al. 1989) and 2) the crucial value of good hepatic oxygenation after liver transplantation for maintenance of graft function (Sumimoto et al. 1987, Kallinowski et al. 1996). The human liver receives 25% of the cardiac output with a total blood flow of 1 - 2.6 L/min (Doi et al. 1988, Jakab et al. 1992). The HA supplies about 40-50% of the liver's normal oxygen requirement, as the arterial blood has a greater oxygen content than the portal blood which is postcapillary and partly deoxygenated (Tygstrup et al. 1962, Andreen and Irestedt, 1976). Changes in PVBF produce inverse changes in HABF which is known as the "hepatic arterial buffer response" (Lautt, 1985, Jakab et al. 1995) but not the reverse (Doi et al. 1988, Jakab et al. 1995).

Although the porcine liver is being considered for hepatic xenotransplantation in humans, to date there is no basic experimental work in pigs to define the changes in hepatic tissue oxygenation with hepatic vascular inflow occlusion and the relationship between tissue oxygenation and liver blood flow.

7.2 Objectives

1. To apply NIRS for measurement of hepatic tissue oxygenation and perfusion changes in a large animal model with hepatic vascular inflow occlusion.
2. Study the interrelationship between the HA and PV blood flow in regard to hepatic tissue oxygenation.

7.3 Materials and methods

7.3.1 Animal preparation and surgical procedure

Large White Landrace pigs (n = 6, 26 ± 2.5 kg) were used. The surgical operation, anaesthesia and the animals' monitoring were mentioned in detail in Chapter 5.3.1.

In summary, the animals' temperature, heart rate, and blood oxygen saturation were monitored continuously. Catheters were inserted into the left internal jugular vein and left carotid artery for fluid administration and blood pressure monitoring, respectively.

Laparotomy was performed through a transverse subcostal incision. The PV and HA were identified and carefully dissected. The ultrasonic flowmeter perivascular probes were placed around the HA and PV (4 and 6 mm diameter, respectively) for continuous monitoring of the blood flow. The HM was monitored using a dual channel LDF. The LDF probe was applied to a fixed site on the liver surface of

the right lobe to avoid any anatomical variation in the microcirculation. NIRS probes were placed, with a 20 mm separation, on the surface of the right lobe of the liver for continuous monitoring of the hepatic tissue oxygenation and blood volume changes.

For vascular inflow occlusion, specifically designed vascular clamps were placed around the hepatic artery and portal vein (Figure 7.1). These clamps were designed and made by Department of Medical Engineering in the Royal Free Hospital to allow occlusion of the vessels without moving the spectroscopy probes and interrupting tissue oxygenation measurement.



Figure 7.1 Vascular clamps for HA and PV occlusion and release without moving the NIRS and interrupting tissue oxygenation measurement.

7.3.2 Experimental protocol

At the beginning of the experiment 10 minutes were allowed for stabilisation of measurements followed by recording of baseline values. Hepatic tissue oxygenation and blood volume changes were continuously measured by NIRS during reduction in the THBF. Temporary reduction in THBF was achieved by hepatic artery occlusion (HAO) for 3 minutes, portal vein occlusion (PVO) for the same period of time and finally total occlusion (TO) by clamping both vessels for 3 minutes. Intervals of 5-minute were allowed between the different occlusion periods for the recovery of hepatic blood flow measurements. After completion of the experiments the animals were killed by a lethal dose (20 ml, 200 mg/ml) of pentobarbitone sodium.

7.3.3 Data collection and statistical analysis

The values are expressed as mean $\pm$ SD of 6 animals. For comparison between the different vascular occlusion periods, NIRS, LDF, and ultrasonic flowmeter measurements were calculated as 1-minute mean values at the beginning of the experiment (baseline) and at the end of each occlusion period. For statistical analysis one way analysis of the variance (ANOVA) and Student's t-test were used with Bonferroni correction for multiple tests. $p < 0.05$ was considered statistically significant. The relationships between THBF, LDF, and tissue

oxygenation parameters (HbO₂, Hb, HbT, and Cyt Ox) were using Pearson correlation coefficient.

7.4 Results

7.4.1 Hepatic blood flow with vascular inflow occlusion

The baseline THBF was 821 ± 53 ml/min. The hepatic artery supplied $16.5 \pm 2.3\%$ and the portal vein $83.5 \pm 1.9\%$. HAO was associated with an insignificant rise (4%) in PV blood flow and a significant reduction in THBF ($13 \pm 2.4\%$, $p < 0.001$ vs. baseline). PVO resulted in a significant rise in HA blood flow (29%, $p < 0.01$ vs. baseline) and a significant reduction in THBF ($79 \pm 2.6\%$, $p < 0.001$ vs. baseline) (Table 7.1).

7.4.2 HM with vascular inflow occlusion

The baseline HM was 265.2 ± 25.7 flux. HAO was associated with a significant reduction in the HM ($30.6 \pm 3.8\%$, $p < 0.001$ vs. baseline). With PVO a greater reduction occurred in the HM ($61.6 \pm 7.1\%$, $p < 0.001$ vs. baseline) and TO resulted in further reduction in HM ($84.5 \pm 5.6\%$, $p < 0.001$ vs. baseline) (Table 7.1). A significant positive correlation was found between the reduction of HM measured by LDF and the reduction in THBF measured by the ultrasonic flowmeter ($r = 0.96$, $p < 0.0001$) (Figure 7.2)

Table 7.1 Hepatic blood flow and HM with vascular inflow occlusion.

	Baseline	HAO	PVO	TO
HABF (ml/min)	135 ± 19 [†]	0 [†]	173 ± 16 ^{†*}	0 [†]
PVBF (ml/min)	686 ± 38 [†]	719 ± 42 ^{†**}	0 [†]	0 [†]
THBF (ml/min)	821 ± 53 [†]	719 ± 42 ^{†**}	173 ± 16 ^{†**}	0 [†]
↓THBF (%)	0 [†]	13 ± 2.4 ^{†**}	79 ± 2.6 ^{†**}	100 ^{†*}
↓HM (flux)	0 [†]	82.4 ± 17.7 ^{†**}	164.3 ± 29.8 ^{†**}	222.6 ± 30.8 ^{†**}

Values are mean ± SD of 6 pigs. [†]p < 0.01 (ANOVA). **p < 0.001 and *p = not significant vs. baseline (Student's t test).

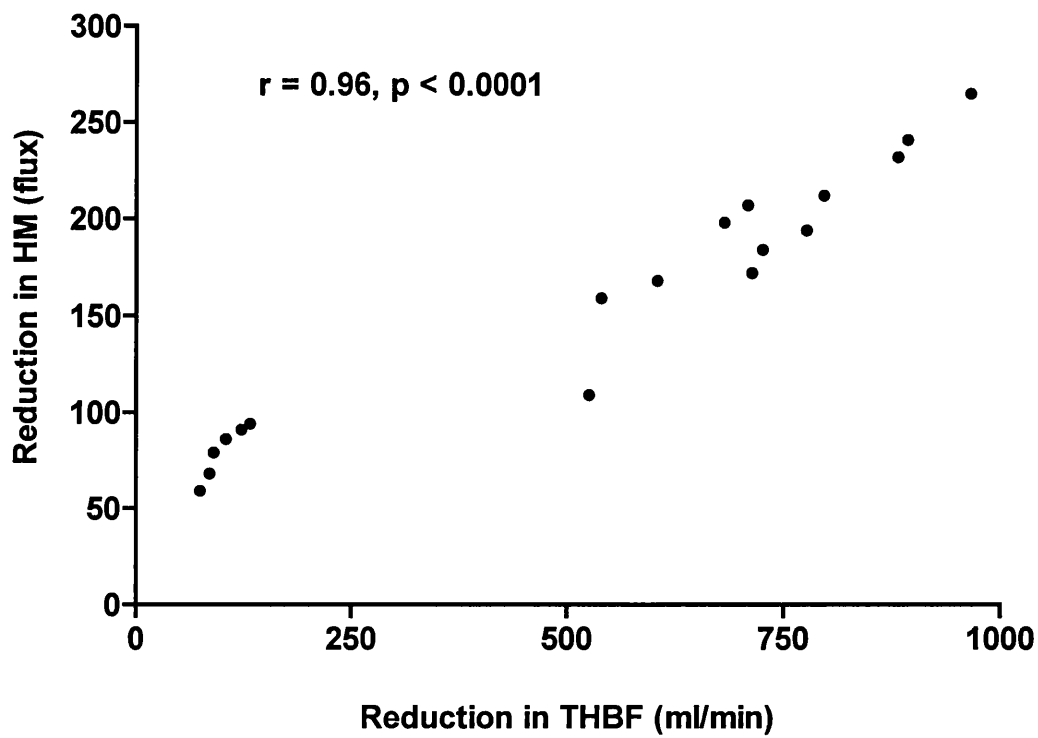


Figure 7.2 The relationship between HM and THBF with vascular inflow occlusion. Each point is the mean of 1-minute values at the end of one vascular occlusion period in one animal.

7.4.3 Hepatic tissue oxygenation and blood volume changes with vascular inflow occlusion measured by NIRS

With HAO there was an immediate significant decrease in HbO₂ (21.48 ± 6.22 $\mu\text{mole/L}$, $p < 0.01$ vs. baseline) and a simultaneous significant increase in Hb (11.61 ± 3.92 $\mu\text{mole/L}$, $p < 0.01$ vs. baseline). A decrease (0.29 ± 0.18) in Cyt Ox was noticed but this reduction was not significant (Figure 7.3A). These changes were associated with a decrease in HbT and HbD (9.88 ± 2.36 , 33.09 ± 10.13 $\mu\text{mole/L}$, respectively) ($p < 0.01$ vs. baseline) (Figure 7.4A).

With PVO a decrease in HbO₂ was observed (33.25 ± 8.74 $\mu\text{mole/L}$, $p < 0.001$ vs. baseline) and a simultaneous increase in Hb (17.53 ± 3.89 $\mu\text{mole/L}$, $p < 0.001$ vs. baseline). A significant decrease (2.92 ± 1.52 $\mu\text{mole/L}$, $p < 0.01$ vs. baseline) in Cyt Ox was noticed (Figure 7.3B). These changes were associated with a decrease in HbT and HbD (15.71 ± 5.02 , 50.78 ± 12.56 $\mu\text{mole/L}$, respectively) ($p < 0.001$ vs. baseline) (Figure 7.4B).

With TO there was a decrease in HbO₂ (67.23 ± 15.76 $\mu\text{mole/L}$, $p < 0.001$ vs. baseline) and a simultaneous increase in Hb (24.80 ± 5.99 $\mu\text{mole/L}$, $p < 0.001$ vs. baseline) (Figure 7.3C). Also, there was a significant decrease in Cyt Ox (6.79 ± 2.78 $\mu\text{mole/L}$, $p < 0.01$ vs. baseline). These changes were associated with a decrease in HbT and HbD (42.43 ± 9.86 , 92.02 ± 21.70 $\mu\text{mole/L}$, respectively) ($p < 0.001$ vs. baseline) (Figure 7.4C). NIRS measurements with hepatic vascular inflow occlusion are in Table 7.2.

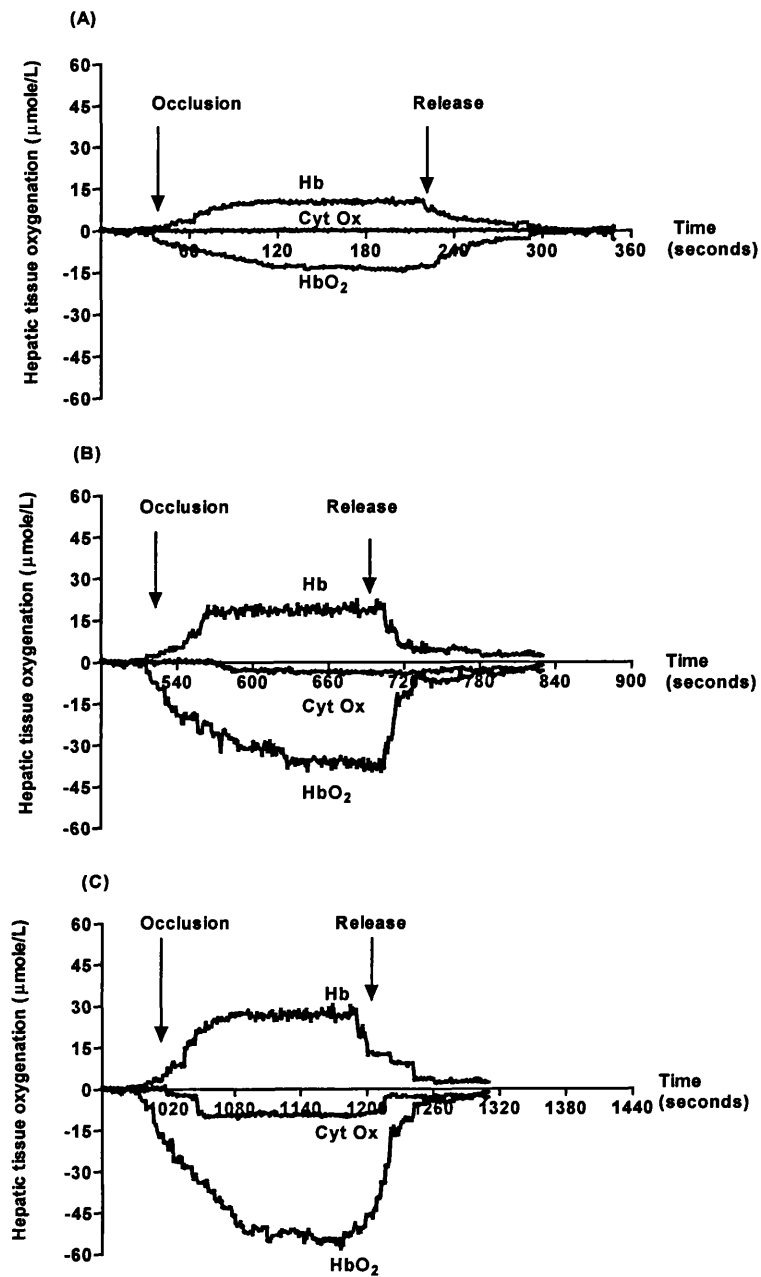


Figure 7.3 Typical examples of hepatic tissue oxygenation changes in one animal with HAO (A), PVO (B), and TO (C).

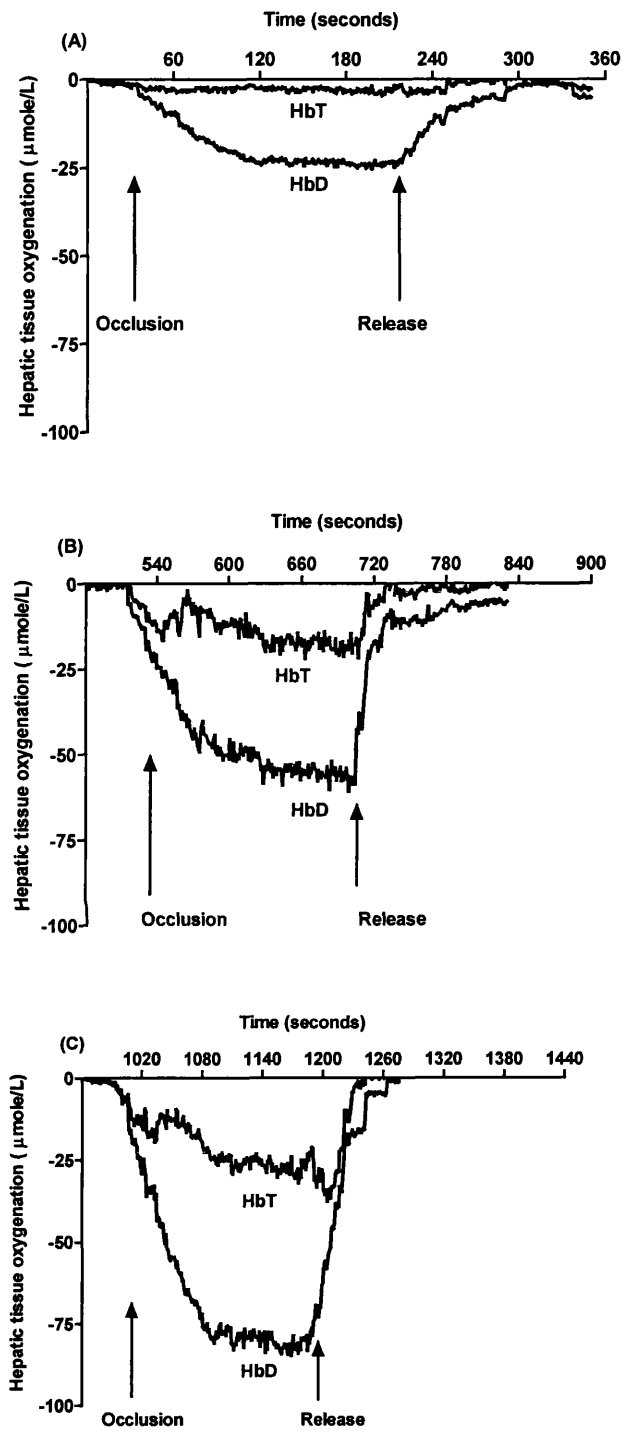


Figure 7.4 Typical examples of hepatic tissue oxygenation changes in one animal with HAO (A), PVO (B), and TO (C).

Table 7.2 Hepatic tissue oxygenation changes ($\mu\text{mole/L}$) with vascular inflow occlusion.

	HAO	PVO	TO
HbO ₂	$-21.48 \pm 6.22^{\dagger*}$	$-33.25 \pm 8.74^{\dagger**}$	$-67.23 \pm 15.76^{\dagger**}$
Hb	$11.61 \pm 3.92^{\dagger*}$	$17.53 \pm 3.89^{\dagger**}$	$24.80 \pm 5.99^{\dagger**}$
Cyt Ox	$-0.29 \pm 0.18^{\dagger}$	$-2.92 \pm 1.52^{\dagger*}$	$-6.79 \pm 2.78^{\dagger*}$
HbT	$-9.88 \pm 2.36^{\dagger*}$	$-15.71 \pm 5.02^{\dagger**}$	$-42.43 \pm 9.86^{\dagger**}$
HbD	$-33.09 \pm 10.13^{\dagger*}$	$-50.78 \pm 12.56^{\dagger**}$	$-92.02 \pm 21.70^{\dagger**}$

Values are means $\pm$ SD of 6 pigs. $^{\dagger}p < 0.01$ (ANOVA). $*p < 0.01$ and

$**p < 0.001$ vs. baseline (Student's t test).

Significant correlations were found between the reduction in THBF and reduction in HbO₂ ($r = 0.84$, $p < 0.001$), increase in Hb ($r = 0.84$, $p < 0.001$), reduction in Cyt Ox ($r = 0.88$, $p < 0.001$) and reduction in HbT ($r = 0.80$, $p < 0.001$) (Figure 7.5A-D). Also, a significant positive correlation was found between the reduction in sinusoidal blood flow measured by LDF and the reduction in hepatic HbT and ($r = 0.85$, $p < 0.0001$) (Figure 7.6).

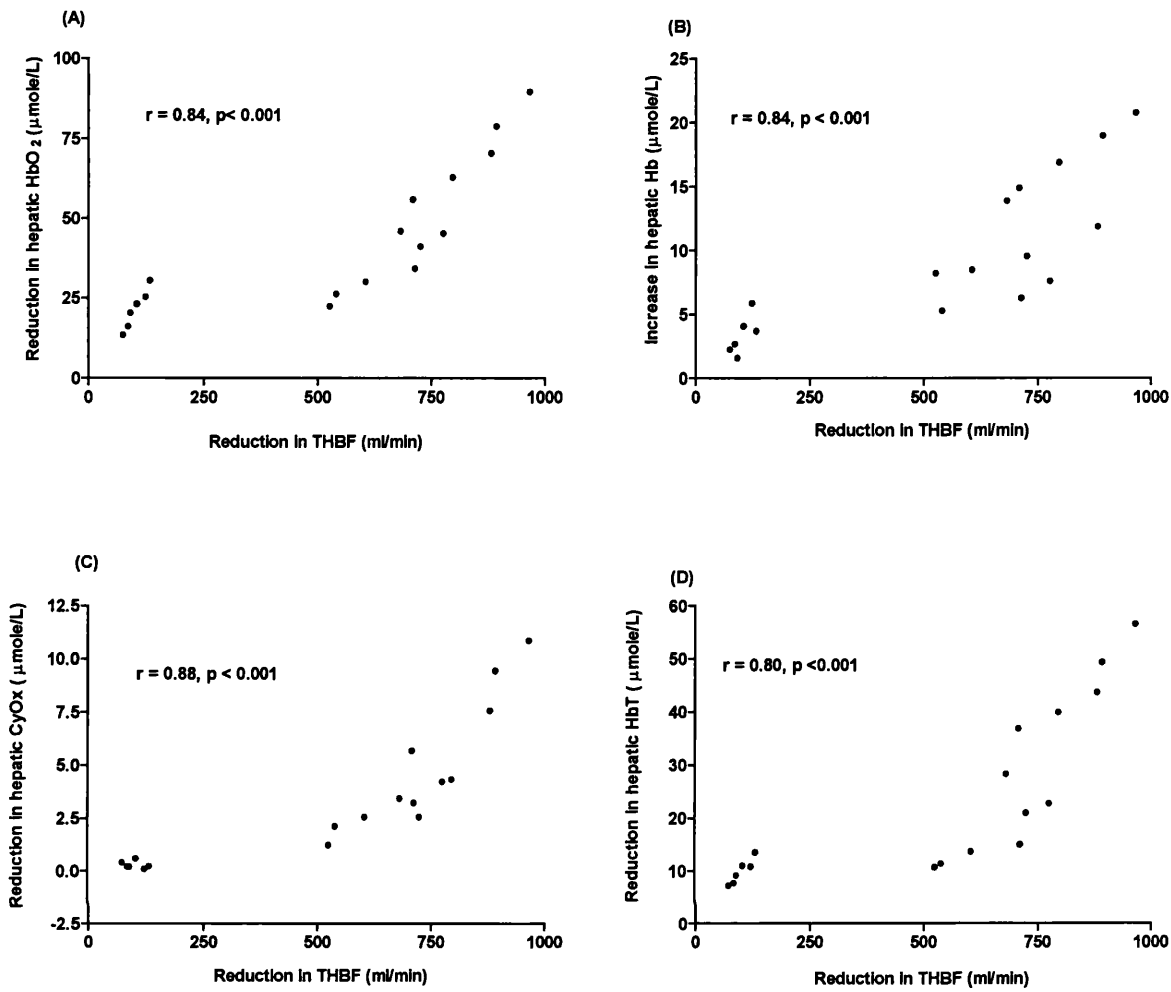


Figure 7.5 The relationship between HbO_2 (A), Hb (B), Cyt OX (C), and HbT (D) and THBF with vascular inflow occlusion. Each point is the mean of 1-minute values at the end of one vascular occlusion period in one animal.

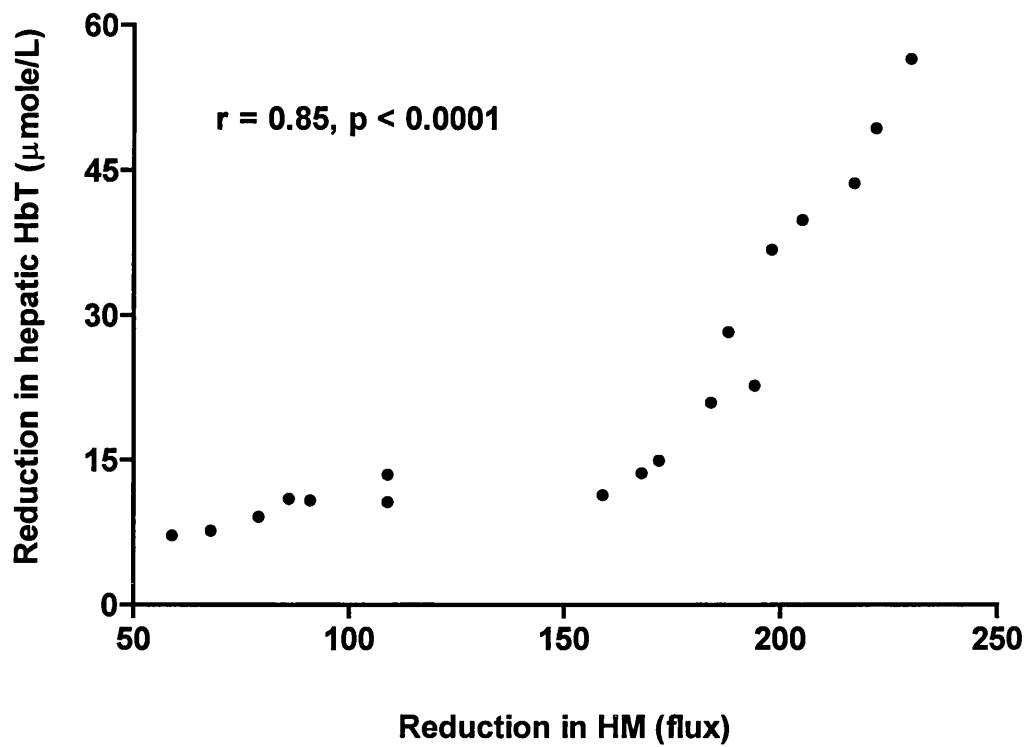


Figure 7.6 The relationship between HM and HbT with vascular inflow occlusion. Each point is the mean of 1-minute values at the end of one vascular occlusion period in one animal.

7.5 Discussion

This study has demonstrated that NIRS can monitor changes in the hepatic tissue oxygenation and blood volume produced by reducing the blood supply to the liver. This is the first study to correlate liver blood flow with the changes in hepatic tissue oxygenation and blood volume parameters, measured by NIRS, utilising an animal model whose liver anatomy and physiology is similar to man (Copper et al. 1991). Although Tokuka and colleagues (Tokuka et al. 1994) observed similar results using NIRS of the rabbit liver, their studies did not involve measurement of the liver blood flow. Also, there are major differences between the liver blood supply in small animals and man. Liver transplantation, for example, can be successfully performed in rodents without restoring the HA inflow (Kamada and Calne, 1979) whereas hepatic artery thrombosis in human liver transplantation results in acute liver failure (Tzakis et al. 1985).

This study has provided further insight into the inter-relationship between the HA and PV blood flow in terms of tissue perfusion and oxygenation. The PV blood flow did not increase significantly with HAO, in agreement with other studies (Andreen and Irestedt, 1976, Jakab et al. 1995). On the other hand, the HA blood flow did increase significantly in response to PVO which supports the presence of a hepatic arterial buffer response (Lautt, 1985, Jakab et al. 1995). More importantly this technique has provided a means of analysing the relationship between liver tissue perfusion, oxygenation and blood supply. With HAO there was a reduction in the HbO_2 with a simultaneous increase in Hb that

reflects a decrease in the hepatic oxygen supply with a concomitant increase in the fraction of oxygen extracted from blood by the tissue (Lautt and Greenway, 1987). The decrease in HbT and HbD is likely to reflect the acute reduction in hepatic blood volume and oxygenation, respectively. These changes in the tissue oxygenation and blood volume with HAO were not compensated for by an increase in the PV inflow which may explain the importance of the hepatic arterial blood supply to the large animal liver (Tzakis et al. 1985). With PVO there was a similar pattern of changes in tissue oxygenation and blood volume as with HAO, whilst the magnitude of change was about twice that with the HAO. This may be due to the blood and oxygen supply to the liver via the PV being greater than that of the HA. Despite the hepatic artery blood flow increasing with PVO, mediated by the hepatic arterial buffer response, no buffer effect was found at the level of liver tissue oxygenation or perfusion. The changes in liver tissue HbO₂, Cyt Ox and HbT after TO were larger than the sum of the changes following the HAO and PVO. This would suggest the presence of a regulatory mechanism for maintaining the hepatic microcirculation that is lost after TO.

The significant correlation found between the liver blood flow measured by the ultrasonic flowmeter and the hepatic HbO₂, Hb and HbT measured by NIRS reflects the value of NIRS as a method of measurement of hepatic tissue blood oxygenation and blood volume. This is the first study to evaluate the relationship between HbT measured by NIRS and liver blood flow. The high level of correlation would suggest that NIRS might have many applications where a non-invasive method of assessing blood flow is required. Also, the significant

correlation between HbT and the HM changes measured by the LDF with the vascular occlusion support the accuracy of the hepatic tissue volume measurement by NIRS.

Measurement of Cyt Ox during hepatic vascular occlusion showed that reduction of Cyt Ox occurred significantly with PVO and TO while no change was associated with HAO. This indicates that Cyt Ox reduction occurs only with a severe degree of hypoxia that did not occur with HAO alone. These results are in accordance with previous studies in rabbit livers in which reduction of Cyt Ox occurred only with a severe reduction in hepatic blood flow following portal vein and total occlusion but with no change following hepatic artery occlusion (Tokuka et al. 1994). Reduction of the Cyt Ox occurred also with severe blood loss (33% of the total blood volume) in rabbits following haemorrhage (Kitai et al. 1993a). This experiment and the hypoxia experiments (Chapters 5 and 6) support the value of Cyt Ox as an indicator of intracellular hypoxia which occurs only with severe hypoxia or marked reduction of the hepatic blood flow.

Chapter 8

**Evaluation of hepatic tissue blood flow and function in a rabbit model of
acute hepatic dysfunction by measuring hepatic indocyanine green
handling using near infrared spectroscopy**

8.1 Introduction

In Chapters 5,6, and 7 the role of NIRS for measuring the changes in hepatic tissue oxygenation and blood volume was investigated. In this experiment NIRS was applied to measure hepatic tissue ICG handling with acute hepatic dysfunction.

ICG is an anionic dye that has been used for many years to measure hepatic blood flow and as a test of liver function (Caeser et al. 1961, Grainger et al. 1983). Conventionally, after a bolus administration of ICG, the hepatic handling of ICG is predicted from its peripheral blood clearance curve (Grainger et al. 1983). Measurement of ICG blood clearance requires repeated blood sampling and a complicated technique to quantify ICG concentration in the samples using a spectrophotometer (Nielsen, 1963).

Ott and colleagues (1994) have suggested that the ICG plasma clearance curve contains no information about the liver-bile interaction (Ott et al. 1994). The close interrelationship between sinusoidal flow and hepatocyte function also represents a great difficulty in the interpretation of blood clearance tests.

Recently Shinohara et al. (Shinohara et al. 1996b) studied the handling of ICG by the rabbit liver using NIRS and produced rate constants for ICG uptake and excretion using a two-compartment model. The uptake rate reflects the dye uptake from blood to hepatocytes which varies with blood flow in the hepatic microcirculation. The excretion rate depends on ICG removal from the liver by cytoplasmic transport and biliary excretion. Direct measurement of hepatic ICG

concentration could provide a more accurate way to study ICG kinetics in the liver which would allow the differentiation between the various hepatic factors which affect blood clearance. Another advantage of direct measurement of hepatic ICG clearance over its blood clearance measurement is to provide a lobar rather than global assessment of liver function.

8.2 Objectives

1. Evaluation of the application of NIRS for direct measurement of hepatic ICG concentration as a dynamic hepatic function test in different experimental conditions associated with impairment of hepatic blood flow, hepatocyte function and biliary excretion.
2. Correlation of the ICG uptake rate measured by NIRS with the hepatic blood flow and HM.

8.3 Materials and methods

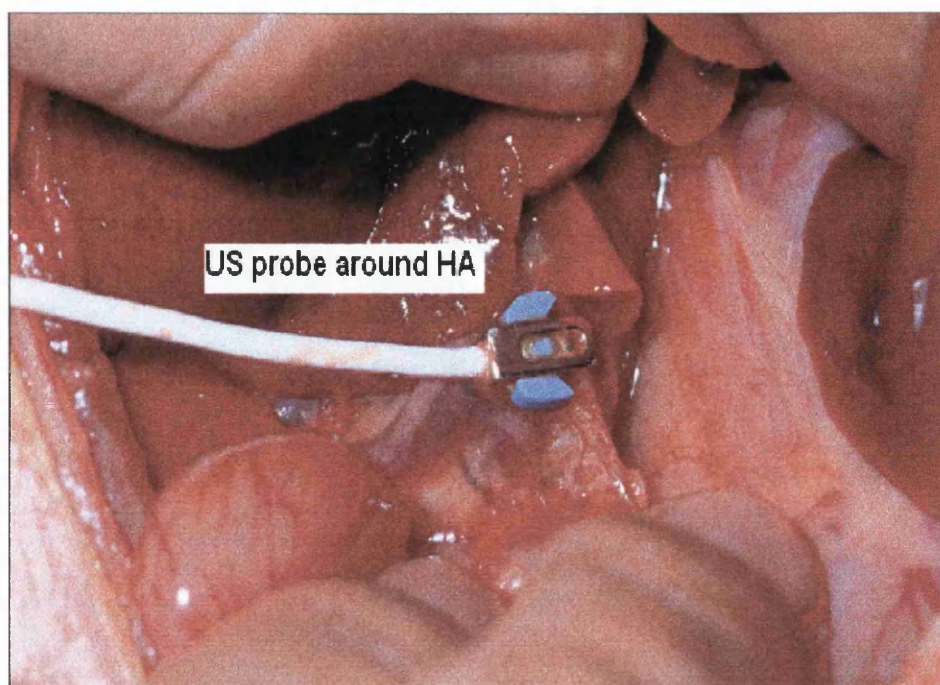
8.3.1 Animal preparation and surgical procedure

Male New Zealand white rabbits (2.9 ± 0.3 kg, $n = 36$) were used in this experiment. Anaesthesia was induced by 0.5 ml/Kg Hypnorm® (fentanyl citrate and fluanisone; Janssen Pharmaceutical Ltd., Oxford, UK) and 2.5 mg/Kg Diazemuls® (diazepam; Dumex Ltd., Hertfordshire, UK) intramuscular and maintained by Halothane (May and Baker Ltd, Dagenham, UK). The animals were placed in supine position on a warming blanket (Harvard apparatus Ltd., Kent, UK) for maintenance of their body temperature between 37-39 °C. The SaO₂ and HR were continuously monitored by pulse oximeter (Ohmeda Biox 3740, Ohmeda Co., Louisville, USA). Polyethylene catheters (PE-50, 0.58 mm inner diameter, Portex, Kent, UK) were inserted into the femoral artery for monitoring of MABP and into the femoral vein for saline infusion (10 ml/kg/hour) to compensate for intraoperative fluid loss.

Laparotomy was performed through a midline incision. The ligamentous attachments from the liver to the diaphragm were divided and the liver was exposed. HA and PV were exposed and dissected. HABF and PVBF were measured using ultrasonic transit time flowmeter with perivascular flow probes around HA and PV (1 and 4 mm diameter, respectively) (Figure 8.1).

Monitoring of the HM was done using LDF. The probe was applied to a fixed site on the left lobe of the liver to avoid any anatomical variation in the microcirculation (Figure 8.2).

(A)



(B)

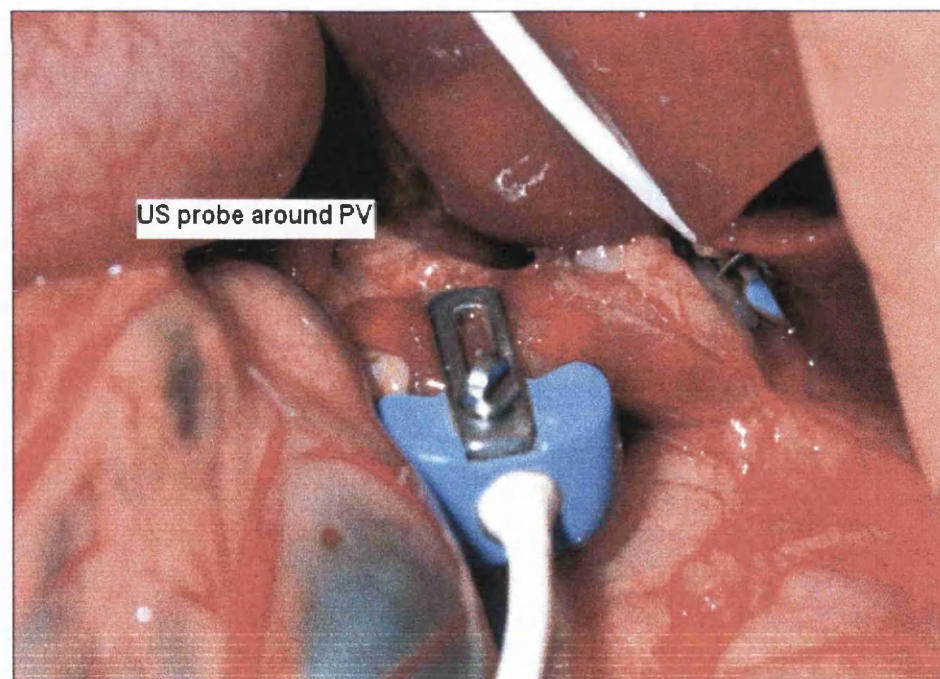


Figure 8.1 Ultrasonic flowmeter probes around HA (A) and PV (B).

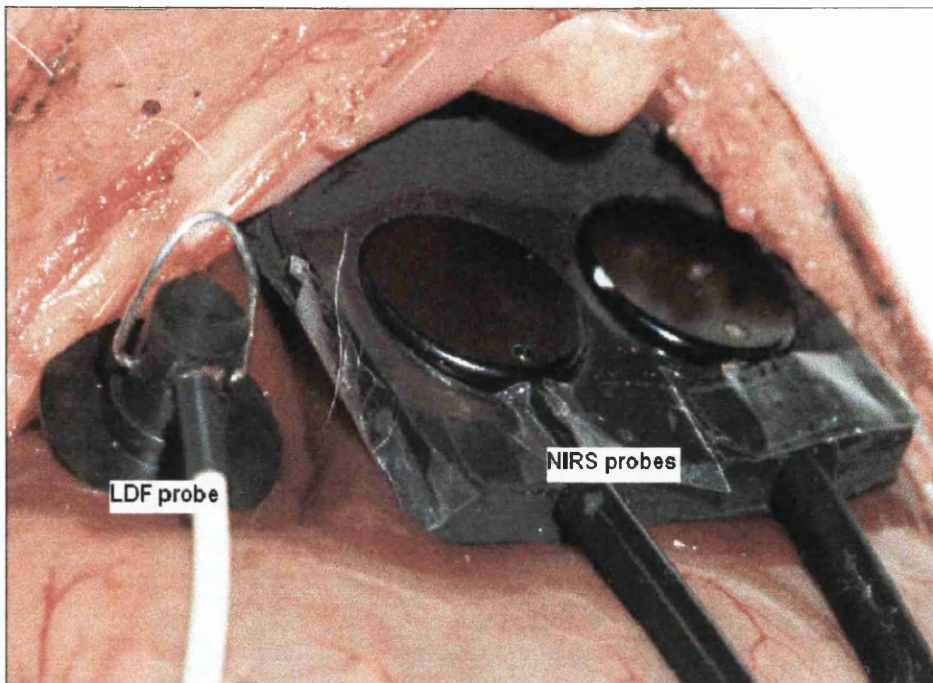


Figure 8.2 NIRS probes and LDF probe over the liver surface.

8.3.2 Measurement and analysis of hepatic ICG concentration curve

For measurement of hepatic ICG clearance in all the groups a bolus of ICG (Cardiogreen, 90% dye content, Sigma, Dorset, UK) 0.5 mg/kg was given. ICG was dissolved in sterile water (1 mg/ml) and given via the femoral vein over 20 seconds in all the groups. ICG was prepared immediately before administration to avoid its degradation by light which results in slower hepatic uptake and excretion which could cause inter-animal variation (Igarashi et al. 1990). In previous studies, two ICG doses were used either 0.5 mg/kg or 5 mg/kg, the high dose being used as a measure of hepatic function independent of hepatic blood flow (Rakich et al. 1987). In this study the low dose was used to ensure that ICG

clearance is controlled by both the blood flow and hepatic function. For continuous measurement of hepatic tissue ICG concentration, the NIRO 500 probes were placed, with a 20 mm separation, on the left lobe of the liver for 30 minutes after ICG injection (Figure 8.2). After measuring the hepatic ICG concentration the animals were killed by exsanguination.

Continuous measurement of hepatic ICG by NIRS produces a concentration-time curve. This curve was analysed to produce two exponential rate constants, representing hepatic ICG uptake from the plasma to the hepatocytes (α) and hepatic ICG excretion from the liver by cytoplasmic transport and biliary excretion (β). These rate constants were calculated by fitting the ICG concentration-time curve to a two-compartment mathematical model as defined by the sum of two exponential equations as previously reported by Shinohara and colleagues (Shinohara et al. 1996b): $ICG(t) = -A \exp(-\alpha t) + B \exp(-\beta t)$

Where $ICG(t)$ is the hepatic concentration of ICG at any time (t), α and β (min^{-1}) are the rate constants for the hepatic ICG uptake and excretion, respectively. A and B are the zero time intercepts, both theoretically equal to the initial hepatic concentration. The assumption is that $\alpha > \beta$ and $A \approx B$.

The fitting to this model was done using a commercial computer package based on the non-linear least square regression (Graph Pad Prism, Graph Pad Software Inc., San Diego, USA). The iterative procedure of the program minimises the reduced sum of squares. The goodness of the fit was evaluated by the R^2 value.

8.3.3 Experimental groups

Controls. In this group (n = 6) hepatic ICG clearance was measured without reduction of the hepatic blood flow or induction of hepatic damage.

HAO. In these animals (n = 6) hepatic blood flow was reduced by occlusion of the HA 30 minutes before measuring hepatic ICG clearance.

PV partial occlusion (PVPO). In these animals (n = 6) hepatic blood flow was reduced by partial (50%) occlusion of the PV, using a specifically designed vascular clamp, 30 minutes before measuring hepatic ICG clearance.

I/R injury. In this group (n = 6) lobar I/R was achieved by occlusion of the vascular pedicles of the median and left lateral lobes of the liver using a microvascular clamp for 30 minutes followed by reperfusion for 60 minutes. This is an established model of hepatic I/R (Vollmar et al. 1994). Hepatic ICG clearance was measured at the end of the reperfusion period.

Colchicine treatment. In this group (n = 6) impairment of the ICG excretion was achieved using Colchicine, a potent microtubule toxin previously shown to reduce ICG excretion from the liver (Mori et al. 1987). Colchicine 2 mg/kg (95%, Sigma, Dorset, UK) was dissolved in 2 ml saline prior to direct injection into the PV using a 27 gauge needle. Hepatic ICG clearance was measured 2 hours after Colchicine administration.

Bile duct ligation (BDL). In this group (n = 6) the common bile duct was ligated for 60 minutes before measuring hepatic ICG clearance.

8.3.4 Data collection and statistical analysis

In the groups with reduction of the hepatic blood flow, the ultrasonic flowmeter, LDF and the NIRS measurements were calculated as 1-minute mean values at the start (baseline) and at the end of the occlusion period. In the I/R group these measurements were calculated as 1-minute mean values before ischaemia (baseline), at the end of ischaemia period and at the end of reperfusion period. The values are expressed as mean $\pm$ SD of 6 animals in each group. For statistical analysis one way analysis of the variance (ANOVA) and Student's t-test were used with Bonferroni correction for multiple tests. $p < 0.05$ was considered statistically significant. The relationships between hepatic ICG uptake rate (α) and THBF and HM were tested using Pearson correlation coefficient.

8.4 Results

8.4.1 Reduction of the hepatic blood flow

HAO significantly reduced the THBF and HM with no significant change of PV blood flow (Table 8.1). Hepatic tissue oxygenation changes, measured by NIRS, showed a significant decrease in HbO₂ with a simultaneous increase in Hb (Table 8.1).

HAO resulted in a significant decrease in ICG uptake rate (α) from control value of $1.85 \pm 0.51 \text{ min}^{-1}$ to $0.37 \pm 0.09 \text{ min}^{-1}$ ($p < 0.001$ vs. controls) with no significant change ($0.08 \pm 0.03 \text{ min}^{-1}$ vs. $0.1 \pm 0.06 \text{ min}^{-1}$) in ICG excretion rate (β) (Table 8.2).

PVPO resulted in a significant decrease in PV blood flow with a simultaneous increase in HA blood flow (Table 8.1). Despite this increase in HA blood flow there was a significant reduction in THBF and HM (Table 8.1). This was associated with a significant decrease in hepatic HbO₂ and a simultaneous increase in Hb (Table 8.1).

PVPO resulted in a significant decrease in ICG uptake rate (α) from the control value of $1.85 \pm 0.51 \text{ min}^{-1}$ to $0.27 \pm 0.14 \text{ min}^{-1}$ ($p < 0.001$ vs. controls) with no significant change ($0.12 \pm 0.08 \text{ min}^{-1}$ vs. $0.1 \pm 0.06 \text{ min}^{-1}$) in ICG excretion rate (β) (Table 8.2).

Table 8.1 Hepatic haemodynamics and oxygenation parameters with vascular occlusion.

	HAO		PVPO	
	Baseline	After occlusion	Baseline	After occlusion
HABF (ml/min)	11 ± 7	0	15 ± 5	20 ± 7**
PVBF (ml/min)	84 ± 11	88 ± 9*	90 ± 9	42 ± 6**
THBF (ml/min)	95 ± 10	88 ± 9*	105 ± 8	62 ± 8**
HM (flux)	239 ± 13	216 ± 16*	229 ± 22	176 ± 19**
HbO ₂ (μmole/L)	0	-12.7 ± 6.6**	0	-26.9 ± 9.2**
Hb (μmole/L)	0	9.7 ± 8.5**	0	20.8 ± 11.2**

Values are mean ± SD of 6 animals in each group. Values are one-minute averages before occlusion (baseline) and at the end of the occlusion periods.

*p < 0.01, **p < 0.001, and *p = not significant vs. baseline (Student's t test).

Table 8.2 Hepatic ICG uptake rate (α) min^{-1} and ICG excretion rate (β) min^{-1} .

Group	ICG uptake rate	ICG excretion rate
Controls	$1.85 \pm 0.51^{\ddagger}$	$0.100 \pm 0.060^{\ddagger}$
HAO	$0.37 \pm 0.09^{\ddagger**}$	$0.080 \pm 0.030^{\ddagger*}$
PVPO	$0.27 \pm 0.14^{\ddagger**}$	$0.120 \pm 0.080^{\ddagger*}$
Lobar I/R	$0.85 \pm 0.59^{\ddagger**}$	$0.020 \pm 0.006^{\ddagger*}$
Colchicine	$1.74 \pm 0.40^{\ddagger*}$	$0.030 \pm 0.010^{\ddagger*}$
BDL	$1.82 \pm 0.77^{\ddagger*}$	$0.002 \pm 0.001^{\ddagger*}$

Values are mean $\pm$ SD of 6 animals in each group. $^{\ddagger}p < 0.05$ (ANOVA).

$**p < 0.001$, $*p < 0.05$, and $^{\circ}p =$ not significant vs. controls (Student's t test).

In the control, HAO, and PVPO groups, THBF correlated significantly with ICG uptake rate (α) ($r = 0.79$, $p < 0.001$) (Figure 8.3).

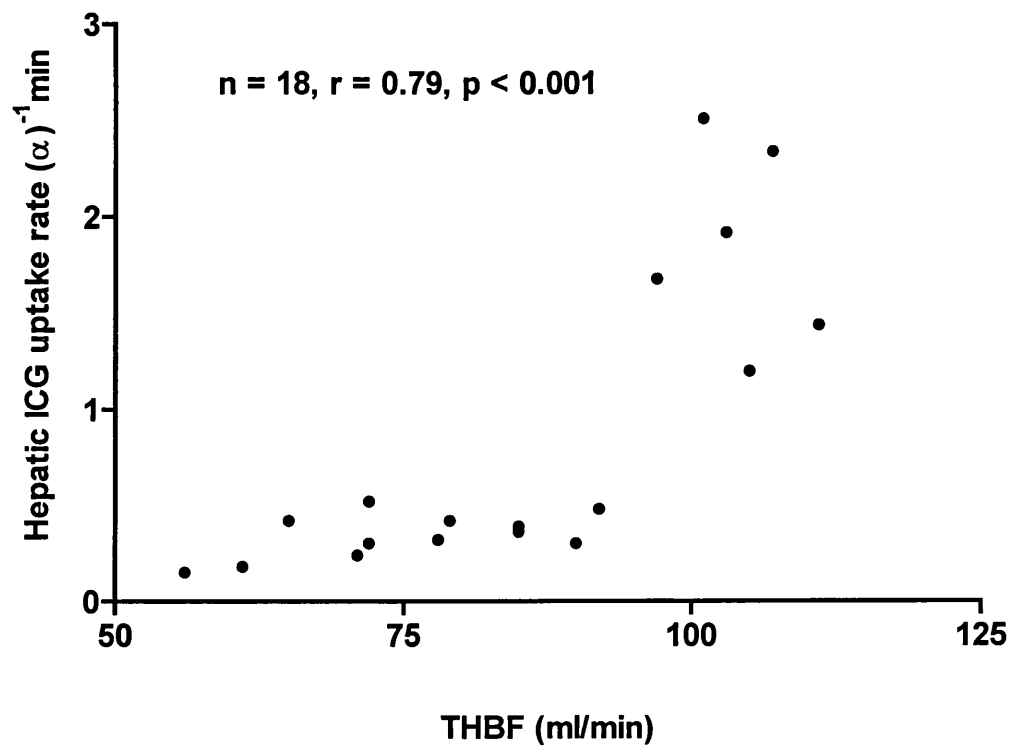


Figure 8.3 Correlation between ICG uptake rate (α) and THBF measured by ultrasonic flowmeter. Each point represent the change observed at the end of one vascular occlusion period in one animal.

8.4.2 I/R Injury

At the end of the ischaemic period the HM was significantly reduced and this did not recover completely following reperfusion (Table 8.3).

Hepatic tissue HbO₂ was significantly reduced at the end of the ischaemic period and the Hb level increased simultaneously. Following reperfusion there was incomplete recovery of tissue oxygenation with a significant decrease in HbO₂ and an increase in Hb (Table 8.3).

After I/R there was a significant reduction in ICG uptake rate (α) from the control value of $1.85 \pm 0.51 \text{ min}^{-1}$ to $0.85 \pm 0.59 \text{ min}^{-1}$ ($p < 0.001$ vs. controls). Also, there was a reduction in ICG excretion rate (β) from the control value of $0.1 \pm 0.06 \text{ min}^{-1}$ to $0.02 \pm 0.006 \text{ min}^{-1}$ ($p < 0.05$ vs. controls) (Table 8.2).

The correlation between HM and ICG uptake rate (α) was investigated in control, HAO, PVO, and I/R groups and a positive correlation ($r = 0.59$, $p < 0.01$) was found between the two parameters (Figure 8.4).

Table 8.3 Changes in HM and tissue oxygenation parameters with lobar I/R.

	Baseline	Ischaemia	Reperfusion
HM (flux)	241 ± 19	43 ± 9**	195 ± 11**
HbO ₂ (μmole/L)	0	-274 ± 38**	-78 ± 13**
Hb (μmole/L)	0	182 ± 37**	43 ± 16**

Values are mean ± SD of 6 animals in each group. Values are one-minute averages before ischaemia (baseline) and at the end of the ischaemia and reperfusion periods. **p < 0.001 vs. baseline (Student's t test).

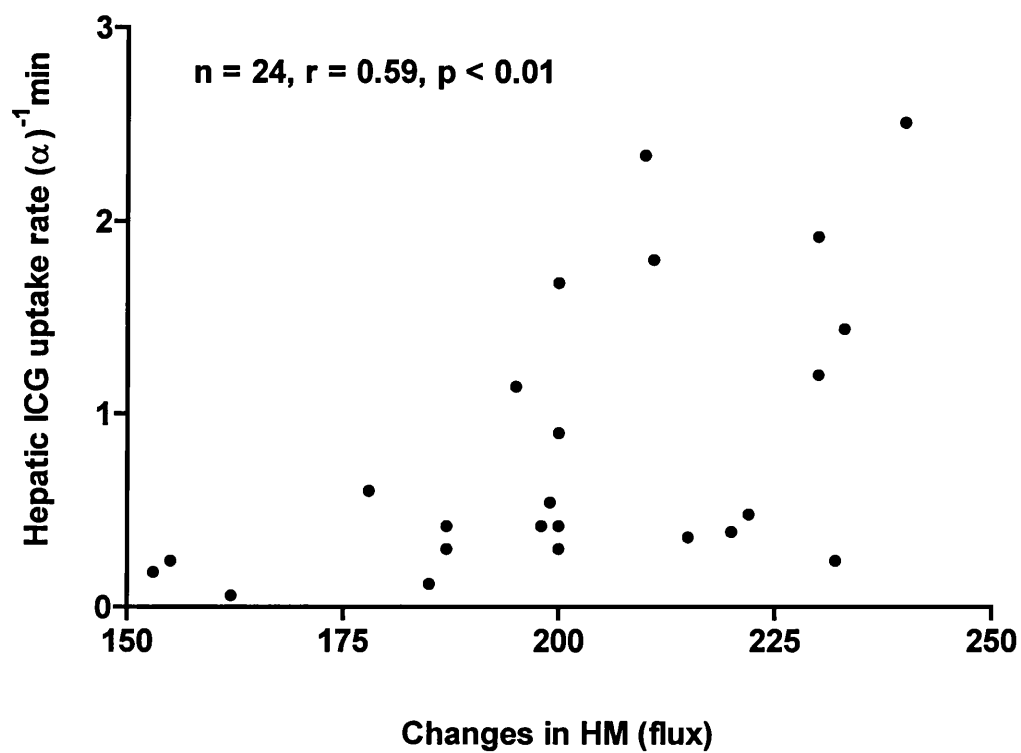


Figure 8.4 Correlation between ICG uptake rate and changes in HM. Each point represents the change at the end of one vascular occlusion period in one animal.

8.4.3 Colchicine treatment

The use of Colchicine resulted in a significant decrease in ICG excretion rate (β) from the control value of $0.1 \pm 0.06 \text{ min}^{-1}$ to $0.03 \pm 0.01 \text{ min}^{-1}$ ($p < 0.05$ vs. controls) with no significant change ($1.74 \pm 0.4 \text{ min}^{-1}$ vs. $1.85 \pm 0.51 \text{ min}^{-1}$) in ICG uptake rate (α) (Table 8.2).

8.4.4 BDL

With BDL there was a significant decrease in ICG excretion rate (β) from the control value of $0.1 \pm 0.06 \text{ min}^{-1}$ to $0.002 \pm 0.001 \text{ min}^{-1}$ ($p < 0.05$ vs. controls) with no significant change ($1.82 \pm 0.77 \text{ min}^{-1}$ vs. $1.85 \pm 0.51 \text{ min}^{-1}$) in ICG uptake rate (α) (Table 8.2).

Typical examples of ICG clearance in one animal in each group and the fitting curves are shown in Figure 8.5.

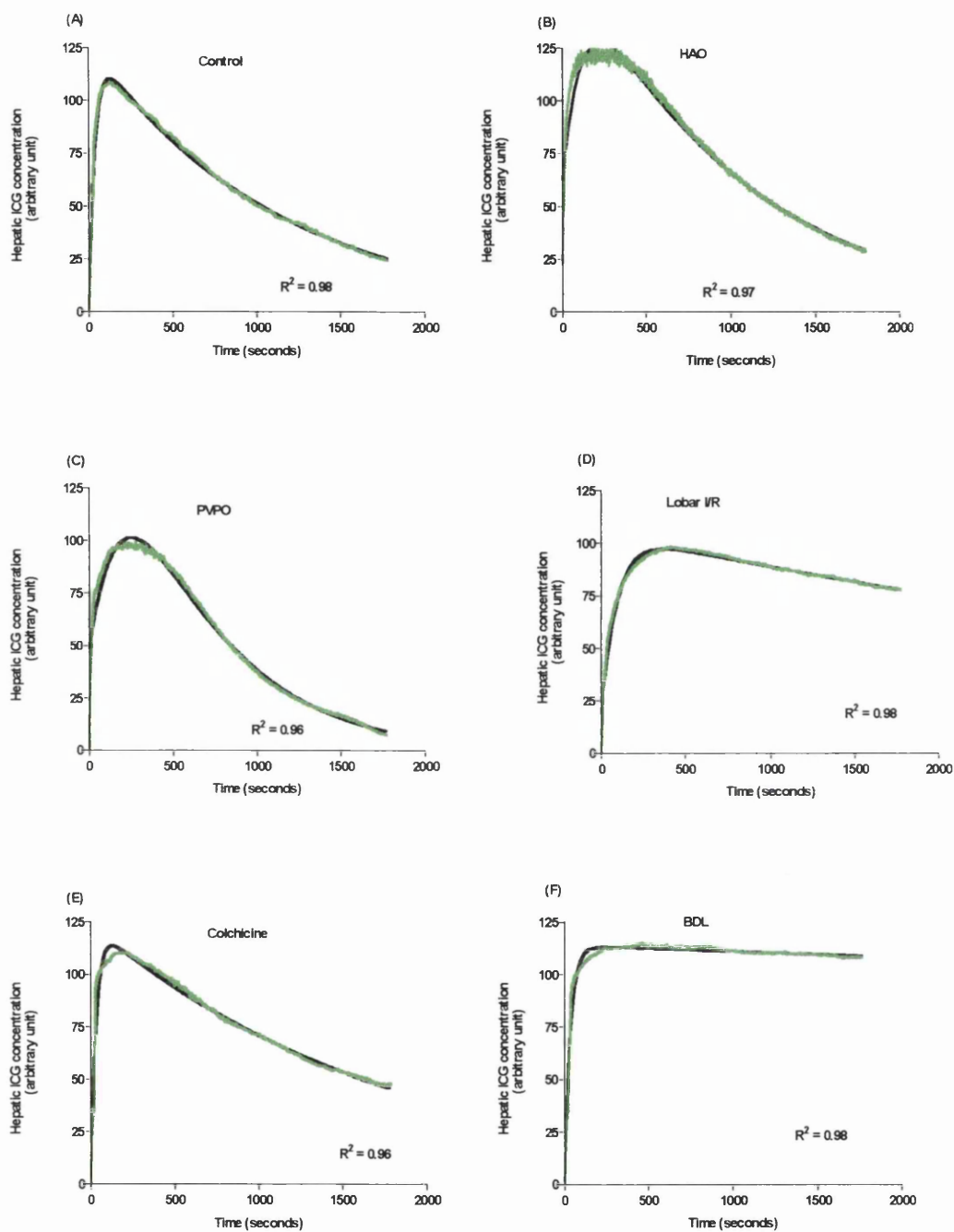


Figure 8.5 Typical examples of ICG concentration time curves and their fitted curves. (A) controls, (B) HAO, (C) PVPO, (D) lobar I/R, (E) Colchicine, and (F) BDL.

8.5 Discussion

ICG is an anionic dye which is highly bound to albumin and α -lipoproteins (Baker and Bradley, 1966, Ott et al. 1992). Experimental and clinical studies have demonstrated the rapid hepatic uptake of ICG, with a hepatic extraction of 90% with normal liver (Caeser et al. 1961). In the space of Disse, ICG dissociates from the blood proteins and enters the hepatocytes via a carrier-mediated pathway (Berk and Stremmel, 1986). The excretion of ICG into the bile canaliculi occurs via ATP-dependent active transport in unconjugated form (Chaudry et al. 1982, Meijer et al. 1988, Sumimoto et al. 1988). Its elimination is exclusively by the liver with no extrahepatic metabolism or excretion (Cherrick et al. 1960). The hepatic ICG uptake is affected by various factors such as hepatic blood flow (Melzer et al. 1992, Lacy et al. 1992), plasma proteins concentration (Ott et al. 1992), hepatocyte volume (Matsui et al. 1996), and the influx across the sinusoidal plasma membrane (Kimura et al. 1993). Its excretion is controlled by the intracellular transport, interaction with cytoplasmic binding proteins and organelles (Sugimoto et al. 1993, Shinohara et al. 1996a), transport across the biliary canalicular membrane, and bile flow (Kimura et al. 1993, Fan et al. 1994). Its excretion across the canalicular plasma membrane is ATP-dependent (Chaudry et al. 1982, Zimniak and Awasthi, 1993).

The direct measurement of hepatic ICG could provide a more accurate index for the ICG kinetics including its uptake and excretion. In this study NIRS has been used to measure directly the hepatic ICG concentration and from its

concentration-time curve the ICG uptake (α) and excretion (β) rates were calculated under different experimental conditions which are known to affect ICG uptake and excretion and which can be encountered in a clinical context. Measuring the ICG uptake rate reflects the effective liver blood flow which is defined as the flow that perfuses functioning sinusoids and is available for metabolic exchange (Henderson et al. 1981). Measuring ICG excretion rate reflects the hepatocyte function and the biliary excretion. Reduction of the THBF by HAO or PVPO was associated with a reduction of hepatic ICG uptake (α) with no change in ICG excretion rate. These results are in agreement with other studies (Andrews et al. 1956, Winkler and Gram, 1965, Krarup and Larsen, 1974) demonstrating that the plasma clearance of dyes is determined mainly by the hepatic blood flow. There is controversy over the contribution of the HA and PV blood flow to the ICG clearance from the plasma. Some experimental observations suggested that the plasma clearance of dyes was principally influenced by the arterial flow to the liver (Andrews et al. 1956), while others showed that the change in clearance was secondary to a decreased total hepatic blood flow from either occlusion of the hepatic artery or shunting of the portal vein flow (Winkler and Gram, 1965, Krarup and Larsen, 1974). The results of this experiment are in accordance with the second view, the reduction of the hepatic ICG uptake reflects a decrease of the total hepatic blood flow, irrespective of whether this is achieved by reduction of HA or PV blood flow. With lobar I/R there was reduction of the HM and tissue oxygenation with a decrease of both the hepatic ICG uptake and excretion rates. I/R injury results in

progressive microcirculatory obstruction (Koo et al. 1992) with the subsequent reduction of hepatic tissue oxygenation (Vollmar et al. 1996) and ICG uptake (Clemens et al. 1985). Possible mechanisms for this microcirculatory obstruction include cellular oedema with subsequent capillary plugging (Leaf, 1973) and leukocyte accumulation and adherence in both liver sinusoids and postsinusoidal venules (Koo et al. 1992). The reduced ICG excretion with I/R could be explained by the reduced cellular ATP production (Kamiike et al. 1982) resulting in impairment of bile excretion (Karwinski et al. 1989). Multiple mechanisms have been suggested for the cellular injury and dysfunction after I/R, including hypoxic depletion of ATP (Marubayashi et al. 1980) with incomplete recovery of the hepatocyte ATP level after reperfusion (Kamiike et al. 1982). The direct measurement of hepatic ICG concentration by NIRS in I/R injury can differentiate between the reduction of hepatic ICG uptake from the microcirculatory impairment and the reduction of ICG excretion due to hepatocellular injury. Colchicine, via its toxic effect on the cellular microtubules, has been shown to inhibit ICG cytoplasmic transport with reduction of its biliary excretion and plasma clearance (Mori et al. 1987). After Colchicine administration there was a decrease in hepatic ICG excretion which confirms the importance of intact cellular microtubules to ICG excretion from the liver and indicates that the ICG excretion rate (β) reflects hepatocellular function.

BDL caused a dramatic reduction in ICG excretion rate (β). The absence of bile flow results in ICG accumulation in the liver, slowed ICG efflux across the bile canalicular membrane and its retention in the hepatocytes (Kimura et al. 1993).

Unlike sulfobromophthalein, ICG does not regurgitate into the hepatic lymph with biliary obstruction (Kimura et al. 1993). This is the first study to quantify the reduction in ICG excretion due to biliary obstruction by NIRS. Further studies are required to define whether the excretion curve could be used to differentiate between hepatic cholestasis and extrahepatic obstruction.

These results have extended and confirmed the findings of Shinohara and colleagues (Shinohara et al. 1996b) regarding the direct measurement of ICG uptake and excretion rates using NIRS in the rabbit liver. In addition, in this study hepatic ICG uptake rate was correlated with hepatic blood flow and HM. A significant positive correlation was found between these parameters, which confirms the use of ICG uptake measured by NIRS as a direct index of liver circulation. The ICG excretion rate was affected by hepatocyte damage and biliary obstruction.

Chapter 9

**Assessment of ischaemia reperfusion injury by measuring hepatic tissue
oxygenation, blood volume and liver function using near infrared
spectroscopy**

9.1 Introduction

One of the major problems accompanying liver transplantation is evaluation of the viability of the grafted liver at an early stage. The ability to assess immediate graft function would help to determine both graft and patient survival and prognosis. Many biochemical markers have been used as indicators of I/R injury and liver damage after transplantation including, determination of liver enzymes in the effluent perfusate (Lange et al. 1996), the liver's ability to restore its ATP content (Higashi et al. 1989), level of purine compounds in the graft perfusate (Nishida et al. 1987), bile flow (Bowers et al. 1987), serum and biliary bile acid analysis (Baumgartner et al. 1995), arterial ketone body ratio in the donor (Taki et al. 1990), and hepatic protein synthesis (Nakagohri et al. 1989). Some of these methods are invasive requiring liver biopsy and others require complicated procedures and considerable time to obtain data. An accurate and readily applicable method of quantifying I/R injury in the liver is required. As mentioned in Chapter 8 preliminary studies on lobar I/R injury suggested that NIRS measurement could provide information on hepatic oxygenation and ICG clearance from which the severity of I/R injury could be determined.

9.2 Objectives

1. The application of NIRS to assess the severity of hepatic I/R injury by measuring hepatic tissue oxygenation and blood volume changes.
2. The application of NIRS to investigate the handling of ICG by the liver after I/R injury.

9.3 Materials and methods

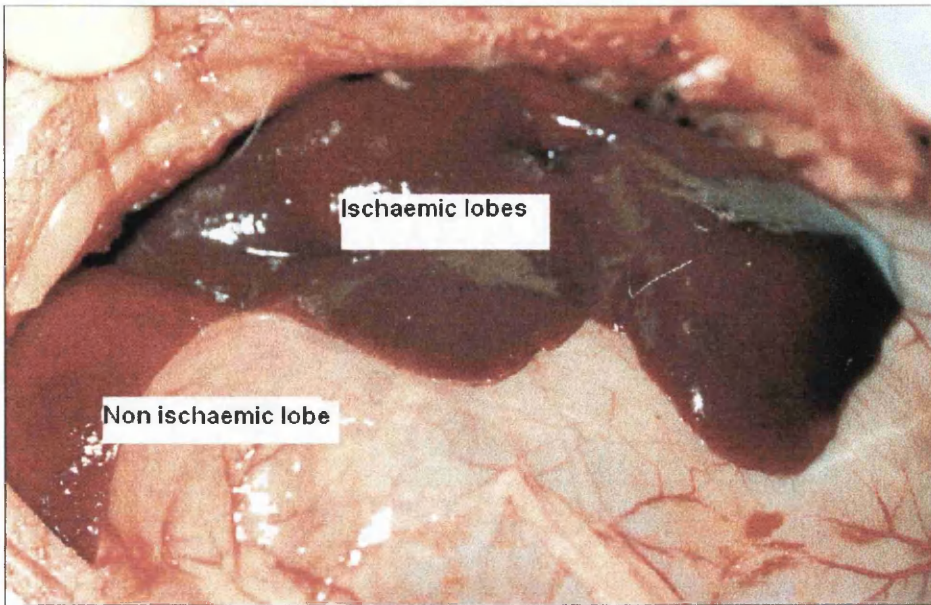
9.3.1 Animal preparation and surgical procedure

New Zealand white rabbits (3.6 ± 0.4 kg, $n = 24$) were used. Anaesthesia was induced by 0.5 ml/Kg Hypnorm and 2.5 mg/Kg intramuscular and maintained by Halothane. The animals were placed in supine position on a warming blanket for maintenance of their body temperature between 37-39 °C. SaO_2 and HR were continuously monitored by a pulse oximeter. Polyethylene catheters were inserted into the femoral artery for monitoring of MABP and for collecting blood samples and into the femoral vein for saline infusion (10 ml/kg/hour) to compensate for intraoperative fluid loss.

Laparotomy was via a transverse incision. Ligamentous attachments from the liver to the diaphragm were divided for complete exposure and mobilisation of the liver. Complete ischaemia of the median and left lateral lobes of the liver was produced by clamping the left PV, HA, and biliary radicles using an atraumatic

microvascular clip (Figure 9.1). This method produces complete ischaemia to the left and median lobes of the liver (about 70% of the liver) while leaving the blood supply to the right and the caudate lobes uninterrupted (Koo et al. 1992, Bowes and Thiernemann, 1998). At the end of the ischaemic period (30, 45 or 60 minutes), the vascular clip was removed and reperfusion was allowed for 60 minutes in all the groups. During I/R periods, the animal's abdomen was covered with a plastic wrap to prevent fluid evaporation. At the end of the experiment the liver was taken and weighed and the animal was killed by exsanguination.

(A)



(B)

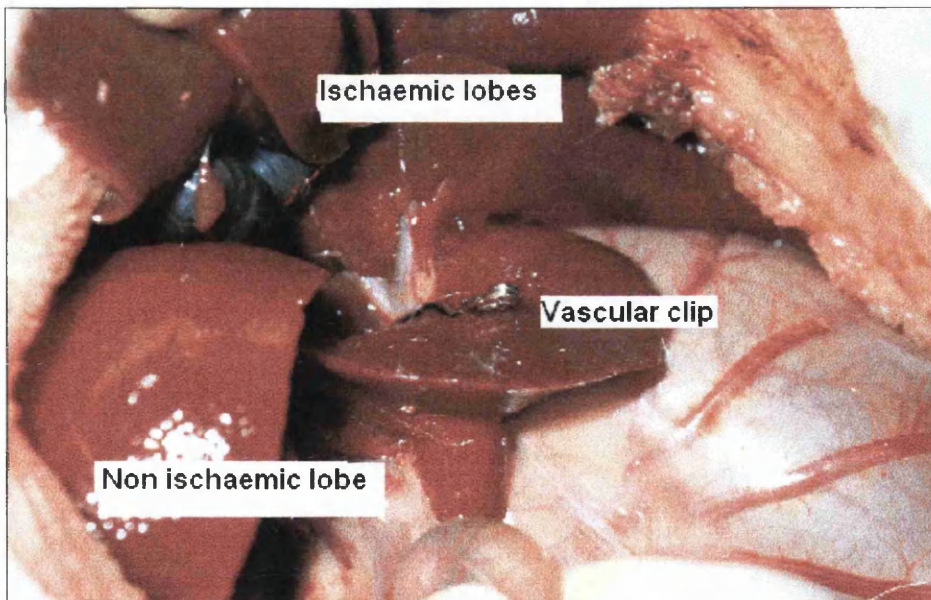


Figure 9.1 (A) The liver ischaemic and non-ischaemic lobes. (B) The under surface of the liver shows the hepatic lobes and microvascular clip around the vascular pedicle.

9.3.2 Measurement of the liver function

Arterial blood samples (2 ml each) were used for liver function tests (ALT, AST, and LDH) measurement before induction of ischaemia (Baseline) and at the end of the reperfusion period. The blood volume taken was replaced by an equal volume of normal saline. The blood samples were taken at the start of the experiment and after reperfusion. The liver function tests were done by a standard spectrophotometric method using an automated clinical chemistry analyser (Hitachi 747, Roche Diagnostics Ltd., Sussex, UK).

9.3.3 Measurement of the bile flow

The common bile duct was cannulated with a polyethylene catheter (PE-50, 0.58 mm inner diameter, Portex, Kent, UK) for continuous collection of bile. Bile was collected in a tube for 15 minutes at three time points: before clamping (baseline), at the end of the lobar ischaemia period and 60 minutes after reperfusion period. Bile volume was expressed as $\mu\text{L}/\text{min}/\text{g}$ of liver wet weight.

9.3.4 Measurement of HM

HM was measured continuously by a surface LDF (flux). The LDF probe was placed on a fixed site of the left (ischaemic) lobe of the liver and was hold in place with a probe holder. For comparison between the different groups, LDF

measurements were calculated as baseline (before clamping), at the end of the ischaemia period and at the end of the reperfusion period. LDF measurements at these relevant time points were collected as a mean of 1-minute data. The amount of reduction in HM after reperfusion was calculated relative to the baseline.

9.3.5 Measurement of hepatic tissue oxygenation and blood volume changes by NIRS

NIRS probes were placed, with a 20 mm separation, on the surface of the left lobe of the liver for continuous measurement of the hepatic tissue oxygenation and blood volume changes. A flexible probe holder was used to ensure a satisfactory contact with the liver surface and a fixed inter-probe spacing. For comparison between the different groups, NIRS measurements were calculated as baseline (before clamping), at the end of the ischaemia period and at the end of the reperfusion period. NIRS measurements at these relevant time points were calculated as a mean of 1-minute data. NIRS measurements during ischaemia and reperfusion were expressed relative to the baseline level before induction of ischaemia.

9.3.6 Measurement and analysis of hepatic ICG concentration curve using NIRS

For measurement of hepatic ICG clearance in all the groups a bolus of ICG 0.5 mg/kg was given to all groups. ICG was given 120 minutes from the start of the experiment in the controls or after 60 minutes of reperfusion in the other groups. ICG was dissolved in sterile water (1 mg/ml) and given via the femoral vein over 20 seconds at the end of the reperfusion period in all groups. Hepatic tissue ICG was measured by NIRS. NIRS probes were placed, on the surface of the left lobe of the liver for continuous measurement of hepatic tissue ICG for 30 minutes after ICG injection.

Continuous measurement of hepatic ICG by NIRS produces a concentration-time curve. This curve was analysed to produce two exponential rate constants, representing hepatic ICG uptake from the plasma to the hepatocytes (α) and hepatic ICG excretion from the liver by cytoplasmic transport and biliary excretion (β). The details of ICG curve analysis are outlined in Chapter 8.3.2.

9.3.7 Experimental groups and protocol

Four groups of animals (n = 6 each) were used. Group A was sham-operated animals (controls). These animals underwent an identical experimental protocol but without clamping of the hepatic blood vessels. For comparison with the other groups, measurements were taken 60 minutes from the start of the experiment in

controls and compared with the ischaemia measurements. Control measurements were also taken after 120 minutes were compared with the 60 minutes postreperfusion measurements in the other groups. Animals of group B, C, and D underwent ischaemia for 30 minutes, 45 minutes or 60 minutes, respectively. A 60-minute reperfusion period was allowed in all the groups.

9.3.8 Data collection and statistical analysis

The values are expressed as mean $\pm$ SD. $p < 0.05$ was considered statistically significant. For statistical analysis one way analysis of the variance (ANOVA) and Student's t-test were used with Bonferroni correction for multiple tests. The relationship between the hepatic tissue oxygenation, blood volume and hepatic ICG uptake and removal rates measured by NIRS with I/R and serum enzyme levels, bile flow and hepatic microcirculation was tested using Pearson correlation coefficient.

9.4 Results

9.4.1 Haemodynamic parameters with I/R

In all animals in the sham-operated control group as well as animals subjected to lobar ischaemia the heart rate and blood pressure did not change significantly throughout the experiment. Also, there was no significant difference between the I/R groups and controls.

9.4.2 Hepatocellular injury with I/R

In all groups the baseline serum enzymes (ALT, AST, and LDH) were within the normal range with no significant difference between the groups (Table 9.1). In all experimental groups 60 minutes after reperfusion, the serum level of these enzymes was significantly increased in comparison to controls (Table 9.1). There was no significant difference in hepatocellular injury between 30 minutes ischaemia (group B) and 45 minutes ischaemia (group C), while there was a significant difference between both these groups and 60 minutes ischaemia (group D) ($p < 0.05$ for groups B and C vs. group D).

Table 9.1 Serum ALT, AST, and LDH levels (U/L).

Serum enzymes	Group A	Group B	Group C	Group D
ALT (baseline)	38 ± 16 [*]	24 ± 18 [*]	25 ± 21 [*]	33 ± 25 [*]
AST (baseline)	49 ± 11 [*]	39 ± 29 [*]	40 ± 19 [*]	42 ± 18 [*]
LDH (baseline)	264 ± 78 [*]	260 ± 69 [*]	255 ± 71 [*]	259 ± 103 [*]
ALT (reperfusion)	36 ± 13 [†]	116 ± 60 ^{†**}	164 ± 60 ^{†**}	427 ± 65 ^{†**}
AST (reperfusion)	51 ± 19 [†]	358 ± 89 ^{†**}	587 ± 68 ^{†**}	2370 ± 389 ^{†**}
LDH (reperfusion)	269 ± 48 [†]	563 ± 83 ^{†**}	787 ± 92 ^{†**}	1400 ± 177 ^{†**}

Values are mean ± SD of 6 animals in each group. Values are before ischaemia (baseline) and after 60 minutes reperfusion in the experimental groups. [†]p < 0.01 and ^{*}p = not significant (ANOVA). ^{**}p < 0.01 vs. group A (Student's t test).

9.4.3 Bile volume with I/R

In group A, bile flow was 4.2 ± 0.8 µL/min/g liver wet weight which did not change significantly through the experiment. Groups B, C, and D had similar baseline values to group A with no significant difference between the groups (Table 9.2). During ischaemia there was almost no bile flow. After reperfusion bile flow recommenced but it remained significantly less than controls in all I/R groups (Table 9.2). There was no significant difference in bile volume between 30 minutes ischaemia (group B) and 45 minutes ischaemia (group C), while there

was significant difference between both groups and 60 minutes ischaemia (group D) ($p < 0.05$ for groups B and C vs. group D).

Table 9.2 Bile volume ($\mu\text{L}/\text{min}/\text{g}$ wet liver weight).

	Group A	Group B	Group C	Group D
Baseline	$4.3 \pm 0.8^*$	$4.1 \pm 0.6^*$	$4.2 \pm 0.6^*$	$3.9 \pm 0.9^*$
Reperfusion	$4.4 \pm 0.7^\dagger$	$2.9 \pm 0.4^{\dagger**}$	$2.3 \pm 0.4^{\dagger**}$	$1.2 \pm 0.5^{\dagger**}$

Values are mean $\pm$ SD of 6 animals in each group. Values are before ischaemia (baseline) and 60 minutes after reperfusion in the experimental groups. $^\dagger p < 0.01$ and $^* p =$ not significant (ANOVA). $^{**} p < 0.01$ vs. group A (Student's t test).

9.4.4 HM measured by LDF with I/R

There was no significant difference in the baseline sinusoidal blood flow measured by LDF between the groups (Table 9.3). With ischaemia there was a significant reduction in HM in all I/R groups in comparison to controls (Table 9.3) with no significant difference between the groups.

Following reperfusion HM remained significantly reduced in all I/R groups in comparison to controls (Table 9.3). There was no significant difference between 30 minutes ischaemia (group B) and 45 minutes ischaemia (group C), while there was a significant difference between the groups and 60 minutes ischaemia

(group D) ($p < 0.01$ for groups B and C vs. group D). Figure 9.2 shows typical examples of LDF measurement of hepatic tissue microcirculation during ischaemia and reperfusion in the experimental groups.

Table 9.3 HM changes with ischaemia and reperfusion measured by LDF (flux).

	Group A	Group B	Group C	Group D
Baseline	$234 \pm 48^*$	$225 \pm 35^*$	$231 \pm 40^*$	$221 \pm 34^*$
Ischaemia	$236 \pm 42^\dagger$	$44 \pm 16^{\dagger**}$	$49 \pm 19^{\dagger**}$	$36 \pm 18^{\dagger**}$
↓HM after reperfusion	$17 \pm 8^\dagger$	$43 \pm 16^{\dagger**}$	$61 \pm 18^{\dagger**}$	$96 \pm 19^{\dagger**}$

Values are mean $\pm$ SD of 6 animals in each group. Values are before ischaemia (baseline), at the end of the ischaemia and 60 minutes after reperfusion in the experimental groups. $^\dagger p < 0.01$ and $^* p =$ not significant (ANOVA). $^{**} p < 0.01$ vs. group A (Student's t test).

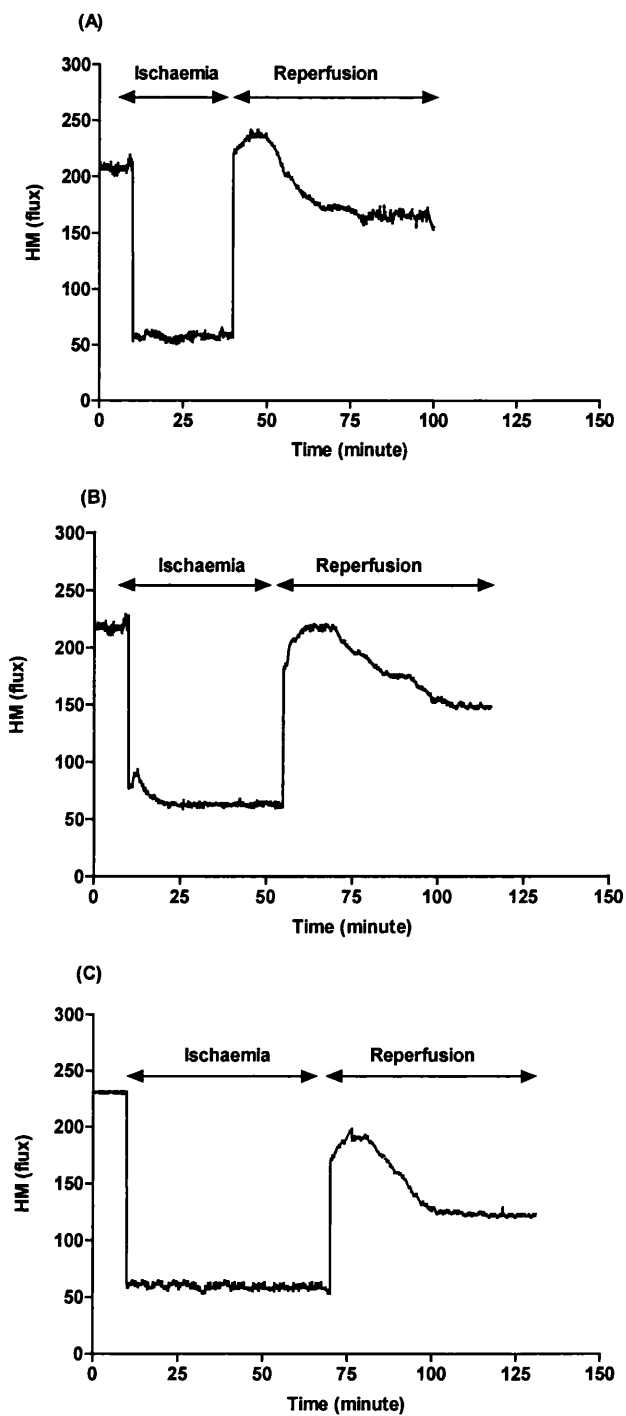


Figure 9.2 Typical examples of LDF measurements with 30 (A), 45 (B), and 60 minutes of ischaemia followed by 60 minutes reperfusion in all groups.

9.4.5 Hepatic tissue oxygenation and blood volume changes measured by NIRS with I/R

Figure 9.3 shows typical examples of hepatic tissue oxygenation changes during ischaemia and after reperfusion in the experimental groups.

With ischaemia, there was an instantaneous significant decrease in HbO₂, Cyt Ox, and HbT (Table 9.4). The changes in these parameters peaked 5-10 minutes after induction of ischaemia. Thereafter, these parameters did not change significantly until the end of ischaemia (Figure 9.3). There was simultaneous increase in Hb (Table 9.4) which also peaked after about 5-10 minutes and again did not change significantly until the end of the ischaemic period (Figure 9.3). For all the changes in tissue oxygenation parameters after ischaemia, there was no significant difference between 30 minutes ischaemia (group B) and 45 minutes ischaemia (group C), while there was significant difference between both groups vs. 60 minutes ischaemia (group D) ($p < 0.05$ for groups B and C vs. group D). After reperfusion, the changes in tissue oxygenation and blood volume occurred in two phases (Figure 9.3). Firstly, there was a significant increase in HbO₂, HbT, and Cyt Ox. However, these parameters did not return to the levels before ischaemia. After 20-30 minutes of reperfusion, these parameters then underwent progressive reduction to the end of the reperfusion period (Table 9.5). Hb changed in a similar pattern but in the opposite direction. At the end of reperfusion period, for all the changes in tissue oxygenation parameters, there was no significant difference between 30 minutes ischaemia (group B) and 45

minutes ischaemia (group C), while there was significant difference between both groups vs. 60 minutes ischaemia (group D) ($p < 0.05$ for groups B and C vs. group D).

Table 9.4 Changes in hepatic oxygenation and blood volume after ischaemia.

	Group A	Group B	Group C	Group D
HbO ₂	-4.26 ± 2.4 [†]	-223.2 ± 33.93 ^{†**}	-240.5 ± 50.25 ^{†**}	-358.7 ± 39.10 ^{†**}
Hb	4.60 ± 5.14 [†]	181.7 ± 24.16 ^{†**}	189.2 ± 42.39 ^{†**}	280.2 ± 30.71 ^{†**}
Cyt Ox	-1.87 ± 1.12 [†]	-9.82 ± 2.62 ^{†*}	-11.67 ± 2.27 ^{†*}	-24.53 ± 5.34 ^{†*}
HbT	0.34 ± 0.23 [†]	-41.5 ± 10.27 ^{†**}	-51.3 ± 16.95 ^{†**}	-78.5 ± 15.53 ^{†**}

Values are mean ± SD of 6 animals in each group. Values at the end of the ischaemia in the experimental groups relative to baseline. [†]p < 0.05 (ANOVA).

^{**}p < 0.01 and ^{*}p < 0.05 vs. group A (Student's t test).

Table 9.5 Changes in hepatic oxygenation and blood volume after reperfusion.

	Group A	Group B	Group C	Group D
HbO ₂	-13.98 ± 5.7 [†]	-80.5 ± 17.13 ^{†**}	-91.33 ± 16.73 ^{†**}	-144.8 ± 29.21 ^{†**}
Hb	9.4 ± 4.5 [†]	59.17 ± 22.78 ^{†**}	68.33 ± 17.94 ^{†**}	103.7 ± 20.11 ^{†**}
Cyt Ox	-1.75 ± 0.52 [†]	-6.98 ± 2.69 ^{†*}	-7.13 ± 3.11 ^{†*}	-14.56 ± 4.52 ^{†*}
HbT	-4.58 ± 1.26 [†]	-21.3 ± 14.76 ^{†**}	-23.0 ± 14.09 ^{†**}	-41.17 ± 13.60 ^{†**}

Values are mean ± SD of 6 animals in each group. Values at the end of 60 minutes reperfusion in the experimental groups relative to baseline. [†]p < 0.05 (ANOVA). ^{**}p < 0.01 and ^{*}p < 0.05 vs. group A (Student's t test).

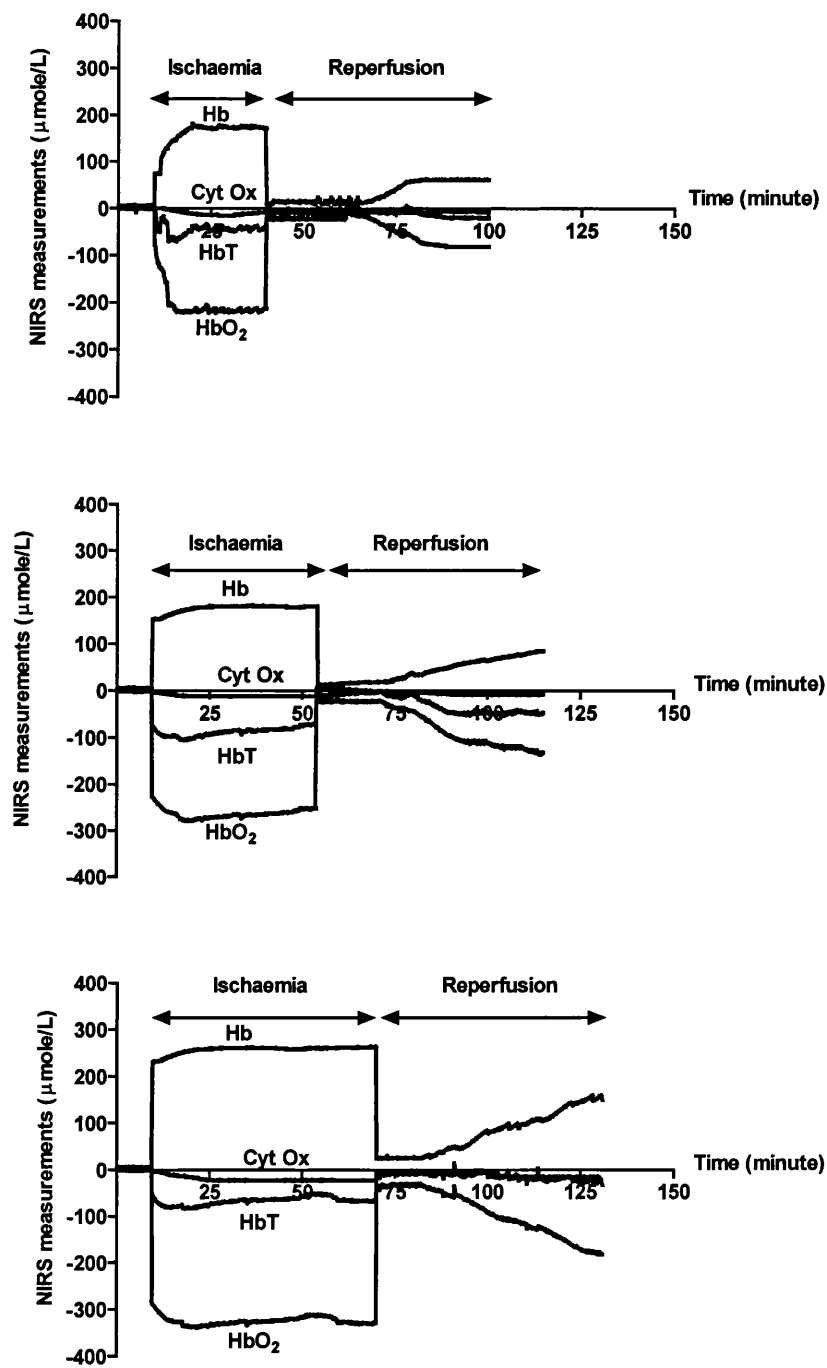


Figure 9.3 Typical examples of NIRS measurements with 30 (A), 45 (B), and 60 minutes (C) of ischaemia followed by 60 minutes reperfusion in all groups.

9.4.6 Correlation of HbO₂ with HM, liver function tests and bile volume

At the end of reperfusion the tissue HbO₂ changes measured by NIRS correlated significantly with the HM changes measured by LDF ($r = 0.94$, $p < 0.001$) (Figure 9.4). The correlations of these parameters in each group (A, B, C, and D) were ($r = 0.81$, 0.75 , 0.81 , and 0.74 , respectively).

HbO₂ correlated with hepatocellular injury parameters. A significant positive correlation was found between the HbO₂ and the serum ALT, AST, and LDH ($r = 0.93$, 0.87 and 0.95 , respectively, $p < 0.001$) (Figure 9.5). The correlation of HbO₂ and ALT in each group (A, B, C, and D) were ($r = 0.96$, 0.98 , 0.98 , and 0.97 , respectively). The correlation of HbO₂ and AST each group (A, B, C, and D) were ($r = 0.923$, 0.98 , 0.76 , and 0.91 , respectively). The correlation of HbO₂ and LDH in each group (A, B, C, and D) were ($r = 0.97$, 0.96 , 0.96 , and 0.92 , respectively). Also, a significant negative correlation was shown between the HbO₂ and the bile volume ($r = 0.91$, $p < 0.001$) (Figure 9.6). The correlation of HbO₂ and the bile volume in each group (A, B, C, and D) were ($r = 0.98$, 0.93 , 0.94 , and 0.90 , respectively).

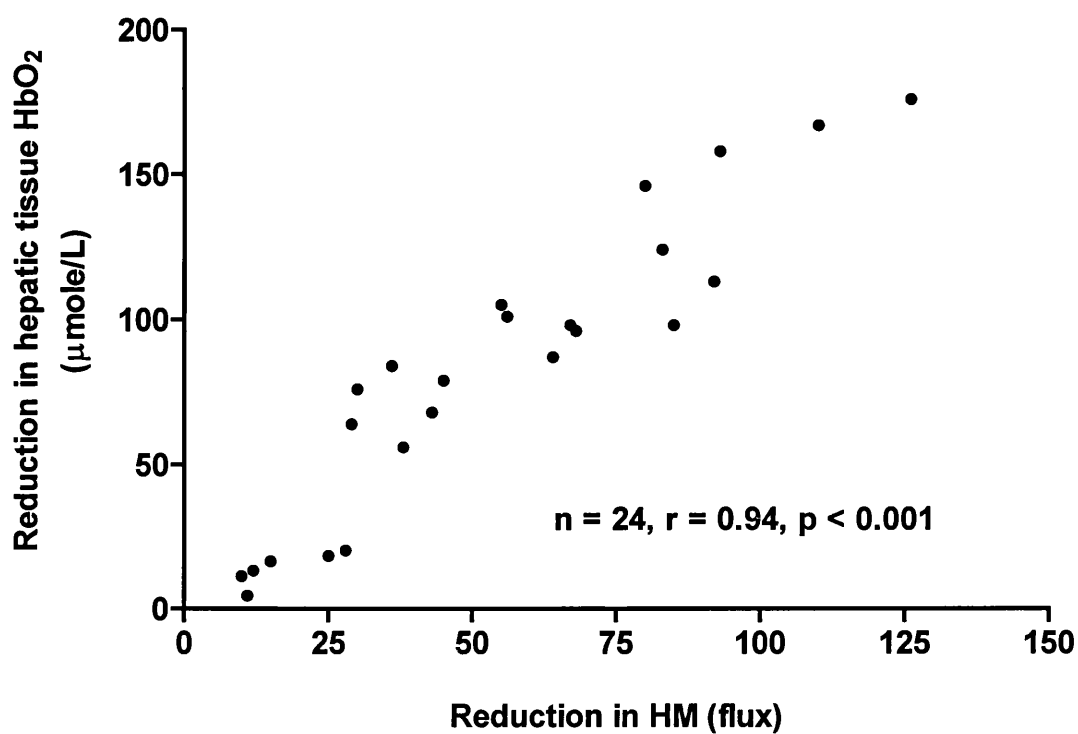


Figure 9.4 Correlation between reduction in hepatic tissue HbO₂ and the reduction in HM after 60 minutes reperfusion in the experimental groups. Each point represents the change observed at the end the reperfusion period in one animal.

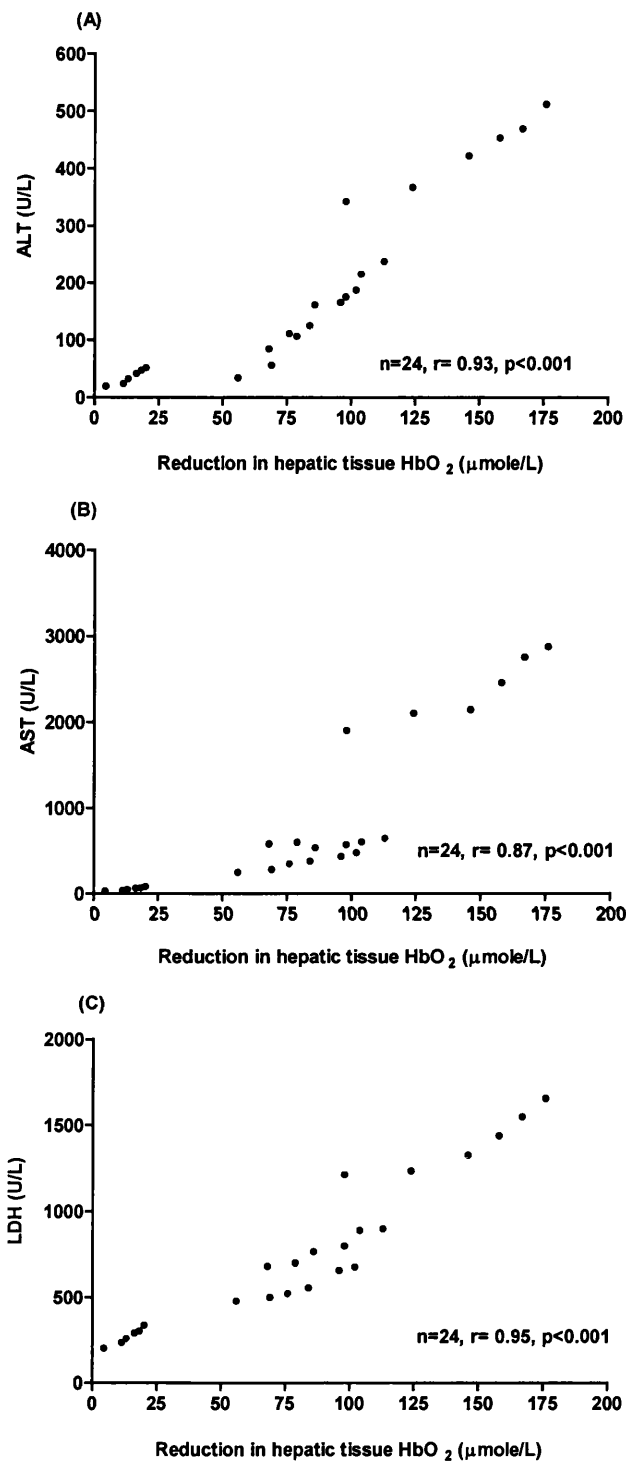


Figure 9.5 Correlation between the reduction in HbO_2 and the increase in serum enzymes ALT (A), AST (B), and LDH (C) after reperfusion in the experimental groups. Each point is the change at the end the reperfusion in one animal.

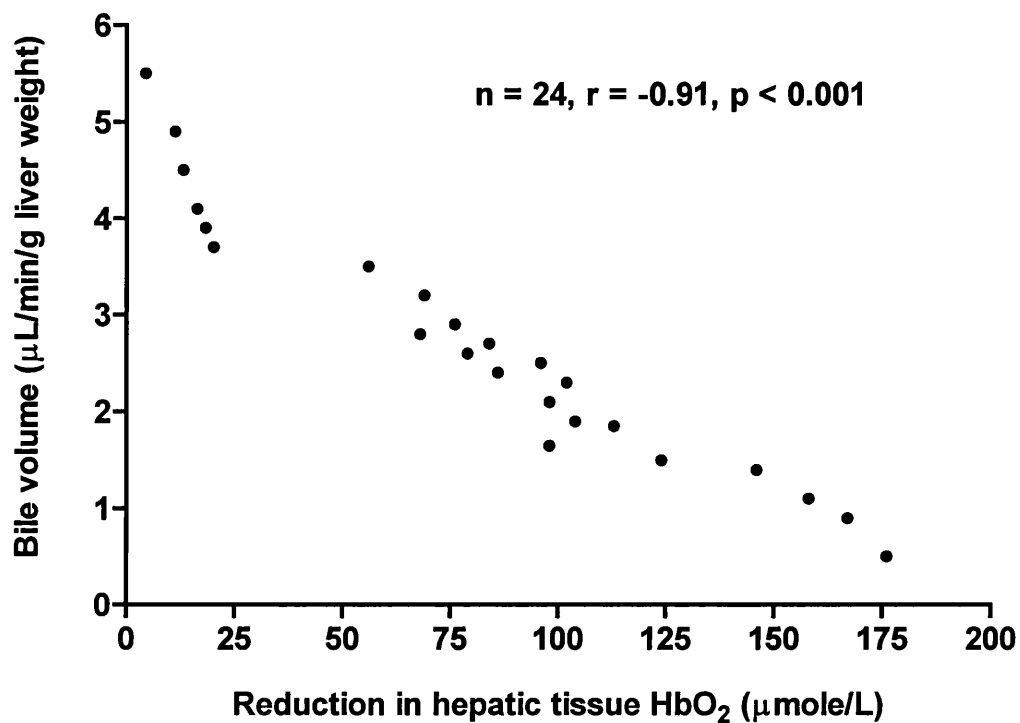


Figure 9.6 Correlation between reduction in hepatic tissue HbO₂ and the bile volume after reperfusion in the experimental groups. Each point represents the change observed at the end the reperfusion period in one animal.

9.4.7 Correlation of Cyt Ox with HM, liver function tests and bile volume

At the end of reperfusion the tissue Cyt Ox measured by NIRS correlated significantly with the HM changes measured by LDF ($r = 0.93$, $p < 0.001$) (Figure 9.7). The correlation of Cyt Ox and LDF in each group (A, B, C, and D) were ($r = 0.68$, 0.69 , 0.79 , and 0.88 , respectively).

Cyt Ox correlated with hepatocellular injury parameters. A significant positive correlation was found between the Cyt Ox level and the serum ALT, AST, and LDH ($r = 0.97$, 0.90 , and 0.85 , respectively, $p < 0.001$) (Figure 9.8). The correlation of Cyt Ox and ALT in each group (A, B, C, and D) were ($r = 0.79$, 0.99 , 0.94 , and 0.95 , respectively). The correlation of Cyt Ox and AST in each group (A, B, C, and D) were ($r = 0.77$, 0.99 , 0.84 , and 0.98 , respectively). The correlation of Cyt Ox and LDH in each group (A, B, C, and D) were ($r = 0.75$, 0.94 , 0.99 , and 0.98 , respectively).

Also, a significant negative correlation was observed between the Cyt Ox and the bile volume ($r = 0.93$, $p < 0.001$) (Figure 9.9). The correlation of Cyt Ox and bile volume in each group (A, B, C, and D) were ($r = 0.72$, 0.99 , 0.99 , and 0.97 , respectively).

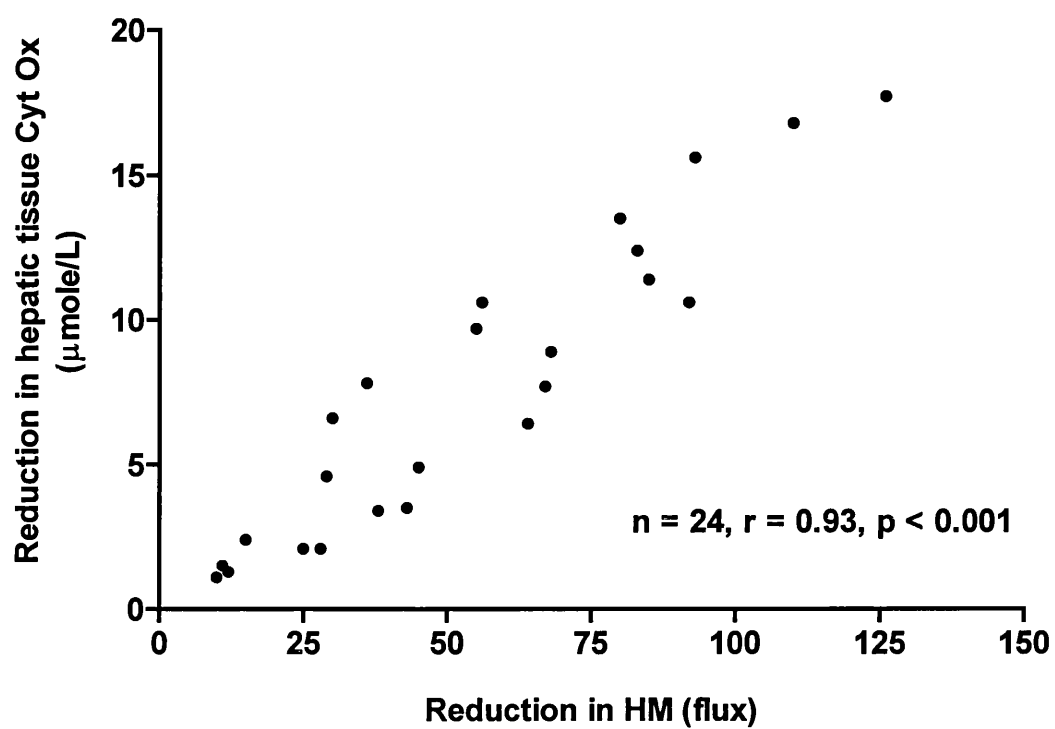


Figure 9.7 Correlation between reduction in hepatic tissue Cyt Ox and the reduction in HM after reperfusion in the experimental groups. Each point represents the change observed at the end the reperfusion period in one animal.

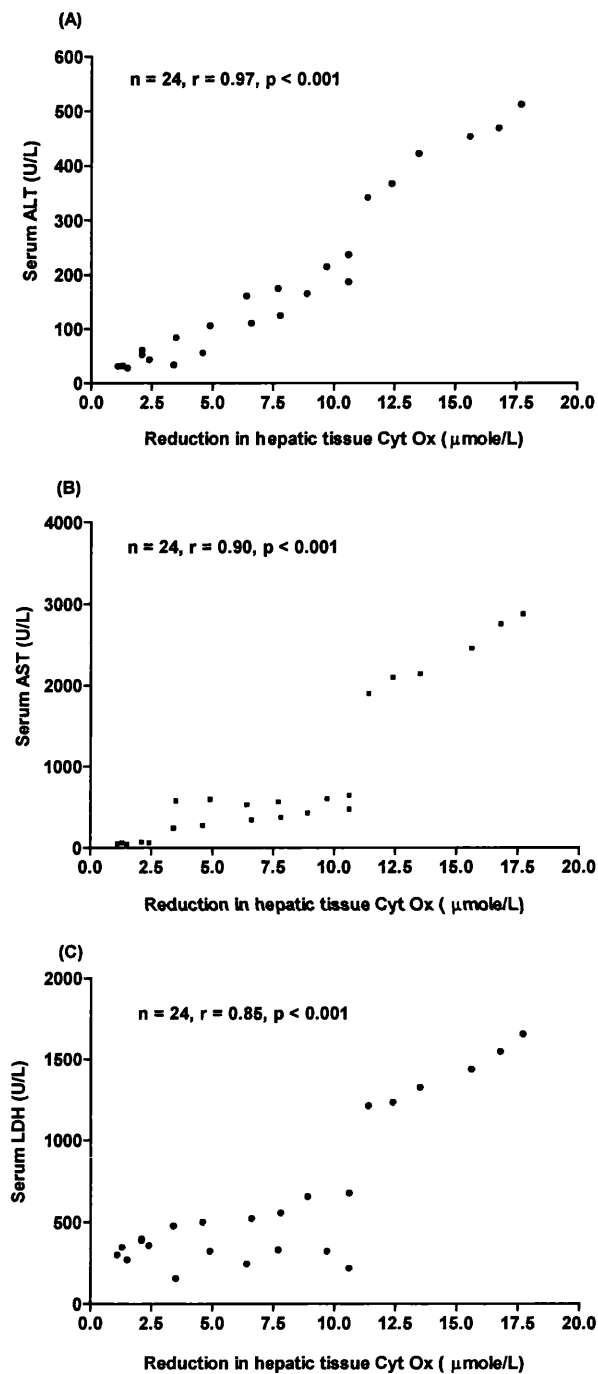


Figure 9.8 Correlation between reduction in Cyt Ox and the increase in serum enzymes ALT (A), AST (B), and LDH (C) after reperfusion in the experimental groups. Each point is the change at the end the reperfusion in one animal.

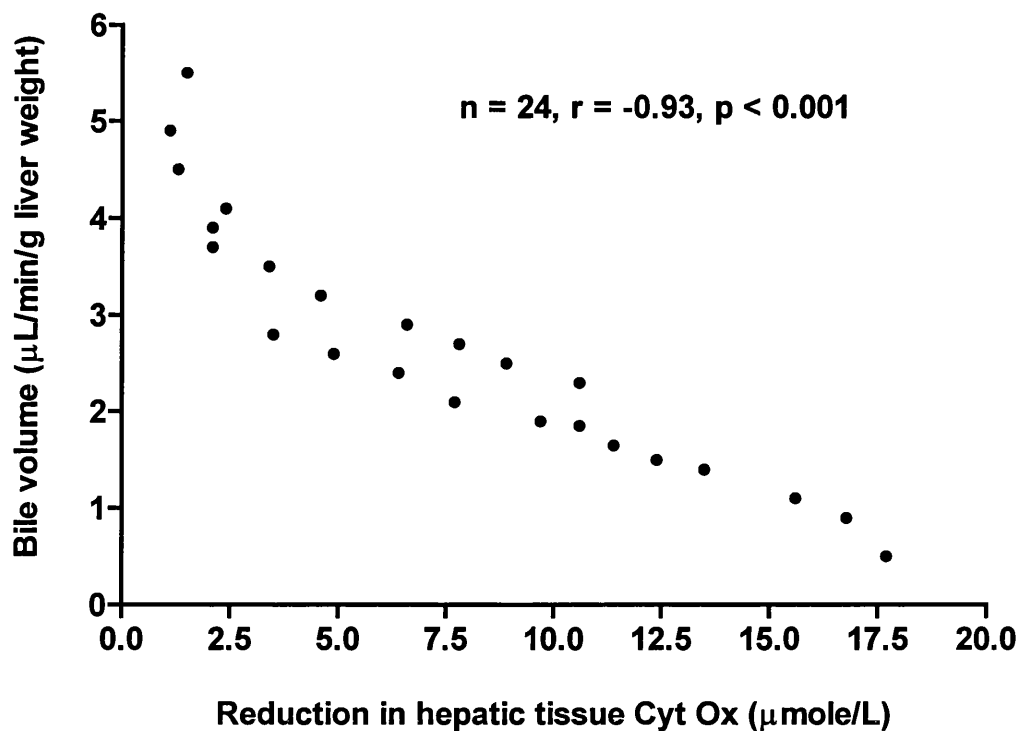


Figure 9.9 Correlation between reduction in hepatic tissue Cyt Ox and the bile volume after reperfusion in the experimental groups. Each point represents the change observed at the end the reperfusion period in one animal.

9.4.8 Correlation of HbT with HM, liver function tests and bile volume

After reperfusion HbT as an indicator to tissue blood volume significantly correlated with the sinusoidal perfusion measured by LDF ($r = 0.80$, $p < 0.001$; Figure 9.10). The correlation of HbT and LDF in each group (A, B, C, and D) were ($r = 0.66$, 0.71 , 0.73 , and 0.69 , respectively).

HbT correlated with hepatocellular injury parameters. A significant positive correlation was found between the HbT and the serum ALT, AST, and LDH ($r = 0.84$, 0.85 , and 0.76 , respectively, $p < 0.001$) (Figure 9.11). The correlation of HbT and ALT in each group (A, B, C, and D) were ($r = 0.80$, 0.76 , 0.78 , and 0.80 , respectively). The correlation of HbT and AST in each group (A, B, C, and D) were ($r = 0.80$, 0.82 , 0.81 , and 0.85 , respectively). The correlation of HbT and LDH in each group (A, B, C, and D) were ($r = 0.69$, 0.71 , 0.74 , and 0.72 , respectively).

Also, a significant negative correlation was shown between the HbT and the bile volume ($r = 0.84$, $p < 0.001$) (Figure 9.12). The correlation of HbT and bile volume in each group (A, B, C, and D) were ($r = 0.82$, 0.86 , 0.79 , and 0.80 , respectively).

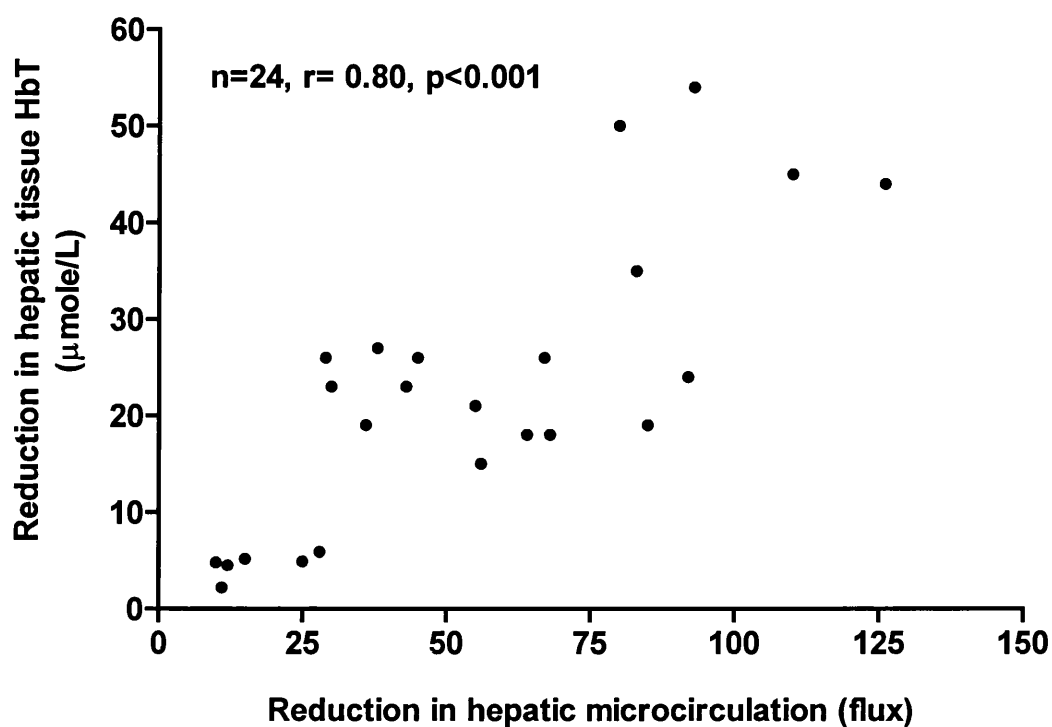


Figure 9.10 Correlation between reduction in hepatic tissue HbT and the reduction in HM after reperfusion in the experimental groups. Each point represents the change observed at the end the reperfusion period in one animal.

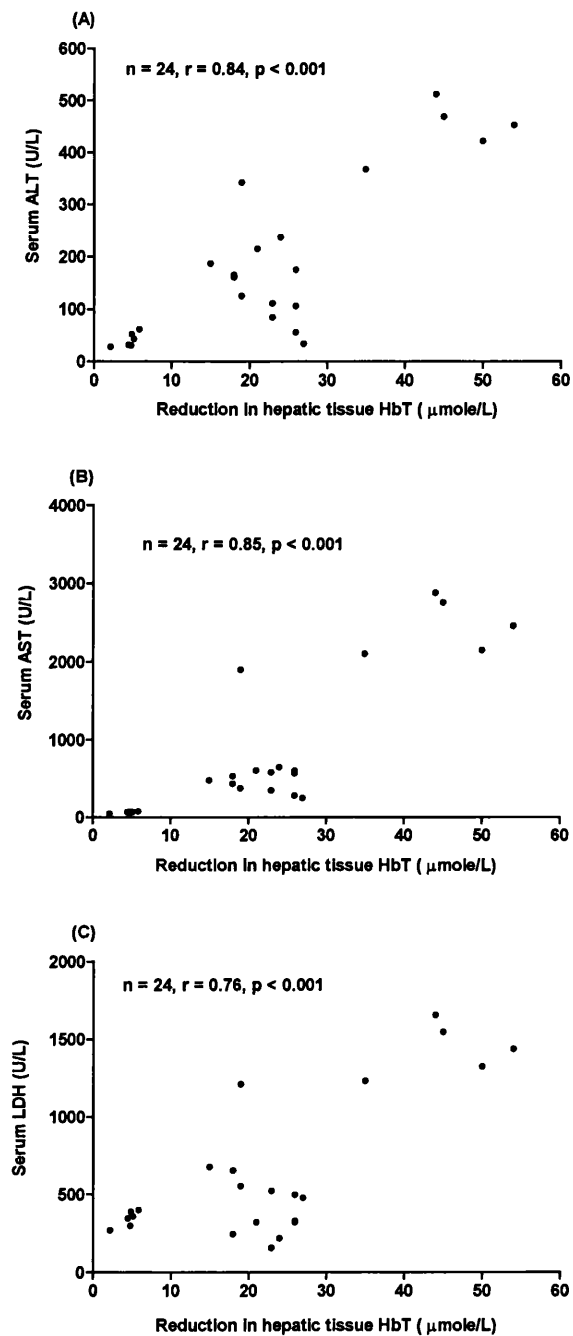


Figure 9.11 Correlation between reduction in HbT and the increase in serum enzymes ALT (A), AST (B), and LDH (C) after reperfusion in the experimental groups. Each point is the change observed at the end the reperfusion period in one animal.

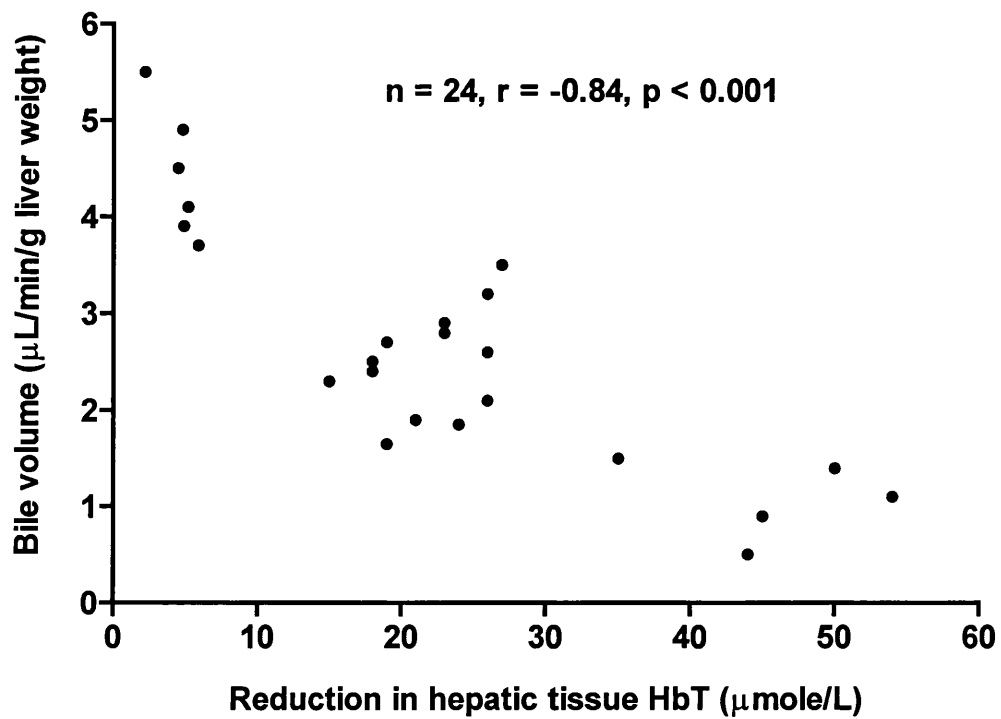


Figure 9.12 Correlation between reduction in hepatic tissue HbT and the bile volume after reperfusion in the experimental groups. Each point represents the change observed at the end the reperfusion period in one animal.

9.4.9 Hepatic ICG measured by NIRS after I/R

After I/R there was a significant reduction in ICG uptake rate (α) from the control value of $2.37 \pm 0.57 \text{ min}^{-1}$ to 1.12 ± 0.39 , 0.85 ± 0.40 , and $0.084 \pm 0.065 \text{ min}^{-1}$ (in groups B, C, and D, respectively) ($p < 0.01$ for each vs. group A) (Table 9.6).

There was no significant difference between group B and group C, while there was a significant difference between these two groups and group D ($p < 0.05$).

ICG excretion rate (β) was reduced from the control value of $0.37 \pm 0.24 \text{ min}^{-1}$ to 0.24 ± 0.23 , 0.15 ± 0.09 , $0.004 \pm 0.003 \text{ min}^{-1}$ (groups B, C, and D, respectively) ($p < 0.05$ for each vs. group A) (Table 9.6). There was no significant difference between group B and group C, while there was a significant difference between these two groups and group D ($p < 0.05$). Examples of ICG clearance curves in all groups are shown in Figure 9.13.

Table 9.6 Hepatic ICG uptake rate (α) min^{-1} and ICG excretion rate (β) min^{-1} calculated from ICG concentration-time curves.

	Group A	Group B	Group C	Group D
α	$2.37 \pm 0.57^{\dagger}$	$1.12 \pm 0.39^{\dagger**}$	$0.85 \pm 0.40^{\dagger**}$	$0.084 \pm 0.065^{\dagger**}$
β	$0.37 \pm 0.24^{\dagger}$	$0.24 \pm 0.23^{\dagger*}$	$0.15 \pm 0.09^{\dagger*}$	$0.004 \pm 0.003^{\dagger*}$

Values are mean $\pm$ SD of 6 animals in each group. ICG given after reperfusion in the experimental groups. $^{\dagger}p < 0.05$ (ANOVA). $^{**}p < 0.01$ and $^{*}p < 0.05$ vs. Group A (Student's t test).

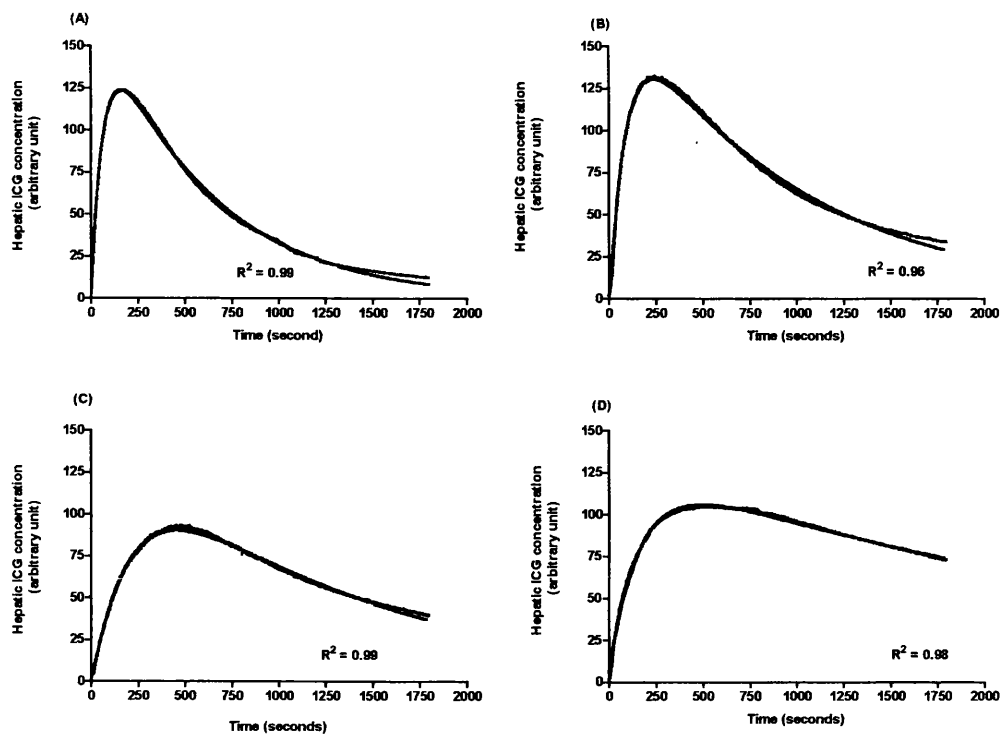


Figure 9.13 Typical examples of ICG concentration-time curves and their fitted curves. (A) controls, (B) 30, (C) 45, and (D) 60 minutes ischaemia followed by 60 minutes reperfusion in all groups.

9.4.10 Correlation between ICG uptake rate (α) and HM

A significant correlation was found ICG uptake rate and the reduction in HM after reperfusion ($r = -0.89$, $p < 0.001$) (Figure 9.14). The correlation of ICG uptake rate and the reduction in HM in each group (A, B, C, and D) were ($r = -0.87$, -0.73 , -0.79 , and -0.65 , respectively).

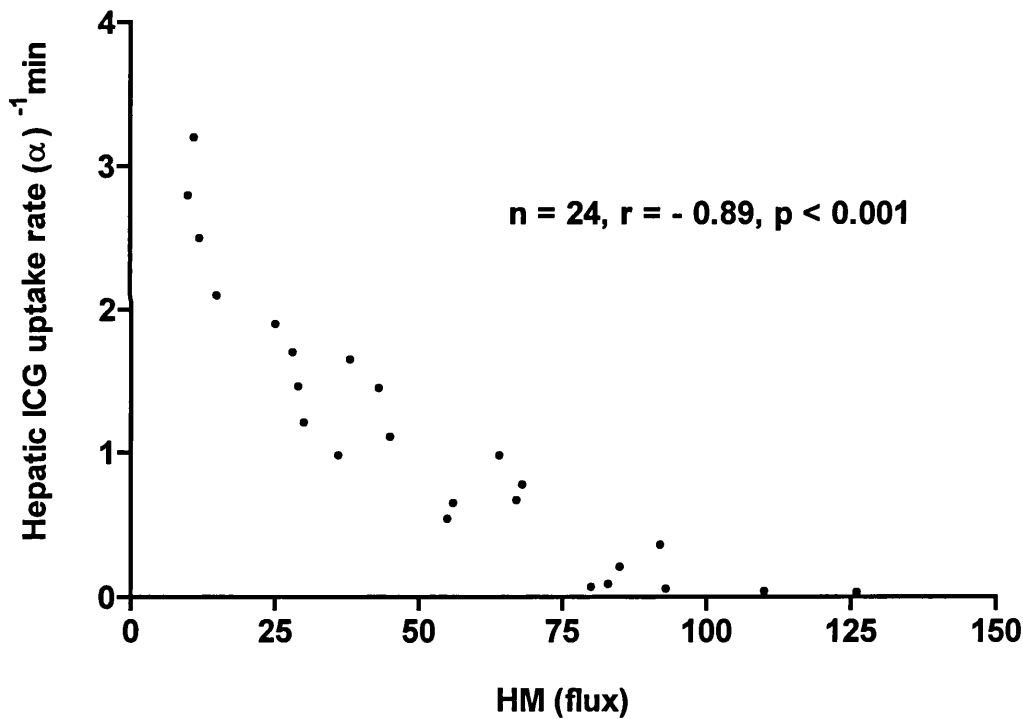


Figure 9.14 Correlation between ICG uptake rate (α) and the reduction in HM after reperfusion in the experimental groups. Each point represents the change at the end the reperfusion period in one animal.

9.4.11 Correlation between ICG excretion rate (β) and liver enzymes and bile volume

A significant correlation was found between ICG excretion rate and ALT, AST, and LDH ($r = -0.73, -0.61, \text{ and } -0.56$, respectively) ($p < 0.001$ for all correlations) (Figure 9.15A-C). The correlation of ICG excretion rate and ALT in each group (A, B, C, and D) were ($r = -0.98, -0.98, -0.97, \text{ and } -0.96$, respectively). The correlation of ICG excretion rate and AST in each group (A, B, C, and D) were ($r = -0.95, -0.97, -0.86, \text{ and } -0.93$, respectively). The correlation of ICG excretion rate and LDH in each group (A, B, C, and D) were ($r = -0.98, -0.90, -0.93 \text{ and } -0.95$, respectively).

Also, a significant correlation ($r = 0.83, p < 0.001$) was observed between ICG excretion rate and bile volume (Figure 9.15D). The correlation of ICG excretion rate and bile volume in each group (A, B, C, and D) were ($r = 0.99, 0.98, 0.98, \text{ and } 0.93$, respectively).

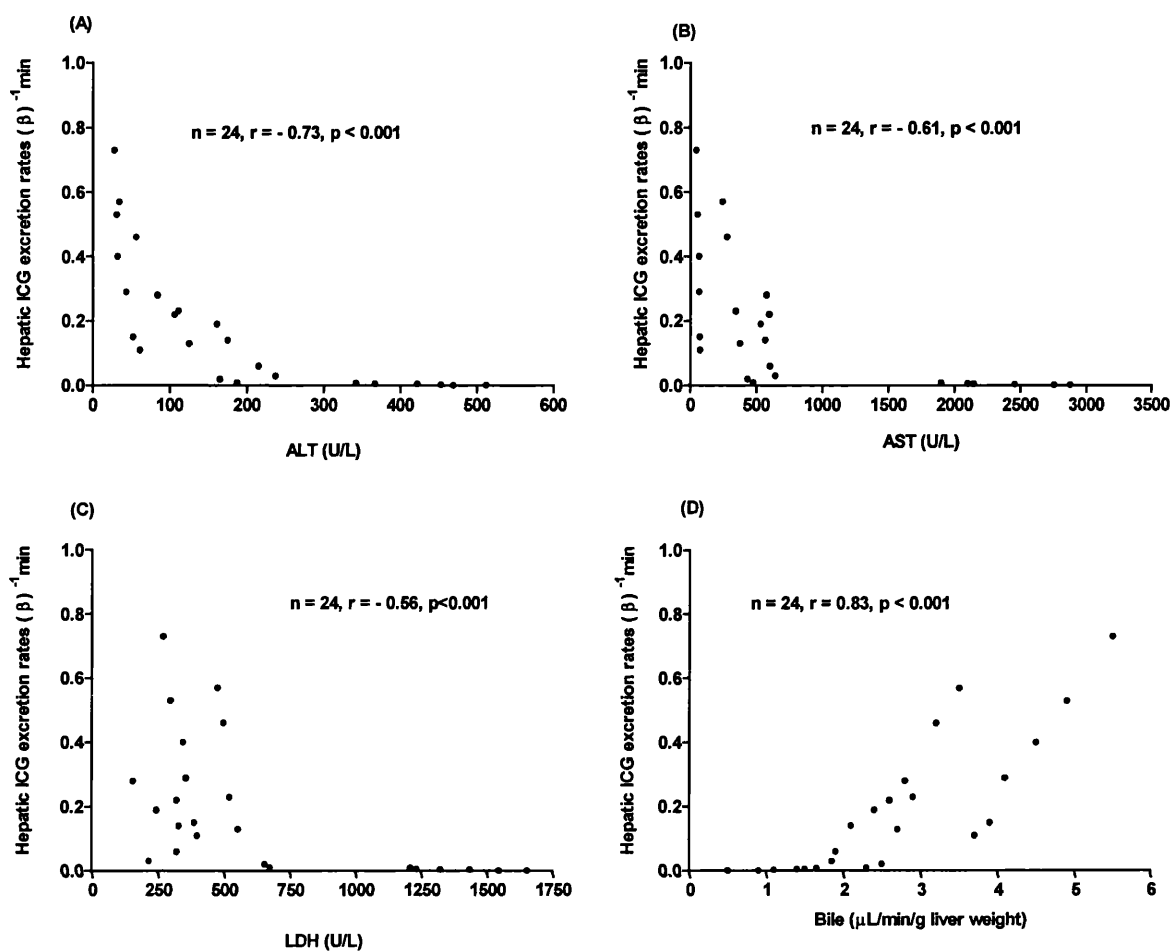


Figure 9.15 Correlation between ICG excretion rate and (A) ALT, (B) AST, (C) LDH, and (D) bile volume after reperfusion in the experimental groups. Each point represents the change at the end the reperfusion period in one animal.

9.5 Discussion

In this study a rat model of complete lobar I/R was used. Blood flow to the median and left lateral lobes was interrupted to induce partial complete hepatic ischaemia while maintaining normal flow to the right and caudate lobes. This maintains the splanchnic blood flow and prevents portal vein stasis and intestinal venous congestion which results in portal bacteraemia with subsequent haemodynamic instability (Bowes and Thiernemann, 1998).

The systemic haemodynamic parameters were not significantly changed in the controls or with this lobar I/R model throughout the experiment which excludes any systemic influence on the extent of the liver injury.

The hepatocellular damage induced by I/R in this study manifested by the significant increase in serum enzyme levels (AST, ALT, and LDH) after reperfusion. This hepatocellular damage correlated with the ischaemia time. The increase in serum enzyme levels occurs secondary to rupture of the plasma membrane with leakage of the cellular enzymes into the circulation (Lemaster et al. 1983, Gores et al. 1990).

In this study bile flow was used as an index of I/R injury and to reflect changes in cellular ATP level (Kamike et al. 1985, Sumimoto et al. 1988). The bile flow decreased with I/R injury and this decrease correlated with the ischaemia time. This reduction of bile flow could be due to decreased hepatic tissue blood flow and oxygenation (Matsumura et al. 1988), reduction of cellular ATP level as bile

production involves ATP-consuming steps (Kamike et al. 1985, Sumimoto et al. 1988) or occlusion of the bile canaliculi by cell swelling (Gores et al. 1990). Hepatic sinusoidal perfusion failure secondary to I/R has been shown to be a key factor in the pathogenesis of I/R injury (Koo et al. 1992, Vollmar et al. 1994). In this study changes in microvascular flow following I/R were measured by LDF which has been applied and validated in many studies (Chavez-Cartaya et al. 1994, Vollmar et al. 1994, Eleftheriadis et al. 1997). LDF signal was observed during ischaemia despite total lobar ischaemia. This has been reported in other studies (Chavez-Cartaya et al. 1994, Vollmar et al. 1994, Eleftheriadis et al. 1997). This paradoxical LDF signal with the cessation of blood inflow is labelled as the physiological zero (Zhong et al. 1998). This signal is due to random wandering motion of residual red blood cells and the influence of breathing movements. After reperfusion there was an increase in blood flow in the hepatic microcirculation but it did not return to baseline values, followed by progressive reduction in the microcirculation. Measurement of hepatic tissue oxygenation is useful to assess the state of tissue perfusion and the balance between oxygen supply and consumption. NIRS was used in this study to monitor continuously hepatic tissue oxygenation and blood volume changes during ischaemia and reperfusion. With ischaemia, there was reduction in HbO₂ and Cyt Ox indicating the decrease in extracellular and intracellular tissue oxygenation, respectively. This reflects the reduced blood and oxygen supply to the tissue with ischaemia. Also, there was reduction of HbT which reflects reduced tissue blood volume with ischaemia. There was a

simultaneous increase in Hb due to tissue extraction of oxygen from haemoglobin.

After reperfusion two phases were recorded. At first return of the blood and oxygen increased HbO₂, Cyt Ox, and HbT with concomitant reduction of Hb. Non of these parameters returned to the baseline before ischaemia. After 20-30 minutes of reperfusion the second phase occurs with progressive reduction of tissue oxygenation (HbO₂, Cyt Ox, and HbT) with simultaneous increase of Hb. The observed reduction of blood flow in the HM and tissue oxygenation after I/R results from the associated sinusoidal perfusion failure (Jaeschke et al. 1990, Menger et al. 1992, Koo et al. 1992, Vollmar et al. 1994).

Several mechanisms may contribute to this impairment of the sinusoidal perfusion including sinusoidal endothelial cell swelling with luminal narrowing (Vollmar et al. 1994) and sinusoidal vasoconstriction mediated by altered endothelin /nitric oxide balance (Nakamura et al. 1995, Pannen et al. 1998).

The increased expression of adhesion molecules with the subsequent leukocyte and endothelial cells interaction may also play a crucial role in this sinusoidal perfusion failure (Vollmar et al. 1995).

The biphasic change in the microcirculation and tissue oxygenation observed in this experiment reflects the time between the start of reperfusion and the occurrence of sinusoidal flow impairment with about 50% of the sinusoids showing cessation of blood flow at 18 minutes (Koo et al. 1992). This biphasic change after reperfusion has been shown in other studies with rat I/R (Goto et al. 1992, Chavez-Cartaya et al. 1994)

A significant correlation was found between the changes in tissue oxygenation (HbO₂ and Cyt Ox) and blood volume (HbT) parameters measured by NIRS and HM measured by LDF which indicate the value of NIRS in monitoring hepatic tissue perfusion with I/R.

Significant correlation was also found between the changes in tissue oxygenation and blood volume and indicators of the severity of I/R injury including serum hepatic enzymes and bile flow. This reinforces the central role of tissue oxygenation to I/R injury and the potential of tissue oxygenation monitoring for assessing the severity of I/R injury.

The role of Cyt Ox in monitoring I/R injury has not been studied before. In this experiment Cyt Ox measurement correlated with parameters of hepatocellular injury and bile flow to a higher degree than HbO₂. Cyt Ox reflects intracellular tissue oxygenation changes and correlates directly with ATP production which is even more fundamental to cell membrane integrity and bile production than the extracellular oxygenation.

The application of direct measurement of hepatic ICG using NIRS to assess the severity of I/R injury has not been investigated before. In this experiment the rate of ICG uptake decreased with I/R and the reduction paralleled the ischaemia time. A significant correlation was found between the changes in ICG uptake rate and the reduction in tissue microcirculation measured by LDF. The reduction of ICG uptake with I/R occurs secondary to impairment of sinusoidal perfusion and tissue blood volume as shown by LDF measurements.

The rate of ICG excretion decreased with I/R and the reduction again paralleled the ischaemia time. A highly significant correlation was observed between the rate of ICG excretion and the liver enzyme levels and bile flow which were used in this experiment as indicators of the severity of tissue injury. This shows the value of the application of a dynamic liver function test as ICG clearance which reflects the changes in the hepatocytes reserve and function. I/R injury can reduce ICG excretion rate via different mechanisms including cellular microtubules damage with the subsequent impairment of ICG removal from hepatocytes into bile canaliculi (Shinohara et al. 1996a) and hepatocyte necrosis (Gonzalez-Flecha et al. 1993) as ICG clearance is significantly correlated with the hepatocyte volume which indicate the hepatic functional reserve (Matsui et al. 1996). Also, it can be reduced secondary to reduction of cellular ATP level after reperfusion (Kamiike et al. 1982, Karwinski et al. 1989) resulting in impairment of bile excretion (Karwinski et al. 1989) and reduction of the bile flow. This study showed the potential of NIRS for evaluating the severity of hepatic I/R by measuring the changes in tissue oxygenation, blood volume and function.

Chapter 10

**The effect of graded steatosis on the hepatic handling of indocyanine
green measured directly by near infrared spectroscopy**

10.1 Introduction

The improved results of liver transplantation have resulted in expanding indications. Unfortunately the limited supply of donor livers has resulted in increased waiting time for liver transplantation. Transplantation therefore tends to occur at more advanced and less cost-effective stages of disease resulting in higher pre-transplantation mortality (Harper and Rosendale, 1996, Everhart et al. 1997). In search for a solution for the donor organ shortage many transplant centres started to use donor livers of "marginal" quality such as fatty livers (De et al. 1996, Fishbein et al. 1997).

Excessive fat accumulation in the liver (steatosis) is a common metabolic disorder seen in humans with an incidence ranging from 6% to 24% in autopsy series (Hilden et al. 1977, Underwood, 1984). High-grade steatosis (> 30% steatotic hepatocytes) was identified in about 50% of donor livers (Garcia et al. 1998). It caused by a wide variety of conditions and diseases such as alcohol, obesity, malnutrition, hyperalimentation, diabetes, pregnancy, and hepatitis but many are idiopathic (Alpers et al. 1993, Burt et al. 1998).

There is an increased risk of initial poor function and PNF of the graft after transplantation of a fatty liver and the risk increases with the severity of steatosis (Urena et al. 1998, Chui et al. 1998). However, some fatty livers can be successfully transplanted and may behave like normal donor livers. The survival of fatty livers after transplantation has been demonstrated both in an experimental animal model (Teramoto et al. 1998) and in the clinical situation

(D'Alessandro et al. 1991). Assessing the severity of steatosis in a donor organ is essential as it is the most important of factors influencing the subsequent function of the graft. Current methods of assessing the severity of steatosis have not proven to be reliable. The percentage of fatty livers diagnosed by gross examination is 17% for mild, 46% for moderate, and 71% for severe steatosis comparing with histological examination (Adam et al. 1991). The use of routine liver function tests such as bilirubin and serum enzymes are of limited diagnostic effectiveness when used alone (Winkel et al. 1975, Karayalcin et al. 1994). The use of dynamic liver tests such as the MEGX test showed no significant difference between normal and steatotic livers (Karayalcin et al. 1994). Frozen section histology has been used (D'Alessandro et al. 1991). This method is subject to sampling errors and it requires the availability of expert pathologists for preparation and examination of the biopsy specimens. The cost of such a service is also considerable and seldom available. NMR has been used to quantify fatty deposition in the liver (Levenson et al. 1991), but this method is not suitable for clinical application as it is cumbersome and requires a complicated process for data analysis. Ultrasound assessment has shown marked differences between different centres as well as between operators in a given centre (Lang et al. 1990, Adam et al. 1991). There is no satisfactory method of establishing the degree of steatosis in donors at the present time and the decision as to whether or not to use a liver is based on gross appearance evaluated by the retrieval surgeon (Trevisani et al. 1996).

10.2 Objectives

1. To study the hepatic handling of ICG with steatosis.
2. The correlation between hepatic blood flow and microcirculation and ICG uptake rate with steatosis.
3. The correlation between liver function tests and ICG excretion rate with steatosis.

10.3 Materials and methods

10.3.1 Induction of fatty liver and experimental groups

Male New Zealand white rabbits (3.1 ± 0.6 Kg) were divided into four groups of 6 each. Group A (controls), were fed on ordinary rabbit chow. Groups B, C, and D were fed with a commercial high cholesterol (2%) rabbit chow, ad libitum for 4, 8, and 12 weeks, respectively.

10.3.2 Animal preparation and surgical procedure

The details about the animals' anaesthesia, preparation and intraoperative monitoring are outlined in chapter 8.3.1. HM was measured by a surface LDF (Figure 10.1). HABF, PVBF and THBF were measured using ultrasonic transit time flowmeter with perivascular flow probes around HA and PV (1 and 4 mm

diameter, respectively). In this experiment, cannulation of the splenic vein allowed the portal venous pressure (PVP) to be measured using a pressure transducer connected with a blood pressure monitor (Instrumentarium Corp., Helsinki, Finland). The haemodynamic parameters (HM, PVPF, HABF, and PVP) were measured for 5 minutes at the beginning of the experiment. Hepatic ICG clearance was then measured using NIRS. At the end of the experiments the liver was removed and weighed and the animals were killed by exsanguination.

10.3.3 Measurement of blood cholesterol and liver function

Arterial blood samples (2 ml each) were used to measure blood cholesterol and liver function (bilirubin, ALT, AST, and albumin). The blood taken was replaced by equal volumes of normal saline. These measurements were done by a standard spectrophotometric method using an automated clinical chemistry analyser (Hitachi 747, Roche Diagnostics Ltd., Sussex, UK) after assessing the baseline haemodynamic parameters.

10.3.4 Measurement and analysis of hepatic ICG concentration curve

For measurement of hepatic ICG clearance in all the groups a bolus of ICG 0.5 mg/kg was given. ICG was dissolved in sterile water (1 mg/ml) and given via the femoral vein over 20 seconds after measuring the liver haemodynamics and blood sampling. Hepatic tissue ICG was measured by NIRS. NIRS probes were placed, with a 20 mm separation, on the surface of the left lobe of the liver for continuous measurement of hepatic tissue ICG for 30 minutes after ICG injection (Figure 10.1).

Continuous measurement of hepatic ICG by NIRS produces ICG concentration-time curve. This curve was analysed to produce two exponential rate constants, representing hepatic ICG uptake from the plasma to the hepatocytes (α) and hepatic ICG excretion from the liver by cytoplasmic transport and biliary excretion (β). The details of ICG curve analysis were outlined in Chapter 8.3.2.

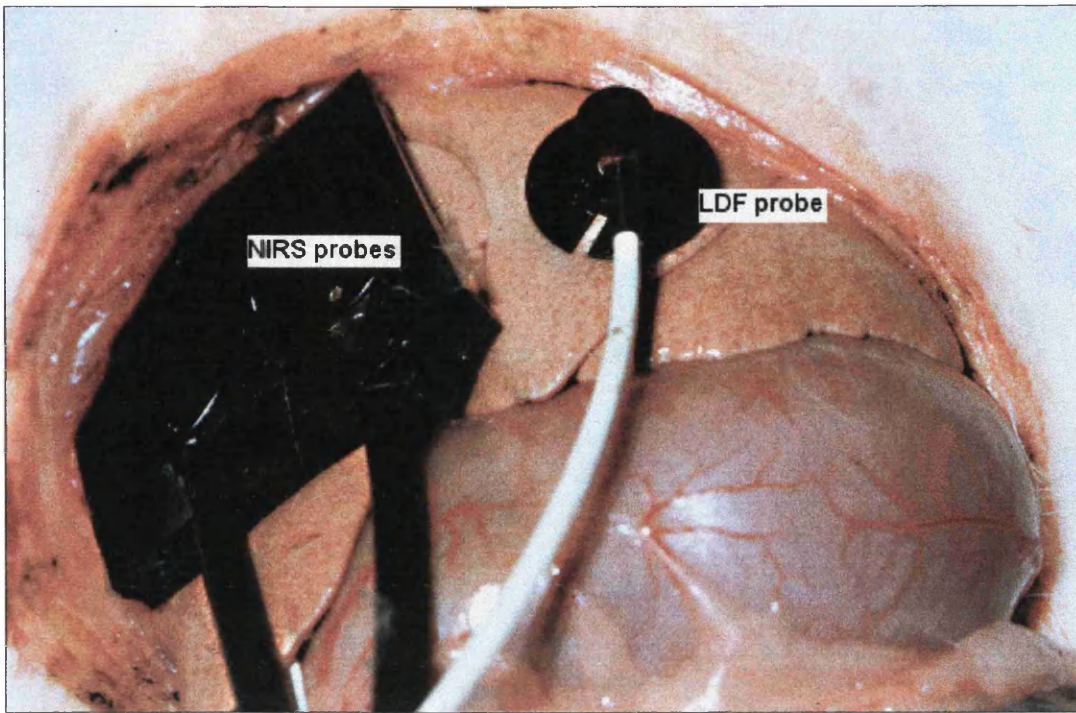


Figure 10.1 NIRS and LDF probes over the liver surface. With steatosis the liver was enlarged, yellowish in colour with a rounded edge.

10.3.5 Liver histological examination

Liver biopsies were taken from the liver at the end of the experiment. The biopsies were fixed in neutral buffered formalin (10%) and embedded in paraffin. Sections were stained with haematoxylin and eosin (H and E) and Chromotrope aniline blue (CAB) for collagen. Sections were reviewed independently to determine the presence and extent of steatosis, inflammation, necrosis and fibrosis. The grade of steatosis was analysed in a semi-quantitative manner: mild

(< 30%), moderate (30-60%), and severe (> 60%) using a clinically applied grading system (Adam et al. 1991).

10.3.6 Data collection and statistical analysis

The values are expressed as mean $\pm$ SD. The haemodynamic parameters were calculated as the mean of 1-minute values. For statistical analysis one way analysis of the variance (ANOVA) and Student's t-test were used with Bonferroni correction for multiple tests. The relationships between hepatic ICG uptake rate (α) and hepatic blood flow, microcirculation, and serum albumin were tested using Pearson correlation coefficient. The relationships between hepatic ICG excretion rate (β) and liver function tests were tested using Pearson correlation coefficient.

10.4 Results

10.4.1 Induction of fatty liver

All the cholesterol-fed animals tolerated the high cholesterol diet with no mortality. The animals maintained a normal body weight (BW) during the cholesterol feeding periods, with no significant difference between the cholesterol-fed groups and controls (Table 10.1). In group B the liver weight (LW) and the LW/BW ratio were similar to controls (Table 10.1). In groups C and D (8 and 12 weeks) the LW and LW/BW ratio were increased significantly in comparison to controls, but with no significant difference between groups C and D (Table 10.1).

At laparotomy all the cholesterol-fed animals showed fatty deposition in the skin, liver and spleen. The liver was enlarged, yellowish in colour, with rounded edge, and firm consistency (Figure 10.1). Some animals in group C and D had ascites and abdominal varices but non in group B.

Table 10.1 BW, LW, and LW/BW ratio in control and fatty animals.

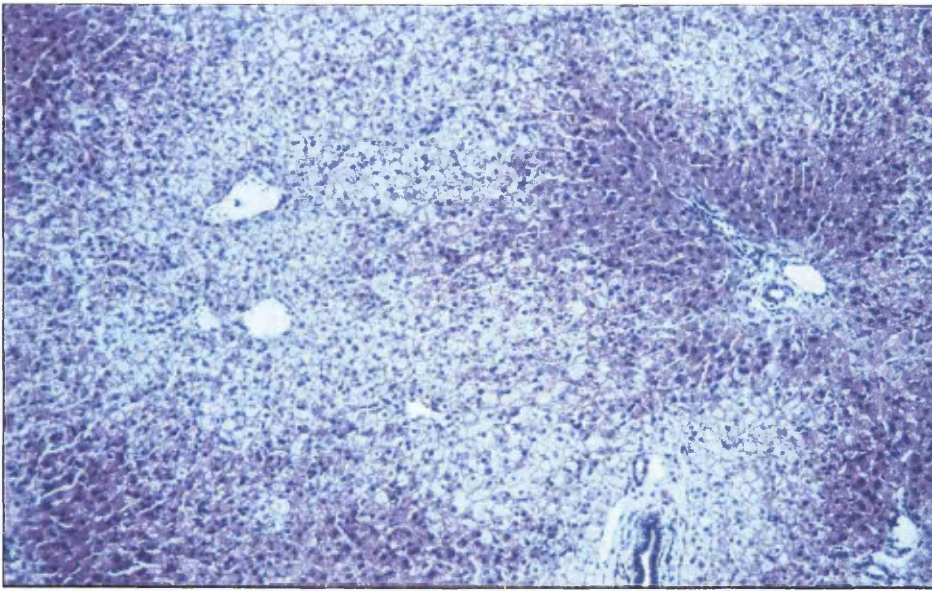
	Group A	Group B	Group C	Group D
BW (Kg)	3 ± 0.7*	3.1 ± 0.5*	3.2 ± 0.8*	3.3 ± 0.5*
LW (g)	97.5 ± 19.6 [†]	99.3 ± 20.5 ^{†*}	140.8 ± 26.4*	152.7 ± 23.8 ^{†*}
LW/BW ratio (%)	3.2 ± 0.3 [†]	3.2 ± 0.2 ^{†*}	4.5 ± 0.3 ^{†*}	4.8 ± 0.3 ^{†*}

Values are mean ± SD of 6 animals in each group. [†]p < 0.05 and *p = not significant (ANOVA). *p < 0.05 vs. group A (Student's t test).

10.4.2 Histological examination

Animals in the control group had normal liver. Animals in group B showed a mild steatosis (<30%). Moderate steatosis was found in both groups C and D. Liver biopsies of fatty animals revealed macroviscous fat accumulation, inflammatory cell infiltrate and necrosis mainly in the periportal areas (Figure 10.2A). The lipid overloading was associated with an increase in hepatic sinusoidal fibrosis demonstrated by the CAB staining only in groups C and D (Figure 10.2B).

(A)



(B)

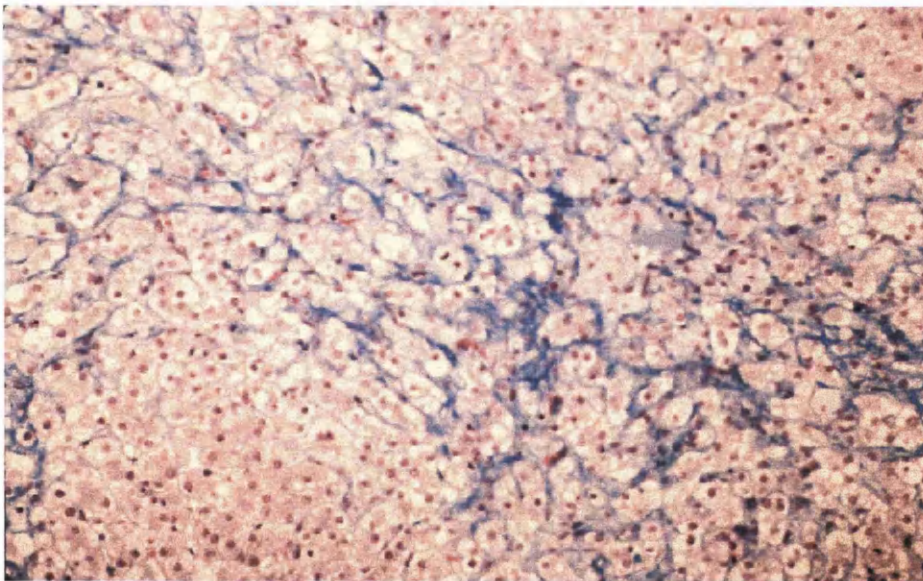


Figure 10.2 Histological features of steatosis with cholesterol feeding for 12 weeks. (A) Macrovesicular fat accumulation in hepatocytes mainly in the pericentral area (H and E x 80). (B) Sinusoidal fibrosis (CAB x 100).

10.4.3 Systemic and hepatic haemodynamics

In the cholesterol-fed animals, there was no significant change in MABP and HR in comparison to controls (Table 10.2). As there was increase in the liver weight in the steatotic groups, the change in blood flow per gram of LW (ml/min/g LW) was examined. With mild steatosis (group B) PVBF, HABF, and THBF did not change significantly in comparison to controls (Table 10.2). With moderate steatosis (groups C and D) there was a significant decrease in PVBF and THBF with significant increase in HABF in comparison to controls (Table 10.2). There was no significant difference in these haemodynamic parameters between 8 and 12 weeks.

HM did not change significantly with mild steatosis (group B) in comparison to controls (Table 10.2). With moderate steatosis (groups C and D) there was a significant decrease in HM in comparison to controls with no significant difference between the two groups (Table 10.2).

PVP did not change significantly with mild steatosis (group B) in comparison to controls (Table 10.2). With moderate steatosis (groups C and D) there was a significant increase in PVP in comparison to controls with no significant difference between the two groups (Table 10.2).

Table 10.2 Systemic and hepatic haemodynamics in controls and fatty animals.

	Group A	Group B	Group C	Group D
MABP (mmHg)	79.3 ± 4.6°	77.5 ± 4.8°	71.8 ± 6.2°	72.8 ± 5.5°
HR (beats/min)	233 ± 11.3°	234 ± 8.8°	243 ± 9.1°	247 ± 10.6°
PVBF (ml/min/g LW)	1.12 ± 0.14 [†]	1.05 ± 0.18 ^{†*}	0.69 ± 0.09 ^{†*}	0.61 ± 0.06 ^{†*}
HABF (ml/min/g LW)	0.12 ± 0.02 [†]	0.11 ± 0.02 ^{†*}	0.16 ± 0.03 ^{†*}	0.17 ± 0.04 ^{†*}
THBF (ml/min/g LW)	1.24 ± 0.12 [†]	1.16 ± 0.17 ^{†*}	0.85 ± 0.09 ^{†*}	0.78 ± 0.07 ^{†*}
HM (flux)	220.3 ± 26.9 [†]	193.3 ± 21.8 ^{†*}	81.8 ± 18.8 ^{†**}	68.5 ± 19.2 ^{†**}
PVP (mmHg)	5.5 ± 1.4 [†]	5.8 ± 2.1 ^{†*}	15 ± 2.2 ^{†**}	16.3 ± 3.5 ^{†**}

Values are mean ± SD of 6 animals in each group. [†]p < 0.05 and °p = not significant (ANOVA). *p < 0.05 and **p < 0.01 vs. group A (Student's t test).

10.4.4 Blood cholesterol changes and liver function tests

In all the cholesterol-fed animals there was a significant increase in serum cholesterol in comparison to controls (Table 10.3). With mild steatosis (group B) there was no significant difference in bilirubin, ALT, AST or albumin in comparison to controls (Table 3). With moderate steatosis (groups C and D) there was a significant increase in bilirubin, ALT, AST with significant decrease in serum albumin. There was no significant difference between groups C and D (Table 3).

Table 10.3 Blood cholesterol and liver function tests in controls and fatty animals.

	Group A	Group B	Group C	Group D
Cholesterol ($\mu\text{mole/L}$)	$2.8 \pm 1.2^{\dagger}$	$31.7 \pm 5.6^{\dagger**}$	$50.8 \pm 7.3^{\dagger**}$	$55 \pm 7.8^{\dagger**}$
Albumin (g/L)	$42.5 \pm 5.8^{\dagger}$	$40.5 \pm 5.2^{\dagger*}$	$24.3 \pm 4.1^{\dagger**}$	$23.7 \pm 4.4^{\dagger**}$
Bilirubin ($\mu\text{mole/L}$)	$8.2 \pm 4.3^{\dagger}$	$8 \pm 4.7^{\dagger*}$	$23.8 \pm 5.4^{\dagger**}$	$26.8 \pm 6.8^{\dagger**}$
ALT (U/L)	$13.7 \pm 5.5^{\dagger}$	$14.2 \pm 6.6^{\dagger*}$	$54.3 \pm 9.8^{\dagger**}$	$57.2 \pm 10.4^{\dagger**}$
AST (U/L)	$17.5 \pm 12.6^{\dagger}$	$18 \pm 12.4^{\dagger*}$	$63.2 \pm 15.8^{\dagger**}$	$75.8 \pm 13.6^{\dagger**}$

Values are mean $\pm$ SD of 6 animals in each group. $^{\dagger}p < 0.01$ (ANOVA). $^*p =$ not significant and $^{**}p < 0.01$ vs. group A (Student's t test).

10.4.5 Hepatic ICG Uptake and excretion measured directly by NIRS

Figure 10.3 shows typical examples of hepatic ICG concentration-time data and their fitting curves in controls and with steatosis. With mild steatosis (group B), ICG uptake (α) and excretion (β) rates did not change significantly (2.42 ± 0.72 , $0.99 \pm 0.21 \text{ min}^{-1}$, respectively) in comparison to controls (Table 10.4).

With moderate steatosis (groups C and D) there was a significant decrease in ICG uptake rate (α) from the control value of $2.59 \pm 0.69 \text{ min}^{-1}$ to 0.78 ± 0.48 , $0.69 \pm 0.38 \text{ min}^{-1}$, respectively ($p < 0.05$ vs. group A) with no significant difference between the two groups (Table 10.4). Also, there was a significant reduction in hepatic ICG excretion rate (β) from the control value of $1.095 \pm 0.23 \text{ min}^{-1}$ to 0.065 ± 0.048 , $0.059 \pm 0.038 \text{ min}^{-1}$, respectively ($p < 0.05$ vs. group A) with no significant difference between the two groups (Table 10.4).

Table 10.4 Hepatic ICG uptake rate (α) min^{-1} and ICG excretion rate (β) min^{-1} calculated from ICG-time concentration curves.

	Group A	Group B	Group C	Group D
α	$2.59 \pm 0.69^{\dagger}$	$2.42 \pm 0.72^{\dagger*}$	$0.78 \pm 0.48^{\dagger*}$	$0.69 \pm 0.38^{\dagger*}$
β	$1.095 \pm 0.23^{\dagger}$	$0.99 \pm 0.21^{\dagger*}$	$0.065 \pm 0.048^{\dagger*}$	$0.059 \pm 0.038^{\dagger*}$

Values are mean $\pm$ SD of 6 animals in each group. $^{\dagger}p < 0.05$ (ANOVA). $^*p =$ not significant and $*p < 0.05$ vs. group A (Student's t test).

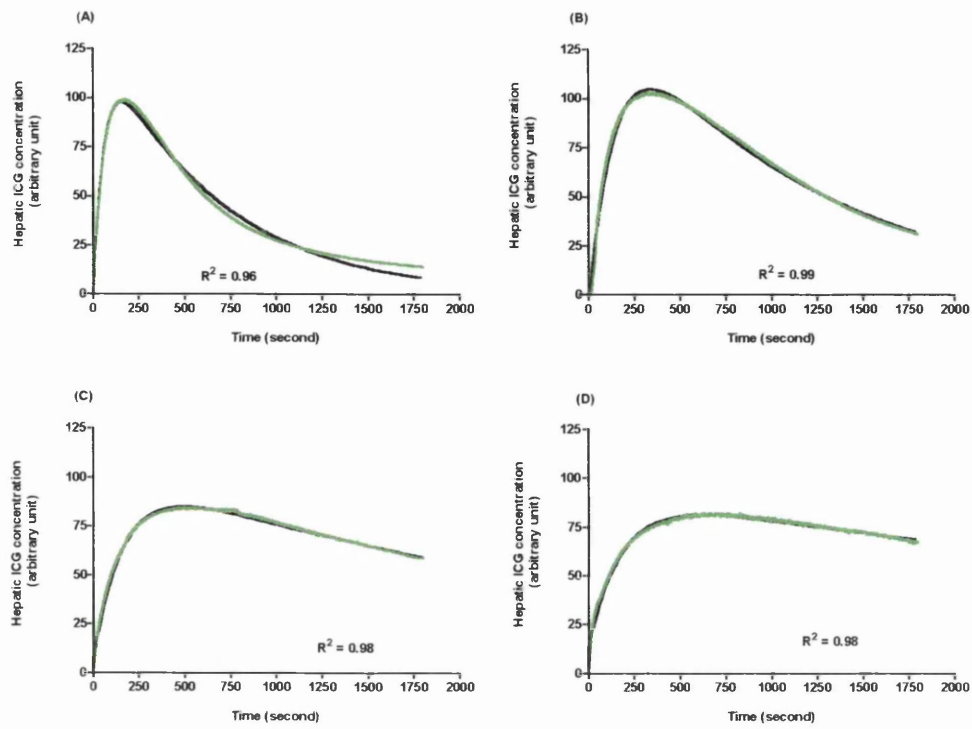


Figure 10.3 Typical examples of ICG concentration-time curves and their fitted curves. (A) controls, cholesterol-fed groups: (B) 4, (C) 8, and (D) 12 weeks.

10.4.6 Correlation between the Hepatic ICG rate of uptake (α) and hepatic haemodynamics

Hepatic ICG rate of uptake (α) significantly correlated with THBF ($r = 0.94$, $p < 0.001$) (Figure 10.4A). The correlation of ICG uptake rate and THBF in each group (A, B, C, and D) were ($r = 0.92, 0.83, 0.79$, and 0.75 , respectively).

Also, a significant correlation was found between ICG uptake rate and HM ($r = 0.96$, $p < 0.001$) (Figure 10.4B). The correlation of ICG uptake rate and HM in each group (A, B, C, and D) were ($r = 0.91, 0.94, 0.93$, and 0.96 , respectively).

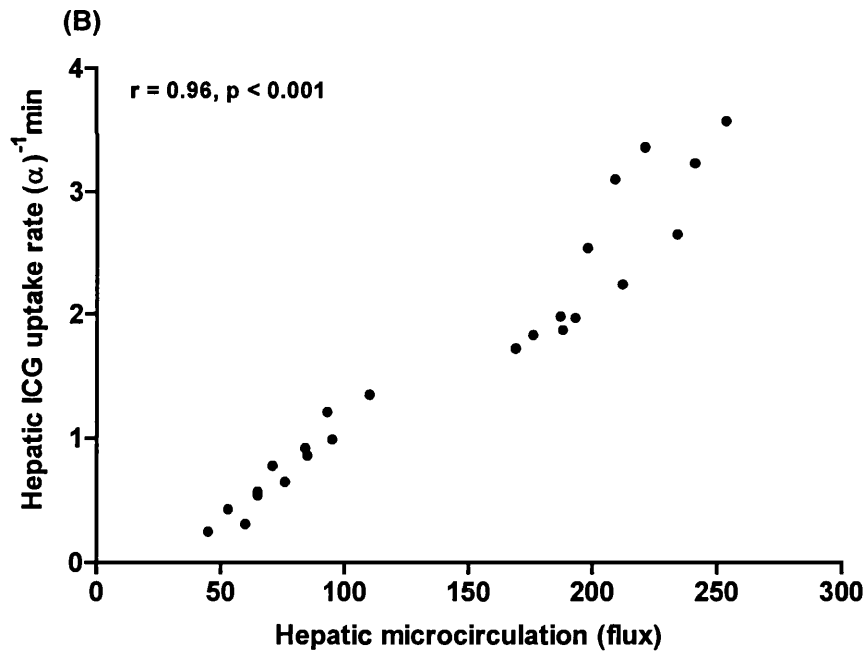
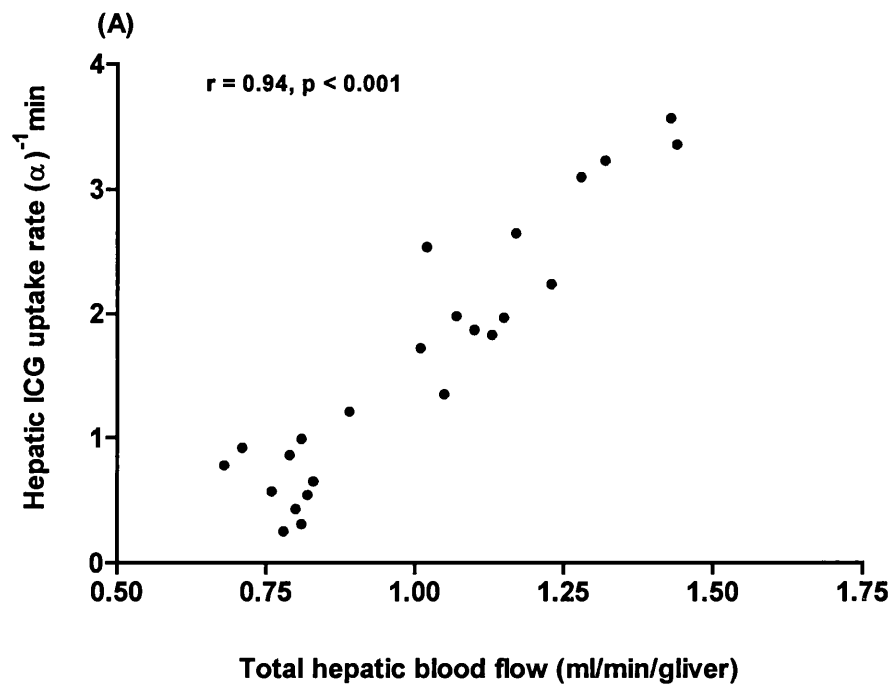


Figure 10.4 Correlation between hepatic ICG uptake rate and THBF (A) and HM (B). Each point represents the change observed in one animal.

10.4.7 Correlation between hepatic ICG excretion rate (β) and liver function tests

Hepatic ICG excretion rate (β) significantly correlated with serum bilirubin ($r = -0.89$), ALT ($r = -0.87$, $p < 0.001$), AST ($r = -0.90$, $p < 0.001$), and albumin ($r = 0.95$, $p < 0.001$) (Figure 10.5). The correlation of ICG excretion rate and bilirubin in each group (A, B, C, and D) were ($r = -0.94$, -0.96 , -0.97 , and -0.95 , respectively). The correlation of ICG excretion rate and ALT in each group (A, B, C, and D) were ($r = -0.93$, -0.94 , -0.90 , and -0.95 , respectively). The correlation of ICG excretion rate and AST in each group (A, B, C, and D) were ($r = -0.90$, -0.87 , -0.93 , and -0.95 , respectively). The correlation of ICG excretion rate and albumin in each group (A, B, C, and D) were ($r = 0.95$, 0.98 , -0.98 , and 0.99 , respectively).

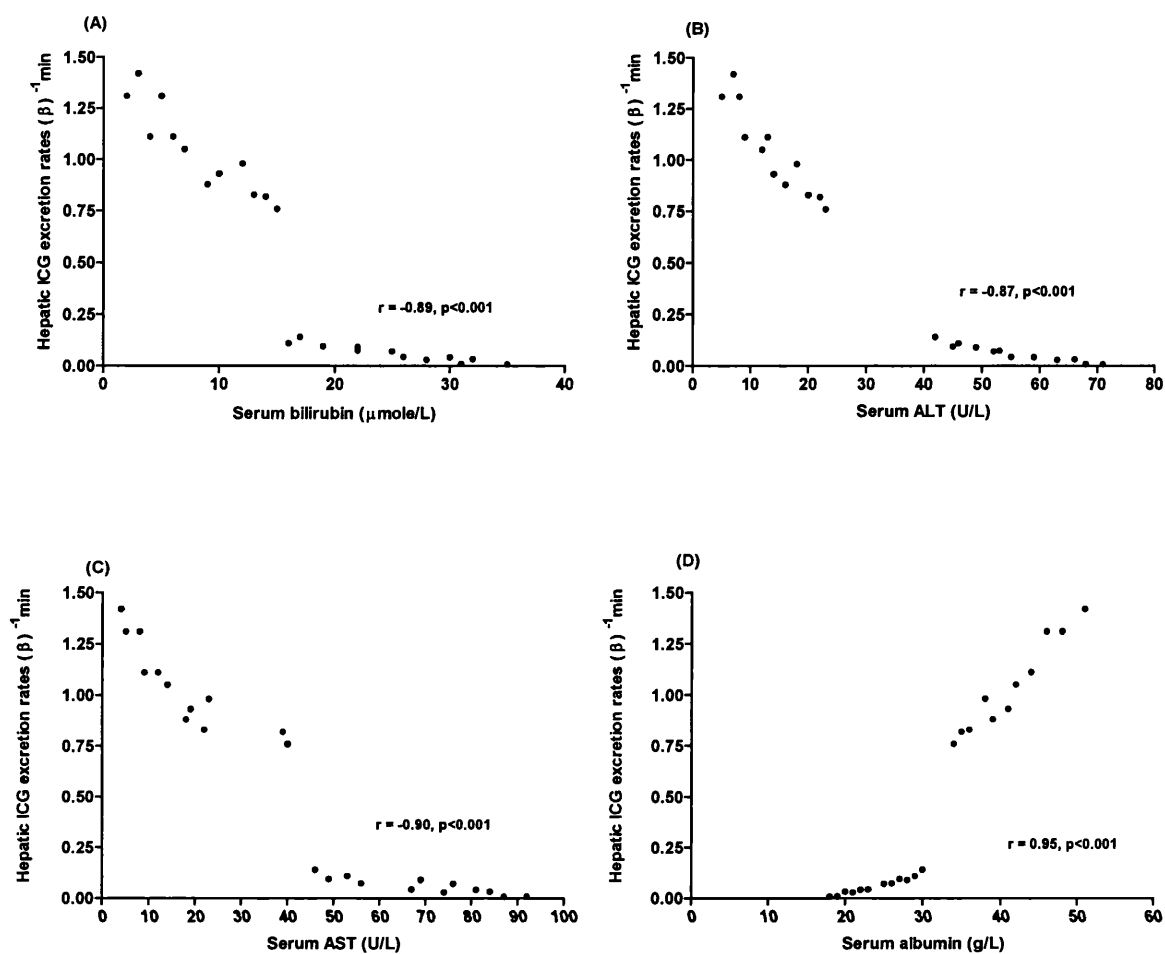


Figure 10.5 Correlation between hepatic ICG excretion rate and serum bilirubin (A), ALT (B), AST (C), and albumin (D). Each point represents the change observed in one animal.

10.5 Discussion

It is generally accepted that transplantation of fatty grafts is associated with a high risk of PNF (Urena et al. 1998, Chui et al. 1998) which underlines the importance of having a readily available method of assessing the degree of liver steatosis in potential donor organs. The current study was designed to investigate the hepatic handling of ICG with steatosis. Also, it correlated the changes in hepatic blood flow, microcirculation and function in fatty liver with the hepatic ICG uptake and excretion rates measured by NIRS.

All the animals tolerated the high cholesterol feeding with no mortality. In this study high cholesterol feeding induced the formation of what is called "cholesterol fatty liver" in which there is accumulation of triglyceride and cholesterol in the liver (Lee and Ho, 1975, Mastai et al. 1996, Wanless et al. 1996). This model different from other studies in which neutral fat and deficient in lipotropic factors results in triglyceride accumulation in the hepatic tissue (Lee and Ho, 1975). Histological examination of the liver showed mild steatosis after 4 weeks cholesterol feeding with moderate steatosis after 8 and 12 weeks of cholesterol feeding. Hepatic fibrosis was observed only with 8 and 12 weeks animals. Rabbits given a high cholesterol (1%) diet together with diethylstilboestrol have been shown to develop more rapid and extensive hepatic fibrosis with fatty infiltration, but associated with 27% mortality after 8 weeks treatment (Mastai et al. 1996, Wanless et al. 1996).

At laparotomy in all the cholesterol-fed groups, the liver was enlarged, yellowish in colour, with rounded edge and firm consistency. In some animals with moderate fatty change there was ascites and abdominal varices suggesting portal hypertension.

Mild steatosis (4 weeks) did not change liver blood flow significantly. With moderate steatosis (8 and 12 weeks) there was a significant decrease in PVBF and THBF with a significant increase in HABF. This was associated with a significant reduction in HM and a significant increase in PVP. Reduction in PVBF and THBF may be due to the development of extrahepatic portosystemic shunts secondary to an increased portal pressure (Kawasaki et al. 1990). The significant increase in HABF could be explained by the ability of the hepatic flow to compensate for the reduced PVBF, a phenomenon labelled as "the hepatic arterial buffer response" (Lautt, 1985, Ayuse et al. 1994).

In this model with moderate steatosis there was a reduction in flow in the HM which previously has been reported in other studies with fatty liver (Sato et al. 1986, Seifalian et al. 1998). The reduction in HM occurs due to sinusoidal compression and decrease in sinusoidal diameter by the enlarged fat-laden hepatocytes (Sato et al. 1986). This change in HM by the enlarged hepatocytes is augmented by an increase in fibroblast numbers with the formation of collagen bundles in the perisinusoidal space which causes further narrowing of the sinusoids (Lee and Ho, 1975, Wanless et al. 1996). The significant increase in PVP with severe fatty changes occurs secondarily to the increased sinusoidal vascular resistance and narrowing or obliteration of the terminal portal venules

due to compression by the enlarged fat-laden hepatocytes augmented by the presence of perisinusoidal fibrosis (Lee and Ho, 1975, Wanless et al. 1996). ICG is highly bound to albumin and α -lipoproteins (Cherrick et al. 1960, Baker and Bradley, 1966). Its elimination is solely by the liver with no extrahepatic metabolism or excretion (Cherrick et al. 1960, Baker and Bradley, 1966). It is excreted in the bile without metabolic alteration or conjugation via transport by glutathione S-transferase and it does not undergo enterohepatic circulation (Cherrick et al. 1960, Baker and Bradley, 1966). The hepatobiliary transport of ICG is affected by various factors such as hepatic blood flow, binding to plasma proteins, influx across the sinusoidal plasma membrane, intracellular transport, ATP-dependent transport across the biliary canalicular membrane and bile flow (Berk and Stremmel, 1986, Zimniak and Awasthi, 1993).

Mild steatosis (4 weeks) did not cause any significant change in ICG uptake and excretion rates. Moderate steatosis (8 and 12 weeks) was associated with a significant reduction in ICG uptake. This may be related to the reduction in THBF and HM with steatosis or the reduced albumin as ICG is transported to the liver albumin bound (Baker and Bradley, 1966, Ott et al. 1992).

A significant positive correlation was found between hepatic ICG uptake rate and both THBF and HM which indicates that direct measurement of ICG uptake by NIRS could be used as an index of hepatic blood flow and microcirculation with hepatic steatosis.

ICG excretion rate decreased significantly with moderate steatosis. Thickening of the sinusoidal wall with membranous formation and proliferation of collagen

fibres with "intrasinusoidal block" decreases the diffusion of protein-bound substances such as ICG into the extravascular space (Inaba et al. 1984, Lee, 1981). Impairment of ICG excretion could also be explained by impairment of its transport across the biliary canalicular membrane due to reduction of hepatic ATP content (Juggi and Prathap, 1979) secondary to the reduction of regional blood volume and decrease regional oxygen (Sato et al. 1986).

The significant negative correlation between ICG excretion rate and serum bilirubin and hepatic enzymes reflects the hepatocellular impairment with steatosis. Also, a significant positive correlation was found with the serum albumin which indicates impairment of the hepatic synthetic function. These significant correlations reflect potential of ICG excretion rate measurement with NIRS as an indicator for the hepatocellular impairment with steatosis.

In conclusion a model of steatosis was established with high cholesterol diet. Hepatic ICG uptake rate correlated with the reduced hepatic blood flow and microcirculation changes in steatosis. Hepatic ICG excretion rate reflects the hepatocellular damage with steatosis.

Chapter 11

Overall thesis discussion and future plans

Liver transplantation is the treatment of choice for patients with end-stage liver disease. The high rate of survival after liver transplantation has resulted in significant expansion of the indications. However, this expansion has not been matched by an increase in the number of potential donors which has created a worldwide problem of organ shortage.

In an effort to improve the use of available donor organs an accurate method is required for identifying donor liver function and viability of the transplanted organ. The aim is to identify grafts that would have the potential to tolerate the inevitable degree of I/R injury which occurs during organ procurement. To date there is no clinically reliable method for monitoring liver graft viability in the peritransplant period.

This study evaluated the application of NIRS as a novel non-invasive technique to assess the changes in hepatic tissue oxygenation, blood volume, and function. A commercially available near infrared spectrometer that has developed for measurement of brain tissue oxygenation was used (Cope, 1991, Wyatt et al. 1986). In collaboration with the Department of Medical Physics at UCL, a modified algorithm was developed for its application to measure changes in hepatic tissue oxygenation. The near infrared light absorption coefficient and DPF of freshly dissected and viable pig's liver was measured for the first time and these data were incorporated in the algorithm of the NIRO 500 for its hepatic application.

Validation of the NIRS measurement of the changes in hepatic tissue oxygenation was initially performed by comparing its measurement with hepatic

vein oxygen pressure with graded hypoxia. The hepatic tissue oxygenation parameters measured by spectroscopy and the hepatic vein oxygen partial pressure represent the balance between hepatic oxygen delivery and oxygen consumption in the tissue (Kitai et al. 1993b, Kainuma et al. 1991, Shimizu et al. 1996). This experiment demonstrated that the extra and intracellular hepatic tissue oxygenation changes with graded hypoxia can be monitored by NIRS and that the spectroscopy data correlate well with hepatic vein blood oxygenation. Mild grades of hypoxia resulted in a reduction in oxygen delivery with a significant decrease in HVPO₂ which was associated with a reduction of tissue HbO₂ but with no significant change in Cyt Ox. Hypoxia which was sufficiently severe to reduce hepatic Cyt Ox was associated with a 50% reduction of HVPO₂ from the baseline. This demonstrates that HVPO₂ does not adequately reflect changes in intracellular oxygenation as monitored in this experiment by the changes in Cyt Ox oxidation. In this study the use of Cyt Ox redox changes as an index of intracellular hypoxia showed that cellular hypoxia occurs with arterial oxygen partial pressure of ≤ 53 mmHg (FiO₂ of $\leq 10\%$). This is in agreement with other studies measuring the critical arterial oxygen partial pressure level at which tissue damage from hypoxia occurs (Huckabee, 1958, Loegering and Critz, 1971, Hobler and Carey, 1973, Ukikusa et al. 1979). These data suggested that NIRS measurement of Cyt Ox could be used as an accurate indicator of cellular hypoxia. The relationship between the level of reduced Cyt Ox and other independent indicators of altered energy metabolism was then investigated. NIRS measurements were correlated with cellular ATP levels with hypoxia. The

changes in hepatic haemoglobin oxygenation did not reflect the temporal changes in cellular ATP. Cyt Ox oxidation was highly correlated and temporally associated with reductions in hepatic ATP. This study confirmed the value of Cyt Ox oxidation changes measured by NIRS as an indicator of intracellular oxygenation and ATP production.

The significance of these data is that a simple non-invasive clinical technique could be used for monitoring cellular ATP. Cellular ATP has a crucial role in cellular homeostasis (Winkler et al. 1986) and correlates with hypoxic liver damage (Higashi et al. 1989, Sumimoto et al. 1988). The currently available methods for ATP measurement included enzymatic assay (Lamprechts and Trautschold, 1963), chromatography (Kamiike et al. 1982) and ^{31}P NMR (McLaughlin et al. 1979). These methods are either invasive requiring tissue biopsy or require a complicated procedure for data collection and analysis. NIRS was applied to measure the changes in hepatic tissue oxygenation in relation to hepatic blood flow and the interrelationship between the HA and PV blood flow in regard to hepatic tissue oxygenation. This was the first study to correlate liver blood flow with the hepatic tissue oxygenation and blood volume parameters, measured by NIRS, utilising an animal model whose liver anatomy and physiology is similar to man (Copper et al. 1991). NIRS measurements with vascular occlusion were consistent and reproducible with a specific pattern of tissue oxygenation and hepatic blood volume changes associated with HA, PV, and both vessel occlusion. There was a significant correlation between HbT and the HM changes measured by the LDF with the vascular occlusion which

supports the accuracy of the hepatic tissue volume measurement by NIRS.

These data would suggest the potential role of NIRS for detection of post-transplantation vascular occlusion as the changes in tissue oxygenation will precede hepatocellular damage and allow early interference and salvage of these donor livers.

The application of NIRS to monitor hepatic ICG handling and tissue concentration was studied. ICG is a synthetic dye that has been used for measuring liver function (Kawasaki et al. 1988, Zoedler et al. 1995) and blood flow (Burggraaf et al. 1996, Perlik et al. 1992) by measuring its blood clearance. Measurement of ICG blood clearance requires repeated blood sampling and a complicated technique to quantify ICG concentration in the samples using a spectrophotometer (Paumgartner et al. 1970, Nielsen, 1963). The close interrelationship between sinusoidal flow and hepatocyte function also represents a great difficulty in the interpretation of blood clearance tests. Ott and colleagues (1994) have suggested that the ICG plasma clearance curve contains no information about the liver-bile interaction (Ott et al. 1994). NIRS allows direct and continuous monitoring of hepatic ICG concentration and calculation of ICG uptake and excretion rates. This allows discrete evaluation of the hepatic microcirculation and function. This technique was applied in an animal model of acute parenchymal impairment. The calculated hepatic ICG uptake rate (α) was found to significantly correlate with the hepatic blood flow and microcirculation measured by the ultrasonic flowmeter and laser Doppler flowmeter, respectively. The measurement of tissue perfusion using ICG uptake rate reflects the effective

liver blood flow which is defined as the flow that perfuses functioning sinusoids and is available for metabolic exchange (Henderson et al. 1981) which is the main pathological change with hepatic I/R injury (Vollmar et al. 1994, Clemens et al. 1985). The hepatic ICG excretion rate (β) reflected the changes in hepatic function and biliary excretion.

The main potential application of NIRS is assessment of the severity of the I/R injury in liver surgery and transplantation. So it was essential to apply this technique to a hepatic I/R model. NIRS has previously been demonstrated to measure the changes in tissue oxygenation with I/R (Kiuchi et al. 1996, Kiuchi et al. 1997). The role of monitoring intracellular oxygenation by measuring the Cyt Ox oxidation has not been investigated. In a rabbit model of lobar total I/R, NIRS was used to assess the tissue oxygenation, blood volume and function changes with different grades of I/R. The tissue oxygenation (HbO₂ and Cyt Ox) and tissue blood volume (HbT) parameters measured by NIRS correlated significantly with the tissue microcirculation changes measured by laser Doppler flowmeter which supports the value of NIRS in monitoring hepatic tissue perfusion. Also, these tissue oxygenation parameters correlated significantly with the hepatocellular damage and bile volume changes after I/R. The measured changes in tissue oxygenation parameters paralleled the ischaemia time.

This experiment showed that measuring intracellular tissue oxygenation changes reflected by Cyt Ox changes was a better indicator of I/R injury than extracellular tissue oxygenation (HbO₂) changes. Cyt Ox changes correlate directly with ATP production which is fundamental to cell integrity and function. This confirms the

relationship between Cyt Ox and ATP production and supports the use of Cyt Ox measurement by NIRS for determining critical cellular hypoxia.

The potential role of NIRS for monitoring I/R injury by direct measurement of hepatic ICG has been shown in other studies (Shinohara et al. 1996b, Shinohara et al. 1996a). In this lobar I/R model, direct measurement of hepatic ICG uptake and excretion rates were used to assess the severity of I/R injury and correlated with the liver microcirculation and function, respectively. The ICG uptake rate reduced in relation with the ischaemia time and correlated significantly with the decrease in the microcirculation blood flow measured by laser Doppler flowmeter. Also, the ICG excretion rate correlated significantly with hepatocellular damage and bile flow changes with I/R. The data of this I/R experiment confirms the crucial role of measuring tissue oxygenation with I/R and supports the potential role of NIRS for monitoring I/R injury.

As a solution of liver organ shortage, fatty livers are being used. There is no clinically reliable method to assess the severity of fatty change. The role of NIRS in assessment of fatty liver has not yet been investigated. A model of hepatic fatty changes with fibrosis was established using a high cholesterol diet.

ICG measurement by NIRS was applied to measure the effect of graded steatosis on hepatic parenchymal perfusion and function. Hepatic ICG uptake rate correlated with the reduction in hepatic blood flow and microcirculation measured by the ultrasonic flowmeter and laser Doppler flowmeter, respectively. Hepatic ICG excretion rate correlated with the liver function tests which reflected the hepatocellular damage with fatty change. By measuring the hepatic ICG

uptake and excretion rates using NIRS in this fatty liver model, it was possible to differentiate between mild and severe fatty changes. NIRS could therefore be useful in selection of fatty donor livers by choosing these with mild impairment of the microcirculation and function.

NIRS has the potential for clinical application in liver transplantation where it could be used:

1. To grade microcirculation and functional disturbances in steatotic donor organs using ICG clearance.
2. Intraoperatively in the recipient operation to assess reperfusion and oxygenation of the graft as an index of the severity of I/R injury.
3. To monitor parenchymal oxygenation as an indicator of acute organ dysfunction and to monitor the response to treatment with vasodilators, N acetylcystine, and steroid etc.

During the hepatic application of the NIRS few problems were encountered. The interference by the effect of operating lights on the measurement requires dimming of the operating light and covering the probes with lightproof black cloth to minimise the light effect. This could represent a minor difficulty in its clinical application. Variation in measurements was found with the re-application of the probes even on the same site of measurement which may be caused by variation of the optical properties of the area under investigation which are tested and accounted for by the spectroscopy with the initial setting. This problem restricts the NIRS application as the probes must be applied and maintained in the same site without movement during the whole procedure which may not be possible in

clinical situation. Absolute quantitation of the measurement will solve such problem.

The close contact between the probes and the liver tissue is required, as the noise/signal ratio would increase with improper contact. This could represent a problem in the postoperative application of the NIRS.

The following aspects in hepatic tissue spectroscopy require further study before its clinical application. The hepatic spectrometer used in these experiments measures absolute concentration changes rather than absolute chromophore concentration. The absolute quantitation of the NIRS measurements is not possible due to the inability to measure the light scattering in the tissue (for details see Chapter 4.1.2). Solving the problem of measuring light scattering in the tissue would allow absolute quantitation and make it possible to choose cut off levels for the measured parameters and assessment of the technique sensitivity and specificity.

The present technology of NIRS requires direct contact between the optical fibres and the liver surface which limits the application of such technique to intraoperative use. NIRS can be used through a small window in the operative wound (Kitai et al. 1996). Such technique could be associated with wound infection which is undesirable in the immunocompromised transplant patient. Improvement of the technique with reduction of the probe size could allow its application during the operation and postoperatively with the passage of probes through the drain site. Also, it could be applied through a laparoscopic port.

NIRS application through the abdominal wall requires improvement of the depth of penetration of the available NIRS technique to avoid monitoring of the anterior abdominal wall oxygenation. This could be achieved by the use of a more powerful light source and sensitive photodetectors.

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Published papers and abstracts

Original Articles

1. Abd El-Hamid El-Desoky, Alexander M. Seifalian, Mark Cope, David T. Delpy, Brian R. Davidson. Changes in tissue oxygenation of the porcine liver measured by near infrared spectroscopy. *Liver Transplantation & Surgery*. 1999; 5: 219-26.
2. A. El-Desoky, A. M. Seifalian, and B.R. Davidson. Effect of graded hypoxia on hepatic tissue oxygenation measured by near infrared spectroscopy. *J.Hepatol*. 1999; 31: 71-76.
3. Abd El-Hamid El-Desoky, Alexander M. Seifalian, Mark Cope, David T. Delpy, Brian R. Davidson. An experimental study of liver dysfunction evaluated by direct indocyanine green clearance using near infrared spectroscopy. *Br. J. Surg*. 1999; 86: 1005-1011.
4. Abd El-Hamid El-Desoky, Long R. Jiao, Roman Havlik, Nagy Habib, Brian R. Davidson, and Alexander M. Seifalian. Measurement of hepatic tissue hypoxia using near infrared spectroscopy: comparison with hepatic vein oxygen partial pressure. *Eur. Surg. Res.* (In press).

Selected presentations and Abstracts

1. A non-invasive technique to assess hepatic tissue oxygenation using near-infrared spectroscopy. A. El-Desoky, A. M. Seifalian, and B.R. Davidson.

Presentation to the 87th Meeting of the Surgical Research Society, London, 8-9 January 1998. Abstract in Br. J. Surg. 1998; 85, 5: 701

2. Quantitative assessment of hepatic Indocyanine Green clearance in acute hepatic dysfunction: An animal model. Abd El-Hamid El-Desoky, Alexander M. Seifalian, Mark Cope, David T. Delpy, Brian R. Davidson. Presentation to the 3rd World Congress of the International Hepato-Pancreato-Biliary Association, Spain, 24–28 May 1998. Abstract in Hepato-gastroenterology 1998, 45, suppl II: CCXXIV.

3. Effect of biliary obstruction on hepatic microcirculation and function. Abd El-Hamid El-Desoky, Alexander M. Seifalian, Mark Cope, David T. Delpy, Brian R. Davidson. Presentation to the 3rd World Congress of the International Hepato-Pancreato-Biliary Association, Spain, 24–28 May 1998. Abstract in Hepato-gastroenterology 1998, 45, suppl II: CCXXIV.

4. New method for assessment of hepatic dysfunction using near-infrared spectroscopy and indocyanine green. A. El-Desoky, A.M. Seifalian, and B.R. Davidson. Presentation to the 88th Meeting of the Surgical Research Society, Dublin, 2-3 July 1998. Abstract in Br. J. Surg. 1998; 85, 11: 1579.
5. The effect of hypoxia on hepatic bioenergetics measured by near infrared and ³¹P-NMR spectroscopy. A A El-Desoky, K K Changani, A M Seifalian, and B R Davidson. Abstract in Hepatology 1998; 28, 4: 764A.
6. In vivo assessment of hepatic tissue oxygenation with graded hypoxia. A. El-Desoky, A.M. Seifalian, and B.R. Davidson. Presentation to the European Congress of the International Hepato-Pancreato-Biliary Association, Budapest, 24-28 May 1999. Abstract in Dig. Surg. 1999; suppl1: 83.
7. In vivo assessment of graded hypoxia on hepatic tissue oxygenation and energy metabolism. A. El-Desoky, A.M. Seifalian, and B.R. Davidson. Presentation to The American Hepato-Pancreato-Biliary Association, Florida, 18-21 February 1999.
8. " The effect of graded steatosis on hepatic parenchymal perfusion and function" A.El-Desoky, A.M. Seifalian, and B.R. Davidson. Presentation to the 3rd annual meeting of European Association for the study of the liver, Rotterdam, Netherlands, 29 April- 3 May 2000.

9. Assessment of hepatic steatosis using indocyanine green measurement by near infrared spectroscopy. A.El-Desoky, A.M. Seifalian, and B.R. Davidson. Presentation to the Surgical Research Society meeting, Cardiff, UK, 23-24 May 2000.

10. Assessment of ischaemia/reperfusion injury by direct measurement of hepatic indocyanine green clearance using near infrared spectroscopy.”

A. El-Desoky, F. Youssef, A. Brozou, A. Seifalian, and B. Davidson.

Presentation to the 4th World Congress of the International Hepato-Pancreato-Biliary Association, Queensland, Australia, 28 May-1 June 2000.

11. Assessment of ischaemia reperfusion injury by measuring hepatic tissue oxygenation and blood volume using near infrared spectroscopy. A. El-Desoky, F. Youssef, A. Brozou, A. Seifalian, and B. Davidson.

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