High-dose insulin therapy to improve liver function in patients with Hepatitis C Virus liver cirrhosis

Mohammed F Shaheen

Division of Hepatopancreaticobiliary and Transplant Surgery, McGill University, Montreal, Canada

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Abstract (English)

Introduction: Hepatitis C Virus (HCV) has been shown to interfere with the hepatocytes' insulin signaling pathway resulting in a state of insulin resistance besides causing oxidative stress leading to a state of chronic liver inflammation. Insulin resistance, along with consequent chronic inflammatory state, will speed up the progression of liver fibrosis. Recently, insulin therapy using the hyperinsulinemic normoglycemic clamp technique has been shown to potentiate the regenerative capacity of the liver and decrease the overall inflammatory state. Therefore, we hypothesize that treating HCV infected patients with high-dose insulin therapy will target the pathophysiology of the disease at both cellular and molecular levels; eventually promoting recovery of liver function and potentially slowing the disease progression. **Methods**: A pilot interventional study was conducted on patients with chronic HCV infection who are not candidates for interferon based therapy. Each patient underwent a minimum of 14 weeks of 6-hours each insulin therapy sessions scheduled twice per week. Hyperinsulinemic normoglycemic clamp technique was used to deliver 2 milliunit of human regular insulin per kilogram of body weight per minute. Testing of liver function, inflammatory markers, metabolic parameters, liver volume, and reported quality of life were collected at the beginning, middle and end of the study period. **Results**: All patients followed by 2 hepatologists were screened; thirteen patients met the inclusion and exclusion criteria and were interviewed. Three patients agreed to participate in the study. One patient successfully completed the protocol. The patient had noted improvements in multiple parameters related to the liver function, liver volume and the inflammatory response with no apparent improvement in metabolic parameters or in the reported quality of life. The second patient didn't complete the study due to hospital

admission secondary to septicemia. The third patient was excluded before the study start date due to hospital admission for variceal bleed. **Discussion**: The application of high-dose insulin therapy in cirrhotic patients is feasible and safe in outpatient settings. Insulin therapy is a novel technique that can alter the systemic inflammatory response and potentially promote liver regeneration and improve liver function in patients with chronic HCV induced liver cirrhosis. Recognizing the limitation of having only one enrolled patient complete the study, further testing is needed to confirm benefits and rule out harm of long term insulin therapy for liver disease. For future work, inclusion criteria need to be revisited to assure adequate sample size while applying the concept in diseases with comparable patho-physiology to chronic hepatitis C infection.

Abstract (French)

Introduction: Il a été démontré que le virus de l'hépatite C (VHC) est capable d'interférer avec la voie de signalisation insulinaire des hépatocytes causant un état de résistance à l'insuline ainsi qu'un stress oxydatif menant éventuellement à un état d'inflammation chronique du foie. La résistance à l'insuline, ainsi que l'état inflammatoire chronique conséquent, vont alors accélérer la progression de la fibrose hépatique. Récemment, la thérapie à l'insuline en utilisant la technique de pince normoglycémique hyperinsulinmique a été prouvée comme étant capable de potentialiser la capacité de régénération du foie et réduire l'état inflammatoire général. Par conséquent, nous émettons l'hypothèse que le traitement des patients infectés par le VHC avec l'insulinothérapie à haute dose ciblera la physiopathologie de la maladie aux niveaux cellulaires et moléculaires; ce qui pourrait éventuellement promouvoir la reprise de la fonction hépatique et potentiellement ralentir la progression de la maladie. Méthodes: Une étude interventionnelle pilote a été menée sur des patients atteints d'infection chronique par le VHC, qui ne sont pas candidats à un traitement à base d'interféron. Chaque patient a subi un minimum de quatorze semaines de séances de thérapie d'insuline de six heures de temps prévues à intervalle de deux fois par semaine. La technique hyperinsulinmique de la pince normoglycémique a été utilisée pour fournir deux milliunités d'insuline humaine régulière par kilogramme de poids corporel par minute. Les tests de la fonction hépatique, les marqueurs inflammatoires, les paramètres métaboliques, le volume du foie, et la qualité de vie reportée ont été recueillis au début, au milieu et à la fin de la période d'étude. Résultats: Tous les patients suivis par deux hépatologues ont été dépistés; treize patients qui répondaient aux critères d'inclusion et d'exclusion ont été interrogés. Trois patients ont accepté de participer à l'étude. Un patient

a complété le protocole avec succès. Le patient a démontré des améliorations dans plusieurs paramètres liés à la fonction hépatique, du volume du foie et de la réponse inflammatoire sans amélioration apparente des paramètres métaboliques ou de la qualité de vie reportée. Le second patient n'a pas complété l'étude en raison d'une hospitalisation secondaire à une septicémie. Le troisième patient a été exclu avant la date de début de l'étude en raison d'une hospitalisation pour hémorragie de varices œsophagiennes. Discussion: L'application de l'insulinothérapie à haute dose chez les patients cirrhotiques est faisable et sécuritaire en milieu ambulatoire. L'insulinothérapie est une nouvelle technique qui pourrait modifier la réponse inflammatoire systémique et potentiellement favoriser la régénération du foie ainsi qu'améliorer la fonction hépatique chez les patients cirrhotiques suite à une infection chronique du VHC. Reconnaissant la limitation d'avoir un seul des patient inscrits seulement complétant l'étude, des travaux supplémentaires sont nécessaires pour confirmer les avantages et reconnaitre les désavantages potentiels de l'insulinothérapie à long terme pour les maladies du foie. Dans les projets futurs, les critères d'inclusion devraient être revus pour assurer un échantillon de taille adéquate tout en appliquant le concept dans des maladies à pathophysiologie comparable à celle d'une infection chronique de l'hépatite C.

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Introduction

Hepatitis C Virus (HCV) causes substantial health burden worldwide. This disease mainly presents in the form of chronic deterioration of the liver function leading to subsequent cirrhosis, hepatocellular carcinoma and death [1, 2]. Although medical treatment with interferon and other antiviral drugs is available, the current standard therapy is still far from effective in clearing the virus and curing the disease. This is due to the medical treatment's ineffectiveness at times, and due to patients' intolerance to medical treatments' serious side effects at others [3]. Considerable portions of patients are left with no other available therapeutic medical options in light of the absence of readily accessible and approved alternatives in a global scale.

Chronic HCV infection progresses over decades starting from mild liver disease, frequently asymptomatic, to marked liver fibrosis with resulting systemic manifestations. It is estimated that 20 to 40% of patients with chronic HCV infection will progress to end-stage liver disease during their life span and around 10 to 20% will die from liver related causes. Patients with advanced liver disease secondary to chronic HCV infection are also at higher likelihood to develop hepatocellular carcinoma [4]. Patients with resulting end-stage liver disease or who develop a worrisome liver lesion are subsequently offered liver transplantation. Although liver transplantation does not resolve the HCV infection, it offers the best chance of prolonged survival [5]. However, because of limited organ supply and the ever expanding waiting list for organ transplantation, the disease sometimes progresses beyond therapy [6].

The natural history of chronic HCV infection is considerably variable between affected individuals. Progression of chronic HCV infection has been an area of research interest for a long time. This is because progression of chronic HCV infection is variable and relatively slow [2, 7]. Knowing the key factors that can influence the speed of the disease progression can serve as an

excellent addition to the current search for better cures. Additionally, trying to attenuate the disease from progressing to end stage liver damage can defer the need of invasive surgical intervention in some patients.

The association between hepatitis C virus progression and the development of metabolic imbalance has been repeatedly noted. The relationship between the two processes is becoming more evident with ongoing research in molecular and cellular biology. It has been noted that HCV contributes directly and indirectly in creating a state of ongoing liver inflammation and systemic metabolic imbalance, leading to insulin resistance, which is a good medium for a faster disease progression [8, 9]. Multiple studies have tried to target the progression of HCV infection by reversing the negative viral effects on the metabolic processes and the state of ongoing cellular stress and inflammation with variable success [10].

The hyperinsulinemic-normoglycemic clamp (HNC) technique has been used by our research group to deliver high-dose insulin without comprising the safety of the patient. We also previously studied its potential therapeutic effects on the hepatocytes. The HNC has shown promising success in supporting liver function, hepatic metabolic processes as well as decreasing hepatocyte apoptosis [11-13].

The goal of this research is to study the potential benefits of high-dose insulin therapy course, using HNC technique, on HCV patients. This will be the first study to adopt the HNC therapy technique and implement it in an outpatient setting. Patients diagnosed with hepatitis C with early cirrhosis planned to receive frequent high-dose insulin therapy sessions at their outpatient visits. During the course of the study, changes in livers metabolic capacity and clinically used scores to reflect the severity of liver disease will be monitored, as well as, markers of systemic inflammation and insulin resistance.

Literature Review

SECTION 1: HCV – an Overview

Virus and mode of transmission

Hepatitis C virus (HCV) belongs to the genus Hepacivirus of the Flaviviridae family. The genome of HCV consists of a positive-stranded RNA encoding for at least ten proteins including structural and non-structural proteins. The virus mainly affects the liver, and uses hepatocytes' resources to drive its replicative process yielding significant cellular stress response. There are at least 6 recognized genotypes of the HCV with multiple subtypes with genotype (1a) predominating in North America [14-16].

It is estimated that between 135 and 180 million people are living with chronic hepatitis C virus infection compromising 2.8% of the world's population. The prevalence of hepatitis C is up to 20% in parts of Africa and around 1-2% in North America. HCV is mainly transmitted through blood and blood-derived products [17-21].

Infection and Natural History

Infected individuals with HCV do not usually mount a clinically evident acute illness. However, the body's immune system recruits inflammatory cells to the infected hepatocytes inducing their necrosis and apoptosis. Unless the virus is successfully cleared by the immune system, the state of ongoing liver inflammation, called chronic hepatitis, leads to more fibrosis and subsequent cirrhosis over time [1, 22]. Around 70-80% of patients will fail to clear the virus and become chronically infected with HCV [23, 24]. The persistent state of hepatic lobular inflammation, cellular necrosis and progressive fibrosis caused by chronic hepatitis C eventually results in liver cirrhosis which is an irreversible process that correlates with severe liver function deterioration[25]. As the

progression of liver dysfunction caused by HCV is variable from one individual to the other, it is estimated that one in five of chronically infected individuals will develop liver cirrhosis over a span of 20 - 30 years [18]. Patients with HCV liver cirrhosis are at high risk of developing hepatocellular carcinoma as well. The cumulative incidence of hepatocellular carcinoma is more than 25% over 10 years in patients with HCV liver cirrhosis [4].

While histopathological assessment remains the gold standard in diagnosing and grading liver fibrosis and cirrhosis, the degree of clinical assessed deterioration of patients suffering from liver cirrhosis is widely accepted when following the progression of disease. In fact, some studies have illustrated the reliability of clinical parameters reflecting the severity of liver dysfunction over histopathological assessment when predicting prognosis [26]. Although no measurable clinical abnormalities can be noted in early stages of chronic HCV infection, multiple systemic symptoms and signs can be noted in later stages of liver cirrhosis. Hepatic encephalopathy, variceal bleeding and ascites are some of the major systemic manifestation of advanced uncompensated cirrhosis. [25, 27]

Hepatitis C Virus Liver Cirrhosis: A Rising Problem

The highest incidence of HCV infection is believed to have had taken place in the 1980's – 1990's. Although, incidences of hepatitis C virus infection has been steadily dropping since its discovery in 1989, HCV related liver cirrhosis and other consequences of chronic HCV disease are expected to rise in the coming years [18]. The natural course of HCV infection is highly variable and depends on both viral and host factors. For instance, older age at diagnosis, male gender, obesity, high alcohol consumption, certain viral co-infections and the presence of insulin resistance are predictors of faster disease progression [1, 7, 27-29]. Predictive models have been developed using

historical data to quantify the expected future burden of the disease. These models speculate that the proportion of hepatitis C virus induced liver cirrhosis is expected to increase exponentially over the coming 10 to 15 years [30]. Consequently, HCV infection is emerging as a leading cause of chronic hepatitis, hepatocellular carcinoma and cirrhosis even in countries where alcoholic liver cirrhosis used to be the predominant cause of cirrhosis [17, 31, 32]. This also suggests that chronic HCV infection will continues to be the main indication of liver transplantation around the globe in the coming several years in the absence of effective therapy to eradicate the virus [5, 6, 33].

Current Management of Chronic HCV Infected Patients

Until recently, the standard medical therapy for HCV is based on PEGylated interferone (IFN) and ribavirin – as a nucleoside analog – aiming to achieve viral clearance. However, the response to this regimen is 40% - 80% depending on the viral genotype, besides considerations of the treatment's severe side effects. Currently, new direct antiviral drugs have emerged out of clinical trials, such as Sofosbuvir and Simeprevir, with promising therapeutic results [3, 34]. These new drugs may potentially change the current situation to a better stand [35].

Surgical management in the form of liver transplantation is considered for patients who suffer decompensated cirrhosis or develop hepatocellular carcinoma. Liver transplantation does not eradicate the HCV; as viral recurrence after liver transplantation is almost universal [36]. Immunosuppressive therapy after liver transplantation is essential to avoid graft rejection but may facilitate chronic HCV disease progression. In fact, the rates of fibrosis and graft dysfunction are noted to increases after liver transplantation in patients with persistent or recurrent HCV infection [37]. However, liver transplantation offers the best survival benefit to those patients with advanced liver disease secondary to HCV infection [38].

SECTION 2: Molecular Pathogenesis of Hepatitis C Virus

Infection of Hepatocytes

Hepatocyte is the cellular working unit in the liver and it is probably one of the most metabolically active cells in the human body. Hepatocytes are consistently, but not exclusively, involved in glucose, amino acid and fatty acid homeostasis. The HCV uses hepatocytes as an avenue to continuously replicate causing severe disruption in their normal function [39-41].

In order to understand the complex intracellular interactions caused by hepatitis C virus infection, multiple studies have used cellular cultures and animal models. Among others, human hepatoma cells like hepatocarcinoma cell line (Huh-7) and human hepatoblastoma cell line (HepG2) have been widely used, as well as transgenic mice models [42, 43]. Still, there is no perfect lab model to study HCV infection as the virus exclusively infects humans and chimpanzees [44].

In this section, the details of pathogenesis of HCV will be examined along with the effect of HCV on normal hepatocyte function. However, before detailing the molecular processes of HCV pathogenesis, it is valuable to examine the rule of cytokines in liver disease.

Role of Inflammatory Cytokines and Adipokines in HCV Liver Disease

Cytokines are regulatory peptides that can exert an autocrine, paracrine or endocrine effect. Cytokines can be produced by all types of nucleated cells in the body [45]. In the liver, several cytokines are of vital importance in liver tissue repair and regeneration as well as in various aspects of inflammatory liver disease [46-48]. Among the different cytokines, TNF-α is one of the earliest and most important cytokines in many liver diseases. TNF-α is a pro-inflammatory cytokine that triggers the production of other cytokines, such as IL-1 and IL-8, and plays an important role in recruiting inflammatory cells and initiating fibrogenesis by activating Kupffer cells to secrete TGF-β [49, 50]. On the other hand, TNF-α plays a role in hepatic regeneration along with local growth

factors and other cytokines, such as IL-6 [46, 51]. Studies have demonstrated a beneficial role of therapeutics targeted to suppress TNF-α during alcohol induced inflammatory liver injury and fatty liver disease with subsequent reduction in other pro-inflammatory and anti-inflammatory cytokines. Subsequently, improvements in the histological and clinical features of the disease have been noted in some studies [52, 53]. The main reported risk associated with anti-TNF-α treatment was that it renders the patient susceptible to severe infections which limit its use [54]. In the setting of HCV infection, anti-TNF-α treatment did not show any evidence of worsening liver function or faster progression of disease which has been a theoretical concern [55, 56].

IL-10 is considered to be an anti-inflammatory cytokine that decreases T-cell secretion of pro-inflammatory cytokines. High levels of IL-10 can reduce signs of hepatic inflammation in HCV infection but potentially provides a suitable environment for the HCV to replicate [57]. Hence, IL-10 based therapies are unfavorable in the setting of chronic HCV infection [50].

IL-6 is a cytokine that has shown to be a key player in inducing hepatic regeneration. Depending on the situation and the levels of other cytokines, IL-6 can play either a pro-inflammatory or an anti-inflammatory role [58, 59].

A recent study retrospectively looked at changes in serum cytokines of 6 patients who were diagnosed with chronic HCV infection – genotype 1 – over a 30-year time period. They demonstrated apparent changes in cytokine profiles between those who experienced faster disease progression in comparison to those who experienced a slow disease progression. IL-8, IP-10, IFN-γ, MCP-1 and MIP-1β were analyzed overtime. Interestingly, MCP-1 was significantly and persistently higher in patients with faster disease progression compared to higher levels of IFN- γ and MIP-1β in patients with slower disease progression [60]. The MCP-1 - monocyte chemoattractant protein-1 – is a chemoattractant cytokine which plays a potent

role in regulating the recruitment, migration and infiltration of monocytes to areas of inflammation. [61]

Adipokines, including adiponectin, leptin, and resistin are proteins excreted from adipose tissue that may be involved in states of liver inflammation, fibrosis and metabolic syndrome [62] [63]. Adiponectin is an inducer of IL-10 production and has an inhibitory effect on TNF-α synthesis, IL-6 and IFN-γ production. [64]. The beneficial effects of adiponectin in liver disease are thought to be mediated by its ability to attenuate TNF-α production and subsequent inflammation as well as its ability to alleviate steatosis [65, 66]. On the other hand, leptin is thought to promote hepatic inflammation and fibrosis in the setting of chronic hepatitis [67]. Leptin is believed to promote hepatic fibrosis by activating hepatic satellate cells and by upregulating TGF-β production leading to activation of Kupffer cells, macrophages and sinusoidal endothelial cells [68]. Plasma resistin has been shown to correlate positively with hepatic fat content and hepatic and systemic insulin resistance [69, 70]. Leptin and resistin are thought to cause insulin resistance by activating SOCS3 - suppressor of cytokine signaling 3 - which in turns inhibits insulin signaling [68, 71]. In vitro, insulin has been shown to suppress the expression and secretion of resistin from adipocytes; however this effect is yet to be verified in vivo [72]. The positive correlation between serum resistin level and hepatic fat content and hepatic insulin resistance have been challenged in the context of HCV infection, suggesting the presence of other mechanisms relating obesity, insulin resistance and hepatic fat content in patients with chronic HCV infection [73]. Resistin is also thought to correlate negatively with the degree of hepatic fibrosis serving as a potential serum marker [74]. Few studies have tried to link adipokine levels to viral response to interferon therapy in order to develop predictive models to treatment response [75, 76]. Although changes in adipokines levels during interferon therapy and after HCV eradication have been used as evidence for its role in chronic

HCV disease pathogenesis, the expression of adipokines has been shown to differ between different studies. It was also shown to be affected by gender, viral genotype and fat content in the liver and the whole body as well as the amount of insulin sensitivity [77, 78]. In fact, the contribution of adipokines in the pathogenesis of HCV infection remains an issue of debate [79-81].

HCV Induced Oxidative Stress and Inflammation

Cellular injury caused by the HCV is mediated through two major mechanisms. One of the mechanisms is an immune-mediated mechanism via T-cell response, which in some instances is able to clear the virus preventing chronic infection [82]. In most instances however, the immune system is not able to clear the virus and will transition into a state of chronic hepatitis. This leads to liver destruction by persistent inflammation, cytokine release and fibrogenesis [83]. Failure of the immune system to clear the infection is believed to be due to the high viral genome variability resulting from the rapid viral turnover process by an imperfect RNA polymerase [41].

The other mechanism is through direct viral cytotoxicity. Besides exhausting hepatocytic resources to create viral replicons through cellular organelles, accumulating viral proteins interacts with multiple cellular signaling pathways causing functional impairment of hepatocytes. This results in significant cellular oxidative stress, lipid peroxidation, alteration in cell cycle, gene expression and eventual DNA damage. [84-86]

Oxidative stress is thought to be the initiator of the destructive cytopathic effects, resulting in the accumulation of reactive oxygen species (ROS) mainly from the endoplasmic reticulum and mitochondria [39]. At early stages, anti-oxidant and adaptive cellular defenses are concurrently induced to encounter high levels of ROS produced by damping the endoplasmic reticulum stress and inhibiting viral protein translation. The balance is eventually lost and cell injury results [85-87]. ROS has been shown to activate the transcriptional activity of some cytokines like TNF-α and IL-

1 which in turn contributes further to cellular injury [86, 88]. Pathological consequences of this process include apoptotic cell death, fat accumulation and inflammation [89]. Upon cellular destruction, a state of necroinflammation results, which in turn enhances the production of ROS and accelerates injury [90]. Additionally, ROS can functions as signaling molecules to activate hepatic stellate cells to produce collagen leading to fibrosis. The resultant inflammation further accelerates fibrosis by activating Kuppfer cell secretion of TGF-β, which is the most important cytokine inducing progressive fibrosis [91]. Furthermore, ROS can contribute to the development of hepatocellular carcinoma by causing oxidative DNA damage to the host cells [92, 93]. Thus, factors inducing more hepatocytic oxidative stress can contribute to faster progression of liver injury in chronic HCV infected patients. Hence, regular alcohol intake is a recognized factor that contributes to faster progression of liver injury in chronic HCV infected patients through further inducing hepatocytic oxidative stress [93].

HCV Effects on Signaling Pathways

In parallel with HCV induced oxidative stress caused by continuous replication, its translated proteins interfere with vital signaling pathways leading to disruption of normal hepatocyte function. For instance, Through the Janus kinase/ Signal Transducer and Activator of Transcription kinases (JAK/STAT) signal transduction pathways, the HCV core protein, a structural protein, modulates the cellular response to IL-6 and IFN-γ, altering the ability of hepatocytes to regenerate and possibly allowing it to evades viral eradication. The HCV core protein can also play a direct role in enhancing fibrosis by upregulating the TGF-β promoter gene in hepatocytes.

The HCV core protein also activates NF-Kappa B (NF-κB) signaling cascade which plays a pivotal role in cellular response to pro-inflammatory cytokines, such as TNF-α and IL-1, leading to further stimulation of inflammation and up regulation of various other cytokines including IL-1, IL-2, IL-

6, IL-8 and TNF-α [94]. An effect on protein phosphatase 2A (PP2A), mitogen-activated protein kinase (MAPK), serum response element (SRE), activator protein 1 (AP-1) and protein 53 (p53) signal transduction pathways have also been suggested in the literature [40, 95, 96].

Furthermore, HCV core protein interferes with cell cycle progression by impairing the phosphorylating ability of cyclin-dependent kinases (CDKs) [84]. Although the viral core protein harbors the most potent effects on signaling pathways, multiple non-structural viral proteins, such as NS3 and NS5A, have also been shown to carry similar effects. The net effects of viral proteins on signal transduction are in direct concordance with its pathogenesis by promoting inflammation, apoptosis and fibrosis. Although some contradictory effects of HCV proteins on signaling pathways were found, it is thought that this serves to balance the proliferation of affected hepatocytes in favor of viral replication [40]. It also serves to utilize cellular machinery away from signals promoting proper cellular growth and function as part of the unfolded protein response (UPR) driven by high levels of cellular stress [97, 98].

The oxidative stress and the inflammatory response that happens in the setting of chronic hepatitis C virus infection play a pivotal role in its pathogenesis. Furthermore, the inflammatory reaction serves as an optimal medium for the HCV to alter the metabolic functions, eventually leading to the development of metabolic syndrome [99].

HCV and Metabolic Syndrome, a Mutual Relationship

Metabolic syndrome refers to the simultaneous occurrence of multiple cardiovascular risk factors, including insulin resistance, obesity, dyslipidemia, visceral adiposity and hypertension. Insulin resistance is believed to be the key player in the pathophysiology of the metabolic syndrome. Insulin resistance is defined as a state where higher insulin levels are needed to sustain a normal metabolic response or when normal insulin levels fails to sustain a normal metabolic response [100].

Through multiple mechanisms, detailed later, HCV leads to high insulin resistance that can eventually leads to the development of Type 2 diabetes mellitus when the body is no longer able to maintain normoglycemia with endogenously secreted insulin [101]. Furthermore, development of hepatic steatosis in the setting of chronic hepatitis C is, in part, related to insulin resistance and a state of hyperinsulinemia. Yet, oxidative stress and the increased levels of proinflammatory cytokines are major players in the pathophysiology linking chronic HCV infection with metabolic syndrome [100, 101].

The important causal link between HCV and liver metabolic syndrome has been repeatedly shown in clinical studies [30, 102]. The link between infection with HCV and the increased risk of type II diabetes mellitus, insulin resistance or hepatic steatosis is well documented [99, 102-104]. Initially, these effects were attributed to the progression of liver dysfunction; however they have been shown to occur -to a lesser extent- even in non-cirrhotic livers [102, 105-107]. These effects vary in predominance in a genotype-dependent fashion. For instances, genotype 1 and 4 are clearly associated with a state of insulin resistance in the early stages of disease progression while genotype 3 causes significantly higher levels of hepatic steatosis with much less effect on insulin resistance [104, 108].

Our understanding of the molecular mechanisms and interactions is enhanced with ongoing studies using genomics and proteomics approaches. Through molecular studies, we now know that HCV lead to altered glucose homeostasis and subsequently metabolic syndrome through both a direct viral effect on the cellular signaling pathways and an indirect effect though creating an inflammatory environment [109-111].

HCV infected human livers were shown to have less responsive IRS-1 – insulin receptor substrate 1 – when compared to non-infected livers. This is believed to be mediated by the direct viral protein modulating the phosphorylation of the serine residues insulin receptors [112]. Multiple other

mechanisms have been suggested through animal and cell culture studies. For instance, increased secretion of TNF-α and other cytokines, as part of the inflammatory response, is believed to hinder insulin signaling [8, 113, 114]. Also, the increased ER stress and elevated levels of ROS can lead to inhibition of the Akt and AMPK pathways, through PP2A and JNK activation, causing various metabolic imbalances and disturbing insulin and IFN-α signaling. In vitro studies have suggested a possible mechanism by which viral core protein causes degradation of the insulin receptors via inducing the suppressor of cytokine signaling (SOCS)-3 and (SOCS)-7 through modulating PPAR - peroxisome proliferator-activated receptors - and mTOR - Mammalian target of rapamycin - signaling pathways in a genotype specific fashion [109, 115, 116]. The resulting insulin resistance prevents glucose uptake into hepatocytes and adipocytes causing a state of hyperinsulinemia and hyperglycemia. This state contributes to imbalance in fat metabolism causing hepatic steatosis [117, 118]. Besides, the hyperglycemia further contributes by increasing the ongoing inflammatory process [119]. Hence, Insulin resistance (IR) is believed to represent the central clinical features associated with chronic HCV infection [30, 120]. In fact, IR has shown to independently attribute to the rapid progression of the liver disease as well as predicts reduced response to the conventional IFN-α based therapy [121].

The relationship between HCV and metabolic syndrome is not confined to how HCV can cause IR and metabolic syndrome. As mentioned, IR and other components of metabolic syndrome, such as type 2 diabetes mellitus (T2DM) and hepatic steatosis, play a pivotal role in accelerating HCV replication and fibrosis progression [103, 121]. In fact, the combined effect of hyperglycemia and hyperinsulinemia, seen with states of insulin resistance, can acts as a direct stimulant to hepatic stellate cells to increase collagen fiber deposition [122, 123]. Also, hepatic steatosis has been linked

with liver vulnerability to injury and increased liver cell apoptosis with subsequent activation of hepatic satellate cell and fibrosis [124].

Increased insulin resistance is associated with reduced response to conventional interferon therapy to achieve sustained viral response [125]. Although the mechanism is not fully clear; the increased levels of members of SOCS family, induced by Hepatitis C virus core protein, is believed to interrupt both insulin signaling and IFN-α signaling. In addition, HCV core protein activation of PP2A potentiates further interference with IFN-α and Insulin signaling [115, 126-128]. Due to the noted relationship between the occurrence of IR and the decreased response to IFN therapy, multiple trials have targeted insulin resistance as a way to improve the response to IFN therapy in chronic hepatitis patients. Life style modifications as well as anti-diabetic drugs such as metformin and thiazolidinediones have been experimented. Despite evidence of possible improvement in some aspects of liver disease, the results were not very encouraging overall [129-135]. Additional studies are needed to verify the advantages of insulin sensitization in this population of patients.

In summary, chronic HCV infection leads to a vicious cycle of chronic hepatic inflammation, and escalating insulin resistance and metabolic imbalance, that in turn lead to a more progressive course of hepatic fibrosis and dysfunction [121].

SECTION 3: Insulin Effects in the Liver

How Does Insulin Work Inside Liver Cells?

Insulin is a potent anabolic hormone that has a vital role in glucose homoeostasis, glycogen formation, lipid and protein synthesis [136]. Insulin promotes hepatocyte growth and proliferation and orchestrates the body's metabolic processes largely taking place in the liver [137]. The hepatocytes are responsible for clearing 80% of endogenously secreted insulin and around 50% of exogenously administered insulin [138]. Insulin induces a complex set of cellular actions by

stimulating multiple signaling pathways. Several Insulin receptor substrates (IRS) have been identified that downstream the diverse insulin, and insulin like growth factor, actions inside the cell in different organs [139]. Deficiency or absence of one or more of these substrates leads to significant growth inhibition and a variety of metabolic derangements particularly in glucose homeostasis [140, 141]. IRS-1, 2 and 4 are important in inducing insulin signaling inside the human hepatocytes and probably plays compensatory but similar roles to one another [142]. Insulin induces a lot of its effects inside the hepatocytes via two main signaling cascades, namely, the MAPK/ERK signaling cascade and the PI3K/Akt signaling cascade. Through the MAPK/ERK signaling cascade, insulin plays a prominent role in hepatic growth which becomes evident after liver resection and also in the sitting of malignant growth [143] [144, 145]. Through the PI3K/Akt signaling pathways, insulin leads to inhibition of apoptosis as well as synthesis of glycogen, fatty acids, triglycerides and proteins via glycogen synthase kinase 3 (GSK3) and mTOR pathways [146]. Furthermore, insulin controls the expression of various genes either positively or negatively in order to exert a variety of effects [147]. In the liver, key mediators of insulin action on gene transcription are Forkhead box protein O (FoxO), Sterol regulatory element-binding protein (SREBP) and Sp1 proteins [148] [149]. Interaction between insulin and other hormones and transcriptional factors is well established in the literature; however this would not be relevant in the current context [150].

Insulin Modulates Inflammatory Response and Alleviates Oxidative Stress

Proinflammatory cytokines, such as TNF-alpha and IL-1, exert their effects through activation of NFkB transcriptional factor leading to further production of inflammatory cytokines and monocyte chemoattractants [151]. Oxidative stress, which is defined by an increased production of ROS, also

affects cellular health through inducing NFkB [152]. The continuous state of inflammation and oxidative stress can lead to cell wall destruction, lipid peroxidation and DNA damage.

In vivo experiments using intravenous insulin infusion have confirmed the antioxidant and anti-inflammatory effects of insulin by illustrating its ability to suppress ROS generation along with the suppression of the major pro-inflammatory transcriptional factor NFkB. [153] The anti-inflammatory effects of insulin is estimated to ensue within few hours of starting an IV infusion. The magnitude of anti-inflammatory effects – estimated by the effect on the CRP level – is believed to be equivalent to 100 – 200 mg of hydrocortisone when insulin is infused at 2 units per hour for 24 hours [154-156].

Over the last few decades, multiple studies have demonstrated the potent antioxidant and anti-inflammatory properties of insulin. Insulin was shown to induce the production of antioxidants such as nitrous oxide (NO) through the activation of PI3/AKT kinases. Insulin also causes inhibition of platelet aggregation and leukocyte adhesion [153, 157, 158]. Moreover, insulin has been shown to modulate inflammatory transcriptional factors and the genes that regulate them [147 159]. Pretreatment with insulin in multiple trials successfully blunted the surge of a variety of pro-inflammaoty cytokines like TNF-α, IL-1 and IL-8 as well as CRP [160, 161 162].

Multiple reports have been constantly showing encouraging effects of insulin in a variety of cardiovascular diseases. In animal studies, insulin has been shown to delay the progression of atherosclerosis due to its antioxidant and anti-inflammatory effects [163]. Besides the use of insulin in the setting of CABG surgery, insulin therapy have shown to be beneficial in managing patients suffering from acute myocardial infarction due to its antithrombotic and profibrinolytic properties potentially decreasing the size of the infarcted myocardium [164 165]. Additionally, the antiapoptotic and antioxidant effects of insulin mediated through PI3/Akt kinases and NO synthase are believed

to contribute to the protective myocardial effect in the setting of acute ischemia [166]. These effects have also been shown in patients undergoing major liver resection. High-dose insulin therapy administered peri-operatively improved hepatic content of glycogen, inhibited apoptosis and modulated inflammatory response [12, 167].

The benefit of using insulin is thought to be dual, that is, in addition to its potent anti-inflammatory properties; insulin also reduces the adverse effects of hyperglycemia.

The Relation between Insulin and Glucose

Unlike the anti-inflammatory properties of insulin, glucose carries pro-inflammatory properties [168]. Hyperglycemia in the setting of suppressed endogenous insulin secretion has been shown to lead to an increase in plasma TNF-α and IL-6, which in turn, interfere with insulin signal transduction through affecting phosphorelation of the IRS and promoting the expression of SOCS-3 [169-172]. In fact, increasing levels of circulating pro-inflammatory cytokines, such as TNF-α, has been clearly associated with increasing insulin resistance in animal models [170, 173, 174]. Besides, hyperglycemia has been associated with higher incidence of morbidity and mortality in severely ill patients [175-177]. For instance, Wahab et al. have demonstrated increased 12-months mortality in patients suffering from acute myocardial infarction if they had hyperglycemia at the same time of their heart attack [178]. Similarly, Williams et al. have demonstrated longer hospital stay and higher short and long term mortality in patients suffering from acute stroke with concomitant hyperglycemia at hospital admission, recommending intensive treatment of hyperglycemia in this population of patients [179].

In the endocrinology literature, intensive insulin therapy has been associated with a delay in macrovascular and microvascular disease progression in diabetic patients, probably though the

combined benefits of insulin and a stricter control of blood sugar [180-183]. Furthermore, intensive insulin therapy has been associated with reduction in IR though the exact mechanism remains uncertain [184]. A possible explanation of this effect is thought to be mediated through insulin's homeostatic effects on blood glucose and lipid metabolism [185, 186].

Positive clinical outcomes where repeatedly demonstrated in studies using insulin as an intervention only when it was coupled with a steady state of normoglycemia. This fact illustrated the importance of achieving normoglycemia and avoiding hyperglycemia to achieve positive outcomes when using insulin based therapies [187-191]. Hence, the dual effect of hyperinsulinemia coupled with normoglycemia is believed to be the optimal combination to achieve better clinical outcomes [192].

Multiple clinical trials in the intensive care settings have shown remarkable morbidity and mortality benefits of maintaining normoglycemia with intensive insulin therapy [189, 193-195]. Besides multiple systemic benefits, Vanhorebeek et al. estimated a 3 – 4 % lower risk of death in populations receiving intensive insulin therapy by reviewing available randomized studies [193]. Unfortunately, due to the significant occurrence of severe hypoglycemia among patients treated with intensive insulin regimens, some trials failed to show overall benefit of intensive insulin therapy suggesting that it can be potentially harmful [196]. Severe hypoglycemia rates, defined as blood sugar of 2.2 mmol/L or less, were associated with intensive insulin therapy in as high as 17% of treated patients [196, 197]. Fortunately, most of the episodes of hypoglycemia were brief with no further long term sequellae [197-199]. Hence, although the most effective method to achieve the full benefit of insulin seems to be through intensive intravenous insulin infusion protocols, preventing severe hypoglycemia appear to be the most important obstacle to overcome in order to achieve the clinical benefits of intensive intravenous insulin therapy [193, 200-202].

Regulation of Insulin Signaling

The regulatory mechanisms of insulin signaling are multiple [203]. Insulin signaling can be inhibited by altering the phosphorylation of its receptor residues (IRS) under various physiological and pathological conditions [204]. In vitro models have demonstrated the potential effects of hyperglycemia to attenuate insulin induced signaling by affecting the association of IRS-1 to the insulin receptor where insulin can auto-reverse this effect [205, 206]. Inflammatory cytokines such as IL-6 have been shown to reduce tyrosine phosphorelation of IRS-1, contributing to the subsequent insulin resistance and development of T2DM in chronic hepatitis [171]. Also, TNF-α have been shown to contribute to the occurrence IR in the setting of chronic HCV infection [207]. High and regular levels of ethanol consumption effects insulin signaling by negatively impacting PI3k/Akt pathway, through causing high cellular oxidative stress, consequently producing hepatic insulin resistance, steatosis, inflammation and apoptosis [208]. Also, the family of suppressors of cytokine signaling (SOCS), that can be induced by inflammation, interacts directly with insulin receptors to inhibit signal transduction and degrades IRS-1 and IRS-2 [209]. SOCS1 can be induced by insulin signaling to facilitate autoregulation while SOCS3 and SOCS7 can be induced by HCV proteins.

The Insulin Clamp Protocol

Insulin is usually used to control blood sugar in hospitals; however, the frequently used insulin sliding scale only reacts to elevated blood sugar readings. Hence, insulin sliding scale does not ensure a steady state of normoglycemia. Intravenous insulin protocols on the other hand are designed to maintain a steady state of normoglycemia by titrating a continuous infusion of intravenous insulin aiming at tighter control of blood sugar.

The idea of intensive intravenous insulin administration where a constant normoglycemia is targeted has been established in the surgical and intensive care literature for long [193]. Although the

concept is the same, different studies have used different protocols with different rates of insulin infusion, different glycemic targets and different monitoring protocols [11-13, 160, 187, 190, 191, 196, 202, 210-214].

In our institution, the concept of hyperinsulinemic-normoglycemic clamp where a fixed rate of insulin is infused and blood sugar is titrated with a concomitant dextrose infusion has been developed and tested for safety and efficacy [215-217]. Although the hyperinsulinemic-normoglycemic clamp can seem very similar to intensive glucose control by intravenous insulin at first glance, there are few important differences between both concepts. An insulin clamp technique aims to deliver higher doses of insulin than physiologically required without rendering the patients hypoglycemic. Hence, patients are given a continuously titrated infusion of dextrose to maintain normoglycemia. On the other hand, intensive glucose control by intravenous insulin protocol aims to achieve normoglycemia targets by calculating an appropriate continuous infusion of insulin as per the patient's physiological requirement. Although both methods aim at achieving normoglycemia they stand to be conceptually different.

The hyperinsulinemic-normoglycemia clamp, referred to as HNC, has been used in patients undergoing cardiac surgery and hepatic surgery. High insulin infusion rates (1-5 milliunit/kg/min) were used with similar glycemic targets arriving to promising clinical outcomes in both patient populations [12, 160, 161, 218].

SECTION 4: Liver Damage and Repair

Clinical Assessment of Liver Damage

The liver plays a central role in metabolizing toxins as well as processing and storing incoming nutrients. Moreover, the liver is responsible for the synthesis of clotting factors and important proteins to maintain vascular volume. Ongoing liver damage in cases of chronic hepatitis will eventually affect the synthetic capacity of the liver leading to problems in hemodynamics, clearance of toxins and clotting function. Additionally, with the changing architecture of the liver's parenchyma as a result of fibrosis, a subsequent narrowing of the portal venous system will cause high venous pressure and further hemodynamic compromise, otherwise known as portal hypertension [219].

Based on the pathophysiological alterations related to ongoing liver dysfunction, two scoring systems have been widely used to reflect the severity of patient's clinical condition. First, The Child-Pugh score which was developed in the 1970s to evaluate the need for surgery in patients with portal hypertension [220]. Since then, it has proven to be of great value in prognosis and – to a lesser extent – in patient selection prior to surgical interventions [221]. The scoring system is based on three biochemical parameters and two objective clinical parameters to grade the severity of liver dysfunction. The severity scale starts with Child-A, reflecting mild liver decompensation, to Child-C reflecting, server liver decompensation. Secondly, the MELD (Model for end-stage liver disease) score which was originally developed to predict survival after the transjugular intrahepatic portosystemic shunt (TIPS) procedure for patient with liver cirrhosis [222]. The MELD score relies solely on biochemical parameters avoiding the subjectivity present in the Child-Pugh scoring. The MELD score is calculated via a complex mathematical formula incorporating these biochemical values. It has proven to be a good prognostic score as well and has since been used globally to

guide organ allocation for patients awaiting liver transplantation [223]. Despite noted differences between both scoring systems, they both act as good prognostic tool with 70-85% accuracy in predicting patient's survival [223, 224]. On the other hand, both scores do not measure actual functional capacity of the liver, but rather relies on measurable markers of global liver dysfunction.

Dynamic liver function tests were established several decades ago as a method to estimate actual liver functional capacity. Though promising, these tests continue to face a very complex challenge due to the huge number of liver functions that are difficult to fully assess. The main principle of dynamic liver function tests is based on quantifying how much cans the liver process of exogenously administered substrates in a given time frame. Substances used are either cleared by the liver, or used by the liver in synthesis of other substances, reflecting, in turn, hepatic extraction ratios and metabolic capacity respectively [225-227]. Potential roles in assessing inborn errors of metabolism and predicting outcomes of transplanted liver grafts have been reported [228, 229]. Despite the ability of dynamic function tests to assess the liver's residual functional capacity, these tests are not widely used in clinical practice.

Various dynamic liver function tests have been reported in the literature testing different microsomal, cytosolic and mitochondrial metabolic pathways [230-235]. Studies have demonstrated the ability of dynamic liver function tests to stratify patients based on the severity of their liver disease with good correlation with histopathological evaluation [236]. Estimation of albumin synthesis to reflect liver synthetic function has gained validity especially in cirrhotic patients [237-239]. The importance of albumin synthesis in this context is due to the fact that albumin is the most abundant circulating protein and accounts for a large portion of hepatic protein synthesis. . Several methods have been implemented to study different aspects of protein and carbohydrate metabolism using labelled isotope tracers. One method is the primed continuous infusion method, where a

priming dose of a labelled isotope tracer is administered intravenously followed by a continuous infusion of the same isotope tracer. When a steady plasma concentration of the isotope is reached, blood samples are obtained to study aspects of protein or carbohydrate metabolism by measuring the relative amount of tracer isotopes in different body compartments or its incorporation into other molecules. Hence, several dynamic liver function tests can illustrate changes in the amount of protein production and breakdown, as well as changes in overall endogenous glucose production from glycogenolysis and gluconeogenesis. [226] The value of monitoring these metabolic parameters stems from the known negative impact of HCV particles and the inflammatory state of chronic hepatitis in interrupting normal insulin signaling. Insulin is known for its role in preserving body proteins by preventing its breakdown, as well as its role in glycogen synthesis and suppression of gluconeogenesis and glycogenolysis.

The development of non-invasive liver function tests has been ongoing for decades but still hindered by its suboptimal sensitivity and specificity. Breath tests are available using carbon labelled substances, such as 13C-aminopyrine and 13C-methacetin, to reflect hepatocellular enzymatic capacity in patients with acute and chronic liver dysfunction [240, 241]. Indocyanine green dye has also been used to measure hepatic flow as it is solely and rapidly removed by the liver. A non-invasive optical finger sensor is used to determine the rate of clearance of indocyanine green using pulsed spectrophotometry [242]. Several studies have shown good correlation between indocyanine green clearance and clinical scoring systems for liver dysfunction, i.e. MELD and Child-Pugh scores [243, 244].

Biochemical liver enzymes, which are secreted in the blood stream as a result of liver injury, are not usually used to follow up patients with chronic viral hepatitis. Still, some biochemical parameters have shown to carry some value to indicate faster disease progression and the degree

of fibrosis [245, 246]. Persistently normal alanine aminotransferase (ALT) levels in patients with chronic hepatitis C infection favored a slower progression of liver disease when compared to patients with elevated ALT levels [247, 248].

The Regenerative Capacity of the Liver

The liver has an innate capacity to self-regenerate after volume loss and self-repair after injury in slow rates [249]. This ability is based on the capacity of hepatocytes to undergo multiple mitosis and increase in size of hepatocytes [250]. Factors such as the Hepatocyte growth factor (HGF), epidermal growth factor (EGF), TGF- α, IL-1, IL-6, TNF-α, estrogen, norepinephrine, serotonin, and insulin have been shown to play a role in promoting this mitogenic effect [251-254]. Besides the multiple effects of insulin to promote hepatocyte metabolic function, insulin plays a substantial role in promoting growth and regeneration even in – otherwise – unfavorable conditions [255]. Multiple cells are involved in coordinating the process of liver regeneration, including endothelial, Stellate, Kupffer, biliary-duct cells and platelets [251].

Liver progenitor cells (LPCs) have been described in human livers. LPCs have higher proliferative potentials than hepatocytes and encompasses some stem cell properties [256]. They consist of a heterogeneous population of non-parenchymal cells found in the portal tracts and periportal regions that have the potential to differentiate into hepatocytes or bile duct epithelium. These cells are of significant importance in cases of significant hepatic injury or when hepatocyte proliferation is impaired [257-260].

HCV has a significant impact on the liver function and histology [9]. Interestingly, studies have documented the ability of the liver to regain biochemical function and improve histological parameters following eradication of the HCV infection. For instance, successful clearance of the

hepatitis C virus with Interferon based therapy have led to cessation of portal inflammation and improved insulin resistance in concordance with improvement in biochemical parameters, histological grading and portal hypertension in different studies [261-263]. Even more, in patients where HCV infection is still persistent, improvements in liver function were sometimes noted in response to treatments aimed at reducing hepatocytic oxidative stress, liver inflammation or insulin resistance [10, 30, 129, 264-266]. Recent researches suggest potential reversibility of liver fibrosis, especially in its early stages, regardless of etiology [267-269].

Alterations in the levels of growth factors involved in the process of liver regeneration have been repeatedly reported in the setting of cirrhosis and chronic viral hepatitis [270, 271]. Expression of certain growth factors, such as HGF and VEGF, tend to increase in patients with chronic hepatitis C virus [271, 272]. Additionally, liver progenitor cells are believed to be activated in livers of patients with chronic viral hepatitis despite the overall impairment in liver's regenerative ability [273, 274]. These phenomena may reflect the body's response to liver injury by initiating regenerative repair processes. The satellite cells play a central role in promotion and termination of liver regeneration [259, 275]. The satellite cells produce an array of angiogeneic factors as wells as growth factors that enhances hepatocytic proliferation. Concomitantly, the satellite cells secrets factors that allow modulation of the extracellular matrix, such as TGF-β, in preparation to terminate further regeneration [249].

Insulin has been continuously associated with hepatic regeneration and has been considered an auxiliary liver mitogen. Animal studies performed by Statzl. et al have demonstrated the clear protective effect of insulin against liver atrophy in otherwise unfavorable environment [255, 276, 277]. Additionally, Fisette et al. has also shown the ability of high-dose insulin infusion to reserve hepatocyte energy and decrease apoptosis after periods of surgical stress [11]. Furthermore, more

recent studies suggest a potential beneficial role of intraportal insulin infusion in promoting liver regeneration [278].

Thus, long term high-dose insulin therapy stands to be a potential effective adjuvant therapy to patients with HCV chronic liver disease as it targets its pathophysiology at both the cellular and molecular levels. Potentially, modulating the inflammatory reaction and assisting the liver to regenerate and regain lost functional capacity.

Our objective is to study the effect of high insulin therapy sessions in patients with non-acutely decompensated Hepatitis C virus induced cirrhosis.

We hypothesis that high-dose insulin therapy sessions will improve liver function, reduces local and systemic inflammatory response and possibly improves systemic insulin resistance in the targeted patient population.

Methods

Study Design and Participants

This is a pilot interventional study. The study was approved by The Biomedical A (BMA) Research Ethics Board (REB) of the McGill University Health Centre (10-086-BMA). The study was also registered in clinicaltrial.gov with a reference number (NCT01271140).

Starting February 2011, all adult patients diagnosed with chronic hepatitis C virus infection who are referred to or followed by the Hepatology group at the Royal Victoria Hospital (MUHC) were considered for the study. The pilot was designed to include the first 5 consecutive consenting patients who meet the following inclusion and exclusion criteria.

Inclusion criteria:-

- Hepatitis C positive adult patients (HCV positive antibody on serum testing)
- MELD score 6-15 at the time of inclusion
- Not receiving – or planned to receive – antiviral therapy

Exclusion criteria:-

- Evidence of hepatocellular carcinoma at the start of the trial either by imaging and or AFP levels above 400
- Hepatitis C Virus genotype 3
- Undetectable HCV RNA in serum
- Co-infection with hepatitis B virus or HIV
- Recent infection requiring a hospital visit or admission within the last 3 months
- Recent evidence of gastrointestinal bleed within the last 3 months

Patient Identification and Consent

Patient meeting the criteria above were identified at the Hepatology outpatient clinic at the Royal Victoria Hospital, Montreal – Canada. The primary physician introduced the study with a brief description of what the study would entail to the patient. If an initial approval was obtained, the study coordinator was contacted and given the necessary patient's information. The research coordinator and the study investigators were made available to introduce and explain the details of the study during the patient's next follow up appointment. An informed consent was obtained (appendix 1 and 2). Also, a detailed timetable was formulated to fit the patient's life style and working schedule. A copy of the timetable was handed to the each patient at the end of the meeting, with the study team contact information for future questions.

Intervention – The HNC Therapy Sessions

The hyperinsulinemic-normoglycemic clamp (HNC) was the primary intervention utilized in this study testing its therapeutic potentials. The sessions of HNC therapy were set to be 6-hour long. Each patient was scheduled to receive two sessions per week for a minimum duration of 14 weeks to a maximum of 24 weeks as per the patients' tolerance. These sessions were set to continue in an uninterrupted fashion for the length of the study. Some visits were dedicated to perform scheduled tests as detailed later. These tests were performed at the beginning of the study, every 7 weeks during the study period, and at the end of the study.

Patients were asked not to eat or drink starting 3 hours prior to the start of each session except for water to avoid unexpected fluctuations in serum glucose levels during the HNC therapy session. During the HNC therapy session patients were also only allowed to drink water until the insulin infusion was stopped.

The newly developed protocol for the HNC therapy sessions that determines the rate of dextrose infusion, monitoring intervals and the target for glucose control is detailed in (appendix 3). The main concepts were primarily adopted from our research group's previous work done using the HNC in the domains of cardiac and liver surgery with some modifications [11, 12, 212].

The concept of HNC was envisioned for use outside the operating rooms and intensive care units for the first time. New challenges accompanying this transition had to be identified and tackled to ensure safety and feasibility of its application. These challenges were mainly related to the change of environment where HNC sessions were administered, the health care personnel who were implementing the HNC protocol, the available resources to establish IV access and monitor blood sugar and ensuring the safe discharge of patients after HNC sessions. The rate of insulin infusion was maintained at 2mu/kg/min - similar to previous trials - and the target serum glucose level was set to be from 4 – 6 mmol/dl [12] .

Patients received 6-hour long sessions of high-dose insulin therapy via the HNC twice a week for the duration of the study. Given the nature of dealing with an awake patient in an outpatient setting we modified the methodology and frequency of blood glucose monitoring, using finger pricks, to ensure the safety of the patients and also to ensure that the patients can go home safely at the end of each session. Using previous experiences with HNC, the modified protocol - used in this study - was meticulously reviewed to accurately reflect the interplay between the intravenously administered insulin and the expected changed in body glucose levels. These changes were detailed over three chronological phases that reflect the start of the HNC application until the end of the session, namely; the initiation phase, the maintenance phase and the termination phase (appendix 3).

To start the insulin and dextrose infusion, a peripheral intravenous cannula was secured in the patient's arm. In cases where the patient agreed to have a peripherally inserted central catheter (PICC), the PICC line port was cleaned with alcohol swaps and flushed with 30 ml of sterile NS prior to use.

The needed intravenous solutions were prepared prior to the start of the session. Insulin was prepared in normal saline (NS) bags diluted in a ratio of 1 unit of Humulin-R® regular insulin (Eli Lilly, Indianapolis, Indiana, USA) to 1 ml of NS. Bags of D10 water or D20 water (D10W®, D20W®; Baxter, Mississauga, Ontario, Canada) were used to administer intravenous dextrose. Phosphate was supplemented in the form of sodium phosphate mixed with the intravenous dextrose bags in the concentration of 15mmol of sodium phosphate (PHOSPHATE-SANDOZ®; Sandoz Canada, Quebec, Canada) in each liter of D10W® or 500ml of D20W®. Potassium was supplemented in the form of potassium chloride tablets. Patients with normal renal function were given 20meq of potassium chloride (Jamp-K20®; Jamp Pharma Corporation, Quebec, Canada) at the start of the session to avoid the unlikely event of symptomatic hypokalemia. In patients with known chronic renal impairment, no potassium supplementation was given in the absence of laboratory evidence of low potassium levels or symptoms suggestive of hypokalemia during the HNC therapy sessions (appendix 3).

Using a multi-channel intravenous infusion pump, both insulin and dextrose solution were setup and lines were primed. Using a connector, both intravenous lines were joined to pass through the same intravenous cannula. This setup procedure was done constantly to ensure the safety of the patient so that if a technical error is to occur; both insulin and dextrose infusion will simultaneously stop.

Blood glucose levels were measured using the finger prick test (Accu-chek® glucose monitor; Roche Diagnostics, Basel, Switzerland). Measurements of blood sugar were obtained in preset time intervals as per the ambulatory HNC protocol and at any time the patient complains of symptoms or shows signs of hypoglycemia such as blurred vision, tingling around the mouth, heart palpitations, shakiness, anxiety, sweating or disturbed consciousness level. Patients were regularly informed of these symptoms and signs to facilitate early discovery of a hypoglycemic episode.

After each session the IV cannula was removed and the patient was allowed to leave the hospital. If a PICC line was in place, a new cap was installed and the catheter was again flushed with 30 – 60 ml of sterile NS then 4 ml of heparinized saline (1000 units/ml) was instilled to prevent clotting. Weekly dressing change for the PICC line was arranged concomitantly during patient's visits.

Assessment Parameters and Timeline

At the first week of the study, each enrolled patient had two scheduled visits to Royal Victoria Hospital in order to perform the baseline tests and investigations prior to the first HNC therapy session as follows: First, a physician performed a brief history and physical examination. Then, blood was withdrawn to test for liver function, kidney function, Hb-A1c, lipid profile, fasting insulin and glucose levels, inflammatory cytokines, lipokines, growth factors and HCV viral load. A baseline abdominal CT was performed to assess radiological liver volume, and to rule out any signs of worrisome hepatic lesions. The baseline insulin resistance and sensitivity measurements were performed. Also, dynamic liver function testing using priming continues infusion with stable isotopes of phenylalanaine, leucine and glucose to assess the liver's synthetic capacity and the efficiency of protein and carbohydrate metabolism was performed as will be detailed later. A liver biopsy was obtained - if the patient consented to it - to look at histological staging of fibrosis, grading of inflammation and percentage of hepatic steatosis. Additionally, two quality of life

questionnaires were used, namely; the EQ-5D and SF36, to reflect the patient's self-reported quality of life.

Each 7 weeks, a similar set of blood investigations were obtained to follow up biochemical parameters including measurement of inflammatory cytokines, lipokines, growth factors, HbA1c, lipid profile, fasting insulin and glucose levels, liver and kidney function tests, measurement of insulin resistance as well as a repeat of the dynamic liver function test. A repeated assessment of the quality of life was also obtained.

At the last week of the study, a set of investigations similar to the ones done in the beginning of the study were performed. This included clinical re-evaluation by a physician, repeat of the blood investigations, abdominal CT and dynamic liver function testing using stable isotopes and quality of life questionnaires. Also, a second liver biopsy was obtained for histopathological analysis upon patient's consent.

Assessment Tools

Multifaceted analysis of liver function was targeted. Several assessment tools were used based on history taking, clinical examination, blood samples, tissue biopsies and radiological investigations. Due to the lack of an ideal test to measure global liver function accurately, multiple tests were used to get a better global view of the liver function and related improvements. Details of the used assessment tools are listed below.

Liver Function:

Child-Pugh scoring system

Measure	1 point	2 points	3 points
Bilirubin (total) µmol/l (mg/dl)	<34 (<2)	34-50 (2-3)	>50 (>3)
Serum albumin g/l	>35	28-35	<28
INR	<1.7	1.71-2.20	> 2.20
Ascites	None	Mild	Severe
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

Child-A = 5 – 6, Child-B = 7 – 9, Child-C = 10 -15

MELD (Model for end-stage liver disease) score

As per UNOS (United Network for Organ Sharing) calculation according to the following formula [279]:

MELD Score = [0.957 x Log_e (creatinine mg/dL) + 0.378 x Log_e (bilirubin mg/dL) + 1.120 x Log_e (INR) + 0.6431] x 10

Liver Enzymes

This includes the peaks and trends of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma-Glutamyl Transpeptidase (GGT) and Alkaline Phosphatase (ALK).

Dynamic Liver Function Tests

Stable isotope tracers for phenylalanine, leucine and glucose were used to estimate changes in whole body protein and glucose metabolism. We used the primed continuous infusion technique of carbon labeled L-[1-13C] leucine, L-[2H5]phenylalanine and [6,6-2H2]glucose (Cambridge Isotope Laboratories, Cambridge, MA). The duration of the study is 6 hours, starting with the infusion of the stable isotopes through a venous cannula secured in one arm, whereas blood samples are withdrawn through the contra-lateral arm through pre-set time points (appendix 4). Whole body oxygen consumption and carbon dioxide production were measured by indirect calorimeter using the open system indirect calorimetry device (Deltatrac Metabolic Monitor, Datex Instrumentarium, Helsinki, Finland).

Percentage of plasma enrichment of the administered isotopes was determined by gas chromatography-mass spectromentery (Analytic Percision AP2003, Manchester, UK). Subsequent calculations using the obtained data are performed to estimate fractional synthesis rates (FSR) of albumin, fibrinogen and total proteins. Also an estimation of total glucose production, total protein synthesis, oxidation and breakdown will be obtained. The preparation of the isotopes, the way of administration and subsequent analysis has been described in a previous publication [280-283].

Liver Biopsies:

Liver biopsies were optional test in this protocol, and so patients consenting to the study may elect not to have it done. All biopsies were obtained through a transcutaneous approach. The liver biopsies were scored based on Batts and Ludwig AJSP 1995 staging and grading system [284]. The same pathologist was asked to score all biopsy slides to minimize variability. Additionally, the amount of steatosis was estimated. The pathologist was blinded to the HNC therapy intervention.

Liver Volume:

Measurement of liver volumes using 3D CT scans volumetrics were used to examine liver volume changes over time as a verdict of liver regeneration [285-287]. It is an established method in calculating liver volume prior to major hepatic resections. [288]

The process of measuring CT liver volumes starts by importing the tri-phasic CT scans on a GE Medical Systems Advantage Windows 4.3 workstation (GE Healthcare, Chalfont St Giles, UK). Then, Measurements are performed on axial views of the porto-venous phase from 2.5 mm thick CT images of the abdomen. The liver is selected by defining the contour of the liver in a series of axial sections excluding major vascular structures. The total liver volume is then computed by multiplying the defined liver contour by the section thickness providing an estimation of total liver volume [289].

Estimation of liver volumes radiologically has shown strong statistical correlation with intraoperative liver weight and water displacement volume [290].

Metabolic Parameters:

Insulin Resistance and Sensitivity

Details about the amount of insulin and dextrose administered during each session of HNC, as well as systemic insulin resistance (IR) calculated by HOMA–IR formula are reported.

Formula for HOMA-IR calculation: fasting glucose (mmol/L) x fasting insulin (mlU/L) / 22.5

Systemic insulin resistance encompasses both hepatic and peripheral insulin resistance [291]. Using data from the dynamic liver function test, the hepatic insulin resistance index will be calculated as per the following formula [292, 293]:

Hepatic IR index = Fasting insulin (µunit/ml) x Endogenous Glucose production (µmol/Kg/min)

Insulin sensitivity is estimated during each session of the HNC. Due to the high dose of insulin infusion, the endogenous production of insulin and glucose becomes negligible during the HNC session. Accordingly, the body will require a certain amount of exogenous dextrose infusion to maintain normoglycemia in the face of continuous, and fixed, insulin infusion in the fasting state. Insulin sensitivity is therefore equal to the amount of exogenous dextrose required to maintain normoglycemia. We estimated insulin sensitivity in the first 2 hours of the maintenance phase of the HNC clamp according to the protocol (appendix 3).

Lipid profile and Weight Change

Lipid profile testing incluses low density lipoproteins (LDL), High density lipoproteins (HDL), cholesterol and triglycerides as well as the cholesterol:HDL ratio. Weight will be measured prior

to each HNC therapy session and BMI will be calculated accordingly. Changes in weight during the length of the study were monitored.

Inflammatory and Growth Markers:

Inflammatory Cytokine and Growth factors

Concentrations of human TNF-α, IL-1β, IL-6, IL-8, IL-10, MCP-1, IP-10, TGF-α, TGF-β, HGF, VEGF and EGF were measured in serum. Milliplex human cytokine kit was used following manufacturer's specifications (HCYTOMAG, HADK1MAG, HAGP1MAG and TGFBMAG kits; Millipore Corp, Bilerica, MA, USA). The samples were analyzed in duplicates by suspension bead array immunoassay with the Luminex 200 X-map instrument (Luminex Corp, Austin, TX, USA) with a reported sensitivity of 0.4pg/mL.

Preparation of the samples consisted of serial dilutions of a reconstituted human cytokine standard to produce a standard curve from 3.2 to 10,000 pg/ml. The standards were mixed 1:1 with 25µl of serum matrix. Serum samples were mixed 1:1 with 25µl of assay buffer and before placement into a place in the 96-well plate. Then, 25µl of diluted antibody coated beads were added to all wells. A plate shaker (Barnstead Int, Dubuque, IO, USA) was used to mix the wells' content for about 16 hours at +4° Celsius. After that, the plate was washed with a wash buffer before and after addition of detection antibodies followed by the addition of 25µl of Streptavidin Phycoerythrin. The plate was sealed and contents allowed to mix using the plate shaker for 30 – 60 minutes during each step. Finally, the fluid was removed and the plate was washed again with wash buffer before the beads were re-suspended in sheath fluid for five minutes. The cytokines were analyzed on the Luminex instrument using MasterPlex CT 1.2 software (MiraiBio Inc, Alameda, CA, USA). Mean fluorescence intensity was obtained from a minimum of 50 beads per sample. Concentrations were

calculated from the standard curve generated by the MasterPlex QT 4.0 analysis software (MiraiBio Inc, Alameda, CA, USA).

Acute Phase Reactants

This includes C-reactive protein and rate of fibrinogen synthesis.

Quality Of Life Questionnaires:

SF-36 and EQ-5

To examine the subjective effects of HNC therapy, two multi-purpose Questionnaires (SF-36 and EQ-D5) were used to reflect on the patients' sense of wellbeing (appendix 5 and 6). Both questionnaires have been extensively used to assess health outcomes and proven to yield reliable results [294-302].

The SF-36 is a very popular generic questionnaire that is composed of 8-subscales to reflect upon physical and mental health. It has been used to assess quality of life across a vast number of medical conditions. The questionnaire generates very useful disease-specific benchmarks in comparison to general population norms. It scores are calibrated so that a score of 50 represents the populations' norm or a state of average health [294]. It has specifically used to measure quality of life in patients with hepatitis C while not on therapy [303, 304]. Furthermore, the scoring of the SF-36 questionnaire has been adjusted to fit the norm of the Canadian population by the International Quality of Life Assessment (IQOLA) [305-307]. We obtained the scores according to the Canadian-population norms through the norm-based algorithm calculator offered by http://www.sf-36.org [308].

The EQ-5D has been translated to many languages and used across continents [302, 309]. It was designed to reflect on health-related quality of life through a descriptive system and a visual analogue scale (EQ-VAS). The descriptive system consists of 5 dimensions, namely, mobility, self-care, usual activity, pain/discomfort and anxiety/depression. In each dimension the patient indicates if he/she experiences no problem, some problems or extreme problems. In addition, it has a 20-cm visual analogue scale (EQ-VAS) to obtain a self-rated overall health value from 0 to 100.

Through population based studies, multiple country-based value sets have been developed that assigns different weights to all 5 dimensions measured by EQ-5D descriptive system. This would allow the responses to the EQ-5D descriptive system to be summed into a summary index value. The value of 1.0 always indicates perfect heath and 0.0 indicates death. As the value sets for Canada is currently in development, we will be using the United States value set to generate the index scores in this study [310].

Both The SF-36 and EQ5-5D questionnaires were used to monitor subjective changes in patients' reported quality of life. The questionnaires will be conducted through an interview with the research coordinator.

HCV RNA and Other Factors Related to Disease Progression

As patients with HCV liver cirrhosis are prone to developing hepatocellular carcinoma (HCC), we used biochemical screening and radiologic investigations to rule out HCC. Only patients with no suspicion of HCC were eligible for enrolment.

Triphasic CT scans were obtained at the beginning and at the end of the study to rule out the presence of any suspicious lesion and Alpha-feto protein was used as a tumor marker of hepatocellular carcinoma [311].

Viral load using HCV PCR technology was assessed at the beginning and at the end of the study.

Estimation of alcohol consumption using the Short Alcohol Dependence Data (SADD) questionnaire [312]. (Appendix 7)

Results

After interviewing a total of thirteen patients, three (23%) patients accepted to get enrolled in this study while the other ten patients either refused (62%) to participate or deferred their decision to a later date (15%). The most prominent reason for refusal was the inability of the patients to invest the required time for the high-dose insulin therapy sessions. Other reasons were related to patients' busy working schedule or the absence of sufficient compensation for their enrolment in the study (figure 1).

One of the three patients who initially agreed to participate got shortly admitted to the Royal Victoria Hospital with the diagnosis of upper gastrointestinal bleed and was therefore excluded prior to the study's starting date. Hence, only two patients were enrolled into the study.

Patient #1

Patient #1 was a 56 year old male who has been diagnosed with chronic HCV infection since 1996 during a medical workup for progressive tiredness. The HCV genotyping revealed genotype 1a. The exact cause of HCV infection is unknown; however the patient attributes it to a work related

injury with a sharp object in the 1980's. No history of intravenous drug use, previous tattoos or history of blood transfusions. No other family member known with chronic HCV infection. Other etiologies for chronic hepatitis were excluded at the time of diagnosis. No other viral co-infections, namely HBV or HIV. Diagnosis of HCV cirrhosis was initially documented on 2005 through a pathological analysis of a liver biopsy. Since 2005, the patient has been getting annual CT scan of the liver to rule out the presence of suspicious lesions. Also the levels of alpha fetoprotein have been repeatedly low. The latest gastroscopy done early in 2011 revealed the absence of significant esophageal varices. The patient was never previously admitted for overt gastrointestinal bleed or medically uncontrolled encephalopathy.

The patient is also known for dyslipidemia and was diagnosed with diabetes mellitus type 2 in 2004; he takes rosuvastatin 5mg daily, metformin 750 mg three time a day, glubyride 10 mg daily and lactulose only upon need. He stopped smoking for more than 30 years now and he is currently an occasional drinker.

The patient had 3 trials of interferon-based therapy to try and clear the virus. The first was on 1997 where he was given interferon monotherapy for a period of 6 months with initial response and early relapse. On 1998, another course of interferon and ribavirin was given for 6 months resulting in partial response. Last course of therapy was in 2006, when he received PEGylated interferon – α 2A with ribavirin for a period of 6 months with only partial response. During the last course of therapy, the patient bared multiple side-effects, most prominently was symptomatic thrombocytopenia and multiple cutaneous side effects.

Upon enrollment, the patient's main complaints were fatigue and somnolence; sleeping more than 12 hours daily on average. His alcohol consumption was minimal, scoring 2 out of 45 in the Alcohol Dependence Data Questionnaire (SADD) indicating low alcohol dependence. His clinical

exam revealed normal vital signs, height of 160 cm and weight of 77 Kg constructing a BMI of 30. No signs of jaundice, edema, venous hypertension, palmer erythema, spider nevi or gynecomastia. Patient was oriented to time, place and person, he however had positive asterixis. The baseline investigations and subsequent investigations are listed in (table 1 and 2).

Course and Results for Patient #1

During the study period which spanned through 14 weeks, the patient received a total of 26 HNC therapy sessions scheduled twice per week with no interruptions; each session lasted for 6 hours. The intravenous insulin infusion ranged from 9.1 to 9.5 units/hour according to changes in body weight. Total units of insulin, Humilin-R®, infused during each session were 53 units in average (range 50 – 58 units). The starting weight was 77 kg and ranged from 76 to 79.5 kg through the time of the study ending at 76 Kg at the last session. The average time required to reach target range of blood sugar, between 4 – 6 mmol/L, during HNC therapy sessions was 101 minutes (range 55 – 145 minutes). Using D10W®, the maximum infusion rate of dextrose ranged from 220 – 380 ml/hour with a mean total of 102.6 mg (range 83.2 – 143.6mg) of dextrose administered each session. A total of 15 blood sugar readings were slightly lower or higher than the target range of 4 – 6 mmol/L, yet not more than 1.5 mmol/L off target range. Two readings clearly deviated beyond acceptable limits. The patient felt tremulous and light headed twice which were attributed to hypoglycemia prompting a measurement of blood sugar revealing a blood sugar of 2.9 and 2.3 mmol/L. Dextrose boluses were administered abruptly according to the protocol without interrupting the insulin drip achieving appropriate response. In both encountered incidences, the blood sugar measurement was scheduled to occur within 5 minutes or less as per the protocol. Another reading of 8.3 mmol/L was encountered and rapid correction was achieved according to

the protocol. No readings below 2.3 mmol/L or above 8.3 mmol/L were recorded after reaching the goal glycemic range during all session (table 4).

At the 14 week time point the patient was given the option to continue for a total of 24 weeks or to stop at this point. The patient requested to end the study at the 14 weeks point as he was offered another trial of HCV therapy with a new direct antiviral drug being tested in a clinical trial.

During the period of the study, a noted decrease in total bilirubin associated with concurrent decrease in liver enzymes (table 1). Also, a decreasing trend in multiple inflammatory cytokines serum levels was noted when compared to baseline. These changes were detected from the first interval blood tests on week 7 and persisted on week 14 (table 2). Serum levels of TNF-α and IL-10 dropped more than 45% whereas serum levels of IL-6, IL-8 and MCP-1 dropped to a lesser degree. Concurrently, HCV viral load measured by PCR technique dropped from 7,244,360 to 4,897,788 copies.

Growth factors, namely VEGF, HB-EGF and TGF-β serum levels increased more than 150% when compared to baseline (table 2). Additionally, a noted increase in liver volume was noted as measured through CT Volumetery comparing the size of the whole liver prior to initiation of HNC session and after the completion of 14 weeks of HNC sessions. Calculations were performed using 2 abdominal CT scans which were performed within 6 months from the start date of the first HNC session and 1 abdominal CT scan performed at the end of the study period. All CT scans were assessed by 4 physicians who are familiar with calculating CT volumetery using a special interactive software package that allows three dimensional visualization of the liver constructed from the axial CT images. The mean measured liver volume from the two baseline-CT scans were 1558 ml and 1527 ml respectively. The measured post-treatment liver volume from the CT scan was 1632ml reflecting an increase of 5.77% when compared to baseline. The measurements

showed high level of consistency between rates with an absolute interclass correlation coefficient of 0.877.

The dynamic liver function tests revealed good synthetic capacity of proteins and albumin throughout the 14 weeks. The amount of total protein breakdown decreased from 167.2 µmol/kg/h to 135.08 µmol/kg/h. Synthesis of fibrinogen and glucose dropped through time as well.

No obvious changes were noted in the serum levels of the measured adipokines, namely; adiponectin, leptin and resistin. Also, no changes were noted in quality of life measured by SF-36 and EQ-D5 quality of life questionnaires (table 3).

Patient #2

The second patient was a 59 year old male who has been diagnosed to have chronic HCV infection since 2005 after undergoing a medical checkup for progressive edema, abdominal distension and elevated liver enzymes. The HCV genotyping revealed genotype 1b. The exact cause of HCV infection is probably related to intravenous drug use with shared needles in 1970's. There was no history of tattooing or previous blood transfusions. No other family member is known to have HCV infection. Other etiologies for chronic hepatitis were excluded at the time of diagnosis by appropriate investigations. No other viral co-infections, namely HBV or HIV. Diagnosis of HCV cirrhosis was initially documented on 2006 through a pathological analysis of a liver biopsy. Since then, the patient has been annually screened to rule out the presence of suspiciously malignant liver lesions. Levels of alpha fetoprotein remained low on serial measurements. The latest gastroscopy done was in 2008 which revealed the presence of grade 1 esophageal varices. Patient was never previously admitted for overt gastrointestinal bleed, spontaneous bacterial peritonitis or medically uncontrolled encephalopathy.

The patient is also known to have spinal stenosis causing him to suffer from chronic back pain. Otherwise, the patient is not known to have other medical comorbidities. Although the patient used to be a daily drinker of alcohol, he stopped drinking heavily since he was diagnosed with HCV in 2005. The patient is currently on diuretics, namely; spirinolactone 150mg daily and furosemide 60mg daily. He also receives Quinine 300mg daily, ursodiol 250mg three times a day and lactulose upon need.

The patient had two trials of interferon based therapy to try and clear the virus. The first was on 2006 where he was given Peginterferon alfa-2a and ribavirin for a period of 6 months achieving only partial response. Although the course was not interrupted, the patient had difficulties due to consequential pancytopenia related to interferone therapy. This was treated with a weekly dose of filgrastim, a human granulocyte colony-stimulating factor, to maintain decent count of platelets and white blood cells. Straight after the first course, another course of therapy with Peginterferon alfa-2b and a higher dose of ribavirin were started. The second course was continued for 3 months only and was stopped due to failure to illustrate viral response besides persistent thrombocytopenia despite supportive medical therapy.

Upon enrollment, the patient's main complaints were consistent of fatigue and bothersome leg edema. He scored 3 out of 45 in the (SADD) indicating low alcohol dependence and has reported recent complete abstinence. His clinical exam revealed normal vital signs, height of 180 cm and weight of 117 Kg constructing a BMI of 36.1. The patient had poor dentition and overall poor body hygiene. He also had evidence of bilateral gynecomastia as well as a clear pitting edema in both lower limbs. There were no signs of jaundice, palmer erythema, spider nevi or caput medusa. The patient had an unremarkable cardiovascular and lung exam; however the jugular venous pressure could not be clinically assessed due to presence of heavy beard. Abdominal exam revealed

the absence of ascites with a negative shifting dullness examination and no clinically demonestratable hepatospleenomegaly. He was slow to respond to questions but nonetheless was oriented to time, place and person, with no signs of asterixis.

The baseline investigations and subsequent investigations are listed in (table 1, 2 and 3).

Course and Results for Patient #2

The patient started off as patient with Child-B cirrhosis and a MELD score of 10. The patient had received three sessions of high-dose insulin therapy scheduled twice per week. The insulin infusion ranged from 13.9 to 14.1 units/hour according to the patient's weight measured prior to each session. In all three sessions the patient's first blood sugar measurement was in the goal range (4 – 6 mmol/L). Using D20W®, the maximum infusion rate of dextrose ranged from 250 – 300 ml/hour with a mean total of 1010 ml (range 940 – 1090 ml) of dextrose administered each session. A total of two blood sugar readings were slightly lower or higher than target range - not more than 1.5 mmol/L - off target range. No readings below 2.6 mmol/L or above 5.8 mmol/L were recorded during all three session (table 4).

Unfortunately, on the second week of the trial when the patient was scheduled to receive the 4th session of high-dose insulin therapy, patient was not feeling well and was febrile and was sent to the emergency department. Patient was subsequently admitted to the hospital with the primary diagnosis of methecillin sensitive staphelococcus aureus (MSSA) septicemia, as was suggested by blood cultures, and was started on appropriate systemic antibiotics. The PICC line which was used for the administration of the high-dose insulin therapy was believed to be the potential source of infection. Unfortunately the PICC line was pulled out without proper culture. The patient had a prolonged and complicated hospital stay spanning over the course of 2 months and 20 days before he was able to return back home in good condition.

This was considered a serious adverse event which required presentation to the ethical board given the fact that the PICC line may have been the primary source of the problem. Although appropriate modifications took place in the study protocol and the PICC line was eliminated as an optional route for intravenous access. This event had a significant negative impact on the continuation of the study.

Discussion

Two patients were enrolled in the clinical study. The first patient had noted decrement in total bilirubin, liver enzymes, systemic inflammatory response and liver growth and with no apparent improvement in metabolic parameters or in the reported quality of life. The second patient didn't complete the study due to a hospital admission secondary to infection which occurred during the second week of the trial. The infection may have been related to the use of a peripherally inserted central catheter (PICC) as a route for insulin infusion.

In regards to the modified HNC protocol implementation in the outpatient setting, we had no recorded events of sever hypoglycemia – blood sugar < 2.2 mmol/L – during a total of 29 high-dose insulin therapy sessions. Blood sugar measurements were in the target range of 4 – 6 mmol/L in 86% of the time.

Chronic Hepatitis C virus infection is not viewed as merely a liver specific disease anymore but also as a metabolic disease that can affect multiple organ systems [313]. Studies describing the pathophysiology of the disease from cellular and molecular levels have enriched our understanding of this complex relation between the HCV, chronic inflammation and metabolic syndrome. HCV related oxidative stress and inflammation are believed to be a key mediator of subsequent increasing resistance and other elements of metabolic syndrome [99, 109]. Insulin resistance and

steatosis being results of HCV infection, then become key predictors of faster HCV disease's progression leading to cirrhosis [314 315].

Recognizing the vicious cycle that is created by the HCV through inducing inflammation and metabolic syndrome, multiple studies have targeted these elements either alongside conventional HCV treatment or when conventional treatment failed. Choi et al. have reviewed the role of oxidative stress and necroinflammation in the pathophysiology of HCV and reviewed previous studies that have used antioxidants as a therapeutic modality for chronic HCV infection [10]. Although some studies couldn't demonstrate beneficial effects, others have demonstrated the ability of different antioxidant regimes to reduce the levels of inflammatory cytokines, elevated liver enzymes and high viral titers as well as show some histological improvement [316]. Yet, these results were not always reproducible [317]. Attenuation of inflammation can also be achieved theoretically through inhibiting pro-inflammatory cytokines or enhancing anti-inflammatory cytokines. Nonetheless these strategies hold the potential concern of disrupting the immune response against the virus. Emerging reports suggest that these concerns may be overestimated but solid evidence is yet to be available [55, 318]. In fact, Knobler and Schatter successfully ameliorated metabolic syndrome in transgenic mice infected with HCV using anti-TNF antibodies [207].

On the other hand, some studies have targeted HCV pathophysiology by aiming to improve elements of metabolic syndrome. Hickman et al. demonstrated beneficial role of weight loss in the context of chronic HCV infection as it resulted in reduced ALT levels as well as improved histological grading of liver fibrosis and steatosis over the span of 3 months [129]. Several authors have used insulin sensitizers before or during interferon therapy with inconsistent result depending on viral genotype and the drug used. Khattab et al. showed an increased viral response when pioglitazone was added to interferon based therapy in patients with chronic hepatitis C genotype

4 though previous trials with piglitazone did not show similar effects [130, 132, 135]. Other studies that used metformin have suggested potential beneficial effect in lowering viral load in absence of interferon therapy [133].

Insulin therapy has shown multiple benefits through its use in different disease processes. Besides insulin's pivotal role in metabolic homeostasis, it has been shown to exert potent anti-inflammatory effects. Furthermore, Hassanain et al. have shown the benefits of peri-operative insulin therapy patients undergoing liver resection. Insulin therapy optimized the surgically reduced liver volume by increasing glycogen content and reducing apoptosis consequently reducing postoperative liver dysfunction [11, 12]. Although insulin therapy has always been a difficult thing to carry out given the serious side effects of hypoglycemia, the HNC concept facilitates the administration of high doses insulin with good safety and efficiency profiles [217, 320].

Many intersection points between HCV pathophysiology and reported benefits of insulin therapy have become evident with ongoing research. This lead us to the original idea of trying to improve liver function of HCV infected patient by sequential HNC therapy sessions; aiming to modulate their ongoing inflammatory reaction, improve their metabolic imbalance caused by the virus and potentially aid their liver into self-recovery. This has not been done before; thus this is the first trial – to our knowledge – to use insulin as a therapeutic drug for HCV patients. It is also the first trial to use the HNC concept in a long term fashion in out-patient settings.

In this pilot study, we combined the theoretical bases of HCV induced liver injury alongside previous observations from previous trials performed by our research group [12, 319]. Consequently, we proposed the concept of liver metabolic support via repeated sessions of high-dose insulin infusion as a potential therapeutic intervention to assist the liver against HCV pathogenesis. The introduction of this new concept required several focused group meetings and preparation of space

and personnel in order to transition to a practical reality. Performing repeated sessions of high-dose insulin therapy in outpatient settings was a great challenge to initiate and overcome. Hence, the choices made regarding the number of sessions and the length of each session were formulated after multi-disciplinary discussions between members of hepatobiliary, hepatology and anesthesia groups at the Royal Victoria Hospital who were largely involved in previous experiences with the HNC concept. Due to the novelty of the idea and approach, there was no available literature that can guide us on the appropriate length of treatment to achieve outcomes. Hence, as a proof of concept, the decision was to generate a protocol that would be intensive enough yet tolerable to patients in order to facilitate detection of outcomes. Acknowledging the absence of proven benefits of high-dose insulin for patients with chronic HCV up till the time of this trial, only patients with no available therapeutic options were allowed to participate. We adopted a strict selection criterion to ensure homogeneity of involved participants and to eliminate confounding variables as much as possible. Decisions on the inclusion and exclusion criteria, as well as the laboratory and imaging investigations to pursue during the study period were also discussed and agreed upon between participating groups.

Obtaining an intravenous access for the patients multiple times every week in an outpatient setting has been one of the issue that received the attention of participating research groups. Several ways to overcome this challenge to facilitate practicality and to enhance patients' compliance had been proposed. Of particular importance, the voluntary option of inserting a temporary PICC line for the participating patients was implemented in early protocols after having a discussion with the patients about its potential harms. Although the option of having a PICC line inserted was available in earlier protocols, this option was later omitted altogether due to encountering a serious adverse

event during the study period where the PICC line may have been primarily responsible for a state of septicemia.

The adoption of strict inclusion criteria to ensure exclusive examination of the effects of insulin therapy on homogeneous population of patients with HCV cirrhosis resulted in a very hard recruitment process; hindering us from reaching our sample size goal. In future projects, inclusion criteria need to be revisited to allow for a wider use of the HNC in patients with liver disease.

Interesting Results

Although one patient only completed this pilot study, there are several interesting aspects to be discussed. Compared to the patient's baseline characteristics, the patient had noted changes observed at blood investigations performed on week 7 and persisted at week 14. Initially, the patient had mildly elevated liver enzymes which normalized through the course of therapy dropping about 25% in levels. There was also an observable drop of total bilirubin levels from levels of 39 µmol/l at baseline to 25-30 µmol/l during the course of therapy. This may reflect definitive liver function improvement or just temporary improvement of cholestasis. Additionally, there was a notable concurrent drop in pro-inflammatory and anti-inflammatory cytokine levels, including TNF-α, IL-8, IL-10 and IL-6. Also, the level of MCP-1 dropped around 41% when compared to the baseline level. The concern of increased viral replicative activity arises with such modulation of cytokines. However, HCV viral load did not increase but rather showed a modest drop. Interestingly, similar effect has been previously reported with the use of insulin sensitizers for HCV infected patients, however, this can also merely reflect insignificant variation [321]. Although there was no noted change in CRP as an acute phase reactant, CRP was not elevated at baseline. Yet, the percentile synthesis of fibrinogen amongst body proteins – another acute phase

reactant – was elevated at base line and dropped adequately throughout the course of treatment. This may signify a beneficial shift of body protein synthesis away from the production of acute phase reactants. Concomitantly, a noted decrement in glucose production has been noted which, alongside the decrement in fibrinogen production, may be explained by the overall anti-inflammatory effect of high-dose insulin sessions. Collectively, these data may suggest a promising role of insulin in modulating the inflammatory reaction in HCV infected patients.

When looking at the metabolic parameters, we unfortunately don't see similar trends. There were no obvious changes in the lipid profile parameters and the HBA1C actually showed slight increment from 7.9 to 8.1 at the end of the study. The insulin resistance measured by the HOMA-IR also increased from 5.39 to 10.99. Although the HNC sessions were not performed as a diagnostic tool to measure insulin resistance or sensitivity, it can be utilized for that purpose. In fact, similar insulin clamp concepts are considered to be the gold standard for determining systemic insulin sensitivity. Insulin sensitivity reflects the amount of dextrose given intravenously per hour to maintain normal blood sugar levels in the fasting state when the patient is on fixed high dose of intravenous insulin. High dose intravenous insulin will block endogenous production of insulin and the amount of glucose that the body utilizes during this state reflects the sensitivity of cells to insulin [322, 323]. Given that the insulin rate was fixed to 2 milliunit/Kg/min during the HNC, we looked at the amount of needed intravenous dextrose to maintain blood sugar level between 4-6 mmol/L. This rate was calculated over 2 hours after the patient reached a steady requirement of intravenous dextrose. A progressively decreasing trend in insulin sensitivity over time was observed, parallel to what was observed by the HOMA-IR calculations (figure 1) [324].

IR in chronic HCV infection is not purely hepatic as one would assume. In fact, IR has been shown to be peripheral as well as hepatic in HCV patients. This is believed to be a result of the insulin

signaling interruption by the HCV particles and the resulting systemic inflammation [292, 325, 326]. Hepatic IR can be calculated from the level of fasting insulin multiplied by the endogenous glucose production – referred to as absolute glucose production –. When calculated in the enrolled patient we note that increase in hepatic IR was mild and not proportionate to the increase in systemic IR. This means that the noted over all elevation in systemic IR was mainly driven by an increase in peripheral IR rather than hepatic IR. We wonder if this difference, yet again, is due to a reduced hepatic inflammatory state. Nonetheless, the overall progressive increase in IR can be explained by the down regulation of insulin receptors in response to high doses of insulin administered, indicating that the amount of administered insulin may have been too high. Unfortunately, given that the patient has been suffering from Type-2 diabetes and dyslipedemia and has been taking medications to control both, these assumptions may perhaps be inaccurate. At this time, the exact insulin dose balancing the positive and negative effects on insulin resistance is not clearly defined but merits further studying.

The dynamic liver function tests revealed good liver ability to synthesis albumin reflected by the measured albumin FSR levels. All measured levels of albumin and total protein FSR were high, reflecting good baseline liver's synthetic functional capacity. Although patients were instructed not to have oral intake at least 3 hours before presenting for the dynamic liver function test session, there was no further nutritional instruction. Hence, fluctuation in the FSR of albumin and total protein is not unexpected and the absolute number is not a good correlate of change in liver's functional capacity in this setting but may rather reflect nutritional intake prior to the test [238, 283]. In fact, patients with advance cirrhosis have a blunted level of FSR for albumin despite stimulating factors [238]. Thus, starting with a good ability to produce albumin makes it difficult to elicit further improvement even by using dynamic liver function tests. Nonetheless, the estimated protein

breakdown decreased 20% from base line which concurs with the long reported protein-protective effects of insulin [327].

Liver regeneration has been a topic of interest for many decades. Insulin's role in liver regeneration has long been recognized, yet the exact role and extent of insulin effect on the regeneration process is not fully revealed [255, 277, 328]. Moreover, the humoral cytokines and growth factors' functional alterations during the state of liver regeneration have been observed and documented through the years [254, 329]. Insulin, HGF, EGF, VEGF, IL-6, TGF-α, TGF-β and several others have important roles to play to orchestrate the complex process of liver regeneration with significant interplay between these growth hormones and cytokines. Several recent reviews describe the complexity of the molecular and cellular mechanistic involved in liver regeneration [249, 250, 329-331]. Interestingly, we have observed several changes in cytokines and growth factors involved in liver regeneration during the period of the study which was associated with a measurable difference in liver volume measured through abdominal CT scans. We have noted a 5.7% increase in liver volume when comparing the liver volume of patient #1 before the start of the trial and 15 weeks later, following the end of the study. CT-based liver volumetery studies have been shown to be an excellent estimate of the actual liver volume with excellent reproducibility [332, 333]. Hence, an observed change of more than 5% by CT volumetry studies by 4 blinded observers suggests the presence of a real change in liver volume, especially in the absence of proportional change in the patient's body weight (figure 2). The real significance of this volume change is not clear, however plausible explanations are entertained. Insulin infusion, with concomitant glucose infusion, can lead to replenished glycogen stores and eventually to accumulation of fat leading to steatosis. Although this can explain the noted increase in size, we failed to see a reflective change in the percentage of steatosis obtained from the liver biopsies. The baseline liver biopsy showed an estimated 20-30%

steatosis while only 10-20% at the biopsy obtained at the end of the study. Also, the insulin clamp method used in this trial focuses in providing only sufficient amounts of glucose to maintain normoglycemia in face of high doses of insulin rather than a high carbohydrate load, therefore not facilitating the optimal biochemical environment that would lead to steatosis[334]. Kawata et al. have noted an association between high-dose insulin and dextrose infusion in rats after hepatectomy with the swelling of hepatocytes that was not related to accumulation of glycogen or triglycerides, but rather reflects the accumulation of intracellular water. This effect was again noted more prominently when high doses of glucose were administered. This effect was immediate and was associated with measurable liver dysfunction. Additionally, these changes were noted in liver histopathological examination appearing as vacuolar degeneration. We did not observe biochemical or histopathological changes suggestive of hepatocytic swelling in patient #1 that can coincides with the measured change in liver volume.

Enhanced capacity of the liver to regenerate is another potential explanation. The state of liver injury induced by hepatitis C is known to initiates the liver regenerative processes but the disease continues to progress beyond the liver's ability to regenerate [273, 274]. High levels of insulin in the portal circulation have always been recognized as an important factor that would assist the liver in its ability to regenerate. Startzl et al. have demonstrated how maintaining normal levels of portal insulin prevented the atrophy of dog livers after performing a surgical portosystemic shunt procedure [255]. Although the ability of insulin to enhance changes in liver volume has never been shown, safe high doses of insulin infusions has always been difficult to achieve [214]. Recently, Xu et al. were able to demonstrate a significant increase in volume of transplanted liver graft after one week when a continuous insulin infusion was delivered to the graft at a fixed rate [278]. Likewise, we maybe are observing the effect of administering high doses of insulin infusion repeatedly over

the course of 14 weeks. On the molecular level, insulin activates common pathways that are also important in regenerative signaling such as MAPK and PI3K/AKT signaling pathways [330]. The modulation of measured serum cytokines and growth factors provides further support for this explanation. We find an increase in levels of some growth factors like HB-EGF, VEGF and TFG-β while others such as IL-6, TNF-α and TGF-α decrease in levels. Unfortunately, due to the complexity of liver regeneration, the interplay between the inflammatory and regenerative signals and having the results of only one patient makes these changes hard to clearly rationalize. Nonetheless, the noted variability of serum levels of various cytokines and growth factors that are involved in the regenerative process during the time of the study, combined with the observed changes in liver volume is an interesting finding that merits further investigations.

It is important to note that all measurements were not obtained directly following insulin treatment, but rather before a scheduled HNC therapy session. These results suggest that insulin can have a persistent effect for at least few days after administration. However when comparing results obtained from 7 weeks versus 14 weeks of therapy we note no apparent cumulative effect.

Looking at both quality of life questionnaires, we do not illustrate significant differences between scores obtained before and after the study period.

Adverse Event

Therapeutics that modulates the inflammatory response such as steroids and anti-TNF have been associated with increased incidence of infectious morbidities [53, 54]. Although high-dose insulin therapy is believed to exert analogous effects to mentioned therapeutics, it has not been linked to infectious comorbidities in clinical trials. On the contrary, trials that have used high-dose insulin infusions have shown similar or reduced subsequent infectious complications to comparison groups [12, 160, 194].

In our early protocol, the presence of a PICC line used for administration of insulin therapy sessions carried a certain risk of developing blood stream infections. It probably served as an entry route for skin flora into the blood stream in our encountered adverse event with the second patient. However, the patient's initial symptoms and later complicated hospital course raises suspicion of other unrecognized sources of infection that may have not been related to the study's intervention or treatment. We consider this adverse event a potential result of using the PICC line in patients with suboptimal health status similar to our targeted study population. Although the PICC line was only optionally inserted for convenience after infection risks and other risks were disclosed to the patient, it was not an essential element for implementing the high-dose insulin sessions. Nonetheless, the option of having a PICC line has been appropriately eliminated in subsequent modification of the study protocol.

The Efficacy of the Ambulatory HNC therapy Protocol

During the course of the study we established the protocol of ambulatory HNC therapy – adopting from the previously reported GIN "Glucose-Insulin Normoglycemia" concept – allowing the administration of high doses of insulin in ambulatory settings (appendix 3) [320]. Previous GIN protocols were formulated as general guide lines for the use of the HNC. These protocols where appropriately brief, given that they were mainly used and managed by anesthetists in the operating rooms or by ICU teams who are familiar with the use of intravenous insulin. Operating the HNC outside the vicinity of the operating rooms and the ICU required a clearer and more detailed protocol that can be easily followed by healthcare workers who are less familiar with the HNC concept. With the collaboration of the Anesthesia group at the Royal Victoria Hospital, an ambulatory HNC protocol was developed tailored to the outpatient environment (appendix 3). The

protocol is sufficiently detailed to be used by any healthcare personnel with minimal amount of training.

Although the protocol remains labor intensive, we were able to demonstrate good safety and efficiency parameters similar to those previously published [320]. It is worth noting that it is not possible to speculate the exact percentage of time where blood sugar was in target range versus off range as blood sugar are not taken at fixed intervals but rather are protocol driven. This means that the number of measurements of blood sugar does not precisely correlate with time. However, having 86% of blood sugar measurements within target range and more than 99% of measurements within or slightly off target range reflects the protocol's safety and efficacy. We have not encountered any incident of severe hypoglycemia, defined as blood sugar less than 2.2 mmol/L, in all 29 sessions of HNC.

Limitations

Even though we have demonstrated exciting results, these have to be interpreted in the appropriate descriptive context. Having the data mostly evolving around a single patient is a huge limitation to any conclusive statements about our observations. These observations should only stimulate further testing of introduced concepts.

Given the novelty of this approach, we have aimed to have a fairly extensive, yet feasible, protocol to help us proof its theoretical bases or otherwise disproof it. However, given the relatively small pool of patients who meet the inclusion and exclusion criteria combined with their suboptimal health condition, it was not easy to find patients who are willing to participate in this trial. Additionally, this project required the collaboration of different healthcare groups from different disciplines. Due to the inherent differences between healthcare disciplines it was difficult to get a universal agreement from all parties to contribute and participate in this research project. For

instance, a significant limitation to access patients was that only 2 hepatologist willingly allowed us to introduce this project to patients they regularly follow in their clinics who meet the inclusion and exclusion criteria. Even more, the complication we encountered with the second patient had a big impact on the continuation of the study despite modifying the protocol to eliminate any potential offending agent (i.e the PICC line).

It is also important to acknowledge the short term application of the therapeutic intervention when viewed in the context of a disease process that takes decades to develop. Ideally, the study was envisioned to extend through 24 weeks but was not possible due to previously described circumstances. This study still represents a proof of concept and feasibility.

During this study period more clinical trials emerged using new protease and polymerase inhibitors targeting the same population of patients we were targeting. Consequently, the targeted population became almost nonexistent. Ultimately, the study had to be stopped prior to reaching the target sample size.

Future implications

Although this trial was prematurely stopped due to the factors previously explained, it has indeed opened the horizon for further research.

The newly developed ambulatory HNC protocol proves the feasibility of administering high doses of intravenous insulin infusion in the outpatient settings. The setup of the HNC sessions and the subsequent monitoring of blood sugar remains cumbersome to perform and can be considered a limitation in the utility of the HNC protocol for long periods. The developed ambulatory HNC protocol can serve as a solid starting point that dictates the instillation approach and the intervals of glucose measurements. Further efforts to simply the protocol without jeopardizing the safety of the patients can improve its applicability and clinical usefulness. Innovations that would allow

less invasive and real time blood glucose monitoring can serve as useful adjuncts to the current protocol.

The ability of high-dose insulin infusion to modulate the generalized inflammatory state in patients with liver disease carries a great potential. Future work should concentrate on measuring optimal length of intervention and possibly applying the concept in diseases with comparable patho-physiology to chronic hepatitis C infection. In future projects, inclusion criteria need to be revisited to allow for a wider use of the HNC in patients with liver disease.

Lastly, the potential regenerative support of high-dose insulin infusion to the liver calls for more investigations and testing. If proven true, insulin infusions can be utilized for patients with major liver resections or cirrhotic undergoing major surgeries particularly combined with our ability to administer high doses of insulin through the HNC protocol safely.

The obtained results also calls for more studies that focuses on changes occurring at the level of the cell, including changes in gene expression and levels of tissue cytokines and growth factors.

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Tables and Figures

Table 1: Clinical Prospective of Liver Function and Viral Load

	Patient 1				Patient 2
	Baseline	7 weeks	14 weeks		Baseline
• CBC					
- Hemoglobin (gm/L)	159	143	151		123
- White cell count (10^9/L)	4.35	4.08	4.62		2.87
- Platelets	74	61	75		117
• Clinical scoring of cirrhosis					
➢ Child-Pugh score	7 (child-B)	6 (child-A)	6 (child-A)		7 (child-B)
➢ MELD score	10	8	9		10
- Total Bilirubin	39	25	30		32
- INR	1.08	1.00	1.02		1.15
- Albumin	39	37	38		24
- Creatinine	65	50	55		74
- Ascites	1	1	1		1
- Encephalopathy	2	2	2		1
• Liver Enzymes					
- ALT	220	134	164		44
- AST	159	108	120		78
- ALP	122	100	90		99
- GGT	120	78	76		36
• Pathology evaluation (according to Batts and Ludwig AJSP 1995)					
- Fibrosis stage	2	NA	2		3-4
- Inflammation grade	4	NA	3-4		2
- Steatosis	20-30%	NA	10-20%		10-20%
• HCV PCR (Viral load)	7,244,360	NA	4,897,788		10,715
• Dynamic liver function test					
- Total protein fractional synthesis (%/day)	23.8 %	26.7%	16.1%		12%
- Albumin fractional synthesis (%/day)	22.7%	27.1%	18.3%		9.7%
- Absolute Albumin breakdown (µmol/kg/h)	167.22	135.29	135.08		109.50
- Fibrinogen fractional synthesis (%/day)	42.9%	28.8%	20.2%		11.2%
- Fibrinogen plasma concentration (g/L)	0.73	1.12	0.88		2.7
- Absolute Glucose synthesis (µmol/kg/min)	19.77	15.64	12.76		6.82

Table 2: Metabolic and Inflammatory Parameters

	Patient 1				Patient 2
	Baseline	7 weeks	14 weeks		Baseline
• Metabolic parameters					
➢ HOMA –IR	5.39	8.32	10.99		5.91
➢ Hepatic insulin resistance index	236.4	232.1	308.3		198
- BMI	30	30	29.7		36.1
- HBA1C	7.9	8	8.1		5.1
- Fasting Glucose (mmol/L)	10.2	12.7	10.3		4.6
- Fasting Insulin (pmol/L)	82.5	102.4	166.7		200.8
• Lipid profile					
- Triglycerides	0.93	2.46	0.71		0.36
- High density lipoproteins	1.11	0.89	1.15		0.94
- Low density lipoproteins	2.04	1.79	1.76		1.22
- Cholesterol	3.57	3.8	3.23		2.32
- Cholesterol / High density lipoprotein	3.2	4.3	2.8		2.5
• Inflammatory Markers, adipokines and growth factors					
- CRP (mg/l)	0.8	1.2	0.9		4.8
- TNF-alpha (pg/ml)	10.4	6.6	3.7		20.9
- IL-1B (pg/ml)	undetectable	undetectable	undetectable		undetectable
- IL-6 (pg/ml)	2.06	1.67	1.66		0.96
- IL-8 (pg/ml)	12.03	5.05	7.89		33.52
- IL-10 (pg/ml)	3.46	0.46	0.52		5.9
- IP-10 (pg/ml)	1259.7	1008.2	1281.6		2993.6
- MCP-1	695.6	421.0	408.4		327.7
- PAI-1	46101	59771	32804		79530
- TGF-α	2.4	1.72	0.74		undetectable
- TGF-β 1	12871.9	18836.5	17074.5		28798.1
- EGF	10.99	3.95	12.58		31.3
- HB-EGF	32.52	48.52	52.38		52.78
- HGF	550	584.68	522.2		1019.81
- VEGF-A	84.27	141.08	153.3		342.53
- Leptin	9696.45	11599.82	7117.26		83044.28
- Adiponectin	$25x10^6$	$29x10^6$	$24x10^6$		$40x10^6$
- Resistin	19091	27023	19646		45072

Table 3: Quality of Life Questionnaires

	Patient 1				Patient 2
	Baseline	7 weeks	14 weeks		Baseline
• QOL questionnaire SF-36					
- Physical health	34	23	32.2		15.9
- Mental Health	50	41	46		53.7
• QOL questionnaire EQ-D5					
➢ Descriptive scale index score (min = -0.11, max=1.0)	0.8	0.77	0.77		0.53
➢ The visual analogue scale (EQ-VAS)	0.50	0.45	0.69		0.50
- mobility	No problems	No problems	No problems		Some problems
- self-care	No problems	No problems	No problems		No problems
- usual activity	No problems	Some problems	Some problems		Extreme problems
- pain/discomfort	Some problems	Some problems	Some problems		Some problems
- anxiety/depression	Some problems	Some problems	Some problems		Some problems

Table 4: Ambulatory HNC Therapy Sessions Efficiency and Safety

	Patient 1		Patient 2
• Number of insulin sessions	26		3
- Length of sessions – (hours)	6 ± 0.25		6±0.2
- Total units of insulin administered per session (units) (mean ± SD)	53 ± 2.4		77.5 ± 2.2
- Total intravenous dextrose administered per session (gm)	102.6±17.4		200±18
- Insulin / body weight (milliunits/kg)	685 ± 32		663 ±13
- Insulin / dextrose ratio (milliunit/gram)	530 ± 84		389 ±29
• Blood glucose monitoring parameters (per session)			
- Total Number of blood sugar measurements (including measurements prior to reaching target range)	12.4 ± 2.3		11 ± 1
- Total number of blood sugar measurements after reaching target range	9.3 ± 2.2		11 ± 1
- Within target range (4-6 mmol/L)	8.2 ± 1.7 (86.6%)		9.3 ± 0.6 (84.9%)
- Number of measurement with minor deviation off target - (2.5-4 mmol/L) and (6-7.5 mmol/L)	1.0 ± 1.1 (12.6%)		1.6 ± 1.5 (15.1%)
- Number of measurements with major deviation off target - (less than 2.5 mmol/L and more than 7.5 mmol/L)	0.1 ± 0.3 (0.8%)		0.0 ± 0.0 (0%)

Figure 1: Patient enrollment

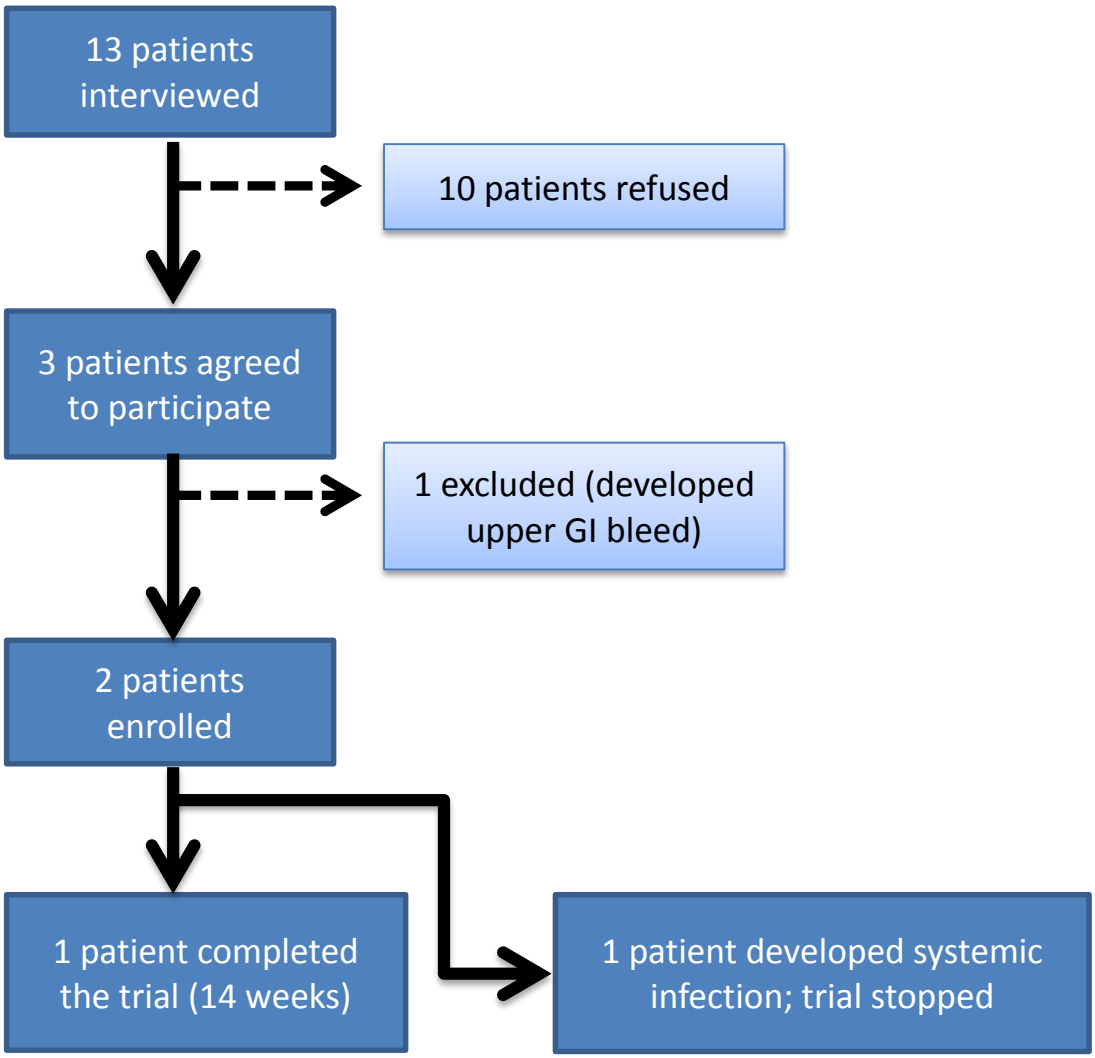
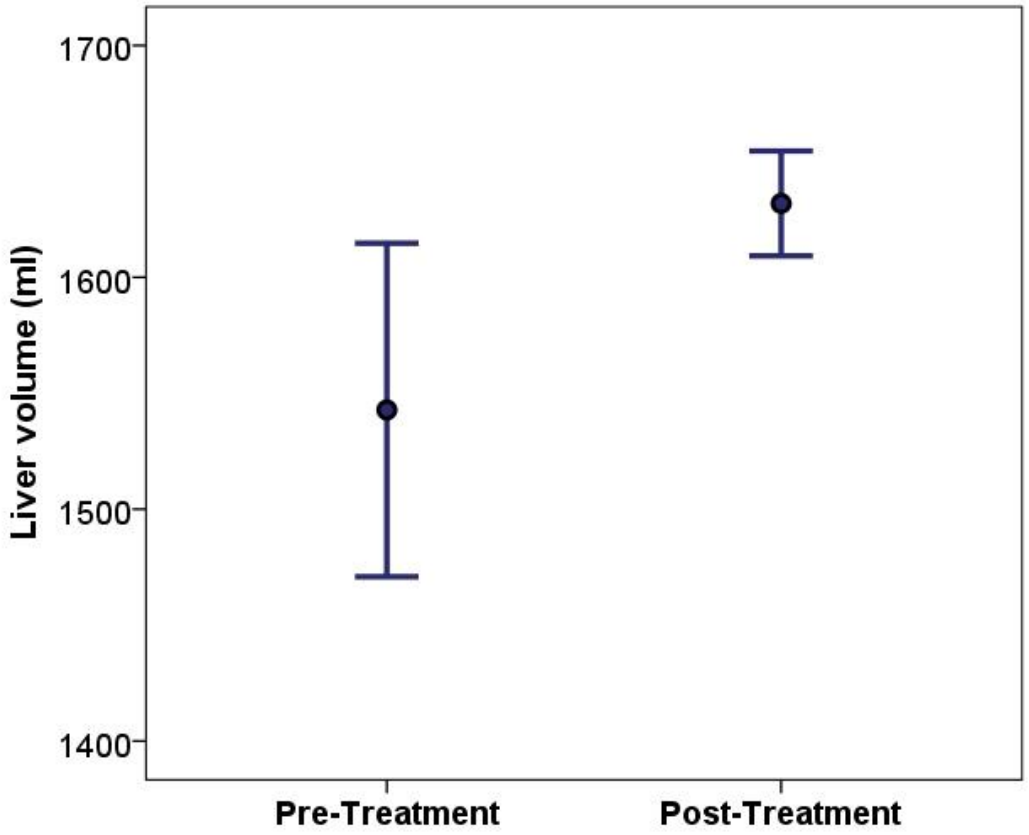
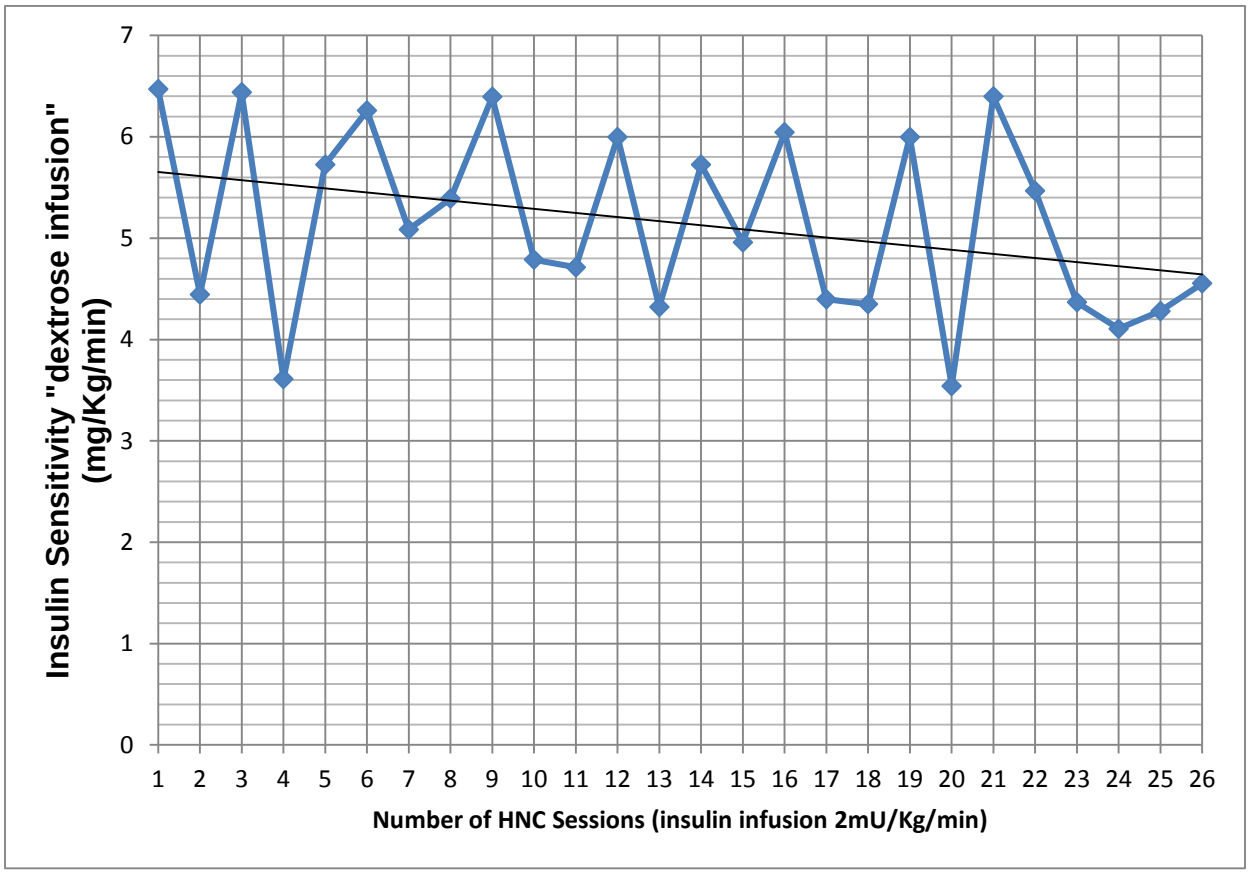


Figure 2: Liver CT Volumetry for a patient #1



- Data is presented as mean ± 2SD.
- A total of 2 pre-treatment CT scans and 1 post-treatment CT scan were assessed by 4 raters blinded to the date of the study. Interclass correlation coefficient (absolute)= 0.877

Figure 3: Insulin to Dextrose ratio over time for patient #1



Appendices

Appendix 1 – English Consent Form

High-Dose Insulin Therapy to Improve Liver Function in Patients With HCV Liver Cirrhosis

Principal Investigator:	Dr.Peter Metrakos MUHC HPB and Transplant Surgery
Co-Investigators:	Dr.Mohammed Shaheen, Dr.Mazen Hassanain, Ms.Ayat Salman, , Dr.Peter Ghali, , Dr.Thomas Schricker, Dr.Ralph Lattermann, Dr.Linda Wykes, Dr.Tatiana Cabrera, Dr.David Valenti
Study Coordinator:	Dr.Mohammed Shaheen

INTRODUCTION

We are inviting you to take part in a research study. Before deciding to participate in the study, it is important that you understand why it is being done and what it will involve. This document provides information on the study, but it may contain words that you do not fully understand. You must ask the study staff to explain anything which is not clear. Please take your time to read this document, discuss it with friends and relatives if you wish. You are also encouraged to ask the study staff as many questions as you may need to help you decide whether to take part or not.

PURPOSE OF THE STUDY

You are invited to this study to test the potential benefits of high insulin therapy using the dextrose/insulin clamp on patients infected with hepatitis C virus. This study will closely follow changes in liver function, including changes in insulin resistance and markers of inflammation as will be clarified later on.

BACKGROUND INFORMATION

What are the functions of the liver?

The human liver is the largest single organ in the body. It has several critical jobs, including the removal of poisons from the body, producing immune agents that are critical for controlling infection and removing bacteria and germs from the blood. The liver also produces proteins necessary for the regulation of blood clotting and makes bile for the absorption of fat and certain vitamins. It plays a big role in storing and making use of the nutrients we eat. A person cannot live without a functioning liver.

What is liver cirrhosis?

Cirrhosis is a long process of inflammation and scarring that happens in the liver. It results in blocked blood flow and impaired liver function. There are many causes of

cirrhosis, for example, heavy alcoholic drinking, exposure to certain toxins, blocked bile ducts and certain infections. One of the most common infections that lead to cirrhosis is a virus called Hepatitis C.

A person can have cirrhosis without immediately feeling symptoms. With time, however, healthy cells are replaced with scar tissue and liver function is reduced gradually. That is why doctors don't only depend on patients' symptoms and signs to assess the extent of his/her disease, but also use various blood tests and radiological studies to help them decide how good the liver is.

What are symptoms of liver deterioration?

As liver function begins to fail, symptoms of exhaustion, loss of appetite, nausea, and weight loss may become evident. The affected individual may also experience weakness, abdominal pain, and spider-like blood vessels on the skin. As cirrhosis progresses, complications may develop. These complications may include edema, ascites (accumulation of fluids in the abdomen), bruising, bleeding, jaundice (yellow coloring of the skin), itching, and gallstones (stones in the gallbladder). Other complications can include toxins in the blood or brain, medication sensitivity, portal hypertension, varices, insulin resistance, type 2 diabetes, and liver cancer. Additionally, a person with cirrhosis may develop problems with other organs. Treatment can delay or stop progression of the disease. The treatments are different

depending on the cause of cirrhosis. When treatment fails to control complications, liver transplantation is then considered.

What is insulin and how can it help?

Insulin is a hormone produced by the pancreas. Its main function is to regulate blood sugar so it does not go high. Cells of the liver respond to insulin by taking up sugar from the blood to utilize it in the proper way.

Hepatitis C virus – in addition to what has been mentioned previously – disturbs the response of liver cells to insulin, making them resistant to take sugar from the blood, eventually causing blood sugar to accumulate and go high. The body's immune system responds to changes in the liver and causes more inflammation and scarring. High dose insulin therapy, known as the dextrose/insulin clamp, has been used in multiple studies. It has been shown that over time it reduces the resistance of the cells to insulin, helping to maintain the blood sugar balance, and also shown to decrease inflammation. In addition, this therapy has been shown to be safe and effective in maintaining normal blood sugar level.

DURATION OF THE STUDY

It is anticipated that approximately 5 research participants will take part in the study. It will be conducted at the Royal Victoria Hospital of McGill University Health Centre. The total study period will be 24 weeks. Insulin therapy sessions will be

given twice a week and blood tests will be required every 2 months to monitor liver function as detailed below.

CONDUCT OF THE STUDY

The study will begin on November 2010. If you are eligible for the study and agree to take part, you will be given a schedule that best fits your lifestyle illustrating the required visits to have tests or receive the insulin session as follows:

Start of the study

At the beginning of the study (week 1), you will have two scheduled visits to Royal Victoria Hospital, in order to do all the required tests and be prepared for the therapy sessions. This will include answering a questionnaire and undergoing a brief physical examination by a doctor. Most of the blood tests are done routinely, and the amount of extra blood that will be taken for analysis in our lab is minimal, not more than 10 teaspoons. In addition, you will undergo a CT and / or MRI of your liver.

In order to assess the competence of your liver in using glucose and proteins, we will use a "stable isotope tracer technique", which is non-radioactive and has been developed for use in humans. Stable isotopes, i.e. substrates like sugar or proteins which have one heavier atom to make them distinguishable from the natural compounds in our body, will be infused into a hand vein over 6 hours and blood samples will be drawn after 3, 4, 5 and 6 hours of isotope infusion from a second

plastic cannula (elastic hollow tube into the veins of your arm), which will be inserted in one of the veins of the arm on the opposite side.

Some of these tests however can be measured by simpler ways, i.e by a fingertip sensor or a breath test, which will be used when applicable.

An optional test that would be of great value in assessing changes in liver scarring before and after the therapy would be a liver biopsy and pressure measurements in liver veins. A liver biopsy (a tiny fragment of the liver weighing around 1 gm) is the most specific test to assess the nature and severity of liver diseases. The pressures in the hepatic veins also reflect the amount of liver scarring.

There are currently several methods available for obtaining liver tissue. One method is taking a liver biopsy from inside the body through the vascular system. The advantage of this method is that it will allow us to measure the pressures inside the liver veins at the same time of the biopsy. A flexible tube (catheter) will be advanced inside the veins of the neck, going down through the big veins entering the heart using the aid of x-rays (fluoroscopy), guiding it to reach a major vein carrying blood from the liver called “the right hepatic vein”. A needle biopsy of the liver will then be performed through the catheter. During the procedure, continuous electrocardiographic monitoring will be maintained to detect any arrhythmias induced by passage of the catheter through the heart. Another method is to obtain the biopsy directly through the skin into the liver. Unfortunately, the pressures in the

liver veins cannot be measured through the skin, and will be obtained through passing a catheter from the neck veins to the liver veins as described above.

Two liver biopsies and two hepatic veins measurements will be obtained, one at the beginning and one at the end of the study. These tests are optional and will not be done unless you consent.

Insulin infusion sessions and follow ups

Every participant will have to undergo two sessions per week of dextrose/insulin clamp for 24 weeks (total of 48 sessions). Each session will be done at a separate day, and will be a six-hour long hospital visit. During this period you will receive constant intravenous insulin infusion combined with the necessary amount of intravenous sugar (dextrose) to maintain a normal blood sugar level through a peripheral venous cannula in the veins of the hand/arm. Sometimes, a small sample of blood (2-3 ml) will be taken to measure blood potassium salts before the infusion. Potassium salts tablets will be provided if levels are low. Also your blood sugar will be monitored on hourly intervals – more if necessary – using finger prick test (Accu-check®) to ensure normal blood sugar throughout. You will be allowed to drink for thirst, but no food shall be allowed. These visits will continue uninterrupted for the length of the study. In some weeks however, tests will be required before the start of the dextrose/insulin clamp session as will be detailed below.

At week 7 and week 14, a brief physical exam will be required in addition to a small quantity of blood that will be taken from your veins to monitor liver function changes and level of inflammation. In addition to the routine blood works, 6-7 extra teaspoons of blood will be taken for the purpose of this study. Additionally, some of the tests done at the beginning of the study will be repeated. These will include answering the same questionnaires and repeating the infusion of the "stable isotope tracers" to follow up changes in liver competence. As mentioned, they will be infused over 6 hours and blood samples will be taken after three, four, five and six hours of infusion, using a second plastic tube that is inserted into a vein in your arm.

End of the study

At week 24 (or if it was necessary to stop earlier), all tests done at the beginning of the study will need to be repeated and then analyzed by the research team, including all the blood work, imaging and, if you consented, the liver biopsies and the hepatic veins pressure measurments, following the same - previously mentioned - procedure.

BLOOD SAMPLES AND BIOPSIES

As mentioned above, extra blood that will be taken for research analysis will not exceed 10 teaspoons at the beginning and the end of the study, and no more than 6 teaspoons at the scheduled follow ups (week 7 and 14). The total amount of blood taken is considered small.

We also seek your permission to take an optional fragment of your liver (1 gram). This will be only done twice, at the beginning and at the end of the study. You don't have to agree to these biopsies to participate in the study. However we encourage you to agree to the biopsies as it will give us a clearer idea about the changes in scarring happening in the liver, as well as changes in the liver's competency to use proteins and fats.

POTENTIAL BENEFITS

The results of this study could lead to the creation of commercial value or value related to intellectual property. By signing on this consent form, you accept the fact that the researcher can apply for a copyright. You also agree that you will not obtain any financial benefit that could emerge from this study.

There may be no personal benefits associated with this study but data collected in this study could provide useful information for the benefit of future patients.

POTENTIAL RISKS

Intravenous materials used

The use of stable isotopes and insulin/glucose intravenously is harmless and safe with no major side effects. However, in the unlikely event that an allergic reaction or an episode of low blood sugar should occur unexpectedly, treatment will be

immediately available. Every precaution will be taken to ensure your safety as a research subject.

Biopsies

The insertion of a needle to obtain a biopsy can cause bleeding and local infection and/or mild pain. Serious complications that require medical attention - such as moderate to large bleed - are rare (less than 1.5 percent). All precautions will be taken to avoid complications before, during and after the procedure. In case any complication occurs, abrupt measures will be taken to abort or minimize harm.

Pressure measurements of liver veins

Slight discomfort can be experienced upon inserting the needle into your vein. Uncommonly, temporary extra heart beats can be felt if the venous catheter comes too close to the heart. It is very rare that the needle enters an adjacent artery instead. If that occurs it is always quickly recognized and corrected. All efforts will be made to avoid complications. The research team will deal with complications if any occur.

CONFIDENTIALITY

The team of researchers at the Royal Victoria Hospital could consult your medical records in order to record data related to this study, including your medical history, physical examination, laboratory results and (or) information to your health.

All information will be treated as strictly confidential. You will be identified by a code that only authorized personnel can access. The data will be stored for 2 years which will cover the period of the study and the time needed for necessary analysis. Subsequently all samples will be discarded. Your identity will not be revealed in the combined results. In case you terminate your participation in the study, all previously collected data will be kept for data analysis purposes but will be destroyed if otherwise specified by you. Only the principal investigator and the clinical research team will have access to the coded list on which your name appears.

All samples will be stored in Dr. Peter Metrakos's laboratory, the LD MacLean at Royal Victoria Hospital, for the duration of this study and will be destroyed at the end of this study.

By your signing on this consent form, you allow us to disclose information relating to your participation in the study to the above persons and also notify your primary physician. Please note that your privacy will be protected to the extent permitted by Canadian laws and regulations.

COMPENSATION:

All participants will be compensated with 25 CAD per visit to compensate for their transportation expenses and a minimum of 4 free meals every month. Neither the McGill University Health Centre (MUHC) Research Institute, nor the researcher can grant compensation in the unlikely event you may suffer injury as a result of your

participation in this study. However, all injuries caused by the study procedures will be managed by the current standards of care and will be financed by the MUHC. Moreover, you do not waive any of your rights by signing the consent form and agreeing to participate in the study.

VOLUNTARY PARTICIPATION

Your participation in the study is strictly voluntary. You can refuse to participate or withdraw at any time, without being asked and without penalty or loss of benefits to which you are otherwise entitled. If you terminate your participation, you will not suffer any prejudice concerning your medical care at this hospital or your participation in any other study.

CONTACTS

If you have any questions, please contact Dr. Mohammed Shaheen, study coordinator, at 514.298.6446 or send an email to mohammed.shaheen2@mail.mcgill.ca .

For questions about your rights as a research subject, please contact the hospital's ombudsman at (514)934-1934 local 35655.

High-Dose Insulin Therapy to Improve Liver Function in Patients with HCV Liver Cirrhosis

CONSENT FORM

I have read the contents of this consent form and agree to participate in this study. I had the opportunity to ask questions and I got satisfactory answers. I was given enough time to consider the information contained in the consent form and ask for advice, if any. I understand that I will receive a signed consent form. By signing the consent form, I do not waive any of my rights.

	Yes	**No**
I have read this information and consent form	☐	☐
I have had the opportunity to ask questions	☐	☐
I agree to participate in the study	☐	☐

Optionally:-

I consent for pressure measurements of hepatic veins at the start and end of the study.	☐	☐
I consent for the optional liver biopsies at the start and end of the study.	☐	☐

_____
Patient Name (print)

_____ _____
Signature Date

_____
Name of the person who explains the contents of the form consent (print)

_____ _____
Signature Date

Appendix 2 – French Consent Form

Insulinothérapie à dose élevée visant à améliorer la fonction hépatique chez les patients présentant une cirrhose du foie causée par le virus de l'hépatite C (VHC)

Chercheur principal : Dr Peter Metrakos
Centre universitaire de santé McGill, chirurgie hépatobiliaire et de transplantation

Cochercheurs : Dr Mohammed Shaheen, Dr Mazen Hassanain, Mme Ayat Salman, Dr Peter Ghali, Dr Thomas Schricker, Dr Ralph Lattermann, Dre Linda Wykes, Dre Tatiana Cabrera, Dr David Valenti

Coordonnateur de l'étude : Dr Mohammed Shaheen

INTRODUCTION

Nous vous invitons à participer à une étude de recherche. Avant de décider de participer à cette étude, il est important que vous compreniez son objectif et en quoi elle consiste. Le présent document fournit des renseignements sur l'étude mais peut contenir des mots que vous ne comprenez pas parfaitement. Vous devez demander au personnel de l'étude d'expliquer tout ce qui n'est pas clair. Veuillez prendre le temps de lire ce document, et d'en discuter si vous le souhaitez avec des amis et des parents. Nous vous encourageons également à poser au personnel de l'étude toutes les questions que vous jugerez utiles pour prendre la décision de participer ou non.

OBJECTIF DE L'ÉTUDE

Nous vous invitons à participer à cette étude afin de vérifier les avantages potentiels de l'insulinothérapie à dose élevée administrée au moyen de la technique du clamp dextrose-insuline chez les patients atteints du virus de l'hépatite C. Cette étude suivra de près les changements dans la fonction hépatique, y compris les

changements dans la résistance à l'insuline et les marqueurs de l'inflammation, comme cela sera précisé plus tard.

RENSEIGNEMENTS DE BASE

Quelles sont les fonctions du foie?

Chez l'humain, le foie est le plus gros organe unique du corps. Il remplit plusieurs fonctions cruciales, y compris l'élimination de poisons du corps, la production d'agents immunitaires qui sont essentiels pour lutter contre les infections et retirer les bactéries et les microbes du sang. Le foie produit en outre les protéines nécessaires à la régulation de la coagulation sanguine et fabrique de la bile pour l'absorption des graisses et de certaines vitamines. Il joue un grand rôle dans le stockage et l'utilisation des éléments nutritifs que nous consommons. Une personne ne peut vivre sans un foie qui fonctionne.

Qu'est-ce que la cirrhose du foie?

La cirrhose est un long processus d'inflammation et de cicatrisation qui survient dans le foie. Elle bloque le flux sanguin et altère la fonction hépatique. Les causes de la cirrhose sont nombreuses et comprennent par exemple la consommation abusive d'alcool, l'exposition à certaines toxines, le blocage des canaux biliaires, et certaines infections. Une des infections qui causent le plus couramment la cirrhose est une infection provoquée par un virus appelé hépatite C.

Une personne peut être atteinte d'une cirrhose sans en ressentir immédiatement les symptômes. Au fil du temps, toutefois, les cellules saines sont remplacées par des tissus cicatriciels et la fonction hépatique diminue graduellement. C'est pourquoi les médecins ne se fondent pas uniquement sur les symptômes et les signes présents chez le patient pour évaluer l'ampleur de la maladie dont celui-ci est atteint, mais effectuent également diverses analyses sanguines et des études radiologiques pour déterminer l'état du foie.

Quels sont les symptômes de la détérioration du foie?

Lorsque la fonction hépatique commence à défaillir, les symptômes suivants peuvent apparaître : épuisement, perte d'appétit, nausées, et perte de poids. La personne

atteinte peut aussi présenter une faiblesse, des douleurs abdominales et des angiomes stellaires cutanés. À mesure que la cirrhose progresse, des complications peuvent survenir, dont les suivantes : œdème, ascite (accumulation de fluides dans l'abdomen), ecchymoses, saignements, jaunisse (jaunissement de la peau), démangeaisons, et calculs biliaires (calculs dans la vésicule biliaire). D'autres complications peuvent survenir, comme la présence de toxines dans le sang ou le cerveau, une sensibilité aux médicaments, une hypertension portale, des varices, une résistance à l'insuline, le diabète de type 2, et le cancer du foie. De plus, une personne atteinte de cirrhose peut développer des problèmes touchant d'autres organes. Le traitement peut retarder ou arrêter la progression de la maladie. Les traitements diffèrent selon la cause de la cirrhose. Lorsque le traitement ne permet pas de maîtriser les complications, une transplantation du foie est alors envisagée.

Qu'est-ce que l'insuline et comment peut-elle aider?

L'insuline est une hormone produite par le pancréas. Sa principale fonction est de réguler la glycémie afin de l'empêcher d'atteindre un niveau élevé. En réaction à l'insuline, les cellules hépatiques absorbent le sucre contenu dans le sang pour l'utiliser de la bonne façon.

Le virus de l'hépatite C – en plus de ce que nous avons mentionné précédemment – perturbe la réponse des cellules hépatiques à l'insuline et les rend résistantes à l'absorption du sucre contenu dans le sang, provoquant ainsi éventuellement une accumulation de sucre dans le sang qui se traduit par une glycémie élevée. Le système immunitaire de l'organisme réagit aux changements survenant dans le foie et intensifie l'inflammation et la cicatrisation.

L'insulinothérapie à dose élevée, qui est connue sous le nom de technique du clamp dextrose-insuline, a été utilisée dans de nombreuses études. Il a été démontré qu'elle réduit la résistance des cellules à l'insuline au fil du temps, contribuant ainsi à maintenir une glycémie équilibrée, et aussi qu'elle réduit l'inflammation. De plus, il a été démontré que ce traitement est sans danger et est efficace pour maintenir la glycémie à un niveau normal.

DURÉE DE L'ÉTUDE

Il est prévu que quelque cinq participants à la recherche prendront part à l'étude. Celle-ci sera menée à l'Hôpital Royal Victoria du Centre universitaire de santé McGill. L'étude durera 24 semaines au total. Les séances d'insulinothérapie auront lieu deux fois par semaine et des analyses sanguines seront nécessaires tous les deux mois pour surveiller la fonction hépatique, tel que précisé ci-après.

DÉROULEMENT DE L'ÉTUDE

L'étude débutera en novembre 2010. Si vous êtes admissible à l'étude et que vous acceptez d'y participer, vous recevrez un calendrier qui convient le mieux à votre mode de vie et indiquant les dates auxquelles vous devrez vous présenter pour subir les tests ou recevoir l'insulinothérapie, comme suit :

Début de l'étude

Au début de l'étude (semaine 1), vous aurez deux visites cédulées à l'Hôpital Royal Victoria afin de subir tous les tests requis et vous préparer aux séances de traitement. À cette occasion, vous devrez notamment répondre à un questionnaire et vous soumettre à un bref examen physique effectué par un médecin. La plupart des analyses sanguines seront réalisées régulièrement, et la quantité supplémentaire de sang qui sera prélevée à des fins d'analyse dans notre laboratoire est minime, soit 10 cuillerées à thé au plus. De plus, vous aurez à passer un scan tomographique ou une résonnance magnétique (IRM) du foie.

Afin d'évaluer la compétence de votre foie à utiliser le glucose et les protéines, nous utiliserons une « technique de traceur d'isotopes stables », qui n'est pas radioactive et a été conçue pour usage chez les humains. Des isotopes stables, c.-à-d. des substrats comme le sucre ou les protéines comportant un atome plus lourd qui les distinguent des composés naturels de notre organisme, vous seront administrés par perfusion dans une veine de la main pendant plus de six heures, et des échantillons de sang seront prélevés après trois, quatre, cinq et six heures de perfusion d'isotopes à partir d'une seconde canule de plastique (tube élastique creux installé dans les veines de votre bras), qui sera insérée dans l'une des veines de votre bras du côté opposé.

Certains de ces tests peuvent cependant être effectués au moyen de méthodes plus simples, c.-à-d. un capteur digital ou une épreuve respiratoire, qui seront utilisées s'il y a lieu.

Certains des tests facultatifs qui seraient très utiles pour évaluer les changements sur le plan de la cicatrisation du foie avant et après le traitement consisteraient à effectuer des biopsies sur le foie ainsi que des mesures de la pression des veines hépatiques. La biopsie du foie (prélèvement d'un petit fragment du foie pesant environ un gramme) est la méthode la plus précise permettant d'évaluer la nature et la gravité des maladies du foie. La mesure de la pression des veines hépatiques aide à refléter la gravité de la cicatrisation du foie.

Il existe actuellement plusieurs méthodes pour obtenir des tissus hépatiques.. Une de ces méthodes consiste à effectuer une biopsie du foie à partir de l'intérieur du corps par le biais du système vasculaire..L'avantage de cette méthode est qu'elle permet de mesurer la pression a l'intérieur des veines hépatique simultanément à la biopsie. Un tube souple (cathéter) sera introduit par les veines du cou, puis sera avancé à travers les veines majeures qui mènent au cœur et, en ayant recours aux rayons X (fluoroscopie), on le guidera jusqu'à une veine principale transportant le sang à partir du foie, appelée « veine hépatique droite ». Une biopsie à l'aiguille du foie sera ensuite effectuée par le biais du cathéter. Pendant cette procédure, vous ferez l'objet d'une surveillance électrocardiographique continue visant à déceler toute arythmie induite par le passage du cathéter dans le cœur. L'alternative est d'obtenir une biopsie du foie directement à travers la peau. Malheureusement, cette méthode ne permet pas de mesurer la pression des veines hépatiques et on devra effectuer ces mesures là en passant un cathéter des veines du cou jusqu'au foie, tel que décrit ci-dessus.

Deux biopsies du foie ainsi que deux mesures de pressions hépatiques seront obtenues, une au début et une à la fin de l'étude. Ces tests sont facultatifs et ne seront pas réalisés à moins que vous n'y consentiez.

Séances de perfusion d'insuline et suivi

Tous les participants devront subir deux séances de perfusion par semaine au moyen de la technique du clamp dextrose-insuline pendant 24 semaines (total de 48 séances). Chaque séance aura lieu au cours d'une journée distincte et exigera un séjour de six heures à l'hôpital. Pendant cette période, vous recevrez une perfusion intraveineuse constante d'insuline qui sera combinée à la quantité de sucre (dextrose) intraveineux nécessaire pour maintenir une glycémie normale à travers une canule périphérique dans une veine de la main/avant-bras..Parfois, un petit échantillon de sang (2 à 3 ml) sera prélevé afin de mesurer les taux sanguins de sels de potassium avant la perfusion. Des comprimés de sels de potassium vous seront fournis si ces taux sont faibles. De plus, nous surveillerons votre glycémie toutes les heures – plus souvent au besoin – à l'aide du test par piqûre du doigt (Accu-check®) afin de nous assurer que votre glycémie demeure normale tout au long de la séance. Vous pourrez boire pour étancher votre soif, mais vous ne pourrez pas manger. Ces séjours se poursuivront sans interruption pendant la durée de l'étude. Au cours de certaines semaines, cependant, il sera nécessaire d'effectuer des tests avant le début de la séance de perfusion au moyen de la technique du clamp dextrose-insuline, tel que précisé ci-après.

À la 7^{e} et 14^{e} semaine, vous subirez un bref examen physique et nous prélèverons une petite quantité de sang à partir de vos veines pour faire le suivi des changements dans la fonction hépatique et du niveau d'inflammation. En plus de procéder aux analyses sanguines régulières, nous prélèverons six à sept cuillerées à thé de sang supplémentaires aux fins de la présente étude. En outre, certains des tests réalisés au début de l'étude seront répétés. À cette fin, vous devrez notamment répondre aux mêmes questionnaires et subir de nouveau la perfusion de « traceurs d'isotopes stables » visant à faire le suivi des changements sur le plan de la compétence hépatique. Tel que mentionné, la perfusion durera six heures et des échantillons de sang seront prélevés après trois, quatre, cinq et six heures de perfusion, à l'aide d'un second tube de plastique qui est inséré dans une veine de votre bras.

Fin de l'étude

À la 24e semaine (ou plus tôt s'il est nécessaire d'arrêter l'étude), tous les tests effectués au début de l'étude devront être répétés et seront ensuite analysés par l'équipe de recherche, ce qui comprend toutes les analyses sanguines, l'imagerie et, si vous avez donné votre consentement, les biopsies du foie et les mesures de pression des veines hépatiques seront réalisées selon la même procédure que celle mentionnée antérieurement.

ÉCHANTILLONS DE SANG ET BIOPSIES

Tel que mentionné ci-dessus, les échantillons supplémentaires de sang qui seront prélevés aux fins d'analyses de recherche au début et à la fin de l'étude ne dépasseront pas dix cuillerées à thé, et ceux prélevés en vue du suivi régulier (7e et 14e semaines) ne dépasseront pas six cuillerées à thé. La quantité totale de sang prélevée est considérée comme étant petite.

Nous vous demandons aussi la permission de procéder au prélèvement facultatif d'un fragment (1 g) de votre foie. Ces prélèvements n'auront lieu que deux fois, soit au début et à la fin de l'étude. Vous n'êtes pas tenu de consentir à ces biopsies pour participer à l'étude. Nous vous encourageons toutefois à consentir aux biopsies, car cela nous donnera une idée plus claire des changements survenant sur le plan de la cicatrisation du foie, ainsi que sur les changements dans la compétence du foie à utiliser les protéines et les graisses.

AVANTAGES POTENTIELS

Les résultats de cette étude pourraient mener à la création de valeur commerciale ou de valeur liée à la propriété intellectuelle. En signant le présent formulaire de consentement, vous acceptez le fait que le chercheur peut déposer une demande de droits d'auteur. Vous convenez en outre que vous n'obtiendrez aucun avantage financier qui pourrait découler de cette étude.

Il se peut que cette étude ne vous procure aucun avantage personnel, mais les données recueillies dans le cadre de celle-ci pourraient fournir des renseignements utiles qui profiteront à de futurs patients.

RISQUES POTENTIELS

Matériel intraveineux utilisé

L'administration d'isotopes stables, d'insuline et de glucose par voie intraveineuse est sans danger et sûre et n'a pas d'effets secondaires majeurs. Cependant, dans le cas peu probable où une réaction allergique ou un épisode d'hypoglycémie surviendrait de façon inattendue, vous recevriez immédiatement les soins requis. Toutes les précautions seront prises pour assurer votre sécurité en tant que sujet de recherche.

Biopsies

L'insertion d'une aiguille à des fins de biopsie peut provoquer un saignement et une infection localisée et/ou une légère douleur. Les complications graves exigeant des soins médicaux – comme un saignement moyen à abondant − sont rares (moins de 1,5 %). Nous prendrons toutes les précautions pour éviter les complications avant, pendant et après la procédure. Si des complications surviennent, nous agirons rapidement pour faire cesser les dommages ou les réduire au minimum.

Mesure de pression des veines hépatiques

Vous pourriez ressentir une légère gêne lors de l'insertion de l'aiguille dans votre veine. Il peut arriver, mais rarement, que vous présentiez temporairement des battements cardiaques supplémentaires si l'intraveineuse passe trop près de votre cœur. Il est très rare que l'aiguille touche une artère adjacente par erreur. Ces cas là sont reconnaissables rapidement d'habitude et la situation corrigée sur le coup. Tous les efforts seront faits pour éviter les complications. L'équipe de recherche traitera toute complication qui surviendra, le cas échéant.

CONFIDENTIALITÉ

L'équipe de chercheurs de l'Hôpital Royal Victoria pourrait consulter vos dossiers médicaux afin de consigner des données liées à la présente étude, y compris vos antécédents médicaux, votre examen physique, vos résultats d'analyses de laboratoire et/ou des renseignements sur votre état de santé.

Tous les renseignements seront traités de manière strictement confidentielle. Vous serez identifié au moyen d'un code auquel seul le personnel autorisé aura accès. Les données seront conservées pendant deux ans, ce qui comprend la période de l'étude ainsi que le temps requis pour effectuer les analyses nécessaires. Par la suite, tous les échantillons seront éliminés. Les résultats combinés ne révèleront pas votre identité. Si vous cessez de participer à l'étude, toutes les données recueillies antérieurement seront conservées aux fins des analyses de données mais elles seront détruites sur indication contraire de votre part. Seuls le chercheur principal et l'équipe de recherche clinique auront accès à la liste codée sur laquelle votre nom figure.

Tous les échantillons seront conservés dans le laboratoire du Dr Peter Metrakos, le laboratoire LD MacLean à l'Hôpital Royal Victoria, pendant la durée de cette étude et seront détruits à la fin de l'étude.

En signant le présent formulaire de consentement, vous nous autorisez à divulguer aux personnes susmentionnées les renseignements liés à votre participation à l'étude et aussi à informer votre médecin de premier recours. Veuillez noter que nous protégerons votre vie privée dans la mesure où les lois et règlements canadiens le permettent.

INDEMNISATION

Tous les participants recevront une indemnisation de 25 $ CAN par visite pour compenser les frais de transport et un minimum de 4 repas gratuits chaque mois. Ni l'Institut de recherche du Centre universitaire de santé McGill (CUSM), ni le chercheur ne peuvent vous accorder une indemnisation dans le cas peu probable où vous subiriez des blessures à la suite de votre participation à cette étude. Toutefois, toutes les blessures causées par les procédures de l'étude seront traitées conformément aux normes actuelles en matière de soins de santé et ce, aux frais du CUSM. De plus, vous ne renoncez à aucun de vos droits en signant ce formulaire de consentement ni en acceptant de participer à cette étude.

PARTICIPATION VOLONTAIRE

Votre participation à cette étude est strictement volontaire. Vous pouvez refuser de participer ou choisir de vous retirer en tout temps, sans que nous vous le demandions et sans être pénalisé ou sans perdre les avantages auxquels vous avez par ailleurs droit. Le fait de cesser de participer ne compromettra pas les soins médicaux que vous recevez à cet hôpital ni votre participation à une autre étude.

PERSONNES-RESSOURCES

Si vous avez des questions, veuillez communiquer avec le Dr Mohammed Shaheen, coordonnateur de l'étude au 514-298-6446 ou envoyer un courriel à mohammed.shaheen2@mail.mcgill.ca.

Si vous avez des questions concernant vos droits à titre de sujet de recherche, veuillez communiquer avec l'ombudsman de l'hôpital au 514-934-1934, poste 35655.

Insulinothérapie à dose élevée visant à améliorer la fonction hépatique chez les patients présentant une cirrhose du foie causée par le virus de l'hépatite C (VHC)

FORMULAIRE DE CONSENTEMENT

J'ai lu le présent formulaire de consentement et j'accepte de participer à cette étude. J'ai eu la possibilité de poser des questions et j'ai obtenu des réponses satisfaisantes. J'ai eu assez de temps pour examiner les renseignements contenus dans ce formulaire de consentement et pour demander des conseils, le cas échéant. Je comprends que je recevrai un formulaire de consentement signé. En signant ce formulaire de consentement, je ne renonce à aucun de mes droits.

	Oui	**Non**
J'ai lu ces renseignements et le présent formulaire de consentement	☐	☐
J'ai eu la possibilité de poser des questions	☐	☐
J'accepte de participer à cette étude	☐	☐
Facultatif : Je consens à la mesure des pressions hépatiques au début de l'étude	☐	☐
Je consens à subir les biopsies facultatives du foie au début et à la fin de l'étude.	☐	☐

_____
Nom du patient (écrire en lettres moulées)

_____ _____
Signature Date

_____
Nom de la personne qui a expliqué le contenu du formulaire de consentement (écrire en lettres moulées)

_____ _____
Signature Date

Appendix 3 – Ambulatory HNC Therapy Protocol

- Dextrose "D10W or D20W" and regular insulin are given using a normoglycemic hyperinsulinemic clamp at 0.12u/kg/hr (2mu/kg/min)
- Duration: 6 hours per session
- Patient will not be allowed to eat during the period of the insulin infusion and for a minimum of 3 hours before the infusion
- Blood sugar goal range is 4 – 6 mmol/l, measured using (Accu-Check) as per the following protocol

❖ Infusion Protocol:

- PHASE I (Initiation):
  - Obtain baseline blood glucose measurement and follow accordingly:

Blood glucose level (mmol/L)	**Dextrose "D10W" infusion rate (ml/hr)** **If "D20W" is used, all the following rates should be reduced by half*
Below 6.0	✓ Start D10W infusion at 80 ml/hr
6.0 – 8.9	✓ Give 2 units of insulin (bolus) ✓ Start D10W infusion at 40ml/hr
9.0 – 12	✓ Give 2 units of insulin (bolus) ✓ Start D10W infusion at 20ml/hr
More than 12.0	✓ Give 4 units of insulin (bolus) ✓ Start D10W infusion at 10ml/hr

  - Check blood sugar in 30 – 45 minutes and follow accordingly:

Blood glucose level (mmol/L)	**Dextrose "D10W" infusion rate (ml/hr)** **If "D20W" is used, all the following rates should be reduced by half*	**Intervals (minutes)**
Below 3.0	✓ Set rate of D10W to 300 ml/hr ✓ Give 40 ml bolus of D10W ✓ Inform research MD on-call	✓ Repeat in 10 minutes ✓ If persists twice, then stop insulin infusion
3.0 – 3.9	✓ Set minimum rate of D10W to 240 ml/hr ✓ Give 40 ml bolus of D10W	✓ Repeat in 10 – 15 minutes ✓ If persists, increase rate by 40 ml/hr
4.0 – 4.5	✓ Set minimum rate of D10W to 180 ml/hr	✓ Repeat in 10 - 15 minutes ✓ If persists twice, then start Phase II (stabilization)
4.6 – 5.5	✓ Set minimum rate of D10W to 140 ml/hr	
5.6 – 6.0	✓ Set minimum rate of D10W to 100 ml/hr	
More than 6.0	✓ Set rate of D10W to 40 ml/hr	✓ Repeat in 10 - 15 minutes

- PHASE II (Stabilization):
  - Check blood sugar in 10 – 15 minutes and follow accordingly:

Blood glucose level (mmol/L)	**Dextrose "D10W" infusion rate (ml/hr)** **If "D20W" is used, all the following rates should be reduced by half*		**Intervals (minutes)**
Below 3.0	✓ Increase rate of D10W by 80 ml/hr ✓ Give 40 ml bolus of D10W ✓ Inform research MD on-call		✓ Repeat in 10 minutes ✓ If persists twice, then stop insulin infusion
3.0 – 3.9	✓ Increase rate of D10W by 60 ml/hr ✓ Give 40 ml bolus of D10W		✓ Repeat in 10 - 15 minutes
4.0 – 4.5	✓ No drop in blood sugar	✓ Maintain infusion rate	✓ Repeat in 20 - 25 minutes ✓ If persists twice, repeat in 40 - 45 minutes
	✓ Blood sugar drop < 0.5 mmol/l	✓ Increase rate by 40 ml/hr	
	✓ Blood sugar drop > 0.5 mmol/l	✓ Increase rate by 60 ml/hr	
4.6 – 5.5	✓ Maintain infusion rate		✓ Repeat in 30 - 35 minutes ✓ If persists twice, repeat in 60 - 80 minutes
5.6 – 6.0	✓ No increment in blood sugar	✓ Maintain infusion rate	
	✓ Blood sugar increase < 0.5 mmol/l	✓ Decrease rate by 20 ml/hr	
	✓ Blood sugar increase > 0.5 mmol/l	✓ Decrease rate by 30 ml/hr	
6.1 – 9.0	✓ Decrease rate of D10W by 40 ml/hr to a minimum of 40 ml/hr		✓ Repeat in 30 - 35 minutes
More than 9.0	✓ Decrease rate of D10W by 60 ml/hr to a minimum of 30 ml/hr		

  - Blood glucose measurement must be obtained any time the patient complains of symptoms of hypoglycemia. i.e. light headedness, sweating, shaking, blurry vision… etc, and must be clearly documented
- PHASE III (Termination):
  - Stop Insulin infusion 30 – 45 minutes before the end of the session maintaining the dextrose infusion rate without change.
  - Blood sugar is measured at the end of the 30 – 45 minutes, then repeated every 10 – 15 minutes until blood sugar level is more than 6 mmol/l
  - Stop Dextrose infusion and keep patient under observation for 20 minutes before discharge.

- ❖ Electrolyte replacement:
    - o Electrolyte replacements will be implied to avoid hypophosphatemia and hypokalemia due to the insulin infusion.
        - ▪ Sodium phosphate (15 mmol) will be added to the dextrose infusion solution per bag of D10W® = 1L capacity or D20® = 500mL capacity.
        - ▪ To avoid the unlikely event of sever hypokalemia, potassium level will be checked before initiating the hyperinsulinemic normoglycemic clamp infusion protocol whenever possible:
            - If potassium level is 3.9 meq/L or more, Infusion protocol will be initiated with no concern
            - If potassium level is less than 3.9 meq/L, oral form of potassium chloride (20 meq) will be given to the patient to swallow
        - ▪ If it is not possible to check the level of potassium at the time of the clamp, the patient will be given 20 meq of potassium chloride orally at the beginning of the session unless he/she is known for chronic kidney impairment.

- ❖ Alerting symptoms: If any of the following sigs are encountered:
    - Blurry vision
    - Tingling around the mouth
    - Cardiac palpitations
    - Tremors or anxiety
    - Excessive sweating
    - Disturbed consciousness level
    - Muscle weakness
    - o Immediate blood sugar measurement should be taken
        - ▪ If blood sugar is off target range act according to protocol with close monitoring
        - ▪ If blood sugar is within normal, please obtain an ECG and a send a blood sample for electrolyte measurements including potassium and phosphate. Ask an MD to interpret the results and react appropriately

Appendix 4 – Dynamic liver function test: protocol

0 min		150 min	160 min	170 min	180 min	240 min	300 min	360 min

[6,6-$^{2}H_2$] glucose	
Priming dose (22µmol/Kg)	Continuous infusion (0.44µmol/kg/min)
L-[1-^{13}C] leucine	
Priming dose (4µmol/Kg)	Continuous infusion (0.06µmol/Kg/min)
L-[$^{2}H_5$] phenylalanine	
Priming dose (4µmol/Kg)	Continuous infusion (0.1µmol/Kg/min)

0 min		150 min	160 min	170 min	180 min	240 min	300 min	360 min

START ▬▬▬▬▬▬▬▬ END

○ ○ ○ ○ ○

• • • • •

▬ Indirect calorimeter

- ○ Measurement of leucine and glucose enrichment in plasma (in the form of [1-^{13}C] α-ketoisocaproate and [6,6-$^{2}H_2$] glucose) and expired $^{13}CO_2$
- • Measurement of L-[$^{2}H_5$] phenylalanine enrichment in total plasma proteins, albumin and fibrinogen.