The effect of perioperative insulin therapy on patients undergoing major liver resections

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A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy in Medicine.

Contributions of Authors

This thesis consists of 3 manuscripts describing novel therapeutic approaches for augmenting liver glycogen, improving postoperative liver function and maintaining normoglycaemia in patients undergoing liver surgery. The third paper highlights the potential pathways explaining the clinical benefits of this therapy. All studies were conducted under the clinical trial registered at clinicaltrial.gov NCT00774098. A list of the papers is presented below followed by contributions of all authors.

1. Hassanain M, Schricker T, Metrakos P, Carvalho G, Vrochides D, Lattermann R. Hepatic protection by peri-operative metabolic support? Nutrition. 2008 Jun 27: 1217-1219.

2. Mazen Hassanain, Peter Metrakos, Alexander Fisette, Suhail A.R. Doi, Ayat Salman, Prosanto Chaudhury, Thomas Schricker, Ralph Lattermann, George Carvalho, Chris Dey, Linda Wykes, Evan Nitschmann, Haneen Molla, and Katherine Cianflone; Insulin Therapy Improves Liver Function and Reduces Complication Rates in Patients Undergoing Major Liver Resection—*submitted to Annals of Surgery*

3. Alexander Fisette & Mazen Hassanain, Peter Metrakos, Suhail A.R. Doi, Ayat Salman, Thomas Schricker, Ralph Lattermann, Linda Wykes, Evan Nitschmann, Jessica Smith, and Katherine Cianflone; Altered Inflammation, Reduced Apoptosis and Increased Cell Proliferation from High-Dose Insulin Therapy Reduces Postoperative Liver Dysfunction and Complications in Liver Resection Patients—*submitted to JCEM*

I conceived the ideas behind this project, designed the protocol for the randomized clinical trial, identified patients eligible for the study and introduced them to the study. As well, I sought IRB approval, took care of the study registration, and managed meetings with the clinical dietician. With regards to patients I handled their admission to the hospital, arranged hospital beds suitable for intravenous hydration, and carried the insulin therapy after the operation. I also supervised the randomization process, collected and analysed all study–related blood and tissue samples; and collected and analyzed all the data.

Conjuring up the rational for the study started and matured with my supervisor Dr. Peter Metrakos. He also helped in identifying and introducing eligible patients to the study. Dr. P. Metrakos reviewed all manuscripts and supervised the writing of the thesis.

Dr. T. Schricker contributed to the development of the idea, shared his experiences with using the clamp during heart surgery and reviewed the first 2 papers. Members of his lab assisted with the analyses by measuring free fatty acid, insulin and glucagon levels.

Dr. R. Lattermann helped by implementing the clamp in the operating room and arranged transport of some patients to the recovery room prior to surgery when we needed to draw blood samples from them. He supervised the writing of the first manuscript and reviewed the second.

Dr. G. Carvalho coached me on how to use the clamp given his extensive experience with using the clamp technique in cardiac surgery, and helped Dr. Lattermann in applying the clamp during

the operation in some patients. He also introduced me to the lab and provided me with the training needed to spin, aliquot and store collected blood samples for the study.

Dr. D. Vrochides was the surgeon for the case report presented in the first manuscript.

Dr. P. Chaudhury assisted in surgical data collection and in reviewing the second paper. He also finalized and instituted the postoperative clinical pathways for all patients.

Ms. A. Salman was the study coordinator. She took care of the consenting process, collecting demographic data, double-checking the accuracy of collected data, completing paper work needed by the ethics board for renewal, and conducting the randomization process.

Collaborators Mr. Alexander Fisette M.Sc., Dr. K. Cianflone, and Dr. S. Paglialunga from the Université Laval Biochemistry Department measured blood levels of growth factors, complements, and adipo-hormones. Dr. Paglialunga participated in analysing samples for the first group of patients only and was included in the abstract authorship list. This group also measured the tissue mRNA expression using the insulin pathway kit, and assisted in reviewing the second and writing the third manuscript.

Dr. L. Wykes and Mr. E. Nitschmann, collaborators from the McGill School of Dietetics and Human Nutrition, helped in measuring serum cytokines using the Luminex 200.

Ms. H. Karasek, B.Sc., and Ms. H. Molla M.Sc. are the clinical dieticians from the McGill University Health Center who followed up the study patients, instructed them on how to ingest the required caloric intake prior to surgery, conducted the 24-hour dietary recall, and revised the intake of the control patients. Ms. Karasek assisted with the early patients only and was included in the published abstract at the AHBPA.

Dr. S. Doi is a clinical epidemiologist who supervised the statistical analysis included in the second and third paper and also performed the principal component analysis for both of these manuscripts. Dr. Doi is a collaborator from the Clinical Epidemiology Unit, School of Population Health, University of Queensland, Brisbane, Australia.

Dr. R. Dey, Dr. A. Khankan, and Dr. D. Valenti are interventional radiologists from the McGill University Health Center who measured all related liver volumes and calculated future liver remnants. Drs. Khankan and Valenti assessed liver parameters for the preliminary group of patients; and were referenced in the published abstract. Dr. Dey did the calculations that were included in the second manuscript.

More detailed information concerning the authors' contributions is included at the beginning of each manuscript.

Acknowledgments

First and foremost I would like to express my deepest gratitude to my wife Haneen Molla for all her help in making this thesis happen. In addition to her never ending moral support, she assisted with the clinical studies by meeting with the study patients as part of the nutrition team. I also want to thank my children Faisal and Joud because without the occasional and much needed distractions garnered from their enthusiastic play and smiles I would have accomplished nothing! I am grateful to my family, mom and dad in Saudi Arabia for their moral and continuous support.

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Abstract

Tissue injury related to liver surgery stimulates the release of stress hormones and proinflammatory cytokines to produce the necessary elements needed for healing. An exaggerated stress response compounded by preoperative fasting limits the liver's capacity to regenerate by depleting its energy stores. This change in metabolism leads to an insulin resistant state in the patient and associated hyperglycaemia. Dextrose supplementation coupled with intravenous insulin given perioperatively affords superior surgical outcomes through preservation of liver glycogen and strict glycaemia control.

The goal of this research was to investigate the benefits of a metabolic support protocol involving carbohydrate loading and insulin infusion provided by a hyperinsulinemic normoglycaemic clamp (HNC), for patients undergoing hepatic resection. Studies were performed in the context of a randomized-controlled clinical trial registered at clinicaltrial.gov NCT00774098.

Initially, 5 patients including 1 with diabetes mellitus were given the HNC protocol while undergoing a major hepatectomy. This feasibility study helped fine-tune the protocol towards improving its safety and reproducibility, and prompted expansion and randomization of the trial to include sixty patients. The goal of the expanded trial was to study the benefits of the protocol in augmenting liver glycogen and reducing postoperative complications. The patients were randomly assigned to either intervention or control groups. Intervention patients received a 24-hour oral carbohydrate-load, followed by an IV dextrose infusion (2 mg/kg/min) in the 24 hours leading to surgery, followed by insulin therapy given intra- and postoperatively. Controls

adhered to routine management. Blood and tissue samples were taken at various time points for monitoring liver glycogen content and function. In the final stage of the project a subset of patients was selected for genetic expression testing. Blood and liver tissue samples were analyzed to identify specific inflammatory, metabolic and cell cycles pathways stimulated by the protocol.

Carbohydrate loading combined with intra- and postoperative use of the HNC protocol augmented liver glycogen and reduced postoperative complications. Diabetic and nondiabetic patients alike benefited from this therapy.

Résumé

Les tissus traumatiques chirurgicaux stimulent la libération d'hormones de stress et de cytokines pro-inflammatoires afin de créer les éléments nécessaires pour la cicatrisation.

Une réponse au stress aggravée par le jeune préopératoire limite la capacité du foie à se régénérer en puisant dans ses réserves d'énergie. Ce changement de métabolisme conduit à un état de résistance insulinémique et d'hyperglycémie chez le patient. Un supplément de dextrose et d'insuline par voie intraveineuse durant la période périopératoire offre des résultats post-chirurgicaux supérieurs tout en laissant le foie préserver son glycogène et maintenir un contrôle glycémique stricte.

Le but de cette étude était d'étudier les avantages reliés à l'utilisation d'un protocole de soutien métabolique impliquant la perfusion de glucides et d'insuline par le biais d'une pince hyperinsulinémique normoglycémiques (HNC) sur des patients cédulés pour une résection hépatique. Cette étude a été réalisée dans le cadre d'une étude clinique randomisé-contrôlées enregistré sous NCT00774098 sur clinicaltrial.gov.

Au départ, 5 patients, dont 1 atteint de diabète Type I, ont été randomisés sur le protocole HNC durant l'hépatectomie majeure. Cette étude de faisabilité nous a aidés à affiner le protocole afin de le reproduire, tout en sécurité, sur un plus grand échantillon. Nous avons généré un essai clinique randomisé incluant 60 patients afin d`étudier les effets bénéfiques reliés à ce type de protocole au niveau de l'accroissement du niveau de glycogène dans le foie et la réduction des complications post opératoires. Les patients ont été randomisés soit sur le protocole HNC ou le groupe de contrôle. Les patients randomisés sur l'étude recevaient, en plus du HNC durant la période intra et post opératoire, des suppléments élevés de glucide par voie orale, suivie d'une perfusion de dextrose par voie intraveineuse à 2 mg / kg / min dans les 24 heures précédant

l'intervention chirurgicale. Le groupe de contrôle était gérer en suivant le protocole standard de l'hôpital. Des échantillons de sang et de foie ont été prélevés à divers moments afin de suivre de près la fonction hépatique et la teneur de glycogène dans le foie. En plus, des tests d'expression génétique ont été faits sur certains de ces échantillons. Les échantillons de sang et de foie étaient analysés pour étudier le cycle métabolique, inflammatoire et cellulaire.

Une surcharge de glucide combiné avec l'utilisation du HNC durant l'opération et la période post-opératoire a augmenté le niveau de glycogène dans le foie et a réduit les complications post-opératoires. Les diabétiques et les non-diabétiques ont bénéficié d'une telle thérapie.

Abbreviations

HCC:	hepatocellular carcinoma
CRCLM:	colorectal cancer liver metastasis
POLD:	postoperative liver dysfunction
FLR:	future liver remnant
ROS:	reactive oxygen species
TNF-α:	Tumour Necrosis factor-α
IL:	Interleukin
TGF-β:	transforming growth factor-β
CRP:	C-reactive protein
MKP:	MAP kinase phosphates
JNK:	c-Jun terminal kinase
IKK:	IκB kinase enzyme complex
PVE:	portal vein embolization
HVE:	hepatic vein embolization
RES:	reticuloendothelial cells system
GSK:	glycogen synthase kinase
MetS:	metabolic syndromes
NAFLD:	nonalcoholic fatty liver disease
NASH:	nonalcoholic steatohepatitis

ATP:	adenosine triphosphate
ACTH:	adrenocorticotrophic hormone
HNC:	hyperinsulinemic normoglycaemic clamp
GIN therapy:	perioperative glucose and insulin administration while maintaining normoglycaemia
CV:	coefficient of variability
SD:	standard deviation
TG:	liver triglycerides
TLV:	total liver volume
PCA:	principle component analysis
PONV:	postoperative nausea and vomiting
DVT:	deep vein thrombosis
ARF:	acute renal failure
UTI:	urinary tract infection
PCR:	polymerase chain reaction
BCL:	B-cell lymphoma
ASP:	acylation stimulating protein
MCP:	monocyte chemotactic protein
LR:	liver resection
CABG:	coronary artery bypass grafting

Introduction

III. Rational

Liver resection is the surgical removal of a portion of the parenchyma and associated bile ducts. This procedure is commonly performed to treat patients with primary or secondary liver tumours and currently represents the only curative option for selected patients with hepatocellular carcinoma (HCC), bile duct cancer (cholangiocarcinoma), gall bladder cancer, and colorectal cancer liver metastasis (CRCLM) (*1-11*).

Surgeons are performing hepatic resections with increasing frequency due to the availability of high resolution imaging techniques that facilitate selection of patients with favourable tumour stage and locations, better understanding of the segmental liver anatomy, and general improvements in operative and anaesthetic techniques (*12*). The postoperative mortality rate now stands at <5% in major hepatobiliary centers (*4, 13-17*).

Improved patient survival following liver surgery especially for patients with CRCLM has extended the indications of hepatectomy to patients with advanced liver involvement, patients with prior liver resections and patients with extrahepatic disease. As well, surgeons now perform this procedure more routinely in patients with benign tumors of the liver, and during liver graft transplants from living donors (*18, 19*).

Liver surgery invokes an adaptive stress response that can lead to complications such as liver dysfunction, hyperglycaemia, infections, longer hospital stays, and mortality. Postoperative complications stem from a multitude of factors, but mostly from exaggerated inflammatory and

metabolic responses culminating in liver dysfunction and hyperglycaemia. Liver dysfunction is a major determinant of postoperative morbidity and mortality. Postoperative liver dysfunction (POLD) occurs in 20%–70% of patients undergoing liver resection (*12, 16, 20-24*). The reported morbidity rate is 30%–70% and more than 80% of these patients have infections (*4, 12, 13, 25, 26*). The degree of POLD correlates linearly with postoperative morbidity (*23, 27*).

Postoperative complications in addition to their harmful effects on the patient reduce the survival benefit from liver surgery. In fact, assessment strategies incorporating liver function laboratory parameters and clinical observations are routinely used to predict patient survival expectancy following a resection (*23, 28, 29*). Major risk factors for developing postoperative liver dysfunction include fatty liver disease (steatosis), low hepatic glycogen content, obesity, preoperative chemotherapy, volume of the future liver remnant (FLR), chronic liver disease, male gender, older age, perioperative bleeding and blood transfusion (*18, 20, 30-34*).

Most risk factors for developing POLD are irreversible, but a select few such as liver glycogen depletion may be reversed through intervention. Preoperative carbohydrate loading delivered orally or intravenously can reverse liver glycogen depletion and potentially reduce the associated risk of POLD. This effect was demonstrated in animal studies where perioperative feeding as opposed to fasting improved the liver's resistance to ischemic injury by increasing hepatic glycogen (*35-37*). Glycogen exerts a protective effect on the liver by maintaining mitochondrial health and thereby inhibiting the production of reactive oxygen species (ROS) known to damage the hepatocyes (*38*). Although augmenting liver glycogen during surgery helps maintain hepatocyte integrity it also causes hyperglycaemia and associated complications.

During the inflammatory response to surgery, endothelial and epithelial cells as well as neutrophils, macrophages and lymphocytes all stimulate the release of proinflammatory mediators TNF-α, Interleukin-1β (IL-1β) and IL-6. An exaggerated response can overwhelm local defence mechanisms and culminate in defence system failure (*39-43*). In particular, up-regulation of cytokines suppresses immune function and potentially renders patients more susceptible to postoperative infection (*44-46*). This proinflammatory process also jeopardizes liver regeneration and function. Heightened release of TNF-α and IL-6 can lead to hepatic apoptosis and liver dysfunction or failure (*47*). Insulin provides a protective effect in patients requiring porta-hepatis clamping for restricting the inflow of blood to the liver (Pringles manoeuvre) during liver resection by suppressing the exaggerated release of IL-6 (*48*).

Surgery related hyperglycaemia represents an important concern for the patient and treating surgeon. More than 90% of patients develop blood glucose values higher than 6.1 mmol/L during critical illness (*49, 50*). Tissue injury from surgery disrupts the feedback mechanism required to maintain hypothalamic-pituitary-adrenal axis homeostasis. This leads to either up-regulation or suppression of hormones involved in regulating blood glucose. Release of epinephrine, glucagon and cortisol in response to injury augments glucose production via glycogenolysis and gluconeogenesis pathways. The same stress response suppresses insulin. The net effect is hyperglycaemia and an insulin resistant state in the patient (*51, 52*). Hyperglycaemia marks an increased risk of complications and mortality in critically ill patients (*53*).

Managing glucose control during surgery presents its own challenges. Surgeons often experience difficulty in controlling blood glucose perioperatively. Resulting hyperglycaemia causes postoperative complications such as infection, end organ dysfunction, longer ICU stays and increased mortality rates (*50, 54-57*). Because patients can't communicate the early warning

signs of hypoglycaemia while under anaesthesia, blood glucose levels must be checked frequently, and patients treated immediately with dextrose infusion when hypoglycaemia is detected (*55*). Infusion protocols used to maintain tight glucose control in the operating room are complex, labour intensive and can be distracting to the anaesthesiologist. Patient welfare may therefore be compromised during complicated surgery as result of the attention required to administer tight glucose control therapy (*54, 58*).

Improved patient management strategies are needed to reduce postoperative complications and enhance the survival benefit of liver resection. Ultimately liver surgeons want to bring more patients to surgery with improved patient outcomes and reduced complications. Reducing postoperative liver dysfunction and improving perioperative glucose control represent important steps in achieving this goal.

IV. Objectives

The goal of my research was to find a more effective and more comprehensive metabolic based protocol for supporting liver integrity and function following major liver resection. I proceeded with a vision of improving liver glycogen content and reducing postoperative complications, while at the same time maintaining strict normolglycaemia. The main objectives of my thesis work were to:

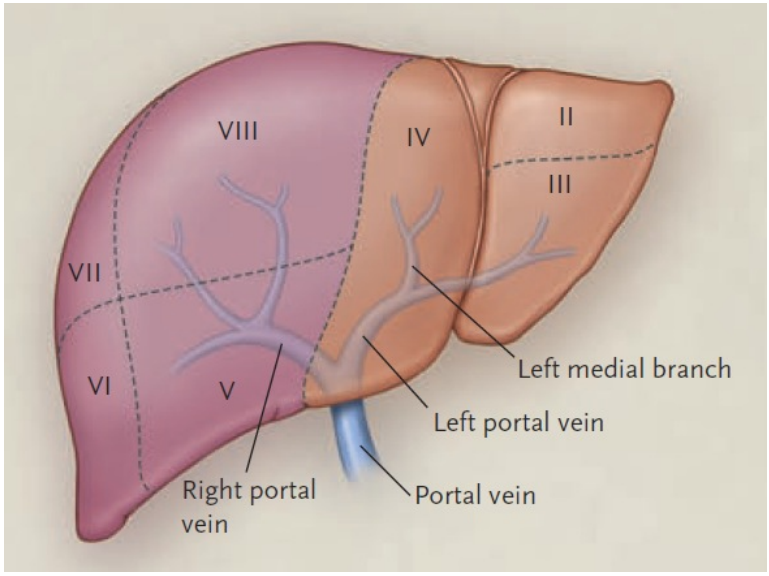
- Improve liver glycogen content during surgery
- Maintain normoglycaemia in patients undergoing liver resection
- Reduce postoperative liver dysfunction after major hepatectomy
- Reduce the incidence of postoperative complications from major liver surgery
- Study the pharmacological mechanism underlying insulin therapy benefits for the surgical patient

Literature Review

V. The liver

The liver is a vital organ located in the right upper quadrant of the abdominal cavity. It is divided into 2 parts; the right lobe which makes up approximately 60 % of its mass and the smaller left lobe (Figure 1). The left lobe is further divided into segments I-IV while the right lobe contains segments V-VIII. A portal vein divides the liver into its separate lobes and functions by transporting blood from the intestines, spleen and pancreas into the liver. The portal vein branches intricately left and right to carry blood further into all segments of the liver. The bile ducts are involved in the transport of bile synthesized by the hepatocytes and are located both extra- and intrahepatically.

Figure 1: Normal Liver Anatomy



VI. What is liver surgery

Liver resection is a surgical procedure that involves removing part of the parenchyma and associated liver ducts. Surgeons consider resection as the method of choice for curative treatment and management of many types of hepatic malignancies. The goal is to completely remove the tumour and preserve the surrounding liver tissue. The liver can withstand this type of operation because of its unique ability to regenerate over a short period of time following resection. Preserving a sufficient amount of functional liver, referred to as the future liver remnant (FLR), is paramount for the patient's survival. Failure to do so leads to death within a few days after surgery (*18*).

Hepatic resection has gained in popularity as a mode of cancer treatment for several reasons. The availability of high resolution imaging techniques like magnetic resonance imaging and computed tomography has made it easier to select patients with favourable tumour load (*59*). Better understanding of the segmental liver anatomy and general improvements in operative and anaesthetic techniques are also important factors for improving perioperative outcome (*12*).

Before the 1980s the mortality rate associated with liver resection stood at more than 10%. Mortality rates have since declined steadily to less than 2%. Improved patient survival following hepatectomy has extended the indications of hepatectomy to patients with advanced liver disease, patients with prior liver resections and patients with extrahepatic disease. As well, this procedure is being performed more frequently in patients with benign tumours of the liver and during liver transplants surgery from living donors.

Despite improvements in patient survival, morbidity rates (postoperative complications) from liver surgery remain high (20% –50%; Table 1), mainly because liver surgeons have focussed their efforts towards preventing mortality from this procedure rather than reducing complications, while at the same time expanding resectability criteria (*4, 13-17, 60*). The variability in the reported morbidity rates is the result of mixing both major and minor liver resection data in some published series. The improved perioperative care was counterbalanced with the expansion of the resectability criteria. This strategy has resulted in a sustained high postoperative complication rate (Table 1).

Table 1. Morbidity and Mortality Rates in Selected Studies

Author	Years	N	Mortality (%)	Morbidity (%)
Fan (*61*)	1994	64	8	55
Cohnert (*14*)	1997	340	4	22
Belghiti (*15*)	2000	747	4.4	22
Jarnigan (*60*)	2002	1803	4	52
Laurent (*16*)	2003	311	3	30
Stewart (*17*)	2004	137	2.9	19.7
Wei (*4*)	2006	423	1.6	17

Indications for liver resection

Liver resection offers the only potential cure for selected patients with hepatocellular carcinoma (HCC), bile duct cancer (cholangiocarcinoma), gall bladder cancer, and colorectal cancer liver metastasis. While cholangiocarcinoma and HCC account for nearly all primary liver cancers, colorectal cancer liver metastasis represents the most common indication for liver surgery.

HCC is diagnosed based on the presence of chronic liver disease, increased serum levels of alpha-fetoprotein, and confirmed tumour features by radiologic imaging. Resection and transplantation treatment options afford the best outcomes in patients at an early disease stage with 5-year survival rates greater than 60% (*1, 2*). In general, the 5-year recurrence rate after resection is more significant in patients with tumours >5 cm compared to those with tumours ≤5cm (43% vs. 32%, respectively) (*59, 62*). Although liver transplantation provides superior results in patients with liver cirrhosis, liver grafts are often not available to meet the timely need of treatment. Surgeons therefore resort to using resection as often as possible and reserving the transplant option for disease recurrence.

Cholangiocarcinoma is a relatively rare, slow-growing tumour originating in the intra- or extrahepatic bile duct (*3*). Although considered minimally malignant, prognosis for this form of cancer is poor, with a best scenario survival rate of 1 year when patients are given supportive care only. Surgical intervention including radical hepatectomy effectively manages intrahepatic and hilar diseases, and is the only potentially curative treatment. The 5-year survival rate for patients undergoing a radical hepatectomy for bile duct cancer remains at 39.7%, with a median survival time of 3.75 years. Postoperative morbidity and mortality rates for this disease are 46.8% and 2.0%, respectively (*3*).

Colorectal cancer is the fourth most common cancer in North America (*4*). Half of all patients with colorectal cancer will develop liver metastases. CRCLM is the leading indication for liver resection and carries the most representation of all cancer forms in the hepatic surgery literature. Liver resection has an impressive track record in prolonging the life of CRCLM patients affording a high 5-year survival rate (60%), while the best chemotherapy treatment regimens offer a median survival of only 22 months (*63*). This disease has therefore pushed the definition of resectability from number and size of lesions to how much liver will remain after a complete resection.

Hepatic resection provides a survival benefit to selected patients suffering from hepatic metastases originating from other forms of malignancies such as breast, ovarian or gastric cancer. For example in one study only 33% of patients with hepatic metastases from breast cancer developed disease recurrence following a hepatectomy (*64*). When chemotherapy was given preoperatively to stabilize the disease the 5-year survival rate after resection rose to 41%.

Transplantation surgery is the only viable surgical treatment option for selected patients with acute liver-failure, advanced cirrhosis, hepatocellular carcinoma, or hilar cholangiocarcinoma (*18, 19*). This mode of treatment has progressed significantly over the last decade. Surgeons are now able to successfully transplant a portion of liver, or partial liver graft, resected from a living donor. Liver grafting from a live donor permits transplantation that is ideally timed with the medical indication. Donors are selected based on their physical well being as first priority, to minimize risks and optimize recipient outcomes. The left lateral lobe of the donor liver (e.g. segments II and III) is used more often for transplants in children while the larger right donor lobe is used more frequently for adult recipients (*19, 65*). The current mortality rate for a right hemihepatectomy in a living donor is 0.2% to 0.5% and the rate of complications is 15% to 30%

(*18, 66*). Transplant surgeons aim for a FLR >35% of the original liver for minimizing postoperative complications (*18, 67*). Donors typically recuperate 80%–100% of their initial liver volume.

Liver surgeons also perform resections for patients with liver adenoma who are at risk of rupture and/or malignant transformation, for patients with liver cystadenoma at risk of malignant transformation; and for relieving symptoms of local compression and/or significant local discomfort in patients with focal nodular hyperplasia, haemangioma or polycystic liver disease.

VII. Types of liver surgery

Hepatic surgery has evolved over the years (*60*). Types of resections performed routinely include wedge resection whereby tumour is removed with a surrounding margin of about one-half inch of normal liver, segmented-oriented resections (segments 1 to 8), and lobe resections: right (right hepatectomy segments 5 to 8) or left (left hepatectomy segments 2 to 4). Surgeons also perform extended resections where segments of the adjacent lobe are also removed: A right trisegmentectomy includes segments 1 or 4 in addition to segments 5–8, and a left trisegmentectomy removes segments 1 or 5 and 8 along with segments 2–4.

Liver resection is further classified into either major or minor surgery. An operation taking 3 or more liver segments is considered a major resection while taking less than 3 is deemed minor (*27*). Surgeons and oncologists view a negative surgical margin i.e. oncologic resection as the most important determinant for disease free survival (*3, 68*).

Staged resections

A staged approach to liver resection is recommended for patients with multiple bilobar liver metastases. The method involves initial resection of tumour burden on one side of the liver followed by a second resection a few weeks later to clear the other side of the liver (*69*). Compensatory regeneration of the remnant liver after the first resection allows safer removal of tissue during the second hepatectomy. Staged resections in which 2 operations are performed with sufficient time between them to allow regeneration of the FLR, are now performed more routinely in patients with bilateral lobe disease since complete resection provides an almost similar survival benefit as a single hepatectomy (*5-11, 70*).

VIII. Postoperative liver regeneration

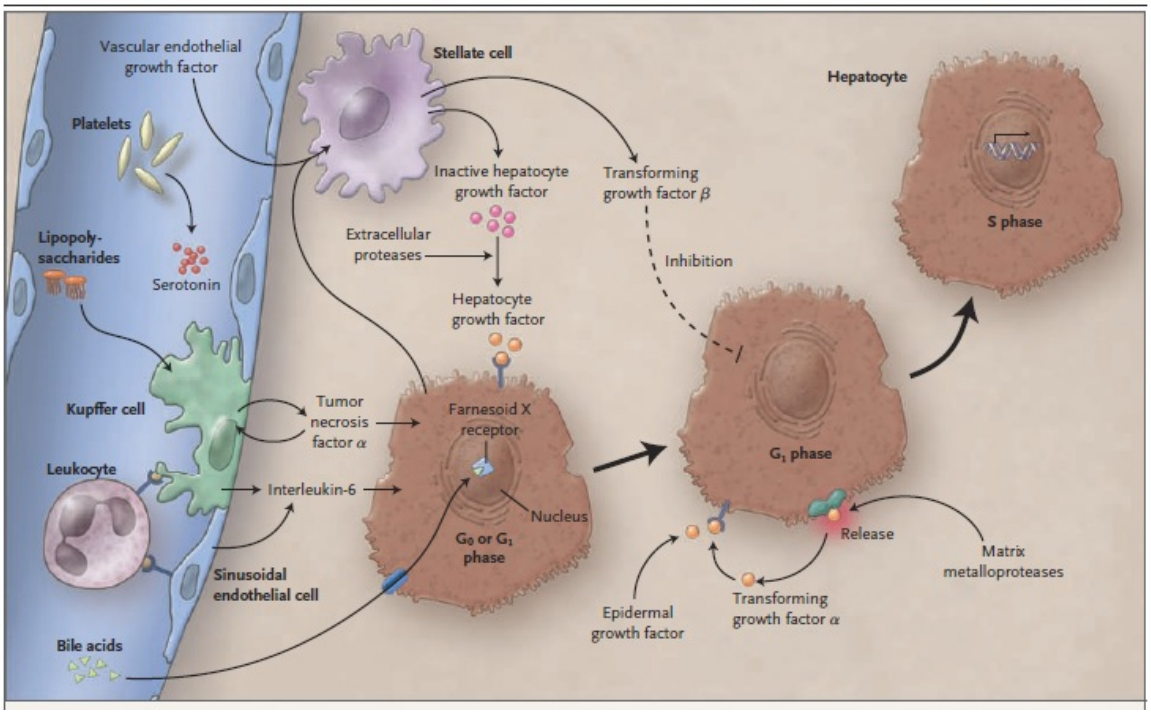
Liver function recovery following liver resection stems from the liver's remarkable capacity to regenerate after injury over a short period of time. Reported times to achieving full recovery of hepatic function are conflicting. Some reports indicate full recovery within 1 week of surgery while others suggest the process can take up to 6 months (*19, 71-75*). Regardless, normal liver function signalled by a return of laboratory parameters to normal levels occurs quickly and thoroughly.

The anatomical structure of a liver that has undergone a hepatectomy does not return to its original state during the restoration process (*76, 77*). Recovery of the liver volume happens through replication of intrahepatic cells followed by cell size increase rather than regeneration of the individual segments (*18*). Replication of hepatocytes begins on day 1 post surgery. Other

cells involved in the process such as endothelial, Kupffer, and biliary-duct cells replicate in a more delayed fashion (*18*). The level of replication and synchronization of different hepatic cell types correlates with the extent of tissue removal and / or damage.

Liver regeneration and acute inflammation involve similar molecular mediators. Hepatocytes start out in the quiescent G_0 phase then move into the active G_1 phase after resection. Cytokines derived mainly from Kupffer cells, tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) are then released in an orchestrated fashion to initiate the cell cycle. (*78, 79*) Transforming growth factor β (TGF-β), which normally inhibits hepatocyte DNA synthesis, is blocked during the hepatocyte proliferative phase. At the end of the regeneration process TGF-β regains its function and helps the hepatocytes return to their quiescent state (Figure 2) (*18, 43, 77*).

Figure 2: Pathway for Liver Regeneration Following a Major



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Full and synchronized regeneration of the hepatocytes depends on a balanced interplay between various cellular and physiological processes. Inability to sustain hepatocyte regeneration after liver surgery leads to inadequate recovery of liver volume and ultimately liver failure (*18*). Certain factors govern the regeneration process. For example, failure to activate the proinflammatory signal cascade delays onset of regeneration (*80*). The neurotransmitter serotonin, peripherally circulated by platelets, also appears to be a co-mitogen that is essential for hepatocyte regeneration (*81*). Bile flow represents a third factor affecting liver regeneration. Low bile flow correlated with reduced hepatocyte proliferation in animal studies (*82*).

IX. Adaptive stress response

Surgery invokes an adaptive stress response to maintain homeostasis in the patient. Under normal circumstances this response affords recovery benefits for the patient. Exaggerated responses however, lead to complications including liver dysfunction, infections, morbidity, longer hospital stays and mortality.

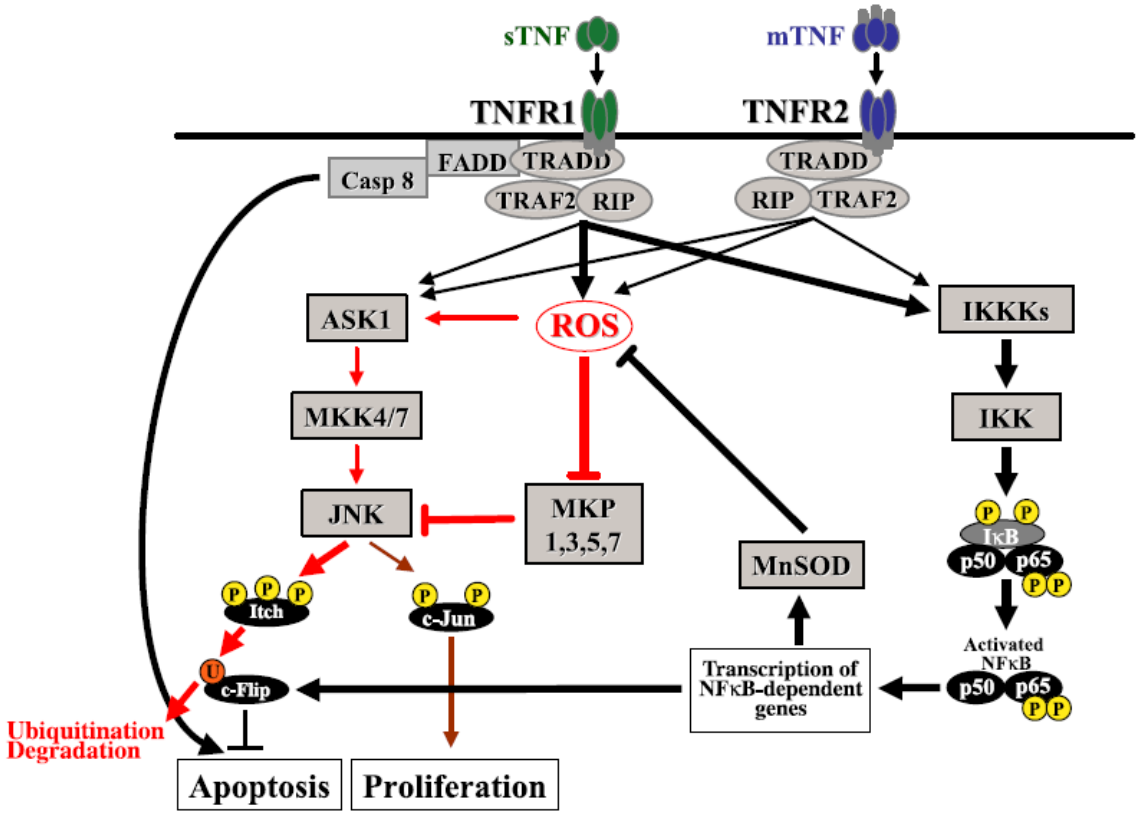
The stress response originates from 3 major systems including the endocrine, sympathetic nervous and acute phase response systems (*43, 83*). Endothelial and epithelial cells as well as neutrophils, macrophages and lymphocytes all stimulate the release of pro-inflammatory mediators TNF-α, IL-1β and IL-6 in response to tissue injury. If sufficiently severe, tissue injuries can overwhelm the local defence mechanisms leading to over-expression of protective biomolecules and persistent up-regulation of cytokines. The process ultimately culminates in a failure of the host defence system marked by for example, higher levels of C-reactive protein (CRP), IL-6 and acute phase protein in the patient (*39-42*). In particular, upregulation of cytokines can suppress immune function and potentially render patients more susceptible to postoperative infection (*44, 46, 84*).

Neutrophils and macrophages also respond to the proinflammatory cascade by releasing granular enzymes and producing reactive oxygen species (ROS) that can lead to organ dysfunction (*44, 85*). Lan and colleagues compared stress biomarkers in 2 patient groups undergoing a partial right hepatectomy, including healthy donors and patients with diseased livers, in order to evaluate the acute phase response following surgery (*43*). Significant increases in IL-6 and CPR were observed in both patient groups indicating the onset of liver regeneration. The cytokine IL-

6 was higher in patients with diseased liver before surgery and remained higher postoperatively suggesting that IL-6 elevation is an indicator for increased risk of developing postoperative complications (*43*). A second study looked at the effect of circulating cytokines, chemokines and stress hormones on postoperative infection and liver dysfunction. Interleukin-6 and Interleukin-10 were cited as the 2 most important cytokines in mediating these postoperative events (*44*).

Tumour necrosis factor-α plays a particularly important role in dictating the fate of hepatocytes. Postoperatively it restores functional liver mass by inducing cell proliferation and liver regeneration. However, under certain conditions it also acts as a mediator of hepatocyte death (*47, 86*). Soluble and membrane bound TNF-α bind to and activate their TNF-R1 and TNF-R2 receptors, respectively resulting in downstream activation of the JNK (c-Jun terminal kinase) and IKK (IκB kinase enzyme complex) pathways (Figure 3). TNF-α induced ROS produced during the stress response help prolong JNK activation by oxidizing and inactivating several members of the MAP kinase phosphates (MKPs). Activation of the JNK pathway for short periods of time contributes to cell proliferation while prolonged activation shifts the balance towards cell death. IKK activation by contrast exerts a protective role. The IKK-β subunit protects the hepatocytes from TNF-α induced apoptosis and initiates the transcription of proinflammatory and proproliferative mediators in Kupffer cells (Figure 3).

Figure 3: Role of JNK and IKK in TNF-α Induced Cell Death and Proliferation



With permission: In Schwabe RF, Brenner DA. Mechanisms of Liver Injury. I. TNF-alpha-induced liver injury: role of IKK, JNK, and ROS pathways. Am J Physiol Gastrointest Liver Physiol. 2006 Apr 1;290(4):G583-9.

X. Volume of the FLR

Volume of the FLR is another factor in deciding the fate of the liver. A small FLR (smaller than a minimum threshold volume) cannot sustain metabolic, synthetic and detoxifying functions, and liver failure ensues (*18*). Clinical signs of this "small-for-size syndrome" include jaundice, coagulopathy, encephalopathy, ascites; and renal and pulmonary failure (*18, 86*).

There are different schools of thought on exactly how much liver must remain following a hepatectomy to minimize surgery associated liver dysfunction, morbidity and mortality. Quality

of the parenchyma represents an important factor in deciding how much liver can safely be resected; better liver quality means more tissue can safely be resected. Some hepatobiliary centers have shown that resection of up to 80% of the liver can be performed safely on normal livers (*6, 8, 22*). Others recommend resection accompanied by preoperative portal vein embolization to help grow the FLR volume if removal of more than 75% of the liver is expected (*87*). A rule of thumb is that resection of more than 75% of the liver may proceed with lower risk only in younger patients (≤40 years) with normal liver parenchyma (*18*).

Embolization for growing the FLR

Because of the relationship between postoperative complications and the size of the FLR, hepatic surgeons have focussed considerable effort towards increasing the volume of the FLR. These techniques enable resection in patients initially considered unresectable because of a marginal FLR (*86, 88-91*). Methods used for growing the FLR although successful, carry an increased risk of potential adverse events.

Preoperative portal vein embolization (PVE) is an invasive technique used help grow the liver prior to surgery. The surgeon or intervening radiologist blocks off blood inflow to the diseased part of the liver and thereby selectively induces atrophy in the diseased, embolized lobe of the liver while increasing the volume of the nonembolized FLR via compensatory hypertrophy (*86, 88*).

Surgeons sometimes use another invasive technique called hepatic vein embolization (HVE) in sequence with PVE to further enhance regeneration of the FLR. Following a PVE the intervening radiologist stimulates further growth in the FLR by additionally blocking blood outflow to the

diseased part of the liver. Hwang and colleagues demonstrated some evidence of stronger compensatory regeneration using this technique (*92*).

Embolization procedures are invasive and carry potential risk of complications. The success of these techniques depends on the ability of the FLR to grow which is affected by the quality of the liver parenchyma. A number of technical and anatomical factors may prevent the surgeon from performing an embolization. Furthermore, induction of FLR growth prior to cancer resection is known to stimulate the growth of the cancer and promote metastasis (*88*). Hepatic vein embolization has also been shown to cause liver damage (*92*). Hospitals should therefore aim to minimize patient waiting times between embolization and resection as much as possible (*88*).

Measuring the FLR

Preoperative estimation of the FLR is typically done with the assistance of computed tomography (*86, 87*). This type of measurement affords an assessment of the liver remnant before resection takes place. In one commonly used method proposed by Vauthey and colleagues a preoperative computed tomography scan of the abdomen including the whole liver is first taken in a single breath-hold. The radiologist virtually reconstructs the liver using specialized software then estimates the total liver volume on the basis of body surface and the following equation (1) (*86, 87*):

$$\text{Liver volume } (cm^3) = 706 \times \text{body surface area } (m^2) + 2.4 \quad (1)$$

Volume of the FLR is determined from the volume of the parenchyma left behind in the virtual image.

XI. Postoperative liver dysfunction (POLD)

Postoperative liver dysfunction results from damage to the liver. The severity of POLD following liver surgery depends on a number of factors such as disease state of the patient, the amount of liver tissue removed, amount of blood loss, operative techniques used, intraoperative ischemia and postoperative management (*15, 60*). Since the liver is involved in many body functions such as digestion, metabolism and immune defence it is not surprising that liver failure compounds postoperative complications and may even be fatal (*43, 93, 94*).

About 20% of patients undergoing liver resection develop postoperative infection complications. Major liver resection compromises the reticuloendothelial cells system (RES), made up in part by liver Kupffer cells, and lowers the body's innate immune defence. Therefore, preserving a functioning FLR is necessary to sustain adequate RES phagocytosis capacity (*86, 94*) and failure to do so predisposes the patient to infection (*86, 94*).

XII. Risk factors for developing POLD

Some of the risk factors cited for postoperative liver failure following a major resection include old age, cirrhosis, fibrosis, hepatitis, intraoperative blood loss, and ischemia (Pringles), obstructive cholestatis, preoperative chemotherapy and hepatic steatosis (*18*). Some of these factors are reversible while others are not, e.g. age and gender. A number of risk factors including chemotherapy induced steatohepatitis, obesity and associated non-alcoholic fatty liver disease and steatohepatitis are on the rise.

Gender

Males are at greater risk of developing POLD. In a study (n=244) that examined preoperative risks factors in patients undergoing liver resection for malignant disease 8 male participants died postoperatively compared to only 2 females (*14*). The investigators cited multiple organ failure as the leading cause of death. Other causes included ischemia and bleeding.

Age

Studies have shown that older patients are at higher risk of developing POLD. For example, in a study conducted at two Italian centers between 1998 and 2006 that included 1271 hepatectomies, elderly patients who underwent liver resection at 1 of the 2 centers demonstrated a significantly increased risk of death following postoperative liver dysfunction (*95*). The Median age for patients who died from liver failure was 64.5 compared to 59 years for the survivors. The investigators suggested that increase in comorbidities, especially cirrhosis, and reduced hepatic functional reserve could explain the higher mortality rate in older patients. A separate study classified postoperative complications in 146 patients who underwent liver resection for malignant or benign lesions, as surgical or medically related (*86*). Risk factors for both were analyzed. Univariate analysis showed that age over 60 years is a risk factor for either class of postoperative complications (*30*).

Decline of hepatocyte regenerative capacity also contributes to increased risk of POLD in the elderly. Results from a study in mice demonstrated that glycogen synthase kinase 3β (GSK3β), an enzyme normally involved in the inactivation of glycogen synthase, also accelerates liver

proliferation. The investigators found a reduction of this critical regulator in older mice and an associated decrease in proliferative capacity of the older liver (*96*).

Bleeding and blood transfusion

Operative bleeding and/or blood transfusions are other important risk factors for POLD. Blood loss during hepatic surgery significantly raises the risk of postoperative mortality and morbidity. Blood transfusions cause complications by suppressing patient immunity (*30*). Surgeons use clamping procedures such as the Pringle manoeuvre during parenchymal transection to control the bleeding and protect the parenchyma (*24, 30*).

Steatosis

Fatty liver disease (steatosis) is on the rise in Western countries, affecting 6% – 11% of individuals in the general population, and is the most common parenchymal disorder in nontumour-bearing livers (*20, 86*). Prevalence of steatosis correlates with alcohol use, obesity, metabolic disorders such as diabetes mellitus II, and previous use of chemotherapy (*20, 86*). Males are more likely to be affected by the disease (*20*). Steatosis leads to poor liver quality and may result in liver inflammation, fibrosis, and cirrhosis. This disease is strongly associated with the metabolic syndromes (MetS) and overall insulin resistance (*34*). In surgical patients, steatosis leads to a greater rate of intra- and postoperative complications, particularly infections, the need for transfusions, increased sensitivity to ischemia and longer operative times. One study reported mortality rates that scaled positively with severity of the disease. Mild and severe disease associated with 3% and 14% mortality rates, respectively (*20, 86*).

The Pathology Committee of the NASH Clinical Research Network devised and validated a semi quantitative scoring system for assessing histological features of non-alcoholic fatty liver disease (NAFLD). The goal was to find a rating system for diagnosis of nonalcohol steatohepatitis (NASH), a progressive form of fatty liver disease. Histological features from 5 broad categories, including steatosis, inflammation, hepatocellular injury, fibrosis, and miscellaneous features were evaluated in adult and paediatric NASH patients by a team of pathologists using simple histochemical staining. Agreement between pathologists with regards to weighting of features recognized in NAFLD was statistically analyzed and a NAFLD activity score (NAS) proposed. A NAS >5 correlates with a diagnosis of NASH while a score of less than 3 associates with absence of NASH (*97*).

Preoperative chemotherapy

Preoperative down-staging chemotherapy can render previously unresectable patients to a resectable status and improve their chance of survival. Reports of up to 60% of patients with initially unresectable metastases have been brought to surgery using a combination chemotherapy regimen comprising Fluorouracil (5-FU) plus oxaliplatin and/or irinitotican (*32, 98-100*). Chemotherapy is also used as a neoadjuvant treatment for resectable patients in many hepatobiliary centers because of its potential survival benefit (*31, 101*). The rate of postoperative morbidity but not mortality is increased in patients undergoing liver surgery combined with preoperative chemotherapy (*31, 32, 101*).

Liver damage caused by preoperative chemotherapy correlates directly with duration of treatment. Few clinical consequences other than mild liver dysfunction (low platelet count, prolonged prothrombin time and increased fatty degeneration postoperative) develop as a result of this damage when patients receive appropriate amount of treatment (*31-33*). Hepatic histological lesions occur frequently from chemotherapy and the nature of these lesions depends on the type of chemotherapy used. As an example, oxaliplatin based chemotherapy can cause severe vascular lesions that lead to operative bleeding requiring transfusion. Studies have identified a correlation between morbidity rate and number of chemotherapy treatment cycles (*32, 101*).

Volume and quality of the future liver remnant (FLR)

A clear correlation exists between FLR volume and POLD; the smaller the FLR the more likely the patient will experience POLD (*102*). Increase in portal pressure and flow, endothelial and Kupffer cell injury and the release of proinflammatory cytokines are all linked to small liver remnant size (*103*). Different groups have proposed a critical FLR, defined by percentage of original liver remaining after surgery, as a predictor of postoperative hepatic dysfunction and this percentage changes depending on the initial health of the liver. Patients with normal liver function recover well with a minimal FLR of 26.5%. The critical FLR for patients who have impaired liver function preoperatively or have received neoadjuvant chemotherapy is 31% (*23, 102*).

Failure to regenerate and increased liver apoptosis is another cause of POLD even when the FLR has normal function (*18*). This complication is typically seen in patients with preoperative cholestasis.

Platelet count

Studies have shown that platelets play an essential role in liver regeneration following liver resection and that platelet-derived serotonin helps mediate this process (*81, 104, 105*). Conversely, depletion of platelets suppresses hepatic regeneration (*81, 104, 105*).

Low platelet count determined either pre- or postoperatively impacts negatively on patient outcome after hepatectomy. Alkozai et al found an almost 5-fold increased risk of delayed postoperative recovery in patients with low platelet count immediately after surgery (OR, 4.9; 95% CI, 1.7–13.9; $P = 0.01$) and concluded that postoperative platelet count is an independent

predictor of both POLD and postoperative mortality (*86*). Others have demonstrated a similar association between low preoperative platelet count and poor patient recovery (*12, 60, 106*).

Other factors

Staged resections and re-resections for recurrences pose increased risk of developing POLD. Although morbidity rates remains steady between multiple hepatectomies the severity of complications increases with each resection. Also, mortality rates are higher in patients who have undergone a staged resection compared to those who have had only one hepatectomy (*11, 69, 107*).

XIII. Measuring POLD

There is no consistent definition of POLD in the literature but diagnosis is consistently based on abnormal values in laboratory parameters and clinical observations. Common laboratory parameters for measuring liver function include total bilirubin, aspartate aminotransferase and alkaline phosphatise levels, coagulation profile, platelet count and prothrombin time (INR). Total bilirubin levels and INR are the most widely accepted biochemical indicators of hepatic insufficiency. Some assessment methods like the Child-Turcotte-Pugh classification system and Model for End Stage Liver Disease (MELD) use preoperative biochemical and clinical markers to predict hepatic insufficiency (cirrhosis) related mortality. The Child-Turcotte-Pugh classification system incorporates 5 clinical measures of liver disease to grade patient survival

expectancy (Table 2). One-year survival expectancies are 100% and 45% for scores of 5–6 (Class A) and 10–15 (class C), respectively (*18, 28*).

Table 2: Child-Turcotte-Pugh Classification System

Measure	1 point	2 points	3 points	Units
Bilirubin (total)	<34	34–51	>51	mmol/L
Serum albumin	>3.5	2.8–3.5	<2.8	mg/dL
Prothrombin time (INR ratio*)	<1.7	1.7–2.3	> 2.3	no unit
Ascites	None	Suppressed with medication	Refractory	no unit
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)	no unit

* International normalized ratio

The MELD system is a mathematical algorithm used to score severity of chronic liver disease as a predictor of survival (2):

MELD = 3.8 x log(e)(bilirubin mg/dL) + 11.2 x log(e)(INR) + 9.6 log(e)(creatinine mg/dL) + 0.643 (2)

A score >30 signifies poor outcome whereas pretransplant mortality is unlikely when the score <9. MELD has been investigated mostly in cirrhotic patients (*27, 29*).

Other surgical teams have proposed standard definitions of hepatic insufficiency measured postoperatively as an indicator of patient prognosis. One study determined that a peak postoperative bilirubin level >120 μmol/L is the single most important predictor of major complications and prolonged hospital stays (*27*). Balzan and colleagues proposed a "50–50" criteria (prothrombin time <50% and serum bilirubin >51 μmol/L) on day 5 post hepatectomy as a reliable predictor of liver failure (*27, 108*). Schindl and colleagues developed a system for scoring postoperative liver function based on the levels of lactic acid, total bilirubin, INR and degree of encephalopathy (Table 3) (*23*).

For our clinical trial we chose to use the later scoring system (Schindl et al) as it provides an objective method for studying postoperative liver function and also grades POLD by degree of disease severity rather than simply returning a black and white categorization, using the formal systems. This helped us in studying the effect of our insulin therapy on liver function.

Table 3: Liver Function as Per Score Generated by Schindl et al[1]

Total serum bilirubin (µmol/l)	<20	20–60	>60
Prothrombin time (INR)	<4.0 (1.8)	4.0–6.0 (1.8–2.3)	>6.0 (2.3)
Serum lactate (mmol/l)	<1.5	1.5–3.5	>3.5
Encephalopathy grade	None	1 and 2	3 and 4
Given points	0	1	2

[1]No dysfunction when score is equal to 0 points, mild when 1–2, moderate when 3–4, and severe dysfunction when >4 points.

XIV. Quality of the FLR

The quality of the FLR is a major determinant of the postoperative liver function status. Although there are multiple basic science publications describing the status of the liver parenchyma very few surgical investigations have focussed on developing techniques to improve FLR quality.

One study showed a link between the amount of glycogen stored in the liver and quality of the liver in adults with alcoholic or biliary cirrhosis. These patients had reduced hepatic glycogen stores compared to healthy individuals with normal liver function. The 2 main factors cited for this observation are loss of hepatocytes and impaired hepatocellular glycogen metabolism. When hepatic glycogen stores are exhausted, the body resorts to using amino acids derived from skeletal muscles and fatty acids derived from adipose tissue to stimulate the production of glucose via alternative pathways. This may contribute to muscle wasting and increase systemic inflammation in patients with liver disease (*86, 109*).

XV. Protective effect of liver glycogen

Glycogen is the body's main form of carbohydrate storage residing primarily in the muscles and liver. Glycogen made by the liver readily reconverts to glucose to meet the body's metabolic needs. During liver surgery hepatocellular glycogen fuels the ATP production needed to sustain hepatocellular integrity via an anoxic glycolysis pathway. In the absence of sufficient liver glycogen ATP levels rapidly deplete and the tissues succumb to irreversible cell injury and

necrosis (*38, 110, 111*). Both fatty degeneration and low glycogen concentration reduce the livers tolerance to ischemia-reperfusion injury (*111-116*).

Studies in animals have shown that preoperative feeding as opposed to fasting increase the livers resistance to ischemic injury (*36, 37, 115*). Investigators cited increased hepatic glycogen as the main reason for this improvement (*35, 111*). Glycogen was shown to exert a protective effect on the liver by maintaining mitochondrial health and thereby inhibiting the production of ROS known to damage the hepatocytes (*38*).

Human trials have also demonstrated higher glycogen content in patients receiving glucose infusion (*117-119*). In one randomized-controlled study including 16 patients, preoperative glucose infusion in fasting patients undergoing abdominal surgery resulted in a 65% increase in liver glycogen content during surgery. This higher glycogen content combined with enhanced FDPase activity suggests that gluconeogenesis represents a favoured route for glycogen formation. These patients however, had a significant high blood glucose level during the infusion (*118*).

A second study looked at glycogen degradation in recipients through various stages of liver transplantation to assess whether intraportal glucose infusion given perioperatively could improve the transplanted livers. Glucose infusion resulted in rapid glycogenesis during the transplant surgery and appeared to exert a protective effect against prolonged ischemia by lowering peak transaminase levels, particularly in young donors (*119*).

Schricker and colleagues showed that a dextrose infusion given to colorectal surgical patients, which started the day before and ended the day after surgery, prevented postoperative loss of body protein (*117*). In a follow up randomized-controlled trial by the same team, patients

received IV glucose starting at the time of incision. Although a decrease in amino acid oxidation was observed after surgery for the patients receiving glucose therapy, a higher proportion of patients in this group developed hyperglycaemia during the acute period of surgical stress. This led the team to question whether the antioxidative benefits outweigh the risks (*120*).

XVI. The catabolic response to surgery

Patients undergoing major surgery frequently encounter what is known as the catabolic response characterized by a series of hormonal and metabolic changes that can culminate in insulin resistance and associated hyperglycaemia (*51, 52*). The stress response initiates within minutes of beginning surgery by stimulating the release of adrenal hormones and pituitary hormones from the hypothalamic-pituitary-adrenal axis, and glucagon from the pancreas. Glucagon stimulates the breakdown of glycogen in the liver leading to increased circulation of glucose and lactose. Glycogen stores are first converted to glucose-6-phosphate by glycogen phosphorylase A and then into glucose by glucose-6-phosphatase; a process referred to as glycogenolysis. Other anabolic hormones like insulin and testosterone are suppressed during the stress response.

Studies in humans and animals have shown that stopping blood flow through the portal vein (Pringle manoeuvre) during hepatic surgery causes a rapid release of glucose stores from the hepatocytes after unclamping. This hyperglycaemia develops in response to procedure induced hypoxia which causes glycogen stores to be broken down into glucose via an anaerobic glycogenolysis pathway (*86, 93, 121*).

Tissue injury further stimulates the hypothalamus to release adrenocorticotrophic hormone (ACTH). This event initiates cortisol production within a few minutes of beginning surgery. The enhanced cortisol secretion fails to inhibit further production of ACTH and high serum concentrations of ACTH and cortisol persist. The magnitude and duration of this increase correlates with the severity of the injury.

Under normal homeostasis cortisol increases blood sugar through gluconeogenesis, suppresses the immune system, and assists with protein and carbohydrate metabolism. During surgery, the enhanced metabolic effects of cortisol cause a breakdown of skeletal muscle protein and thereby provide gluconeogenic precursors and amino acids for protein synthesis in the liver. Lipolysis in adipose tissue is increased and glucose utilization becomes impaired by this process leading to further hyperglycaemia. This impaired glucose utilization is known as the 'anti-insulin effect' (*51, 52*).

XVII. Risks associated with hyperglycaemia

Hyperglycaemia resulting from the catabolic response marks increased risk of morbidity, postoperative complications (for example surgical site infection) and mortality in critically ill patients, for diabetic and non-diabetic patients alike (*53*). More than 90% of patients develop blood glucose values >6.1 mmol/L during critical illness (*49, 50*). Several studies have shown that circulating levels of proinflammatory cytokines such as TNF-α, IL-1β and IL-18 increase during acute hyperglycaemia. As well, the release of certain cytokines post injury, in particular TNF-α, can induce a peripheral state of insulin resistance that in turn leads to hyperglycaemia

(*49*). TNF–α reduces uptake of glucose into the peripheral tissues, increasing lipolysis and decreasing lipoprotein lipase activity (*49, 86*). Collectively, these effects contribute to an insulin resistance state in the patient and cause blood glucose levels to rise. Stimulation of the cytokine pro-inflammatory response further elevates blood glucose levels through increased release of stress hormones (*49, 86*).

Several professional organizations including the American Diabetes Association and the American Association of Clinical Endocrinologists recommend intensive insulin therapy to maintain tight glucose control in critically ill patients (*86, 122*). Despite these recommendations, widespread adoption of intensive insulin therapy has been hindered by concerns about validity of some studies, risk of severe hypoglycaemia, increased mortality rates, difficulty in achieving normoglycaemia in critically ill patients and increased resources required for managing the therapy (*86, 123*).

There is some debate over what constitutes the ideal target blood glucose levels for reducing hyperglycaemia related complications in critically ill patients and whether insulin therapy is a viable option. A number of recommendations for ideal blood glucose target levels have been put forward based on clinical studies. Upper limits of <8 mmol/L, <12 mmol/L and <6.1 mmol/L have all been cited as ideal thresholds (*50, 86, 124*). While some studies have shown tight glucose control reduces in-hospital mortality others have failed to confirm a mortality benefit with intensive insulin therapy among critically ill patients (*50, 124*).

A landmark trial conducted by Van den Berghe et al. (2001) reported reductions in hospital mortalities by 30% among critically ill patients receiving tight-glucose control therapy. Most pronounced survival benefits occurred in a subgroup of patients having sepsis and/or organ

failure (*50*). Few interventions in critically ill patients had met with this kind of success and consequently results of the Van den Berghe trial were enthusiastically incorporated into guidelines.

Several large study centres have since failed to replicate the mortality benefit seen in the Van den Berghe trial (*125-128*). A recent meta-analysis evaluating the benefits and risks of tight-glucose control in critically ill patients concluded a mortality benefit with this therapy but a greater incidence of associated hypoglycaemia (*125*). In fact hypoglycaemia represents a consistent finding among most trials.

The recently published Normoglycaemia in Intensive Care Evaluation —Survival Using Glucose Algorithm Regulation (NICE-SUGAR) study, which evaluated intensive insulin therapy as a mode of hyperglycaemia treatment in the ICU, reported 1580 deaths out of 6104 patients enrolled in the study. However this study included all patient types and not strictly those who received surgery (*123*). Griesdale and colleagues showed an intensive insulin therapy benefit for surgical patients and concluded that some, but not all patients should receive insulin therapy (*122*). Van den Berghe also reported more pronounced insulin therapy benefits for surgical patients in reducing organ failure and improving survival rate (*50, 126*).

Managing glucose control

Managing glucose control during surgery presents its own challenges. Cardiac surgeons have often described their difficulties in controlling blood glucose perioperatively and failure to do so can lead to complications such as infection, renal failure, need for ventilator support, longer ICU stays and increased morbidity and mortality rates (*50, 54-57*). For example in one study 40% of patients undergoing a cardiopulmonary bypass needed treatment for hypoglycaemia (3.3

mmol/L) in the cardiac ICU after surgery despite receiving tight-glucose therapy during the operation (*56*).

Because patients can't communicate the early warning signs of hypoglycaemia while under anaesthesia (headache, lack of concentration, tremor, muscle weakness, visual disturbances, hunger, sweating, irritation) blood glucose levels must be checked frequently, and dextrose infusion administered immediately when hypoglycaemia is detected. Most anaesthesiologists administer dextrose when blood glucose levels decrease below 3.3 mmol/L (60 mg/dL) (*55*).

Infusion protocols used in the operating room are complex, labour intensive and can be distracting to the anaesthesiologist. Patient welfare may therefore be compromised during complicated surgery as result of the attention required to administer tight-glucose control therapy (*54, 55, 58*). Commonly used protocols used for controlling glucose during cardiac surgery include the Portland protocol (automated continuous intravenous insulin) and the hyperinsulinemic normoglycaemic clamp technique (*54, 58*). Both are effective at maintaining glucose at reasonable levels.

Administration of IV insulin for glucose maintenance also runs the risk of causing hypokalemia since potassium is transported intracellularly with glucose and insulin. Therefore, it is important to check serum potassium concentrations when providing insulin, both during and after surgery (*55*).

XVIII. Benefits of insulin therapy

Early studies on the use of insulin therapy in surgical patients focussed on its metabolic effects such as increasing cardiac, hepatic and muscle glycogen content and inhibiting lipolysis in the adipose tissue with associated release of free fatty acids and adiponectine. More recent evidence has since emerged suggesting that insulin protects the organ by counteracting the inflammatory response following injury (*129, 130*). While onset of hyperglycaemia post surgery induces the release of proinflammatory cytokines TNF-α, IL-1β and IL-18, insulin significantly lowers these cytokines. Enhanced production of IL-2 and IL-4 needed for combating the IL-1/TNF-α proinflammatory pathway was observed in an animal study that looked at the effect of insulin in systemic inflammation (*49, 131*). Insulin is also a well known inhibitor of glycogen synthase kinase-3β (GSK-3β) involved in glucose regulation. Blocking the action of (GSK-3β) with a potent and selective inhibitor exerted a protective effect in rats undergoing surgical procedures (*132*).

A pilot study aimed at maintaining normoglycaemia during cardiac surgery using a hyperinsulinemic normoglycaemic clamp (HNC) technique was conducted in 2004 (*54, 86*). Patients received a fixed calculated IV insulin infusion based on body weight. Intravenous dextrose, adjusted according to blood glucose levels, was given to maintain normoglycaemia during the protocol. The investigators hypothesized that the HNC method would preserve normoglycaemia during open-heart surgery requiring a cardiopulmonary bypass. Favourable glucose regulation was achieved in a predictable and reliable fashion perioperatively; but implementing the clamp technique proved to be labour intensive. No conclusion was made about the benefits of perioperative tight-glucose control in reducing morbidities in patients undergoing

cardiac surgery during this study. Three years later the same team evaluated the benefits of using the clamp technique during elective coronary bypass grafting in a randomized controlled setting. Patients receiving the fixed-dose insulin therapy experienced earlier metabolic recovery of the heart, better myocardial protection and functional recovery compared to the control group (*129, 133*).

XIX. Classifying postoperative complications

Complications from surgery are defined as any deviation from the normal postoperative course. Dindo and colleagues proposed a general surgical complication classification system in 1992 that was later refined in 2004, and more recently validated as a reliable tool (*134, 135*). Their goal was to find a simple, reproducible, flexible, and general rating system applicable to all cultural backgrounds (*18, 134*). The method differentiates negative surgical outcomes into 3 classes including complications, failure to cure and sequelae (after effect of surgery). The classification system consists of 5 severity grades: Grade 1 is any deviation from normal postoperative course, Grade 2 means requiring pharmacological treatment, Grade 3 means requiring surgical intervention, Grade 4 is associated with life threatening complications and Grade 5 refers to death of a patient (*134*).

Hepatic surgery has a high incidence of complications when compared to other types of visceral operations. This bias hasn't changed despite improvements in liver surgery. According to published figures, operative mortality and morbidity rates stand at 1% – 4% and 15% – 35%, respectively for standard resections. As well, postoperative complications affect the cost of patient care due to associated longer hospital stays and higher reoperative rates.

Dixon and colleagues recently evaluated the effect of postoperative complications on disease recurrence rates and overall survival in patients undergoing hepatic resection. They found that only 13% of patients experiencing postoperative morbidity survived 5-years disease free after surgery compared to 26% of patients without postoperative complications (P = 0.001) (*136*). Sepsis proved to be an independent predictor for disease-free and overall survival in this study. Number of tumours >8, presence of an inflammatory response to tumour and receiving a blood transfusion were other factors associated with poor prognosis for disease free survival (*136*).

Thesis Manuscript I–*published*

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XX. Background for work in manuscript I

I started my PhD journey with a feasibility study where I looked at the practicality of applying the hyperinsulinemic normoglycaemic clamp (HNC) based metabolic protocol in patients undergoing major liver resections. My rational for trying this protocol on liver surgical patients stems from the recent reported drop in postoperative complications when applying the clamp protocol following open cardiac surgery. The cardiac data revealed positive effects from the clamp in managing cardiac function and the systemic inflammatory response. In considering application of the protocol during liver resections, I predicted such therapy would improve liver glycogen storage and postoperative liver function (*50, 54, 129, 133*). My goal was to assess whether or not the protocol is safe, able to maintain normal glycaemia and can be applied postoperatively in patients undergoing major liver resections.

After obtaining the McGill University Health Center ethics board approval, we performed 5 consecutive major liver resections while using the metabolic protocol.

Patients were first introduced to the study at the outpatient clinic with help of the hepatobiliary treating surgeons. The research coordinator then obtained consent and applied the randomization process. We noticed during this pilot work that obtaining patient consent and applying randomization was more easily handled at the preoperative clinic. Using this approach has

helped us resolve issues related to patient's waiting time and space limitations and also gave patients more time to understand the protocol. After consenting, the intervention patients met with the study nutritionist to go over the diet regimen they should follow on the day prior to the operation. We decided to make this appointment during the week prior to the surgery as some patients forgot protocol details while others applied this protocol on all days pertaining to their operation.

As part of our protocol we also wanted to carry out a dietary recall of all meals taken prior to the surgery to standardize dietary intake. This step proved impractical for 2 reasons; first patients did not record all intakes, and second the dieticians encountered limited availability of time and space to meet with the controls at the recovery room on the day prior to surgery.

Performing the HNC protocol during the operation proceeded easily since the study team included 3 anaesthetists who are all experts in using the clamp protocol. The clamp team provided the clinical treatment during the operation, working independently from the anaesthesia team. Similarly, the study team assisted independently from the surgeons, by collecting tissue and blood samples, documenting all operative variables, and timing all events. Giving the study team responsibility for supervising patients once arrived at the operating room also facilitated standardization of both anaesthesia and surgery, and reduced opportunity for protocol violations. We noticed that application of the HNC postoperatively in either the critical care unit or recovery room went relatively smoothly. Nursing teams attended relevant educational sessions prior to the study and an on-call person from the clamp team remained available for questions. However, applying the HNC on the regular surgical floors required the help of a separate study nurse hired by the study team specifically for managing the clamp. We organized on-call, 8-hour shifts for study nurses to cover the intervention patients during the HNC after surgery.

Blood glucose levels were measured every 5–10 min in the operating room and every 30–60 min postoperatively while applying the HNC. The protocol was verified as both safe and effective at controlling blood glucose levels both during and after surgery. To our knowledge this represents the first time this protocol has been used successfully during the postoperative period. We have not published data from this trial. The study included one type II diabetic patient with controlled blood glucose levels prior to surgery.

The demographics of the selected patients and their mean glucose levels before, during and after surgery are listed in Tables 4 and 5, respectively.

Table 4: Demographics of the 5 Patients in the Feasibility Study

Variables	Results
Sex male (%)	1 (20%)
BMI median	26.6 (19.0–30.0)
Number of resected liver segments	4 (3–6)
Duration of operation (hours)	3.35 (2.25–4.10)
Blood loss mL	1000 (500–1750)

Data are expressed as *n (%)* or median (with range).

Table 5: Glucose Results for Patients in the Feasibility Study

Variables	Blood glucose (mmol/L)
Pre-operative levels	5.3 (4.7–10.1)
Intraoperative	5.2 (5.0–5.6)
Postoperative during the clamp	4.7 (3.5–4.8)
Postoperative 48 hours	6.7 (5.3–13.2)

Data are expressed as median (with range).

None of the study patients experienced severely high or low levels of blood glucose levels while on the protocol. No incidences of hypoglycaemia appeared in the period following cessation of the insulin therapy, as a possible consequence of prolonged insulin action.

As a next step in this feasibility study I applied this protocol on a patient undergoing a major hepatectomy who lost extensive hepatic tissue during the operation. This patient was a 63-year old Caucasian woman with hilar cholangiocarcinoma. An unexpected atrophic right lobe was discovered while performing a left hepatectomy. Events that transpired in the operating room led to almost 90% removal of the hepatic parenchyma. Effectively very little functioning liver remained and we expected she would succumb to acute liver failure. We applied the damage control principals during the operation and notified the family of her poor prognosis. The patient continued receiving the insulin infusion (HNC) treatment and had an unexpected outcome as detailed below.

XXI. Manuscript I text

HEPATIC PROTECTION BY PERI-OPERATIVE METABOLIC SUPPORT?

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Running head: Glucose/insulin therapy for hepatic surgery

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Abstract

Objective: We report the case of a 63-y-old woman undergoing left hepatectomy for hilar cholangiocarcinoma who was at high risk of post-operative liver failure due to an atrophic right liver lobe. She participated in a randomized clinical trial investigating the effect of perioperative glucose infusion on hepatic function after major liver resection.

Methods: Intravenous glucose was initiated the night before the operation at 2 mg/kg/min. During and after the operation, glucose was administered together with a continuous insulin infusion until the first postoperative day. Post-operative liver function was assessed by the score proposed by Schindl, evaluating total serum bilirubin and plasma lactate concentrations, prothrombin time, and the grade of encephalopathy.

Results: The patient's liver dysfunction was classified as "mild" on postoperative day one as "none" on postoperative day two. Postoperative liver function scores were better than those observed in a control group of patients who underwent hepatic resection of similar magnitude without glucose/insulin therapy.

Conclusion: Perioperative glucose/insulin administration was associated with a surprisingly small deterioration of liver function after left lobe liver resection in the presence of an atrophic right lobe. A randomized clinical trial will have to determine whether glucose/insulin therapy can improve hepatic function after major liver resections.

Key words: surgery, liver resection, glucose, liver glycogen, liver function

Introduction

Advances in anaesthetic and surgical care have expanded the indications for major hepatectomy, i.e. the removal of three or more liver segments. Although this operation carries a relatively low mortality rate it is still associated with significant morbidity.[1,2] Depending on the extent of the resection and the definition of liver dysfunction the incidence of postoperative liver failure ranges between 20 and 70%.[2,3,4]

Low hepatic glycogen content, a consequence of preoperative fasting and surgical trauma, has been shown to limit the liver's capacity to regenerate in response to injury.[5,6] Conversely, maintaining hepatic glycogen stores by providing intravenous glucose perioperatively improved surgical outcomes.[5,7,8]

Case report

We describe a patient who underwent left hepatectomy for hilar cholangiocarcinoma and participated in a randomized clinical trial designed to study the effect of glucose infusion on hepatic function after major liver resection. Her clinical course was complicated by the unexpected finding of an atrophic right liver lobe during surgery leaving the patient with minimal vital liver tissue and a high risk of postoperative liver failure.

DB is a 63 year old, jaundiced Caucasian woman with a body mass index of 26 kg/m^2. She presented with severe jaundice and her blood tests showed: total bilirubin 386 μmol/L, alanine transaminase (ALT) 50 U/L, aspartate transaminase (AST) 55 U/L, international normalized ratio (INR) 1.17, and albumin 27 g/L. Renal function parameters and blood cell count were normal. A tri-phasic CT scan demonstrated evidence of intrahepatic duct dilatation with a cut-off sign at the proximal common bile duct (CBD). The magnetic resonance

cholangiopancreatography (MRCP) confirmed the presence of a proximal CBD mass with extension into the left duct system. An endoscopic retrograde cholangiopancreatography (ERCP) revealed similar findings and a covered plastic stent was inserted. Four weeks later the patient's total bilirubin decreased to 46 μmol/L and she was scheduled for resection of the left hepatic and caudate lobe. The patient was consented to participate in a study on the effect of perioperative intravenous glucose administration on liver function after liver resection and was randomized to the treatment group. As per study protocol, on the day before surgery, she received three meals providing a total of 35 kcal/kg (60% carbohydrate, 20% protein, and 20% fat). At 20:00, continuous infusion of glucose 10% was initiated at a rate of 2 mg/kg/min. On arrival in the operating room, an epidural catheter was inserted at the thoracic level T6/7 and bilateral segmental sensory block was produced with bupivacaine 0.5%. After induction of general anaesthesia the glucose 10% solution was replaced by glucose 20% with potassium phosphate 30 mmol/L and intravenous insulin commenced at 2 mU/kg/min in order to avoid hyperglycaemia. Blood glucose was measured every 5-10 min and the glucose infusion rate was adjusted to maintain normoglycaemia, i.e. a blood glucose between 4 and 6 mmol/L.

The laparotomy did not reveal any evidence of distant metastasis. Porto-hepatic dissection was performed and the portal triad was identified. In accordance with the preoperative imaging results a small solid mass was palpated at the proximal CBD extending into the left duct system.

The decision was made to proceed with the planned procedure, i.e. resection of the left hepatic and caudate lobe. The left liver lobe was mobilized, and the left vascular inflow was taken down together with the adjacent lymphatic tissue. Thereafter, the CBD was resected just above the duodenum and portal lymph node dissection was carried out. This facilitated better examination of the proximal bile duct, which revealed cancer extension into the secondary radicals of the

right system. There was no bile return from the right side and mucus discharge was noticed. The right liver lobe also appeared atrophic. It had to be concluded that the cancer was unresectable. The patient unfortunately had lost her functioning left liver lobe during prior dissection and the caudate lobe was the only vital segment. In light of the small amount of remaining viable liver tissue the surgical team considered urgent listing for liver transplantation, but decided to use the damage control principles. In order to minimize further trauma to the vital liver tissue, the bile ducts (anterior right and posterior right) were externally drained, the abdomen was packed open, and the patient was transferred intubated to the intensive care unit. The duration of surgery was three hours and fifty-five minutes. The estimated blood loss was 500 ml and a total of 4500 ml of crystalloid fluid was administered. The patient's relatives were informed of the high likelihood of liver failure and the potential urgent need of a liver transplant.

Epidural analgesia was maintained by the continuous infusion of bupivacaine 0.1% with fentanyl 3 μg/mL at a rate of 8-12 mL/h. On arrival in the intensive care unit, the insulin infusion rate was reduced to 1 mU/kg/min and the glucose infusion adjusted accordingly to maintain normoglycaemia, i.e. blood glucose between 4 and 6 mmol/L. In the first two hours after the operation there was no bile production. ALT peaked to 472 U/L, AST 519 U/L, INR 1.17, lactate 1.4 mmol/L, total bilirubin 47 μmol/L, alkaline phosphatase 233 U/L, and white blood cells (WBC) 13 x 10^9/L. In the following eight hours 100 mL of bile were produced. After twelve hours the patient was extubated with no signs of encephalopathy. The ALT and AST were approximately 400 U/L, INR 1.59, lactate 1.5 mmol/L, total bilirubin 44.5 μmol/L, alkaline phosphatase 218 U/L, and WBC 11.4 x 10^9/L.

By the end of postoperative day one the glucose and insulin infusions were stopped, oral food intake was commenced and well tolerated. The total amount of glucose administered was 108 g before and 170 g during and after the operation.

On postoperative day three, 400 mL of bile were produced every 8 hours and there was no evidence of encephalopathy. ALT and AST were approximately 200 U/L, INR 1.13, normal lactate, total bilirubin of 27 μmol/L, and WBC of 12 x 10^9/L. The patient returned to the operating room for reconstruction of her bile duct system. The abdomen was closed and a drain was left at the anastomosis site.

By postoperative day 7, the patient had normal liver enzymes, normal coagulation profile, and a total bilirubin of 24 μmol/L. The drain was removed on day 10 and the patient was discharged on day 15.

Discussion

The patient's postoperative liver function scores were better than those observed in a control group of patients who underwent hepatic resection of similar magnitude without receiving glucose/insulin therapy (table 1). This surprisingly uncomplicated recovery despite the minimal amount of remaining viable liver tissue lends further support to the hypothesis that the preoperative restoration of hepatic glycogen stores may prevent or attenuate hepatic dysfunction after extensive liver resection. Results from animal studies indicate that the preservation of hepatic glycogen increases the liver's tolerance to oxidative and ischemic stress.[5,6,10,11,12] In perfused rat livers, hepatocyte integrity after continuous perfusion and warm ischemia was notably impaired in glycogen depleted animals compared to animals with preserved hepatic glycogen.[5] Fasting was also associated with increased lipid peroxidation in response to liver

reperfusion which may contribute to the induction of organ failure.[11] Furthermore maintenance of hepatic glycogen content reduced the oxidative damage in both normal and fatty rat livers exposed to ischemia-reperfusion injury.[12] In fact, the extent of oxidative damage in mitochondria positively correlated with prolonged food deprivation. A beneficial effect of preserving hepatic glycogen has also been demonstrated in allogenic liver transplantation in humans as reflected in improved graft function.[13,14]

The continuous infusion of insulin may have also contributed to the patient's remarkable clinical course. Insulin, especially when administered at higher doses, exerts non-metabolic effects including anti-inflammatory, anti-oxidative and cardioprotective effects with particular benefits for patients exposed to major surgical trauma.[15]

Table 1. Liver dysfunction score as proposed by Schindl et al. [3] in patient DB versus control patients*

	Segments removed	6 h after surgery	POD 1	POD 2	POD 7
Patient DB	4	1	1	0	0
Control (n=5)	4.1	2.4 ±1.3	1.8 ± 1.9	1.6 ± 0.5	2.0 ± 2.8

Control, no metabolic therapy; POD, postoperative day. * Values are means ± SDs. Severity of hepatic dysfunction as assessed by the score proposed by Schindl et al: 0, none;1–2, mild; 3–4, moderate; >4, severe.

It is of further interest to note that this patient received epidural anaesthesia during surgery followed by postoperative epidural analgesia using local anaesthetic. Neuraxial blockade has long been recognized to suppress the counterregulatory endocrine responses to abdominal surgery, thereby reducing glycogenolysis and gluconeogenesis and facilitating the formation of hepatic glycogen.[16]

Conclusion

In conclusion, perioperative glucose/insulin administration was associated with an unexpectedly small deterioration of liver function after left lobe liver resection in the presence of an atrophic

right lobe. A large randomized clinical trial is necessary to determine whether glucose/insulin therapy can improve hepatic function after major liver resections.

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XXII. Transition to manuscript II

After the feasibility study we instituted a randomized-controlled trial (RCT) with two major objectives:

Objective 1

First we wanted to see if glucose and insulin administration while maintaining normoglycaemia using the HNC protocol coupled with preoperative carbohydrate loading could augment liver glycogen stores and improve postoperative liver function. For this part of the study we recruited 60 patients scheduled to undergo liver surgery. Patients were subdivided into 2 groups; receiving HNC (intervention) or receiving conventional insulin titration therapy (control). I measured the liver glycogen content of each patient from two separate liver biopsies taken intraoperatively and rated their liver function using a score generated by the method of Schindl et al (*23*). The preliminary results were reported as an oral presentation at the American Hepatobiliary Association (AHPBA) meeting.

1. HPB Abstract

HPB: The Official Journal of the International Hepato Pacreato Biliary Association, Feb2008 Supplement 2, Vol. 10 Issue s2, p1-27, 27p; DOI: 10.1080/13651820801953510; (*AN 31159626*) Subjects: CONGRESSES & conventions; FLORIDA; Convention and Trade Show Organizers; FORT Lauderdale (Fla.)

IMPROVING GLYCOGEN LIVER CONTENT IMPROVES POSTOPERATIVE LIVER FUNCTION IN PATIENTS UNDERGOING MAJOR LIVER RESECTIONS

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Abstracts 47

Purpose. To prevent liver dysfunction after major hepatectomy

Design. Open-label clinical trial of 40 patients undergoing major hepatectomies (3 or more segments) for the treatment of malignancy randomized to peri-operative carbohydrate (CHO) load vs. standard care.

Method. Patients in the intervention-group (n=21) received high calorie, CHO rich meals the day before the operation (35 kcal/kg, 60% CHO) followed by intravenous dextrose 10% (2 mg/kg/h) for 16 hours until arrival at the operating theatre. Intraoperatively a hyperinsulinemic normoglycaemic clamp of 2 units/kg/min was initiated. At the end of the procedure the clamp was decreased to 1 unit/kg/min and continued until 16 hours post-operatively. Dextrose 20%, supplemented with 30 mmol/L potassium-phosphate was titrated to maintain blood glucose between 4 and 6 mmol/L. The control group received routine management: fasting from midnight until 16 hours after the operation. A blood glucose level above 10 mmol/L was treated with intravenous insulin as per standard sliding scale. The preoperative preparation, intra and postoperative management, including pain control were standardized. Glycogen content in liver

tissue was measured upon exposure of the liver and prior to closure of the fascia. Postoperative liver function was compared using the Schindl functional score (lactic acid, total bilirubin, INR, and encephalopathy).

Results. There were no significant differences between groups in body mass index, percentage of residual liver volume, operative duration, intraoperative blood loss, intraoperative fluid therapy, timing of both liver biopsies, grade of steatohepatitis and fibrosis, the duration of inflow occlusion when performed, pre-operative portal embolization, underlying pathology, and the preoperative liver function tests. The intervention group patients were older 54.2 ± 9.4 vs. 63.8 ± 14.6 ($P=0.01$) and had a lower average blood glucose level 7.5 ± 0.8 vs. 5.8 ± 0.5 mmol/L ($P<0.001$). The initial liver glycogen content was 238.5 ± 94.3 in the control group vs. 380.7 ± 114.4 mmol/kg in the intervention group ($P=0.003$), and after hepatectomy it was 178.7 ± 46.3 vs. 305 ± 125.6 mmol/kg ($P=0.009$). Schindl functional scores in the control group ranged from 1 to 7 and from 0 to 4 in the intervention group. The average score was 3.15 ± 1.6 in the control group vs. 1.82 ± 0.9 in the intervention group ($P=0.02$).

Conclusion. Elevated liver glycogen content achieved by perioperative CHO loading is associated with a lower rate and severity of postoperative liver dysfunction. This may result in an improved post-operative morbidity.

Objective 2

Given the most recent publications questioning the benefit of protocols targeting strict blood glucose control and warning of the potential risk of increased mortality due to hypoglycaemia we wanted to be sure that insulin infusion using the HNC protocol is safe and achieves strict glucose

control when compared to routine sliding scale management. Therefore, the second objective of this study was to see if insulin infusion in the form of HNC therapy could maintain normoglycaemia in patients undergoing a hepatectomy without the increased risk of hypoglycaemia, in a randomized-controlled trial setting. A total of 56 patients divided into intervention and control groups were given either insulin infusion (intervention) or standard therapy (control) during and after surgery. We looked at fluctuations in glycaemia between readings taken at 30–60 min or 10–15 min intervals for standard and insulin infusion patients, respectively and compared intra- and postoperative glucose levels. The data was further segregated based on the presence or absence of diabetes mellitus (*137*).

In this trial there were no significant differences in the characteristics of the two study groups. Seven patients in each group had type 2 diabetes mellitus. A total of 268 plasma potassium levels and 1719 blood glucose levels were recorded, 422 during surgery and 1297 in the ICU.

In the control group the mean blood glucose gradually increased during surgery in non-diabetic patients and remained high in the ICU at approximately 9.0 mmol/L (P=0.029) (Figs. 4 and 6). Diabetic patients showed a mean blood glucose concentration above 8.0 mmol/L before surgery. Glycaemia slightly increased to 10.0 mmol/L toward the end of surgery (P=0.102) and remained between 9.0 and 12.0 mmol/L in the ICU (Figs. 5 and 7).

The mean blood glucose in the HNC therapy group always remained within the normoglycaemic target range. Blood glucose levels were lower during and after surgery [during surgery, P=0.003 in non-diabetics (Fig. 4), P=0.002 in diabetics (Fig. 5); after surgery, P<0.001 (Figs. 6 and 7)].

The oscillation of blood glucose was smaller in the HNC therapy group as compared to the control therapy group (SD, P=0.046 in non-diabetics; P=0.050 in diabetics during surgery.). This

was especially pronounced in non-diabetic patients after surgery (SD, $P<0.001$; CV, $P=0.027$).

Figure 4: Time dependence of intraoperative blood glucose concentrations in non-diabetic patients. Data are mean blood glucose concentration ± SD (mmol/L).

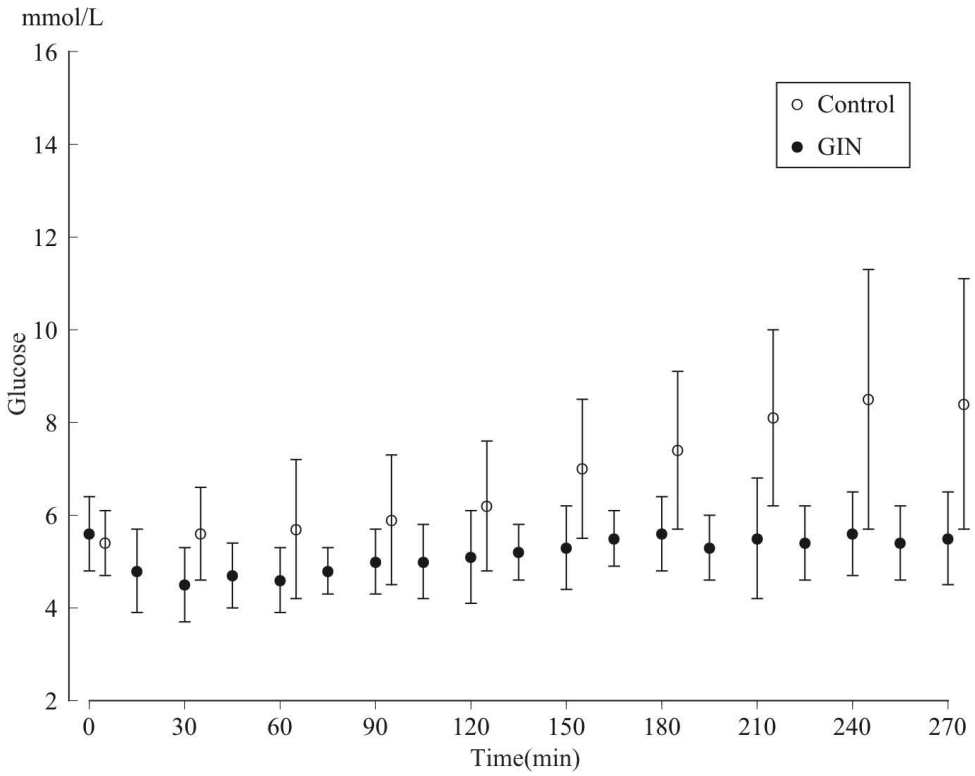

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Figure 5: Time dependence of intraoperative blood glucose concentrations in diabetic patients. Data are mean blood glucose concentration ± SD (mmol/L)

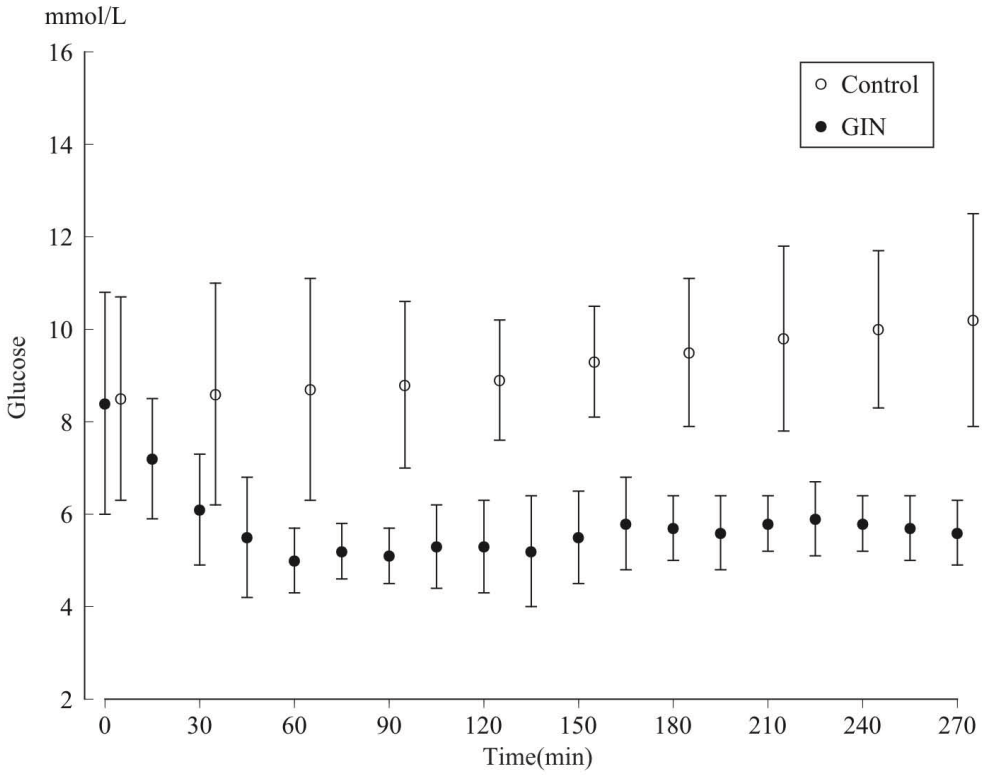

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Figure 6: Time dependence of postoperative blood glucose concentrations in non-diabetic patients. Data are mean blood glucose concentration ± SD (mmol/L).

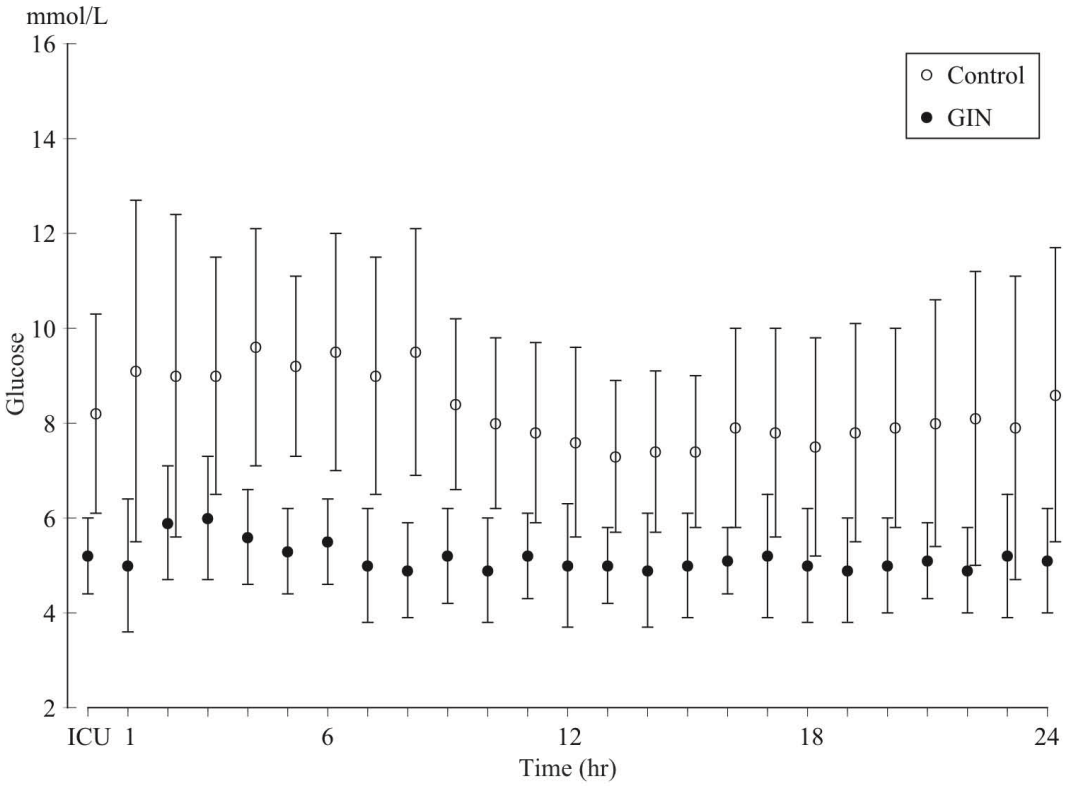

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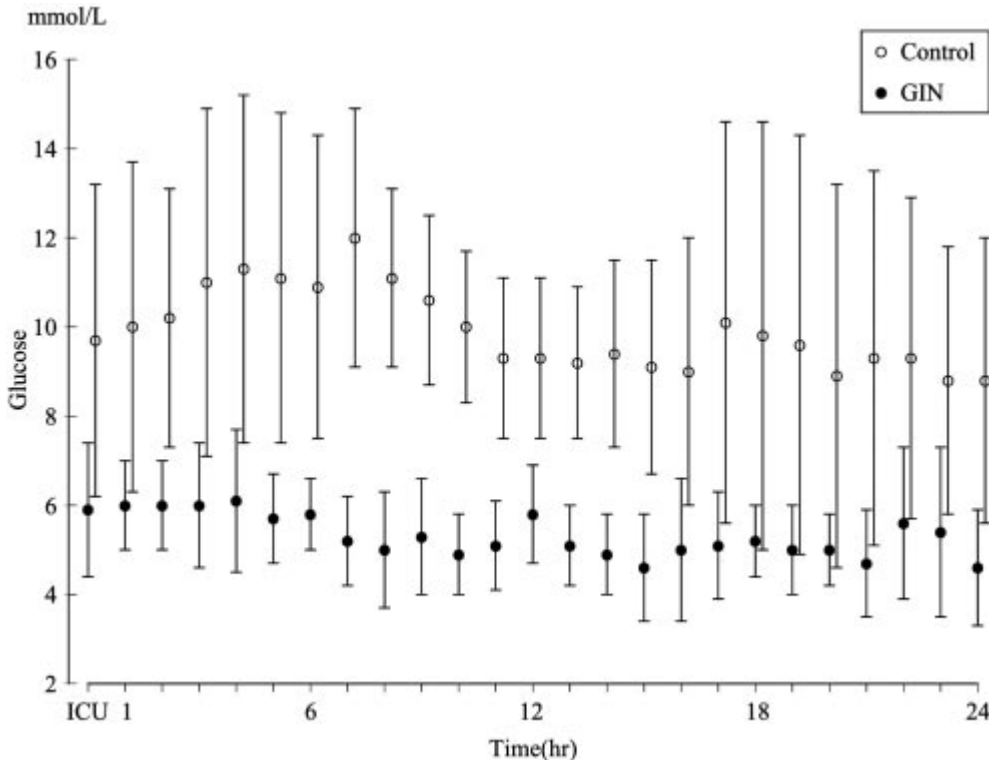
Figure 7: Time dependence of postoperative blood glucose concentrations in diabetic patients. Data are mean blood glucose concentration ± SD (mmol/L).



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Based on these results we concluded that major liver resection is associated with a moderate to severe hyperglycaemic response. We also demonstrated that insulin therapy given with the hyperinsulinemic normoglycaemic technique in safe for patients undergoing major liver resections with minimal (almost nil) risk of developing severe hypoglycaemia. This work has encouraged us to proceed with the clinical trial whereby we examined the effect of this therapy on the clinical outcomes of patients undergoing major liver resections.

Thesis Manuscript II–*submitted*

INSULIN THERAPY IMPROVES LIVER FUNCTION AND REDUCES COMPLICATIONS IN PATIENTS UNDERGOING MAJOR LIVER SURGERY

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XXIII. Background for work in manuscript II

Given the fact that insulin infusion can maintain strict normoglycaemia during and immediately after liver resections, and that preoperative carbohydrate load can augment liver glycogen content and improve liver function, we wanted to test the influence of such a metabolic support protocol in affording stronger clinical outcomes. The third aim of my research was thus to verify if the metabolic support protocol would reduce postoperative adverse events. We continued the random-controlled trial this time recruiting 60 consenting patients >18 years. Selection was based on sample size calculations obtained from our previous preliminary results.

The randomized clinical trial was designed as an open-label trial. Blinding of the intervention was discussed at length with involved clinicians. We determined that it would be impossible for us to blind therapy to either the investigators or to the patient. Instead, we established

independent study and treating teams, standardized most steps to both groups, used common pathways for postoperative course, organized an autopilot method to collect data, and reviewed all results with an independent study coordinator for accuracy. Analyses of postoperative complications were segregated into different categories including infectious vs. noninfectious, and severity as per the Clavien score. The results were collated into manuscript II and submitted for publication to the Annals of Surgery Journal.

XXIV. Manuscript II text

INSULIN THERAPY IMPROVES LIVER FUNCTION AND REDUCES COMPLICATION RATES IN PATIENTS UNDERGOING MAJOR LIVER RESECTIONS

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Trial Registration Clinicaltrial.gov Identifier: NCT00774098.

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Author Contributions: Drs. Hassanain and Metrakos conjured up the rational for the study and are responsible for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Drs. Hassanain, Metrakos, Cianflone and Schricker.

Data acquisition and Manuscript contribution:

Surgical and clinical data: Drs. Chaudhury and Hassanain, and Ms. Salman

Nutrition protocol: Ms. Molla and Dr. Wykes

Glucose insulin clamp: Drs. Schricker, Lattermann, and Carvalho

Cytokine assays: Mr. Nitschmann and Dr. Wykes

Liver glycogen analysis: Mr. Fisette and Dr. Cianflone

Liver volume calculations: Dr. Dey

Statistical analyses including the performance of the principal component analysis: Dr. Doi

Interpretation of data: Drs. Hassanain, Metrakos, Cianflone, and Doi; and Mr. Fisette.

Critical revision of the manuscript for important intellectual content: Drs. Chaudhury, Schricker, and Cianflone

Obtained funding: Drs. Metrakos and Hassanain

Study supervision: Dr. Metrakos

Writing assistance: Dr. Katharine Carpenter

No conflict of interest (applied to all investigators/authors)

Financial Disclosures: none

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Role of the Sponsors: The centers providing financial support for this trial had no role in the design and conduct of the study; interpretation of the data; or preparation of the manuscript.

Additional Contributions: We gratefully acknowledge valuable contributions from the Department of Clinical Nutrition at the McGill University Health Center, Montreal Canada.

ABSTRACT

Background: Liver resection currently represents the only curative option for patients with hepatic malignancies. Postoperative liver dysfunction is the major source of morbidity and mortality associated with these patients. In this study we test the benefits of a metabolic support protocol based on insulin infusion for reducing postoperative liver dysfunction and complications following major hepatic resection.

Method: Consecutive consenting patients requiring major liver resection were randomized to receive preoperative carbohydrate loading followed by intra and postoperative insulin therapy using the hyperinsulinemic normoglycaemic clamp protocol (intervention group) or standard therapy (control group). In the intervention group patients followed a strict dietary regimen for the 24 hours leading to surgery. Intravenous dextrose infusion was started at a rate of 2 mg/kg/min the night before and continued until the time of operation. Initiation of hyperinsulinemic therapy at 2 mU/kg/min coincided with induction of anaesthesia then continued postoperatively at 1 mU/kg/min for 24 hours. Patients were given dextrose to maintain normoglycaemia (65-110 mg/dL). The control group fasted from midnight and received a conventional insulin sliding scale during and after surgery.

Results: We studied 60 patients. Patients were similar when comparing their demographic data. The intervention patients showed a significant increase in both liver glycogen content and liver

function. Liver glycogen content measured from tissue biopsies taken initially during surgery was 430 (188-723) and 278 (48-620) mmol/kg for intervention and control patients respectively. Liver dysfunction scores were better for intervention patients ranging from 2-8 in the control group and 0-4 in the intervention group. Multivariate analysis revealed a significant reduction in overall complications and in postoperative infections in the intervention group. The incidence of any complication decreased from 26% in the control group to 17% for the intervention patients. Overall infection rates were 14% and 8% in intervention and control groups, respectively. None of the intervention group patients experienced a very high (>180 mg/dL) or very low (<40 mg/dL) blood glucose level. Several patients in the control group had either very low (36 mg/dL; n=1) or high (mean 207 (185-270) mg/dL; n=7) blood glucose readings. There were no mortalities in either group.

Conclusions: The glucose/insulin protocol improves liver glycogen content, and reduces postoperative liver dysfunction and overall complications in liver resection patients. It is safe and can be easily applied at a step-down setting

Keywords: liver resection, liver dysfunction, insulin therapy, postoperative complications

INTRODUCTION

Background

Liver resection (LR) is the only treatment of colorectal cancer liver metastasis (CRCLM) and other hepatic malignancies that can provide long-term survival and cure in selected patients.[1-3] As a result of improved imaging, better perioperative care and more effective chemotherapy

there has been an increase in liver resections. If the future liver remnant (FLR) left behind after a complete oncologic LR is judged adequate to sustain life then patients are considered resectable.

Overall operative mortality from LR has dropped to ≤2% for routine and about 7% for the complex LRs, resepectively.[4-5] However, morbidity rates remain high (17%–55%) and more than 80% of these are infections. [4, 6-9] Postoperative liver dysfunction (POLD) is of particular concern as it occurs in 20%–70% of patients (Table S8). POLD and related disturbances in the metabolic and immune body systems correlates linearly with postoperative morbidity. [6, 9-12]

While strategies used to improve the volume of the FLR have been exploited, little has been done to protect and/or enhance FLR functional quality. In fact, liver quality has deteriorated because of the rising incidence of non-alcoholic fatty liver disease [13] and the increased use of preoperative chemotherapy. [2, 14-16] If we can improve the function of the FLR then more patients will receive the benefits of LR with acceptable morbidity rates.

Perioperative fasting combined with the operative stress response promotes insulin resistance that diminishes liver glycogen, releases free fatty acids and promotes hyperglycemia.[17-18] Studies have confirmed that there is a rapid and profound transition in glucose concentration during LR.[17] Evidence suggests that such hyperglycemia in surgical patients is both a marker and a cause of adverse outcomes for diabetic and non-diabetic patients alike.[19-20] Tight-glucose control reduces postoperative morbidity, but may also increase hypoglycemic episodes and associated

risk of mortality.[21] The hyperinsulinemic normoglycemic reduces these effects and furthermore, is proven safe and effective in achieving tight perioperative-glucose control. [22] In this protocol patients receive a fixed intravenous (IV) insulin infusion based on body weight. An IV dextrose drip, titrated to blood glucose readings maintains the targeted blood glucose level. [19, 22]

Objectives

In the present study we carry out a randomized-controlled trial of patients undergoing major liver resections, comparing a novel metabolic support protocol that consists of preoperative carbohydrate loading followed by a hyperinsulinemic normoglycemic clamp to standard perioperative management of glucose. The primary objective of this study is to determine if preoperative carbohydrate loading and perioperative insulin therapy can augment liver glycogen content and decrease postoperative liver dysfunction. The secondary objective is to determine if this metabolic support decreases postoperative complications.

METHODS

Study Design and Participants

With the approval of the McGill University Health Center's (MUHC) ethics board we performed an open-label randomized-controlled trial that included all adult (>18 years old) patients scheduled for a major LR (≥3 liver segments) between June 2007 and September 2009. The study is registered at clinicaltrial.gov NCT00774098.

Exclusion criteria included all patients with type 1 Diabetes Mellitus, uncontrolled blood glucose levels (fasting level >180 mg/dL), known chronic liver disease (Child-Pugh B or C) or renal failure (need for dialysis), patients with significant anemia (<10 g/dL), patients on oral beta-blockers, calcium-channel blockers, or acetylsalicylic acid, or patients undergoing a simultaneous resection of other viscera.

Interventions

Fluid and Insulin Management

Control group

Patients were NPO from midnight onward except for water and medications. IV normal saline (NS 0.9%) infusion was started just before anaesthetic induction, and titrated to hemodynamic parameters and urine output. Arterial-blood glucose levels were checked at induction, and every 30 min thereafter with an Accu-Chek® glucose monitor (Roche Diagnostics, Switzerland). A blood glucose level above 110 mg/dL was treated with a 2U bolus of IV insulin (Humulin® R regular insulin, Eli Lilly and Company, Indianapolis, IN) followed by a 1 U/hour drip infusion adjusted according to a standard sliding scale.[23] Patients were cared for in a step-down unit for the first 24 hours (Table S1).

Intervention group

Intervention patients were instructed by a clinical dietician to follow a clearly written dietary regimen on the day (24 hours) prior to surgery. The three meals were composed of food elements

tailored to each patient. The meals provided 35 kcal/kg (ideal body weight) of which 60% was carbohydrate, 20% fat, and 20% protein. Meals were spaced 5-hours apart, and patients ate their last meal at 7 pm.[23] Compliance was checked by the study dietician. Patients who failed to complete their diet as instructed were requested at the clinical dietician's discretion to compensate with food supplements at suppertime. At 8 pm, patients began receiving IV dextrose 10% (D10W®) infused at a rate of 2 mg/kg/min (ideal body weight). Blood glucose levels were checked every 3 hours during the dextrose infusion. Subcutaneous insulin (Humulin® R regular insulin, Eli Lilly and Company, Indianapolis, IN) was administered when needed to maintain blood glucose between 72–180 mg/dL as per a sliding scale. In the operating room, the blood glucose level was checked after the insertion of the epidural catheter and prior to intubation. A 2U bolus of IV insulin was given if blood glucose level is higher then 110 mg/dL, followed by an IV infusion of 2 mU/kg/min (0.12 U/kg/hour). Dextrose 20% (D20W®) was started when arterial-blood glucose levels fell below 110 mg/dL and then was titrated to maintain blood glucose between 63–110 mg/dL. Blood glucose levels were measured at 5–10 min intervals with an Accu-Chek® glucose monitor (Roche Diagnostics, Switzerland) to ensure euglycemia. Caution was exercised at the time of parenchymal transection and during transfusion of blood products. Following surgery, the insulin infusion was reduced to 1 mU/kg/min (0.06 U/kg/hour) and continued at the step-down unit to complete the 24 hours of insulin therapy. After the operation, the arterial blood glucose level was checked every 60 min and the dextrose infusion was adjusted as per a given sliding scale.[23] In the event that an intervention patient received a blood product, the blood glucose level was checked every 30 min during the transfusion. After 24 hours, the insulin therapy was stopped and the dextrose infusion was weaned off over 30 min.

Diabetic patients resumed their preoperative treatment. Normal saline (NS 0.9%) infusion was given and adjusted to hemodynamic parameters and urine output (Tables S2, 3, 4).

Operative Details and Liver Samples

All patients received preoperative antibiotic and anti-thrombosis prophylaxis. Diabetic patients on oral hypoglycemics were asked to discontinue their therapy 24 hours prior to surgery, and those on insulin were asked to omit their evening dose. Anaesthesia induction and maintenance, including the use of epidural analgesia, were according to standard protocols.23 Phenylephrin was given to patients with low intraoperative blood pressure. Perioperative steroid and dextrose containing solutions were not used.

The first liver biopsy (300 mg) was taken with a knife from the FLR after completion of the liver mobilization and the intraoperative ultrasound but prior to parenchymal transection. The transection was performed with the ERBE Helix Hydro-Jet®, locking plastic clips and Endo-GIA staplers. Vascular inflow occlusion was rarely employed and only for hilar lesions. A second liver sample (300 mg) was taken after completing the resection, again from the FLR. The time of sample collection was documented relative to the time of skin incision.

Liver samples were snap frozen in liquid nitrogen within 5 min of procurement and stored at –80 °C until processed. Glycogen content in the liver sample was determined by subjecting it to acid

hydrolysis with 1 M HCl at 100 °C for 3 hours, neutralizing the extracts with 2M TRIS-KOH then assaying the supernatant for glucose using a Glucose Assay Kit (Sigma®).

Postoperative Clinical Pathway

Patients were given clear liquids on their first postoperative day, followed with regular diet within 24 hours as tolerated. Normal Saline (NS 0.9%®) was used for IV hydration in the first 24 hours until tolerance of oral intake. Patients were discharged when tolerating a solid diet and not requiring nursing assistance. Drains were removed on postoperative day 2 unless a bile leak was detected in which case removal was delayed until the leak stopped.

Assessments and tests

Serum and plasma samples were taken immediately preceding skin incision, just prior to parenchymal transection, after abdominal closure, and 24 hours after surgery. The arterial lactic acid, base deficit, venous insulin glucagon, and human IL-1β, IL-6, IL-8, IL-10, MCP-1 and TNF-α were measured from these samples. Cytokines were analyzed by suspension bead array immunoassay using a Luminex 200 X-map instrument (Luminex Corp, Austin, TX, USA). The cytokines were measured using a Milliplex human cytokine kit following manufacturer's specifications (MPXHCYTO-60k, Millipore Corp, Bilerica, MA, USA). All samples were measured in duplicate and the kit had a sensitivity of 0.4 pg/ml.

Additional samples were taken at 30 days before surgery (or at least three weeks after their last cycle of chemotherapy, in patients receiving preoperative therapy), and 6 h, 12 h, 24 h, 48 h, 72

h, 7 days and 30 days postoperatively to monitor complete blood count, liver enzymes and function test, coagulation profile, albumin, fibrinogen, pre-albumin, electrolytes, fasting serum blood glucose, renal function, CRP (C-reactive protein), and cortisol levels. A liver volumetric analysis was performed on all patients with a triphasic CT-scan at least 6 weeks prior to surgery or 3–4 weeks after a portal vein embolization. Standardized total liver volume (TLV) and future liver remnant (sFLR) were calculated.[24]

Definitions of outcomes

Liver glycogen measured from liver biopsies as described above, and expressed as μmoles of glucose per gram (g) of liver wet weight. Postoperative liver function was scored using the system developed by Schindl et al.[25] This system grades liver dysfunction according to the levels of lactic acid, total bilirubin, INR and encephalopathy (Table S5). Total scores of 0, 1–2, 3–4, or >4 are used to classify liver dysfunction as absent, mild, moderate or severe, respectively.[9, 25] Surgical morbidity in the 30 days following the operation were ranked as per Clavien (Dindo et al).[26] Morbidities were classified as either infectious or non-infectious (Table S6).

Sample Size

Using 30 patients in each group with an alpha error of 0.05 and an absolute reduction of 35% from an average of 70% of total moderate and sever liver dysfunction (relative reduction of 50%) will achieve at least 80% power for 2 means of proportion as calculated by nQuery advisor 6.01

program. At McGill 80 major liver resections are done per year, and so we anticipate the need of 2 years to complete this study

Randomization

Randomization was carried out with the assistance of an independent randomization center blinded to the study and orchestrated by the study coordinator, after obtaining consent. The surgical team was not involved in the randomization process.

The study coordinator collected and stored all data. Data collection and accuracy were double checked by an independent clinical research assistant. All demographics, operative data, and postoperative variables including glucose levels, degree of hepatic steatosis, steatohepatitis and fibrosis, complications, and divergence from the postoperative care pathway were collected prospectively.

Statistics

The data were expressed as means ± SD or medians and range if not normally distributed. Demographic, operative and postoperative variables were compared using the unpaired t-test or Mann-Whitney U test for continuous data. Proportions were compared by the Chi-square or Fisher Exact tests.

Since a large number of study variables were collected (174) we applied the principle component analysis (PCA) method of multivariate analysis to reduce the 174 study variables to a smaller set of uncorrelated variables that capture most of the information (variance) in the original data. Missing values were replaced with the mean. The PCs were rotated using an orthogonal rotation

- varimax normalized. We retained only components that extracted at least as much variance as the equivalent of 5 original variables (eigenvalues greater than or equal to 5). Sample factor scores (values) for each component were saved and used in a subsequent logistic regression analysis.

A P-value less than or equal to 0.05 was considered significant. All analyses were done using the Statistica version 9 statistical package.

RESULTS

Sixty of the 70 patients assessed for eligibility were randomized to the protocol including 31 patients in the insulin therapy group (intervention group) and 29 patients in the control group. We excluded 4 patients: 1 patient was unresectable and 3 had protocol violations; 2 intervention patients didn't receive their preoperative IV treatment due to hospital bed shortage, and 1 control patient received IV steroid at induction (figure 1). There was no significant difference in patient demographic, preoperative and operative data between the 2 groups (Table 1).

Figure 1: Patient distribution. Seventy patients were assessed for eligibility for the study and 60 were randomized. After randomization, 4 patients were excluded, 1 for unresectable disease and 3 for protocol violations.

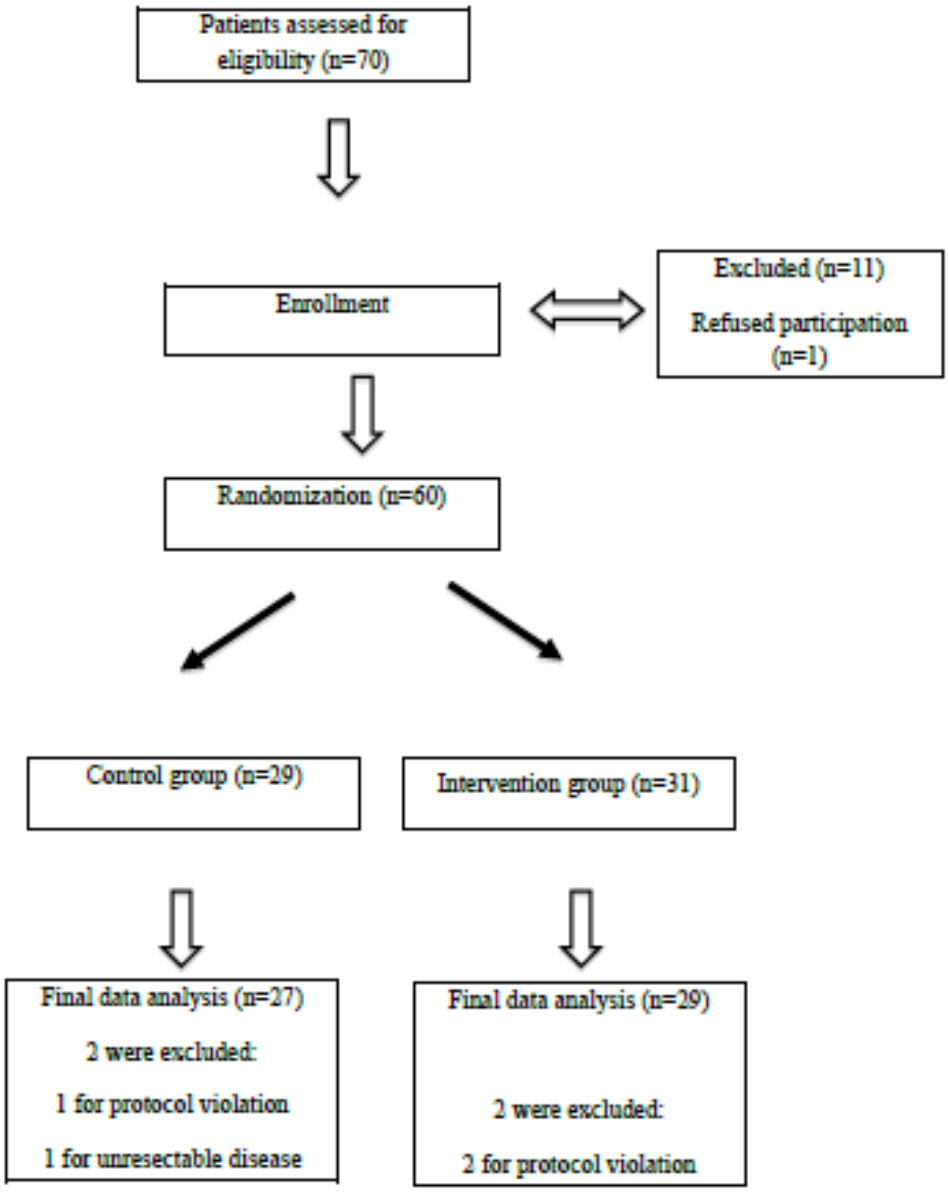


Table 1: a) General Demographics and Preoperative Characteristics of Patients in Control and Intervention Groups

Variables	Control group	Intervention group	*P*-value
Age (years)	55.5	64.0	0.09
Gender (males, n %)	16 (59)	12 (41)	0.17
Diabetes Mellitus, *n (%)*	7 (26)	7 (24)	0.26
Hypertension, n *(%)*	3 (11)	7 (24)	0.20
Heart disease, *n (%)*	0.0 (0.0)	3.0 (10.3)	0.086
Hyperlipidemia, *n (%)*	1.0 (3.7)	2.0 (6.8)	0.59
Body mass index kg/m2	26.5 (19.6–35.2)	25.5 (18.8–30.8)	0.94
Baseline lactic acid (mmol/L)	0.8 (0.4–1.9)	0.6 (0.1–1.4)	0.84
Baseline total bilirubin (µmol/L)	15.0 (7.5-121.0)	13.0 (8.0–133.0)	0.83
Baseline INR	1.0 (0.9–1.9)	1.0 (0.9–1.9)	0.37
Baseline creatinine (µmol/L)	73 (53–117)	72 (45–117)	0.55

Data are expressed as *n (%)* or median (with range).

Table 1: b) Operative Characteristics of Patients in Control and Intervention Groups

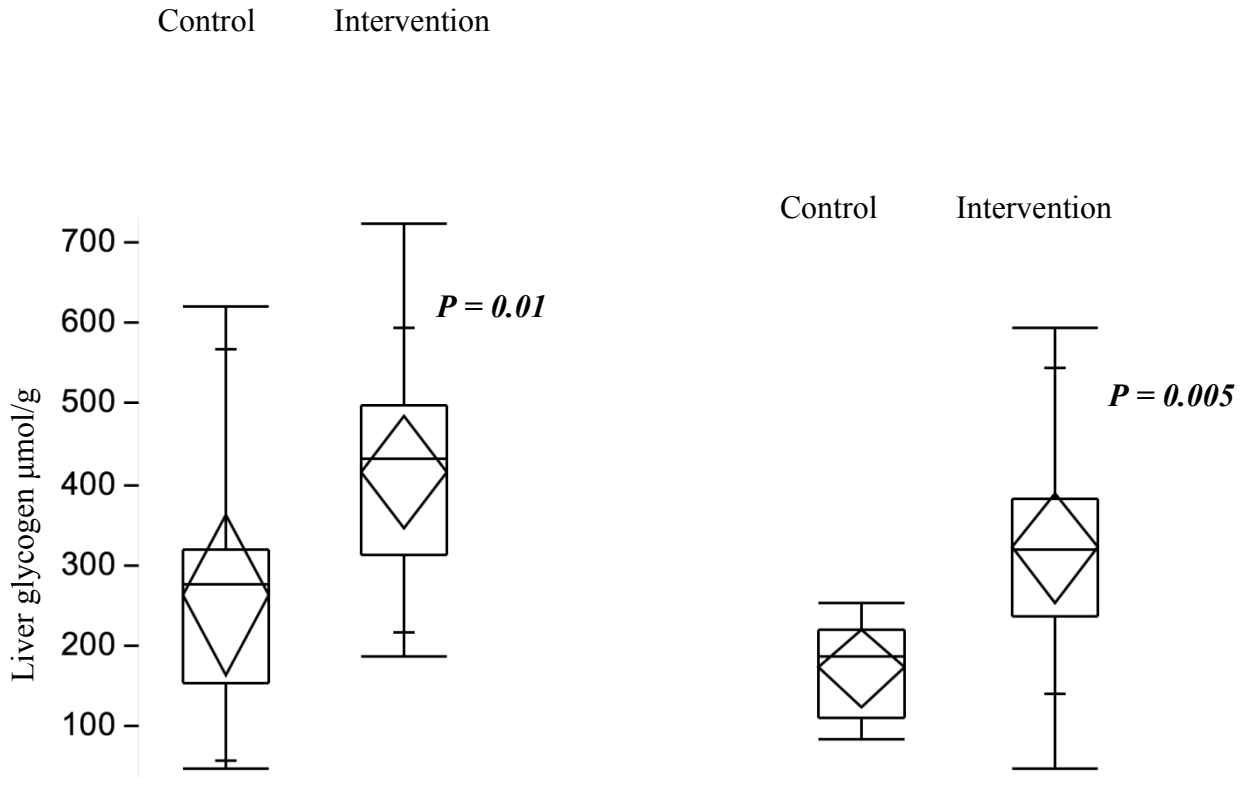
Characteristics	Control group	Intervention group	*P*-value
Rehepatectomy, *n (%)*	3 (11)	6 (20.6)	0.33
Trisegmentectomy, *n (%)*	6 (22)	11 (38)	0.20
Duration of NPO (hours)	13.5 (8.5–21.0)	12.0 (9.0–18.0)	0.26
Number of segments resected	4 (3–5)	4 (3–6)	0.49
Total liver volume (ml)	1623 (1064–2082)	1626 (1013–213)	0.06
Standardized FLR (%)	44 (21–84)	39 (17–88)	0.92
OR duration (hours)	3.0 (2.3–6.0)	3.0 (1.5–7.0)	0.44
Number of pringles	1.0 ± 3.7	3.0 ± 10.3	
Pringles duration (min)	18.00 ± 6.02	19.00 ± 5.32	0.76
Blood loss (ml)	1425 (500–5325)	1155 (400–3400)	0.11
Blood transfusion, *n (%)*	11.0 (40.7)	12.0 (41.4)	0.97
Blood transfusion (unit)	0 (0–8)	0 (0–4)	0.72
Crystalloid (L)	3.0 (1.0–6.0)	3.0 (0.5–5.5)	0.15
Colloid (L)	1.0 (0.0–1.5)	1.0 (0.0–1.0)	0.80
Fibrosis grade (0–4)	0.0 (0.0–4.0)	0.0 (0.0–3.0)	0.26
Steatohepatitis grade (0–8)	0.0 (0.0–4.0)	0.0 (0.0–2.0)	0.44

Data are expressed as *n (%)* or median (with range).

Primary outcomes

Measurements relating to the study's primary outcome are summarized in Figures 2 and 3 (Table S6). Patients on our protocol had increased liver glycogen content before and after resection compared to the control patients (430 (188-722) and 306 (37-580) vs. 278 (48-620) and 187 (83-255) mmol/kg, P-value of 0.01 and 0.005 respectively) and demonstrated less POLD according to Schindl Scores, 2 (0–4) vs. 3 (2–8), P-value of 0.03 (Figures 2 and 3). Sample timing and other tissue analyses did not reveal any significant difference.

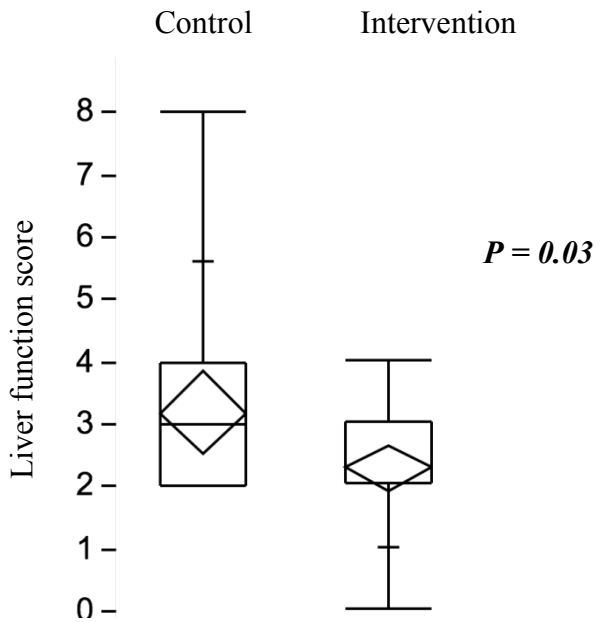
Figure 2: Quantile box plots for liver function score (Schindl et al) of patients in control and intervention groups (*P*=0.02).



a) Initial liver glycogen

b) Post-hepatectomy liver glycogen

Figure 3: Quantile box plots for liver function score (Schindl et al) of patients in control and intervention groups (*P*=0.02).



Secondary outcomes

Our protocol achieved a significant reduction in overall complications and in postoperative infections. The rate of overall complication events decreased from 26 in 27 patients to 18 in 29 patients in control and intervention groups, respectively. Infections Clavien grade 2 or higher occurred in 26% of patients in the control group and 3.4% in the intervention group. There was no mortality in either group (Table S7).

Blood glucose

Patients had statistically lower blood glucose levels while receiving insulin therapy, (Table 2). The statistical difference persisted regardless of whether the patient was diabetic or not.[23] The

intervention group patients reached targeted blood glucose levels more often and experienced fewer fluctuations compared to the control group, P=0.03 and 0.04 respectively.[23]

Table 2: Blood Glucose Level Comparison at Different Time Intervals

Blood Glucose level mmol/L	Control	Intervention	*P*-value
At baseline	5.4 (4.2–15.0)	5.5 (3.8–9.3)	0.17
Prior to surgery	6.3 (4.2–14.7)	6.2 (4.0–10.0)	0.51
Intra-operative	7.5 (5.4–13.7)	4.5 (2.3–7.9)	<0.001
Post-operative 48 hours	6.7 (4.0–9.0)	6.6 (4.3–14.5)	0.12
Post-operative 7 days	5.2 (4.0–10.0)	6.4 (4.5–11.0)	0.41
Post-operative 30 days	6.0 (4.5–12.7)	5.3 (3.0–11.0)	0.13

Data are expressed as median (with ranges).

Blood glucose levels measured at baseline, prior to surgery; and at 48 hours, 7 days, and 1 month post surgery were statistically similar between the 2 groups. None of the intervention group patients experienced a very high (>180 mg/dL) or very low (<40 mg/dL) blood glucose level. One patient in the control group developed a very low (36 mg/dL) level. There were 7 other control patients with at least 10 high blood glucose readings (mean 207 (185–270) mg/dL).

Multivariate analysis

PCA extracted 10 components from the data. The proportion of variance accounted for by these factors was approximately 52%. Of these 10 components, only PC1, PC3, PC6 and PC9 were determined to be independently associated with study intervention, infection or complications via logistic regression analysis (Table 3). The beta coefficients indicate the relative strengths of the associations in each analysis. PC1 contained postoperative levels of ALK, GGT, platelet count and serum IL-8 and may be interpreted as liver repair activity. It was positively associated with study intervention (Beta=3.10). PC3 consisted of perioperative blood glucose levels. Higher glycemic levels correlated negatively with the study intervention (Beta=–4.27). PC9 contained FLR volume and higher FLR correlated negatively with the intervention (Beta=–1.13). PC6 contained markers of POLD, total bilirubin, INR, and IL-6. The interaction variables suggested that the association between intervention and liver repair activity was blunted by either a rise in glycemic levels or an increase in residual liver volumes. POLD and higher glycemic levels associated positively with infection (Beta=1.99 and 1.49, respectively) but only higher glycemic levels were associated with all complications (Beta=0.64).

Certain groups of low yield laboratory test values were missing in up to 50% of the total values, completely at random. Missing data points were replaced with variable means. Replacing values with means is the most conservative course for assessing the data, short of dropping cases from analysis; but may restrict emergence of potential findings and/or data relationships by decreasing variability and item correlations.

Table 3: The independent Associates of Intervention, Infections and All Complications in Total Patient Group

Dominant content of each PC	Beta (Exp beta)	P-value
Associates of intervention		
PC1. Higher liver repair activity	3.19 (24.29)	0.014
PC6. Higher glycemic levels	–4.27 (0.01)	0.001
PC9. Higher residual liver volumes	–1.13 (0.32)	0.071
PC1*PC6	3.68 (39.65)	0.024
PC1*PC9	2.99 (19.89)	0.073
Associates of all infection		
PC3. POLD	1.986	0.025
PC6. Higher glycemic levels	1.488	0.004
Associates of all complications		
PC6. Higher glycemic levels	0.639	0.042

The collected variables were transformed into 10 principal components (PC). *P* values and beta coefficients are presented from a forward stepwise logistic regression run.

DISCUSSION

Perioperative dextrose supplementation augments liver glycogen stores and provides a protective effect on the hepatocyte,[27-33] but when administered alone[18] may compound hyperglycemia and increase postoperative morbidity. [19-20] Tight glucose control reduces postoperative morbidity, but also causes significant hypoglycemic complications and mortality.[21] Insulin therapy with a hyperinsulinemic normoglycemia protocol counteracts both these effects by sustaining serum glucose homeostasis, inhibiting liver glycogen and inhibiting peripheral fat breakdown. There is evidence that it may also exert an anti-apoptotic effect on hepatocytes and decrease inflammation.[34-35] We have previously reported that the hyperinsulinemic normoglycemia protocol can reduce the inflammatory response in coronary artery bypass grafting patients.[34] Our patients on the insulin therapy had significantly lower blood glucose levels, and experienced fewer fluctuations, with no incidence of severe hypoglycemia (Table 2). Our protocol thus permits replenishment of liver glycogen stores while maintaining normoglycemia, limiting the complications associated with hyperglycemia. Intervention patients in our trial also exhibited a lower rate of morbidity (there was no mortality).

In-vivo studies have demonstrated a linear relationship between liver glycogen content and liver function. Glycogen is essential for maintaining hepatocellular integrity and functions by supplying glucose for ATP generation.[31] ATP depletion leads to a series of events causing cell injury and necrosis that can be reversed with glucose supplementation.[27-31, 33, 36-37]. Our protocol achieved a significant increase in liver glycogen content and reduced POLD compared to standard glucose management (Figures 2 and 3). This intervention also decreased the postoperative complication rates (P-value 0.004) and held true for the infectious subset of complications (P-value 0.04) (Table 3).

Normal liver function is important for keeping metabolic and immune system functions intact. Failure of the liver to regenerate and/or insufficient functional FLR following surgery leads to POLD and increases the likelihood of postoperative infections and mortality.[9, 38] We observed a strong parallel between infection complications and both POLD and higher glycemic levels (Table 3). That our protocol reduced the incidence of postoperative infection is expected since it improves both POLD and glycemia control. Increased liver repair activity from insulin therapy was also enhanced by both good glycemic control and smaller FLR's (i.e. Larger volume LRs), which is consistent with in-vivo findings.[1]

Both glycemia control and hepatocellular glycogen level maintenance are necessary factors in maintaining hepatocellular integrity, and function during LR. Our protocol reduced POLD and increased liver glycogen content by maintaining a balance between these 2 factors (Figures 2 and 3). Results from this trial therefore, emphasize the importance of tight glucose control for improving FLR function.

Finally, insulin as a mode of treatment may exert its own therapeutic benefits. For example insulin improved survival of wild-type neonatal hepatocyte by preventing caspase-3 activation, pro-apoptotic gene expression and DNA laddering.[35, 39] Because insulin is a potent anti-inflammatory it may regulate the perioperative inflammatory response caused by surgery, liver manipulation, and resection.[34, 40-42] Further studies are needed to fully understand the mechanism by which this treatment strategy fulfills its clinical benefits.

CONCLUSION

We demonstrated that our novel glucose/insulin protocol achieves elevation in liver glycogen content, improves postoperative liver function and reduces overall postoperative complication

rates for patients undergoing major liver resection. The protocol appears to be safe and can be easily applied at a step-down setting.

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XXV. Supplementary material for manuscript II

Table S1: Standard Step-down Unit Insulin Infusion Sliding Scale

If blood glucose mg/dL	Action
<63	Stop insulin infusion. Give dextrose 20% (D20W ®) 10ml infusion and re-check level in 10 min
63.0–143.0	Maintain current infusion rate
144.0–180.0	Increase insulin infusion by 1 units / hour
>180.0	Increase insulin infusion by 2 units / hour

Table S2: Subcutaneous Insulin Sliding Scale for Intervention Patients Prior to Surgery

Blood glucose level	Insulin dose	Additional instructions
< 72 mg/dL	None	Call on-call MD
73.0–180.0 mg/dL	None	
181.0–216.0 mg/dL	2 units	
217.0–260.0 mg/dL	4 units	
261.0–288.0 mg/dL	6 units	
280.0–325.0 mg/dL	10 units	
330.0–360.0 mg/dL	12 units	
>360mg/dL	14 units	Call on-call MD

Table S3: Standard Insulin Infusion Sliding Scale While in the Operating Room

If blood glucose mg/dL	Action
<63	Stop insulin infusion. Give dextrose 20% (D20W ®) 10ml infusion and re-check level in 10 min
63.0–110.0	Maintain current infusion rate
111.0–143.0	Increase insulin infusion by 1unit / hour
144.0–180.0	Increase insulin infusion by 2 units / hour
>180.0	Increase insulin infusion by 3 units / hour

Table S4: Dextrose Sliding Scale during the Insulin Infusion in the Postoperative Period

Blood glucose level mg/dL	Dextrose infusion	Additional instructions
<63	↑ D20W by 15 ml/h, and give 20 ml of D20W bolus	Call research MD on-call
63.0–74.0	↑ D20W by 10 ml/h, and give 10 ml of D20W bolus	
75.0–81.0	↑ D20W by 5 ml/h	
82.0–98.0	Maintain same rate	
99.0–110.0	↓ D20W by 5 ml/h	
111.0–116.0	↓ D20W by 10ml/h	
>126	↓ D20W by 50%	Call research MD on-call

Table S5: Liver Function as Per Score Generated by Schindl et al[1]

Total serum bilirubin (μmol/L)	<20	20–60	>60
Prothrombin time (INR)	<4.0 (1.8)	4.0–6.0 (1.8–2.3)	>6.0 (2.3)
Serum lactate (mmol/L)	<1.5	1.5–3.5	>3.5
Encephalopathy grade	None	1 and 2	3 and 4
Given points	0	1	2

[1]No dysfunction when score is equal to 0 points, mild when 1–2, moderate when 3–4, and Severe dysfunction when >4 points.

Table S6: Criteria for Classifying Morbidities as Infectious or Noninfectious

Noninfectious Characteristic	Description
Blood Glucose	>10 mmol/L (hyperglycemia)
	<2 mmol/L (hypoglycemia)
PONV	Persistent beyond day 2 postoperative
Bleeding	Requiring surgical intervention
	Hemoglobin drop >4 mg/dL
Cardiac event	Symptomatic arrhythmia
	Blood troponin >0.5, with ECG changes
Pleural effusion	Tapping required for patient relief
Abdominal	Ascites: dyspnea or leaking through abdominal wall
Acute renal failure	Serum creatine >2 x upper limit of normal
Bile leak	Body fluid bilirubin content >2 x upper limit of serum level
Infectious Characteristic	**Description**
Pneumonia	Pneumonic or atelactic changes on chest radiographs with positive sputum culture
Wound infection	Erythema and indurations associated with positive bacterial culture
Intra-abdominal abscess	Collection of pus in the abdomen with or without necrotic material associated with a positive bacterial culture
Urinary tract infection	Urinary symptoms with urine culture positive for bacterial growth >105 colony forming units/ml
Central line sepsis	Positive culture of the catheter tip >15 colony forming units in the presence of a febrile episode

PONV: postoperative nausea and vomiting

Table S7: Distribution of Complications between Intervention and Control Groups

Complication	Type of complication	Intervention group (n)	Degree as per Clavien for each patient	Control group (n)	Degree as per Clavien for each patient	P-value
Non-infections	PONV	2	1, 3	5	1, 1, 1, 1, 1	NS
	Ascitis/ Plural effusion	2	3, 1	2	1, 3	NS
	DVT			1	4	NS
	Bile leak	2	1, 1	3	1, 1, 1	NS
	ARF	2	1, 1	1	4	NS
	Cardiac event	1	3			NS
Infections	Wound infection	5	1, 1, 1, 1, 1	4	1, 1, 3b, 1	NS
	Central line infection			3	2, 2, 2	NS
	UTI	2	1, 1	2	1, 1	NS
	Pneumonia			1	2	NS
	Intra-abdominal abscess	1	3	1	2	NS
	Other			3	4, 2, 2	NS

PONV: postoperative nausea and vomiting, DVT: deep vein thrombosis, ARF: acute renal failure, UTI: urinary tract infection

Table S8: Morbidity and Mortality Rates in Selected Studies[1]

Author	Years	N	Mortality (%)	Morbidity (%)
Fan	1994	64	8	55
Cohnert	1997	340	4	22
Belghiti	2000	747	4,4	22
Jarnigan	2002	1803	4	52
Laurent	2003	311	3	30
Stewart	2004	137	2.9	19.7
Wei	2006	423	1.6	17
Palaveciono	2009	1157	NA	26

[1]Numbers are for overall or major liver resections when specified.

Table S9: Tissue Analysis of Patients in Control and intervention Groups

Characteristics	Control group N=27 Median	Range	Intervention group N=29 Median	Range	P-value
Liver sample #1 time (hours from skin incision)	1.0	0.5–3.0	1.1	0.5-3.4	0.88
Liver sample #2 time (hours from skin incision)	2.8	1.5–5.5	2.8	1.0–6.5	0.64
Liver glycogen sample #1 (μmol/g)	278.0	48–620	430.0	188–722	0.01
Liver glycogen sample #2 (μmol/g)	187.0	83-255	306.0	37-580	0.005

Thesis Manuscript III–*submitted*

Altered Inflammation, Reduced Apoptosis and Increased Cell Proliferation from High-Dose Insulin Therapy Reduces Postoperative Liver Dysfunction and Complications in Liver Resection; Alexander Fisette & Mazen Hassanain, Peter Metrakos, Suhail A. R. Doi, Ayat Salman, Thomas Schricker, Ralph Lattermann, Linda Wykes, Evan Nitschmann, Jessica Smith and Katherine Cianflone—*submitted to JCEM*

XXVI. Background for work in manuscript III

As the final aim for my thesis work I wanted to study the protocol's potential influence on inflammation, metabolism and liver regeneration pathways following liver surgery. The objective of this part of the thesis is to define the possible mechanisms by which the protocol has produced its effects. This information will provide viable incite towards developing targeted therapies tailored to patients' pre- and intraoperative status. For this part of the study tissue and serum samples were collected from surgical patients and analyzed. A number of biomarkers served to assess effects of the protocol including:

1. Inflammation: Serum levels of IL-4, 6, 8, 10, MCP-1, TNF-α, and C-RP
2. Metabolism: Glycogen content in liver and muscle tissue samples, triglycerides, protein levels, serum cortisol, glucagon, insulin, glucose, free fatty acids, adiponectine, and adipsin

3. Liver regeneration: Growth hormone, TGF-β, and gene array of tissue samples representing different cell cycle stages

This project was carried out in collaboration with a team from the Université Laval Biochemistry Department.

XXVII. Manuscript III text

ALTERED INFLAMMATION, REDUCED APOPTOTOSIS AND INCREASED CELL PROLIFERATION FROM HIGH-DOSE INSULIN THERAPY REDUCES POSTOPERATIVE LIVER DYSFUNCTION AND COMPLICATIONS IN LIVER RESECTION PATIENTS

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*Shared first co-authorship

Abbreviated Title

Insulin reduces complications from liver resection

Key Words

Insulin, liver resection, and liver dysfunction

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Disclosure Summary

The authors do not have any financial or commercial interest in the subject matter, materials, or equipment discussed or in competing materials.

Clinical Trial Registration Number

The study is registered at clinicaltrial.gov NCT00774098.

Author Contributions: Drs. Fisette, Hassanain and Cianflone conjured up the rational for the study and are responsible for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Drs. Hassanain, Metrakos, and Cianflone; and Mr. Fisette.

Data acquisition and Manuscript contribution:

Surgical and clinical data: Dr. Hassanain and Ms. Salman

Glucose insulin clamp: Drs. Schricker, and Lattermann

Cytokine assays: Mr. Nitschmann and Dr. Wykes

Liver glycogen, growth factors, cytokine, and mRNA analyses, and complement measurements: Mr. Fisette and. Cianflone

Statistics including the application of the principal component analysis: Dr. Doi

Interpretation of data: Drs. Hassanain, Metrakos, Cianflone, Doi, and Mr. Fisette

Critical revision of the manuscript for important intellectual content: Drs. Chaudhury, Schricker, and Cianflone

Obtained funding: Drs. Metrakos and Hassanain

Study supervision: Dr. Metrakos.

Writing assistance: Dr. Katharine Carpenter

ABSTRACT

Background: An exaggerated inflammatory response in patients undergoing liver resection (LR) coupled with poor nutrition diminishes the liver's regeneration capacity, and increases the risk of postoperative complications.

Objectives: The study aimed to correlate circulating or genetic markers of inflammation, proliferation and apoptosis with clinical outcomes in patients undergoing LR.

Design: Sixty consecutive consenting LR patients were randomized to receive perioperative dextrose infusion plus insulin therapy (intervention), or standard therapy. Ten patients were randomly chosen from each group for biochemical and genetic assessment.

Main Outcome Measures: mRNA for 100 genes related to insulin signalling, inflammation, proliferation, complement system and apoptosis were quantified using real-time PCR. Cytokines were analyzed with a suspension bead array immunoassay and adiponectin, adipsin GH, TGF-β, C3, C5a and ASP were measured with ELISA. We applied principle component analysis (PCA) to compartmentalize our large number of study variables.

Results: Proinflammatory mediators IL-6, IL-8, TNF-α, and MCP-1 increased acutely in both groups perioperatively, but more significantly in intervention patients. Upregulation of protective anti-apoptotic genes BCL2 ($P = 0.01$) and BCL2L1 ($P = 0.02$) occurred in intervention patients while control patients had higher levels of pro-apoptotic genes, BAX ($P = 0.04$), Caspase 8 ($P = 0.004$) and Caspase 9 ($P = 0.01$). PCA revealed the following associations: Tight-glucose control with activation of glucose/insulin genes preoperatively ($P = 0.03$), and with increased proinflammatory factors intraoperatively ($P = 0.05$), preoperative inflammation with liver dysfunction rate ($P < 0.1$), preoperative activation of insulin-responsive genes with infection (P

= 0.07), and increased proliferative gene activation and decreased apoptosis-related gene expression with reductions in complications, postoperatively ($P = 0.07$).

Conclusion: The benefits of our protocol are mediated through upregulation of insulin-responsive genes prior to and proliferative genes after surgery, as well as altered levels of proinflammatory factors throughout the operation.

INTRODUCTION

Liver resection (LR) is the only treatment capable of providing long-term survival and cure for selected patients with colorectal cancer liver metastasis (CRCLM) and other hepatic malignancies (*1-3*). The indications for liver surgery have expanded over the years due to advances in perioperative care and availability of detailed imaging methods for accurately assessing tumour load (*3-5*). Mortality from hepatic surgery has declined remarkably over the past 15 years to less than 2% for the routine resection, but postoperative morbidity rates remain high at 20%–50% (*6-12*).

The liver's capacity to regenerate following major hepatic surgery depends on the magnitude of the resection, the severity of the surgical stress response and the levels of glycogen stored in the hepatocytes (*13-20*). Tissue trauma from surgery triggers an inflammatory response that causes endothelial and epithelial cells as well as neutrophils, macrophages and lymphocytes to stimulate the secretion of proinflammatory mediators TNF-α, IL-1β and IL-6. Severe trauma can lead to persistent upregulation of cytokines and failure of the host defence system marked by increased levels of C-reactive protein (C-RP), IL-6 and acute phase protein in the patient (*13, 19-21*). Suppressed immune function and liver dysfunction brought about by continued upregulation of cytokines, particularly TNF-α and IL-6, renders patients more susceptible to postoperative infection, morbidity and mortality. The complement system also contributes to the stress response and has been shown to play an important role in the initiation of hepatocyte regeneration in animal models (*22-28*). For example, He at al. proposed existence of a complement activation threshold in deciding regenerative capacity of the liver. According to their results inhibition of C3a activation reduced hepatocyte regeneration whereas increasing C3a/ASP production enhanced the hepatic proliferative response in mice (*29*).

Hepatic surgeons want to see initiation of a proliferative response after partial hepatectomy rather than necrosis and apoptosis, which originate from hepatic dysfunction related complications (*30-35*). Adequate nutrition coupled with proper orchestrated interplay between pro- and anti-inflammatory mediators maintains cellular integrity by ensuring a continued energy supply for hepatocyte regeneration (*18, 36-37*). An exaggerated proinflammatory response compounded by preoperative fasting diminishes the livers energy stores and culminates in an insulin resistant state in the patient (*38-39*). This puts the patient at higher risk for developing hyperglycaemia and leads to postoperative liver dysfunction, increased morbidity and complications, especially infections (*40*).

In theory, insulin therapy should reduce trauma related insulin resistance, increase glycogen stores, provide an anti-inflammatory effect and improve the immune systems defence against infection (*41-43*). Potential benefits of intensive insulin therapy to target tight glucose control particularly for surgical patients have been published by Van den Berg and others (*42, 44-45*).

We recently initiated a randomized-controlled trial that looked at the potential benefits of insulin therapy combined with preoperative carbohydrate loading for reducing episodes of hypo- and/or hyperglycaemia in patients undergoing hepatic surgery. Our protocol involves oral and intravenous carbohydrates given preoperatively coupled with perioperative application of the hyperinsulinemic normoglycaemic clamp protocol (*46*). Significantly better glucose management accompanied by reduced postoperative liver dysfunction and overall complications occurred for intervention patients in this trial compared to control patients who received routine management and a conventional sliding scale insulin therapy.

The aim of our study was to develop a better understanding of the mechanisms through which these positive clinical outcomes were obtained. Alterations in circulating or genetic molecular markers resulting from the adaptive stress response to surgery were assessed and independently correlated with clinical parameters.

MATERIALS AND METHODS

Patients

Sixty consenting patients undergoing liver surgery were randomized to receive nutritional therapy coupled with tight glucose control (intervention) or standard insulin therapy (control). Intervention patients were given a 24-hour preoperative carbohydrate load at 35kcal/kg/day followed by dextrose infusion at 2mg/kg/h for a total of 8 hours. Insulin therapy was then initiated in these patients with the hyperinsulinemic normoglycaemic clamp and involved administering 1.2U/kg/h intraoperatively and 0.06U/kg/h postoperatively for a total of 24 hours. Controls fasted preoperatively for 8 hours then received standard sliding scale insulin therapy. Ten patients were randomly selected from each group for inflammatory mediator, and genetic expression testing.

Assessments and tests

Blood samples were taken from study patients during the morning of the operation just prior to starting the insulin clamp. A second sample was taken 2 hours into the operation and a third one at the end of surgery. We withdrew the last sample 24 hours from the time of first withdrawal. Blood samples were immediately divided into 1 mL sub samples and stored at –80 °C. Complement factors C3 and C5a, Adiponectin, Adipsin, growth hormone and TGF-β were measured with commercial ELISA kits (BD OptEIA human C5a ELISA kit, BD Biosciences, C3 Complement Assay, Kamiya Biomedicals, Custom Human Quantibody Array, Raybiotech) and ASP (C3adesARG) was quantified with an in-house sandwich ELISA, following previously published methodology (*47*).

Cytokine analysis

Human IL-6, IL-8, IL-10, MCP-1 and TNF-α were measured by suspension bead array immunoassay with a Luminex 200 X-map instrument (Luminex Corp, Austin, TX, USA). Analysis of the cytokines was carried out using a Milliplex human cytokine kit following manufacturer's specifications (MPXHCYTO-60k, Millipore Corp, Bilerica, MA, USA). All samples were analyzed in duplicate and the kit had a sensitivity of 0.4 pg/mL. Concentrations were calculated from the standard curve generated by the MasterPlex QT 4.0 analysis software (MiraiBio Inc, Alameda, CA, USA).

Tissue biopsies

We removed 2 tissue samples from the liver FLR; one at the time of incision and one at the end of surgery. Samples were snap-frozen and stored at –80 °C. mRNA was extracted and purified from the frozen tissues using RNeasy mini kits (Qiagen, Gaithersburg, MD, USA) then reverse

transcribed into DNA with a RT2 First Strand kit (SA Biosciences, Frederick, MD, USA). Both steps followed manufacturer's instructions. We quantified 84 genes from each sample using Human Insulin Pathway PCR array cycle time (Ct) measurements (SA Biosciences, Frederick, MD, USA). Genes for C5L2, C3aR, C5aR, Caspases 8 and 9, BAX and BCL2 were measured individually with QuantiTect primers (Qiagen, Gaithersburg, MD, USA). Relative gene expression was calculated and corrected from measurement of 5 housekeeping genes included in the PCR array. All procedures followed MIQE guidelines (*48*).

Statistics

The data were expressed as means ± SD or medians and interquartile range if not normally distributed. Demographic, operative and postoperative variables were compared using the unpaired t-test or Mann-Whitney U test for continuous data. Proportions were compared by the Chi-square or Fisher Exact tests. We applied a principle component analysis (PCA; Statistica version 9 statistical package) to reduce our large number of study variables (136) to a smaller set of uncorrelated variables containing most of the information in the original data. Missing values were replaced with the mean. The PCs were rotated using an orthogonal rotation - varimax normalized and only those components with eigenvalues ≥ 5 were retained. Sample factor scores (values) for each component were saved and used in a subsequent logistic regression analysis. We considered a *P*-value ≤ 0.1 as significant.

RESULTS AND DISCUSSION

Baseline data

Intervention and control patients had comparable demographics with a few exceptions, and both groups exhibited similar baseline liver function and perioperative characteristics (Table 1 and Supplementary Tables S1–S2). We found a greater incidence of hypertension and a borderline higher serum creatinine (renal dysfunction) at baseline in our study cohort. Intervention patients were older and there were non-significantly more males in this group (Table 1). Since older age is a well-known risk factor for liver dysfunction this difference may have created some biases in the study data. In addition, the smaller sample size used in the trial segment described here may explain why the demographics of the original randomized groups were significantly more alike (*46*).

Clinical outcomes

We noted better clinical outcome markers in the intervention patients. These data were reported previously and will only be summarized here (*49*). Intervention patients had better liver function scores as per Schindl (*50*), 2 (range 0–4) compared to 3 (range 2–8) for the controls, ($P = 0.03$) and the rate of overall complication events decreased from 26 in 27 patients to 18 in 29 patients in control and intervention groups, respectively. Infections Clavien grade 2 or higher occurred in 26% of patients in the control group and 3.4% in the intervention group. PCA established significant associations between liver repair activity and intervention, and between postoperative liver dysfunction and infections.

Table1. Demography of patients

Variable	Intervention (n=10)	Control (n=10)	P value
Age	71 (67–82)	53 (45–59)	*0.006*
DM	1.0 (10%)	None	0.38
HTN	4.0 (40%)	None	*0.04*
IHD	2.0 (20%)	None	0.2
High Cholesterol	2.0 (20%)	None	0.2
Sex male %	5.0 (50%)	2.0 (20%)	0.3
BMI	26.7 (25.5–30)	25.4 (21.0–25)	0.34

Data are reported as median and (interquartile range) or number and (percentage).

Table S1. Laboratory tests at baseline: hepatic and renal function

Variable	Intervention	Control	P value
FLR (mL)	764 (523–1014)	578 (521–639)	0.3
TLV (mL)	1765 (1573–1792)	1440 (1232–1678)	0.1
FLR/TLV ratio	40 (29–75)	41 (33.6–48.7)	0.9
Base deficit	1.8 (0.1–4.0)	0.7 (-0.7–1.4)	0.2
Lactic acid mg/dL	9.0 (5.9–10.8)	7.2 (6.3–8.1)	0.2
[mmol/L]	[1.0 (0.65–1.2)]	[0.8 (0.7–0.9)]	
Total Bilirubin mg/dL	0.8 (0.7–4.4)	1.2 (1.0–1.9)	0.3
[μmol/L]	[13 (12–76)]	[21 (17–32)]	
INR	1.0 (1–1)	1.0 (1–1)	0.5
Creatinine mg/dL	1.1 (1.1–1.4)	0.9 (0.8–1.0)	0.05
[μmol/L]	[84 (81–104)]	[69 (58–75)]	

Data are reported as median and (interquartile range).

Table S2: Intraoperative characteristics

Variable	Intervention	Control	P value
Trisegmentectomy	3.0 (30%)	2.0 (20%)	0.9
# of segments removed	4.0 (3.0–4.5)	4.0 (3.5–5.0)	0.7
Duration of OR	3.0 (2.6–3.4)	3.0 (2.7–3.8)	0.7
Blood loss	1100 (500–1800)	1300 (750–2500)	0.4
Blood Transfusion	None	2.0 (20%)	0.1
Units of RBC given	0.0 (0–0)	0.0 (0–2)	0.1
Crystalloid (L)	3.0 (1.5–4.0)	3.8 (2.5–4.0)	0.4
Colloid (mL)	500 (400–1000)	500 (500–1000)	0.7
Pringles (min)	0.0 (0–10)	0.0 (0–7)	0.8
Fibrosis (0–4)	0.0 (0–0)	0.0 (0–2)	0.3
Steatohepatitis (0–3)	1.0 (1–1)	0.0 (0–2)	0.4

Data are reported as median and (interquartile range) or number and (percentage).

Primary outcomes

Complement system is not affected

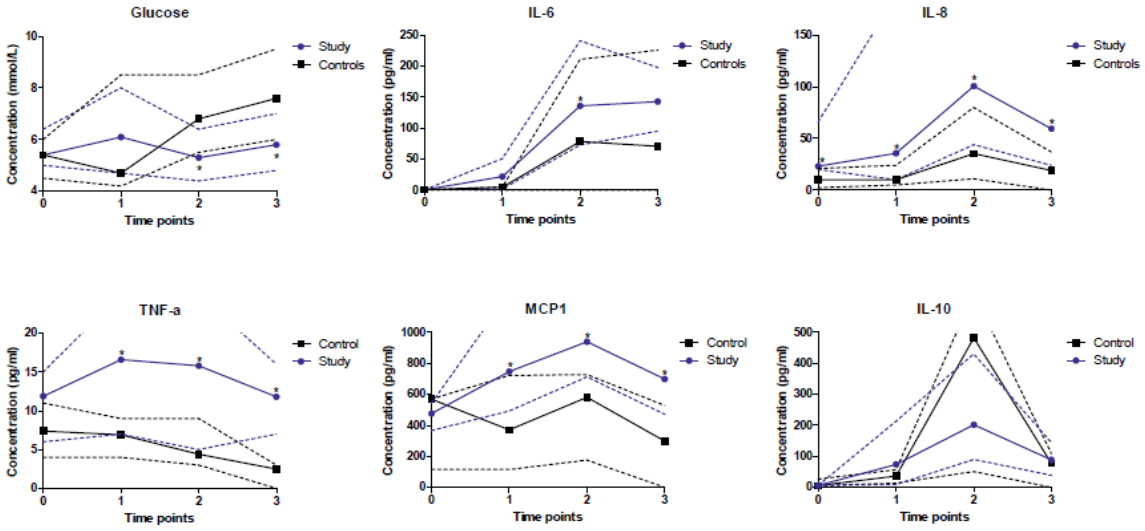
Studies reporting association between blood glucose levels and complement activation are conflicting (*51-56*). In some studies increased complement function associated with higher circulating glucose levels while other studies have shown an opposite trend. Regardless, alteration in complement function consistently resulted from either hyper- or hypoglycaemia. That we did not observe any major intergroup differences in levels of circulating or genetic parameters related to the complement system (C3, ASP, C5a, C5aR, C3aR, C5L2) indicates that neither group experienced blood glucose fluctuations sufficiently out of normal range or often enough to influence complement function. Absence of complement function alterations in adult coronary artery bypass grafting (CABG) patients receiving strict glucose control was also observed by Hoedemakers, in agreement with our findings (*57*). The absence of modulation in complement activation in addition to lack of a relationship between complement factors and clinical outcomes suggests that the beneficial effects of our protocol are not related to the complement system.

A recent study that looked at changes in complement function occurring after LR reported increases in C3a, and C5b9 by 34% and 112%, respectively, while C4a and C5a levels decreased by 25% and 30%, respectively (26). The authors did not mention whether or not patients received glucose control therapy. In our study, we also found changes in complement factors after surgery: while C3 levels decreased significantly by –43%, ($P < 0.0001$), C5a levels were mildly but not significantly reduced (–27%, $P = 0.39$) in intervention and control patients alike.

Inflammatory mediators and glucose balance

Proinflammatory mediators such as IL-6, IL-8, TNF-α and MCP-1 increased acutely for both the intervention and control groups during the resection while IL-1β was undetectable, as would be expected for patients undergoing major surgery (*30, 33, 58*). However, the intervention group exhibited significantly higher levels of proinflammatory cytokines at most data collection time points after initiation of surgery, as seen in Figure 1. We observed an opposite trend in circulating levels of glucose and the anti-inflammatory factor IL-10. While initial glucose levels were comparable in intervention and control patients, a rise in serum glucose concentration was detected throughout the surgery for the controls only (Supplementary Table S3).

Figure 1: Perioperative concentration of glucose and cytokines; T0 = baseline, T1 = 2h into surg., T2 = end of surg., T4 = 24h after surg. Values are shown as mean ± range.



Interestingly, PCA analysis associated these two findings: a component (PC7), representing activation of glucose/insulin-sensitive genes prior to surgery and perioperative release of circulating proinflammatory molecules, negatively associated with the control group. This would suggest that a more pronounced stimulation of glucose metabolism by insulin leads to stable

blood glucose throughout the surgery and permits a stronger acute rise in proinflammatory factors known to initiate liver regeneration.

Table S3: Glucose levels at different time intervals in mg/dL [mmol/L]

Variable		Intervention	Control	P value
Prior to surgery	Baseline	97 (93–108) ([5.4 (5.2–6.0)]	97 (83–108) [5.4 (4.6–6.0)]	0.8
	Preinduction	110 (85–117) [6.1 (4.7–6.5)]	85 (79–135) [4.7 (4.4–7.5)]	0.6
During the surgery	1 hour	95 (94–108) [5.3(5.2–6.0)]	123 (110–144) [6.8 (6.1–8.0)]	*0.02*
	3 hours	105 (94–117) [5.8 (5.2–6.2)]	137 (114–162) [7.6 (6.3–9.0)]	*0.04*

Data are reported as median and (interquartile range).

Preoperative status influences the clinical outcomes

Published results consistently show a strong association between preoperative state of the patient and major clinical outcome parameters. We have shown previously in this clinical trial that hepatic glycogen levels in intervention patients were elevated compared to control subjects prior (+64%, $P = 0.006$) and after (+36%, $P = 0.04$) the surgery (*49*). In the present investigation PCA analysis revealed that other preoperative parameters are associated with clinical benefits. A component (PC4) representing activation of insulin-responsive genes related to PI3K and MAPK pathways prior to surgery, negatively associated with the presence of infectious complications. Additionally, a group of variables (PC8) describing preoperative circulating levels of inflammatory related molecules such as IL-6, TNF-α and IL-10 correlated with postoperative liver dysfunction ($r=0.526$, $P < 0.01$). These observations suggest that the preoperative state of the patient independently associates with clinical outcomes and strongly advocates further studies for investigating a potential causal relationship.

Postoperative status influences the clinical outcomes

Cell death that occurs through apoptosis represents a major complication from LR (*32, 34*). Results for the intervention patients clearly show that the insulin therapy protocol provides an anti-apoptotic protective effect on the hepatocytes. Genes protecting against programmed cell death such as BCL2 ($P = 0.01$), BCL2L1 ($P = 0.02$) were increased in intervention patient liver biopsies. By contrast, proapoptotic genes such as BAX ($P = 0.04$), Caspase 8 ($P = 0.004$) and Caspase 9 ($P = 0.01$) were higher in control patients (Figure 2). These findings suggest that the intervention protocol achieves its clinical benefits partly through a reduction in hepatocytes apoptosis.

PCA analysis classified into one independent component (PC1) a vast set of factors describing proliferation and survival at the end of surgery that mainly occurs through PI3K and MAPK pathways. This component negatively associated with the presence of complications therefore emphasizing the importance of MAPK and PI3K pathway mediated cell proliferation for reducing postoperative complications.

A summary of associations observed between clinical outcomes and biological markers throughout the surgery is summarized in Figure 3. Many of the published risk factors for POLD and complications are unchangeable like age or male gender, or unavoidable such as major resection or blood transfusion. This study explores a potential method that can alter and modify LR response and promote improvements in surgical outcome. We intend to explore the long-term benefit of this therapy on cancer patients in a larger trial setting.

Figure 2. Relative apoptotic gene expression levels after liver resection for intervention (open bars) and control (opaque bars) patients.

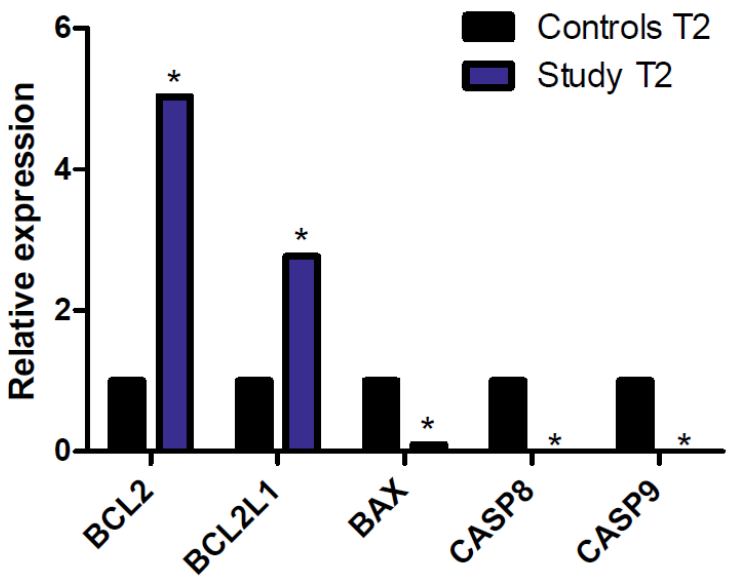
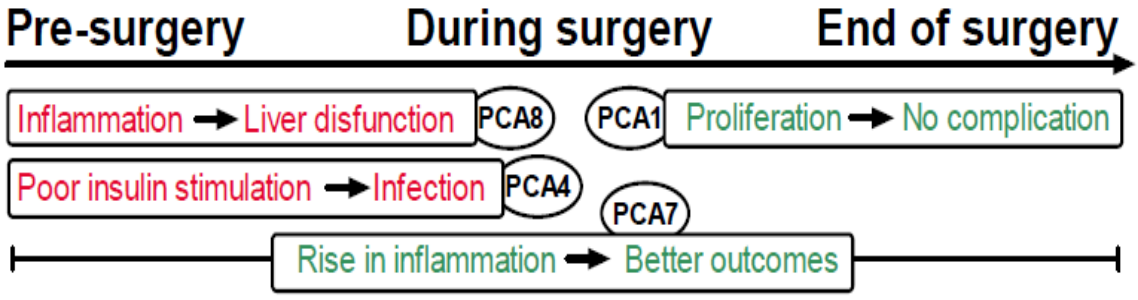


Figure 3. Associations between clinical outcomes and biological markers in LR patients at all stages of surgery.



CONCLUSIONS

The results presented in this study link several clinical outcomes with biological parameters in patients undergoing major liver resection. In particular, insulin therapy reduced postoperative liver dysfunction by suppressing inflammation and associated apoptosis. These results are genuine since our study design does not allow pre-emptive assumptions regarding the effect of intervention on any specific parameter towards altering its clinical outcome.

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Conclusions

For my thesis work I investigated the use of the HNC protocol combined with preoperative carbohydrate loading for maintaining liver glycogen content and sustaining normoglycaemia in patients undergoing major liver resection. This is the first published succefull work aiming to improve the quality of the FLR.

An initial feasibility study showed significant promise in using the HNC protocol in patients undergoing major liver resection. Benefits of the HNC therapy were exemplified by the remarkable recovery of a 63-year-old Caucasian woman who received the HNC protocol during a hepatectomy for hilar cholangiocarcinoma. The operation left her with very little viable tissue. By the end of postoperative day 1 her liver dysfunction was classified as only 'mild'. This assessment was further downgraded to 'no liver dysfunction' by postoperative day 2. This case report prompted initiation of a large randomized clinical trial for determining the benefits of insulin/glucose therapy administered via the HNC protocol. We wanted to see if tight-glucose control by the clamp technique could improve patient outcomes after hepatic resection.

All subsequent studies proceeded under the umbrella of a randomized-controlled trial involving consenting patients >18 years scheduled to undergo liver surgery. Baseline liver function and operative characteristics were similar among participants, and the study included both diabetics and nondiabetics.

Results from the trial demonstrated that major liver surgery induces a stress response that can culminate in moderate to severe hyperglycaemia. We achieved better glucose control overall

with no incidence of sever hypoglycaemia when using the HNC protocol during and after surgery compared to using standard insulin therapy. The mean glucose in patients on the HNC protocol consistently remained within normal target range (3.6–6.1 mmol/L) both during and after surgery. Control patients on standard insulin therapy experienced a gradual increase in mean glucose that plateaued between 9–12 mmol/L perioperatively. Diabetic controls developed slightly more extreme hyperglycaemia than the nondiabetics.

None of the intervention patients experienced severe hyper- (>10 mmol/L) or hypoglycaemia (<2.2 mmol/L) while on the protocol. Fewer fluctuations in blood glucose occurred among intervention patients, but mild hypoglycaemia was more prevalent in this study population postoperatively.

Some of my most exciting results emerged from the trial when we combined the protocol with preoperative carbohydrate loading. This led to significant improvements in parameters necessary for maintaining hepatocyte integrity following tissue trauma. The extended protocol improved postoperative liver function, augmented liver glycogen and reduced the incidence of postoperative complications significantly.

Liver function scores as per Schindl (*23*) were significantly better for the intervention patients. Scores for patients on the protocol ranged from 0 to 4 signifying mild-to-moderate dysfunction while control patients received scores from 2 to 8 indicating moderate-to-severe POLD.

The combined protocol also significantly improved the content of liver glycogen available for hepatocyte regeneration during surgery and reduced overall complications. The mean liver glycogen content measured from liver biopsies taken perioperatively was 430 (188–723) and 278 (48–620) mmol/kg for intervention and control patients, respectively. Adverse events occurred in

62% of intervention patients while almost all controls had an adverse event. Infections Clavien grade 2 or higher occurred in 26% of patients in the control group and 3.4% in the intervention group. The protocol was deemed safe, reliable and easy to apply at a step-down unit.

The third manuscript is very critical in indicating the potential influence of insulin therapy in reducing postoperative complications from liver surgery. When administered at high doses insulin provides anti-inflammatory, antioxidative, and cardioprotective effects especially in patients exposed to major surgical trauma.

The last stage of the study involved determining alterations in biochemical and genetic markers related to the surgical adaptive stress response. Results from serum and liver biopsy samples taken from LR patients at different time points throughout their surgery demonstrated that insulin therapy delivered with the HNC protocol reduces postoperative liver dysfunction by suppressing inflammation and associated apoptosis. The analyses showed that protective anti-apoptotic genes BCL2 ($P = 0.01$) and BCL2L1 ($P = 0.02$) are upregulated in intervention patients while control patients have higher levels of pro-apoptotic genes, BAX ($P = 0.04$), Caspase 8 ($P = 0.004$) and Caspase 9 ($P = 0.01$). As well, proinflammatory mediators IL-6, IL-8, TNF-α, and MCP-1 increased acutely in both groups perioperatively, but more significantly in intervention patients.

Given the promising results from our clinical trial in showing the benefits of the HNC protocol combined with carbohydrate loading i.e. safe reduction of POLD and augmented liver glycogen while achieving normolglycaemia, This protocol can potentially be applied to patients with acute liver failure, patients undergoing liver resections, liver transplantation, or living liver donation in order to preserve their liver function.

Further work can is required to dissect the effect of the two components of the metabolic support protocol in patients undergoing major liver resection. Such work can delineate the differential effect of preoperative carbohydrate load and the effect of perioperative insulin therapy. Furthermore, a randomized clinical trial is needed to examine the effect of the reduction in postoperative adverse events in improving the overall cancer survival.

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