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β-BLOCKADE IN LIVER CIRRHOSIS: EFFECTS ON ENERGY METABOLISM AND GLUCOSE TOLERANCE

Wai Gin Lee

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy,
The University of Auckland,
2014
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The undersigned hereby certify that:
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- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

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Abstract

Aims: Liver cirrhosis is a common cause of morbidity and mortality. Elevated resting energy expenditure (REE) is associated with a poorer outcome, even within the normal range of REE. β-blockers are commonly taken by patients with liver cirrhosis for the prevention of variceal haemorrhage and these patients are less likely to have an elevated REE. The primary aim of this thesis was to determine if treatment with nadolol (non-cardioselective β-blocker) reduced REE and if the treatment resulted in detrimental effects on the insulin sensitivity, β-cell function and glucose tolerance of patients with liver cirrhosis who have an existing predisposition for impaired glucose metabolism.

Methods: The published literature was systematically reviewed to determine the prevalence of diabetes in patients with liver cirrhosis according to aetiology and other sources of potential variation. Analysis and reporting of the systematic review was based initially on the QUORUM and subsequently the PRISMA guidelines. In a double-blind, randomised, controlled, cross-over trial, 23 stable patients with liver cirrhosis not already taking β-blockers were randomly allocated to 3-month treatment with nadolol (titrated to decrease resting pulse rate by 20% or to below 60 beats per minute) or visually-identical placebo. After a 1-month washout period these patients were crossed to the alternate treatment for a further 3 months. Changes in REE, body composition, insulin sensitivity, β-cell function and glucose tolerance were measured at the beginning and the end of each period of treatment. Insulin sensitivity, β-cell function and glucose tolerance were also measured in 6 healthy volunteers. REE was measured by indirect calorimetry using a ventilated hood. Patients were rested for at least 30min in a thermoneutral environment prior to measurement of REE. Concurrently, total body protein (TBP) was derived from measurement of total body nitrogen using prompt-γ in vivo neutron activation analysis while total body fat and fat free mass (FFM) was derived by software analysis of whole-body dual-energy X-ray absorptiometry (DEXA) scanning. Insulin sensitivity was measured using the gold standard hyperinsulinaemic euglycaemic clamp (which was used to derive the insulin sensitivity index adjusted for FFM (ISI_{FFM}), combined with an intravenous glucose tolerance test (IVGTT) to allow concurrent measurement of β-cell function. In addition to the IVGTT, β-cell function was also measured using the disposition index, and a mathematical model for β-cell function (Mari model) derived from glucose, insulin and C-peptide measurements during a standard 75g 2 hour oral glucose tolerance test (OGTT). Insulin secretion in the β-cell function model was derived by C-peptide deconvolution due to the variable kinetics of insulin metabolism that may be more profound in patients with liver cirrhosis. Glucose tolerance was also determined from the OGTT using the 2006 World Health Organisation guidelines.
**Findings:** Diabetes was most prevalent in patients with cryptogenic, hepatitis C (HCV) and alcoholic cirrhosis. REE did not differ significantly on nadolol and placebo due to the study sample of mainly compensated patients with normal REE. Nevertheless, REE during treatment with nadolol was reduced compared to baseline and the reduction correlated with both baseline REE, and the degree by which REE was elevated over expected REE. Nadolol reduced the insulin sensitivity and β-cell function of the patients but the small size of the study precluded demonstrating a significant reduction in glucose tolerance. Unexpectedly, an early increase in β-cell function of compensated cirrhotic patients with normal insulin sensitivity was observed when compared to healthy volunteers.

**Conclusions:** β-blockade may have a role in reducing REE in cirrhotic patients with an abnormally elevated REE although a lack of effect was seen in this cohort of primarily normometabolic patients. However, the potential benefit of this treatment needs to be balanced against the detrimental effects on the insulin sensitivity and β-cell function of these patients which may lead to a poorer outcome. Lastly, an increase in β-cell function may precede and possibly even precipitate the reduction of insulin sensitivity in patients with liver cirrhosis but these findings will need to be confirmed in a larger and more representative cohort of patients.
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## Abbreviations

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<tbody>
<tr>
<td>Abca-1</td>
<td>ATP-binding cassette transporter subfamily A member 1</td>
</tr>
<tr>
<td>AIR</td>
<td>Acute insulin response</td>
</tr>
<tr>
<td>ALD</td>
<td>Alcoholic liver disease</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine-tri-phosphate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>FFM</td>
<td>Fat-free mass</td>
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<tr>
<td>GLUT2</td>
<td>Glucose transporter 2</td>
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<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
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<tr>
<td>HGP</td>
<td>Hepatic glucose production</td>
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<tr>
<td>HOMA</td>
<td>Homeostasis model assessment</td>
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<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
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<tr>
<td>IRS-1</td>
<td>Insulin receptor substrate-1</td>
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<tr>
<td>IVGTT</td>
<td>Intravenous glucose tolerance test</td>
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<tr>
<td>MELD</td>
<td>Model end-stage liver disease</td>
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<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>Pdx-1</td>
<td>Pancreatic and duodenal homeobox-1</td>
</tr>
<tr>
<td>PI3-kinase</td>
<td>Phosphatidylinositol 3-kinase</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>QUICKI</td>
<td>Quantitative insulin sensitivity check index</td>
</tr>
<tr>
<td>REE</td>
<td>Resting energy expenditure</td>
</tr>
<tr>
<td>TBP</td>
<td>Total body protein</td>
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<tr>
<td>TBW</td>
<td>Total body water</td>
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Chapter 1. Introduction

This chapter summarises the clinical framework that underpins the research described in this thesis. It outlines the nutritional and metabolic consequences of end-stage liver disease that contribute to morbidity and mortality, the current role of \( \beta \)-adrenoreceptor antagonists (\( \beta \)-blockers) in the management of portal hypertension, and postulates other potential benefits (reducing REE) and harms (reducing insulin sensitivity and glucose tolerance) that may be associated with the use of \( \beta \)-blockers in this patient population. These postulated benefits and harms form the basis of all the investigations described in this thesis.

1.1. Liver cirrhosis is a common cause of morbidity and mortality

Cirrhosis of the liver is the final common pathway of chronic liver disease. The most common causes of liver cirrhosis in New Zealand are chronic hepatitis B (HBV) and HCV followed by non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD) and cholestatic liver disease. Progressive fibrosis disrupts the liver vasculature and forms islands of hepatocytes in patients with cirrhosis and the change is generally irreversible. The increase in intrahepatic vascular resistance from the architectural distortion and from increased sinusoidal tone\(^1\) leads to many of the complications of cirrhosis including portal hypertension. The mortality associated with liver cirrhosis has declined in New Zealand from 6.1% (1980-2) to 2.8% per 100 000 men (2000-2)\(^2\) as a result of improved access to liver transplantation and advances in the supportive treatment for the complications of liver cirrhosis, primarily by non-selective \( \beta \)-blockade. However, portal hypertension continues to account for around a third of deaths from liver cirrhosis\(^3\) while other causes of death include fulminant hepatic failure, hepatocellular carcinoma, cardiovascular disease and infections.

Liver transplantation is the only effective treatment for liver cirrhosis\(^4\). The availability of donor organs is limited in New Zealand where an estimated 400 adults die per annum from end-stage liver disease (personal communication), whilst over 30 successfully undergo liver transplantation every year\(^5\). Liver transplantation is not available in developing countries where the highest prevalence of chronic liver disease is found. For these reasons there is a strong need for new treatments of end-stage liver disease that may delay or reduce the need for liver transplantation.
1.2. Detrimental nutritional and metabolic changes in liver cirrhosis

Liver cirrhosis is associated with detrimental changes in body composition, physiological function and energy metabolism. Malnutrition is common and results from the progressive loss of body protein and lean body mass with worsening severity of liver disease\textsuperscript{6-8}. In addition to the functional impairment, malnutrition is associated with an increased risk of the complications of liver cirrhosis\textsuperscript{9} and poorer survival\textsuperscript{10,11}.

REE is elevated in up to 35% of patients with liver cirrhosis\textsuperscript{6,12,13}. An elevated REE (determined by the ratio of measured REE over predicted REE) is known as hypermetabolism and is partly determined by the metabolic and haemodynamic derangements of liver cirrhosis\textsuperscript{14,15}. Hypermetabolic patients with liver cirrhosis are at increased risk of mortality\textsuperscript{6,12,13,16,17}. However, a recent publication suggests that even within the normal range, patients with a higher REE have an increased mortality compared to patients with a lower REE\textsuperscript{6}.

1.3. β-blockade may reduce REE and the prevalence of hypermetabolism

β-blockers are commonly used in liver cirrhosis for the primary and secondary prevention of gastrointestinal haemorrhage from oesophageal varices\textsuperscript{18}. Mathur \textit{et al} showed that cirrhotic patients not receiving β-blockers were 3 times more likely to be hypermetabolic than those receiving β-blockers\textsuperscript{6}. An early study showed that short-term intravenous infusion of β-blockers also reduced REE by around 5% in hypermetabolic patients, and around 2.5% in normometabolic patients\textsuperscript{13}. Oral β-blockade has not been tested in cirrhotic patients but has been studied in paediatric patients with severe burns. Treatment with oral propranolol reduced REE in these children and also reversed the protein catabolism associated with severe burns\textsuperscript{19}.

These observations provide a strong rationale for a study to investigate the potential benefits of β-blockade on REE in cirrhotic patients. To summarise: hypermetabolism and malnutrition are common in liver cirrhosis and are associated with a poorer outcome, cirrhotic patients taking β-blockers are less likely to be hypermetabolic, β-blockers reduce REE and reverse muscle-protein loss in severe catabolic illness, and they also reduce REE in cirrhotic patients treated with short term intravenous therapy. Lastly, a reduction of REE in cirrhotic patients following β-blockade may translate into a survival benefit by using a readily available and cost-effective medication.
1.4. β-blockade reduces insulin sensitivity and glucose tolerance

In general, β-blockers have an established safety profile in liver cirrhosis. However, β-blockade is strongly associated with a reduction of insulin sensitivity and the development of new-onset diabetes in certain patients with an existing predisposition such as hypertension and type 2 diabetes mellitus. It is estimated that patients taking β-blockers have a reduction of insulin sensitivity by 15 – 35% and this translates into a higher incidence of new-onset diabetes mellitus.

1.5. Patients with liver cirrhosis have reduced insulin sensitivity and glucose tolerance

Liver cirrhosis is also characterised by a reduction of insulin sensitivity and an increased prevalence of diabetes mellitus. Cirrhotic patients with diabetes have a poorer survival. Interestingly, poorer survival relates to the complications of liver disease itself and not diabetes. In addition, overall mortality and the risk of post-operative complications following surgery and liver transplantation are increased with concomitant diabetes.

β-blockade in patients with liver cirrhosis may have a similar detrimental effect on insulin sensitivity and glucose tolerance as seen in patients with hypertension or type 2 diabetes. No study to date has examined this potential adverse outcome in cirrhotic patients. A trial to investigate the effect of β-blockade on REE is an ideal opportunity to also determine if β-blockade will have a similar adverse effect on insulin sensitivity and glucose tolerance in patients with liver cirrhosis.
1.6. Little is known about β-cell function in patients with liver cirrhosis

β-cell function (in addition to insulin sensitivity) is an important determinant of glucose tolerance\textsuperscript{26, 33}. Studies that have identified a reduction of glucose tolerance in patients taking β-blockers have not examined the possible contribution of a reduction in β-cell function. Only a small series of papers by a single group have shown a subtle reduction in β-cell function in patients taking β-blockers\textsuperscript{21, 23, 34}. In liver cirrhosis, very little is known about β-cell function at baseline and no studies have investigated the impact of β-blockade. Again, the proposed trial of β-blockade to reduce REE provides an ideal opportunity to better understand this complex phenomenon in patients with liver cirrhosis. The β-cell function of normoglycaemic patients with liver cirrhosis was also compared to healthy controls in an attempt to elucidate changes (if any) in the β-cell function of patients with liver cirrhosis prior to the onset of a reduction in glucose tolerance.

1.7. Thesis outline

Several interesting hypotheses can be postulated from the brief synopsis of the published literature above:

1. The prevalence of diabetes in patients with liver cirrhosis is higher in certain aetiologies of chronic liver disease, and the prevalence increases with increasing severity of liver cirrhosis.

2. Oral β-blockade reduces REE in adult patients with liver cirrhosis and the reduction of REE leads to an improvement of protein calorie malnutrition.

3. Oral β-blockade reduces insulin sensitivity in adult patients with liver cirrhosis and that the reduction of insulin sensitivity leads to a reduction of glucose tolerance.

4. Oral β-blockade worsens β-cell function in adult patients with liver cirrhosis and this reduction contributes the reduction of glucose tolerance.
5. The β-cell function of normoglycaemic patients with liver cirrhosis is not impaired when compared to healthy volunteers.

In order to systematically test the hypotheses, this thesis will firstly expand on the themes discussed thus far as the foundation for subsequent research. In particular, body composition analysis with special consideration of the adaptations of methodology for patients with liver cirrhosis will be discussed followed by the association of protein calorie malnutrition and hypermetabolism with liver cirrhosis and other catabolic diseases. Present understanding of the role of β-blockade in hypermetabolism and protein calorie malnutrition will also be summarised. Further discussion of the known derangements of glucose metabolism (insulin sensitivity, β-cell function and glucose tolerance) in cirrhotic patients will be followed by the association of β-blockade with a reduction of insulin sensitivity, β-cell function and glucose tolerance.

Chapter 3 systematically reviews the prevalence of diabetes mellitus in patients with liver cirrhosis. In particular, the association between the prevalence of diabetes and the aetiology and severity of liver cirrhosis will help contextualise the significance of the studies discussed in subsequent chapters.

Chapter 4 discusses the primary study of this thesis and explores the role of oral β-blockade for reducing REE in patients with liver cirrhosis in the context of a randomised, controlled, cross-over trial. A secondary end-point of the study was the change in total body protein (TBP) following β-blockade as a marker for muscle protein catabolism. All measurements in this study were performed using gold standard methodology in an attempt to reduce the uncertainty associated with less sophisticated approaches to body composition analysis.

Chapter 5 and Chapter 6 discuss changes in insulin sensitivity, glucose tolerance and β-cell function at baseline and following β-blockade in patients with liver cirrhosis. These parameters were measured by the gold standard methodology where possible. Lastly the relevance of the research described in this thesis are summarised and future directions for research are discussed in the concluding chapter.
Chapter 2. Background

2.1. Malnutrition and hypermetabolism in liver cirrhosis

2.1.1. Body composition analysis and malnutrition in liver cirrhosis

The assessment of malnutrition in patients with liver cirrhosis is difficult and malnutrition may be masked by physiological sequelae of liver cirrhosis. Liver cirrhosis is often indolent, asymptomatic and unsuspected until complications of liver disease arise. It is estimated that around 40% of patients with cirrhosis die before the disease is recognised. As a result, malnutrition is often missed in patients with cirrhosis, particularly in the early stages of disease.

Malnutrition is assessed by measuring changes in body composition. The study of body composition can be defined as a branch of human biology that studies various body compartments and their quantitative steady-state relationships. Central to this is developing the methodology for measuring the various compartments in vivo and the influence of various biological factors on these compartments and relationships. The 5 level model proposed by Wang et al. provides a structural framework for the study of body composition. The 5 levels (with increasing complexity) are the atomic, molecular, cellular, tissue-system and whole-body levels where each level has specific compartments that comprise body weight. In essence, these levels are a simplified description of the human body. The whole-body level has the highest complexity as it is at this level that the human body is clearly differentiated from any other living organism (e.g. primates or animals).

Measurements at the whole-body level are the easiest to perform and comprise body weight, skinfold and circumference measurements. Accurate testing for compartments at the atomic or molecular levels is less susceptible to error but require specialised and expensive techniques. The following table summarises some of the common direct measurement techniques currently available for each level.
Table 2-1 Common direct measurement techniques for various compartments of body composition levels

<table>
<thead>
<tr>
<th>Level</th>
<th>Compartment</th>
<th>Direct measurement techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic</td>
<td>TB K</td>
<td>Whole-body $^{40}$K scanner</td>
</tr>
<tr>
<td></td>
<td>TB nitrogen</td>
<td>Prompt-γ neutron activation analysis</td>
</tr>
<tr>
<td></td>
<td>TB Na, Cl, Ca</td>
<td>Delayed-γ neutron activation analysis</td>
</tr>
<tr>
<td>Molecular</td>
<td>TB water</td>
<td>Tracer dilution method (deuterium, tritium)</td>
</tr>
<tr>
<td></td>
<td>Bone mineral content</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>Cellular</td>
<td>Extracellular fluid</td>
<td>Tracer dilution method (radiobromide)</td>
</tr>
<tr>
<td>Tissue-system</td>
<td>Adipose tissue</td>
<td>Computed tomography, magnetic resonance imaging</td>
</tr>
<tr>
<td></td>
<td>(subcutaneous/visceral),</td>
<td></td>
</tr>
<tr>
<td></td>
<td>skeletal muscle mass</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: TB, total body; K, potassium; Na, sodium; Cl, chloride; Ca, calcium.

The accuracy of traditional approaches for assessing malnutrition (based primarily on body weight, skinfold or circumference measurements) may be masked by tissue oedema in patients with liver cirrhosis. Sodium retention and oedema occur early in the course of liver cirrhosis before clinical signs emerge\textsuperscript{38, 39} and the use of serial body weight or body mass index (BMI) measurements would not be appropriate in this patient population. Upper-arm anthropometric measurements such as mid-arm muscle circumference are a commonly used method but up to 30% of healthy controls would be considered under-nourished according to the reference standards of this method\textsuperscript{40, 41}. Tissue oedema in cirrhotic patients would be expected to contribute further error to measurements\textsuperscript{42}. Similarly, bio-electrical impedance analysis (an indirect whole-body measurement technique) should be used with caution in liver cirrhosis\textsuperscript{43-45}. Bio-electrical impedance was not able to accurately detect the change in total body water following large volume paracentesis of ascites in individual patients with liver cirrhosis\textsuperscript{46}. Lastly, liver synthetic function is impaired in liver cirrhosis and visceral protein markers of nutritional status like albumin and retinol-binding protein should be used with caution\textsuperscript{47, 48}.

Mid-arm muscle circumference and bio-electrical impedance determine the fat and lean body compartments using a 2-compartment model of body composition. 2-compartment models are the earliest and simplest models (at the molecular level) and divide the body into fat mass and fat-free...
mass (FFM) by means of prediction equations. However these models are dependent on the assumption that the ratio of components within FFM remain constant and that the hydration fraction of FFM is constant. In cirrhosis, the assumptions are not necessarily valid and results obtained using 2-compartment models differed significantly from 4-compartment models that directly measured or estimated total body water, protein and bone minerals\textsuperscript{49}. Both accuracy and precision of measurements were improved using a 4-compartment model\textsuperscript{50}. With the advent of new technology (Table 2-1), more detailed models of body composition that include all 6 compartments of the molecular level have been developed (fat, water, protein, bone mineral content, non-bone mineral and glycogen) and provide the gold standard measurement of body composition (see below).

A 6-compartment model of body composition was developed by Plank \textit{et al} and allows total body water (TBW) to be calculated using a difference method\textsuperscript{51}. Briefly, TBW is one of six compartments comprising body weight. The other compartments are TBP, total body fat, bone mineral content, glycogen and non-bone mineral content. TBP, total body fat and bone mineral content can be directly measured using contemporary body composition methods. The non-bone mineral and glycogen compartments are small and are estimated from TBP and total mineral content (bone mineral content and non-bone mineral content) respectively using the relative size of these compartments in Reference Man\textsuperscript{52}. Including both these components reduces the systematic error that would otherwise arise in extracting TBW by this method. Nevertheless, measurement of TBW using the difference method is still subject to a small systematic error largely from unmeasured fluctuations in the glycogen compartment but results correlated well with the gold standard tracer dilution method\textsuperscript{51}.

Protein represents a key structural and functional component of the body, and loss of body protein is associated with loss of function\textsuperscript{53, 54}. \textit{In vivo} neutron activation analysis is the gold standard for measurement of protein depletion. This technique was first used in cirrhotic patients in 1993 and TBP levels expressed as a nitrogen index (ratio of measured TBP over predicted) were significantly reduced compared to healthy controls\textsuperscript{55}. The technique of \textit{in vivo} neutron activation analysis was subsequently refined for patients with liver cirrhosis using a 6-compartment model by Plank \textit{et al} to calculate the FFM corrected for abnormal hydration (a common finding in cirrhotic patients)\textsuperscript{56}.

The same group proceeded to comprehensively assess body composition in a cross-section of well-characterised and clinically stable cirrhotic patients using the 6-compartment model (Peng \textit{et al})\textsuperscript{8}. In this study of 268 cirrhotic patients, 51\% of patients were protein depleted compared to 386 healthy volunteers with over twice as many men protein depleted compared to women (63\% and 28\% respectively, p < 0.0001). Over-hydration was seen in 65\% of patients and both protein depletion and over-hydration worsened with increasing severity of cirrhosis (as measured by the Child-Pugh score\textsuperscript{3}). Patients with established protein depletion were more likely to be over-hydrated and to have ascites.
The sex difference in muscle depletion may have been related to higher fat stores in women slowing the catabolism associated with cirrhosis because men actually had a higher energy intake as a proportion of REE compared to women\textsuperscript{57}. The percentage body fat in women was 35.7 ± 1.0\% compared to 23.5 ± 0.6\% in men. Percentage body fat was reduced in Child’s C cirrhotic patients compared to Child’s A patients but there was no difference between patients with or without protein depletion.

2.1.2. Resting energy expenditure and hypermetabolism in liver cirrhosis

The study by Peng et al\textit{a}l also examined energy metabolism in the same cross-section of cirrhotic patients. Hypermetabolism was defined as the ratio of measured REE over predicted greater than 1.22 (which represents 2 standard deviations above the mean of the distribution in healthy volunteers). Forty one of the 268 patients were hypermetabolic (15\%) and hypermetabolism was not associated with sex, severity of disease, aetiology of cirrhosis, protein depletion or the presence of ascites\textsuperscript{8}. This compares to a reported prevalence of hypermetabolism of between 18\% and 34.5\% of cirrhotic patients from several other heterogeneous studies\textsuperscript{6, 12, 13, 16, 58-61}. The presence of ascites was associated with an increased REE in a small study of patients with primarily alcoholic cirrhosis\textsuperscript{62}. An increased REE is also associated with a reduction in insulin sensitivity in patients with liver cirrhosis\textsuperscript{60}.

Many approaches have been used to define hypermetabolism which makes direct comparison between studies difficult\textsuperscript{63}. All studies defined hypermetabolism as the ratio of measured REE (using indirect calorimetry) over predicted REE but the method for predicting REE was not the same between studies. Most studies predicted REE using the equations of Harris and Benedict\textsuperscript{64}.

For men:

\[
REEp \text{ (kcal/day)} = 66.473 + 13.7516 \times \text{body mass (kg)} + 5.0033 \times \text{height (cm)} - 6.755 \times \text{age (yr)}
\]  
(1.1)

For women:

\[
REEp \text{ (kcal/day)} = 655.095 + 9.5634 \times \text{body mass (kg)} + 1.8496 \times \text{height (cm)} - 4.675 \times \text{age (yr)}
\]  
(1.2)
Variables in equations 1.1 and 1.2 are easily measured but significant discrepancies between the measured REE and predicted REE using the Harris-Benedict equation (greater than 10%) have been reported even in healthy volunteers\textsuperscript{65-68}. In liver cirrhosis, over-hydration and loss of FFM would result in a further deviation from the predicted value as FFM is the major determinant of REE\textsuperscript{65, 69}. For these reasons, the use of the Harris-Benedict equation for predicting REE in the research setting should be discouraged because greater accuracy is generally required.

Prediction formulae for REE have been proposed that attempt to overcome the confounding effects of progressive over-hydration and loss of FFM in liver cirrhosis. Measurements were taken from 80 healthy volunteers and a prediction formula for REE was derived using the corrected FFM for abnormal hydration (FFM\textsubscript{c})\textsuperscript{66}.

\[
\text{REE}_{p} \text{ (kcal/day)} = 16.85 \times \text{FFM}_c + 7.25 \tag{1.3}
\]

Measured FFM is the difference between total body weight and total body fat. It can also be represented by the following equation:

\[
\text{FFM} = \text{FFM}_c + \text{TBW} - \text{TBW}_c \tag{1.4}
\]

where (TBW - TBW\textsubscript{c}) represents the deviation of measured TBW from the water that accompanies FFM\textsubscript{c}. Equation 4 can be rearranged thus:

\[
\text{FFM}_c = \text{FFM} (1 - \text{TBW}/\text{FFM}) / (1 - \text{TBW}_c/\text{FFM}_c) \tag{1.5}
\]

where TBW\textsubscript{c}/FFM\textsubscript{c} is the ratio of TBW to FFM in healthy subjects. The value of TBW\textsubscript{c}/FFM\textsubscript{c} is 0.73 as measured in 176 health volunteers using the 6-compartment method\textsuperscript{66}.

The prevalence of hypermetabolism determined using the FFM prediction equation when compared to the Harris-Benedict equation was 15% and 8% respectively in the study by Peng et al\textsuperscript{8}. Of the hypermetabolic patients as assessed by the Harris-Benedict equation, 90% were also hypermetabolic according to the FFM prediction formula. Hypermetabolic patients identified using the Harris-Benedict
The correlation between hypermetabolism and malnutrition (as defined in Section 2.1.3) has not been established in liver cirrhosis. Intuitively, an increased REE would be expected to contribute to the progressive protein catabolism and malnutrition characteristic of liver cirrhosis. The largest studies to date using in vivo neutron activation analysis for measurement of TBP have not shown an association between hypermetabolism and malnutrition\textsuperscript{6, 8}. Despite using the best techniques currently available and also correcting for over-hydration in cirrhotic patients by calculating predicted REE using the FFM prediction equation [equation (3)], the studies were limited as they were cross-sectional in design. Similarly, previous cross-sectional studies failed to show an association between hypermetabolism and malnutrition\textsuperscript{13, 16} although patients with increased REE had a lower body weight and lower muscle mass. A prospective, adequately-powered, longitudinal study controlling for energy intake of the patients will be required to definitively answer this question.

Despite a paucity of evidence in liver cirrhosis, hypermetabolism (increased REE) has been associated with significant protein catabolism in patients suffering from burn injuries. The association was first proposed in 1974 by Wilmore et al\textsuperscript{70} who noted an increased nitrogen excretion in association with hypermetabolism at the U.S. Army Institute of Surgical Research. Subsequently, protein catabolism was measured using labelled essential amino acid tracers in protein kinetic studies and the period of maximum protein catabolism coincided with the period of hypermetabolism in paediatric burn patients\textsuperscript{71}. More definitive evidence of the association between hypermetabolism and protein catabolism was reported in a large cross-sectional study of primarily paediatric burn patients (102 children, 21 adults) that measured protein catabolism in protein kinetic studies using the stable isotope L-[ring-\textsuperscript{2}H\textsubscript{5}] phenylalanine and measuring arterio-venous phenylalanine concentration differences across the leg. In that study, hypermetabolism was an independent predictor of protein catabolism on multivariate analysis\textsuperscript{72}. The ratio of measured REE over predicted was between 1.3 and 1.7 in 34\% of patients, while 27\% of patients had a ratio of measured REE over predicted >1.7. The severity and prevalence of hypermetabolism was markedly higher in the burns population when compared to the cirrhotic population and may explain why an association between hypermetabolism and muscle protein catabolism was identified in that group.
2.1.3. Clinical significance of protein calorie malnutrition and hypermetabolism in liver cirrhosis

Protein calorie malnutrition leads to a poorer clinical outcome with or without liver transplantation in patients with liver disease. Most early studies investigated the impact of malnutrition in patients with alcoholic cirrhosis due to the high prevalence of alcoholic liver disease. Study cohorts included both non-cirrhotic and cirrhotic patients with alcoholic liver disease and showed that malnutrition increased the risk of developing complications of liver disease including the severity of jaundice, the presence of ascites and hepatic encephalopathy and the development of the hepato-renal syndrome. Malnutrition reduced the overall survival (1-month mortality) of alcoholic patients with and without liver cirrhosis. Furthermore when the severity of malnutrition was stratified, patients with moderate malnutrition had a better 6-month survival compared to those with severe malnutrition (defined by a protein-calorie total nutrition score of less than 60%). The protein-calorie total nutrition score is a composite score of 8 parameters including percent ideal weight, anthropometry, delayed cutaneous hypersensitivity testing and several blood tests.

The presence of malnutrition is associated with a 2-fold increase in overall mortality following the onset of liver cirrhosis in patients with chronic liver disease. Cirrhotic patients with oesophageal varices are at higher risk of bleeding or death when they are malnourished. The increase in bleeding risk could not be differentiated with the risk of death, nor was the risk quantifiable due to the small size of the study (n=55). Cox regression analysis in a larger study of 139 cirrhotic patients also identified nutritional status as an independent predictor of survival. Subsequent studies employed anthropometric assessments for malnutrition that was thought to be less influenced by the cirrhotic disease process. However, as discussed in Chapter 2.1.1, mid-arm muscle circumference and triceps skinfold thickness tests have been shown to be confounded by the over-hydration in liver cirrhosis. Both studies confirmed that malnutrition (mid-arm muscle circumference and triceps skinfold thickness below 5th percentile) was an independent predictor of death in cirrhotic patients with a near 4-fold increase in mortality rate at 24 months. On a global scale reduced consumption of total calories and animal protein was associated with poorer survival in patients with liver cirrhosis (by potentially contributing to malnutrition) in 38 countries based on data from the World Health Organisation.

Malnutrition is also predictive of outcome following liver transplantation. The most comprehensive and well-reported study noted an improvement in survival after liver transplantation in patients who had a better nutritional status (measured by body cell mass) at the time of transplant (83% survival with a mean follow-up of 447 days, compared to 47% survival in patients with poorer nutritional status). No significant difference in survival between hypermetabolic and malnourished patients was found. A strength of this study was the measurement of total body potassium using a whole body...
counter (by analysing the γ spectrum emitted from naturally occurring $^{40}\text{K}$) in conjunction with standard anthropometric and bio-electrical impedance analysis. Measurement of total body potassium assumes that potassium is almost entirely intracellular (97%) and is an accurate index of body cell mass (metabolically active tissue in the body). The measurement of total body potassium is a significantly more accurate predictor of malnutrition than bio-electrical impedance analysis$^{83}$.

In a contradictory study of cirrhotic patients awaiting liver transplantation, patients were stratified into a high risk group (comprising hypermetabolic patients or patients with concurrent “moderate hypermetabolism” and malnutrition) and a low risk group. Patients in the high risk group had a reduced 1-year (62% vs. 88%) and 5-year (54% vs. 88%) survival post-transplantation$^{12}$. However, when patients were instead divided into 3 groups consisting of hypermetabolic (measured REE over predicted $>1.2$), moderately hypermetabolic (measured REE over predicted between 1.0 and 1.2) and normometabolic (measured REE over predicted $\leq1.0$) patients, a survival difference between hypermetabolic patients and the other 2 groups was not apparent. Similarly, no difference was seen in survival post-transplantation between well-fed and malnourished candidates. A Type 2 statistical error (with a smaller number of patients in each group) and the added inaccuracy of the measurement of body cell mass using bio-electrical impedance analysis may explain this discrepancy. High risk patients were also more likely to develop a septic complication following transplant but this did not translate to an overall increase in post-op complications.

Merli et al questioned if malnutrition was an independent risk factor for mortality in the belief that malnutrition and worsening liver function are strongly associated. In a multi-centre cohort study, 1053 cirrhotic patients were assessed for malnutrition using mid-arm muscle circumference and triceps skinfold thickness. Muscle depletion and/or a rapid reduction in fat deposits were associated with a higher risk of mortality and the impact of malnutrition was more pronounced in patients with a lower severity of liver disease (Childs A and B). However, malnutrition was not an independent risk factor for mortality following Cox multivariate proportional hazard analysis$^{84}$. The trial was a large, well conducted trial but was limited by the chosen method for measurement of malnutrition. Therefore the role of malnutrition in the prognosis of liver cirrhosis has not been confirmed and further trials are needed.

The evidence for an association between hypermetabolism and a poorer outcome in patients with liver cirrhosis is more established and are in agreement$^{6, 12, 17, 58}$. Unexpectedly, in the paper by Mathur et al, transplant-free survival was reduced even within quartiles of normometabolic patients not on β-blockade$^{6}$. Normometabolic patients with a measured REE over predicted (using the FFM$_{c}$-derived prediction equation) of between 1.08 and 1.21 had a survival rate of 19% at 72 months of follow-up compared to a survival rate of 70% in patients with a ratio between 0.74 and 0.91. This
translates to a 22% increased risk of transplant or death for every 0.1 increase in measured REE over predicted. The implication of this is that normometabolic cirrhotic patients may benefit from lowering of their REE and is a completely novel finding to date.

2.2. The Role of β-blockade in hypermetabolism

2.2.1. Hypermetabolism in burn injuries

Briefly discussed in the concluding paragraph of Section 2.1.2, severe burn injuries are characterised by a marked hypermetabolic response. The recent systematic review by Atiyeh et al discusses the metabolic implications of burn injuries in detail. To summarise, burn injuries result in a combination of hypovolaemic and distributive shock on the basis of generalised microvascular injury and third-spacing of interstitial fluid. These severe injuries instigate a systemic inflammatory response syndrome driven by catecholamines, cortisol and other pro-inflammatory mediators. Plasma catecholamines in particular may increase up to 10-fold and are the prime mediators of the hypermetabolic response. These mediators may drive the increased metabolic response seen in severe burns including increased protein synthesis and breakdown but with a net negative protein balance, gluconeogenesis, urea production and substrate cycling of fatty acids and glucose. These processes are estimated to account for 60% of the increased REE following burn injury and the other 40% derive from uncoupled cellular membrane reactions with altered Na+-K+-ATPase activity and proton leakage across the mitochondrial membrane.

The hypermetabolism associated with burn injuries is marked and the measured REE over predicted may be doubled. Administration of β-blockers was recognised early on to reduce the REE in burn patients despite negative findings in several studies. The oral route of administration is equivalent in efficacy to intravenous β-blocker administration. More recently, 25 paediatric patients with severe burns were treated with oral β-blockade (propranolol) for 2 weeks and assessment showed a significant reduction in REE and more importantly showed a reversal of protein muscle catabolism measured by stable isotope balance studies and body composition analysis. No adverse effects of β-blockade were reported. A subsequent study by the same group treated a smaller cohort of children with severe burns using a combination of recombinant growth hormone and oral propranolol for 15 days confirmed a similar reduction in REE but did not detect a change in body composition. The dose of propranolol in the second study was aimed at reducing the resting heart rate by 15% compared to 20% in the first study. Nevertheless, β-blockade is now considered the most effective treatment for protein muscle catabolism in burn injuries.
2.2.2. Hypermetabolism in liver cirrhosis

β-blockade is used for the primary and secondary prevention of variceal bleeding in patients with liver cirrhosis and gastro-oesophageal varices and has been standard of care for the last 30 years. Non-selective β-blockade (with propranolol or nadolol) is preferred as the medications reduce portal blood flow and hepatic venous pressure gradient by reducing both cardiac output (β₁-blockade) and splanchnic vasoconstriction (β₂-blockade)\(^{101}\). There is mounting evidence that carvedilol (a combined non-selective β- and selective-α₁ blocker) may be as efficacious as non-selective β-blockers in reducing the risk of variceal bleeding but definitive trials are awaited\(^{102-105}\). Treatment with β-blockade reduces the risk of variceal bleeding and improves the survival of cirrhotic patients with gastro-oesophageal varices\(^{18,106,107}\).

Cirrhotic patients not receiving non-selective β-blockers were 3 times more likely to be hypermetabolic than those who were receiving β-blockers\(^{6}\). Cirrhotic patients given a short-term infusion of intravenous β-blockade had a reduction of REE by 5% in hypermetabolic patients and around 2.5% in normometabolic patients\(^{13}\). The mechanism of action is unclear but may be related to a hyperdynamic circulation driven by catecholamine secretion. As discussed in Section 2.1.3, hypermetabolism in cirrhotic patients is associated with a poorer outcome and there is the potential for a survival benefit if a safe and effective treatment for hypermetabolism is found.
2.3. Glucose tolerance in liver cirrhosis

The association between chronic liver disease and diabetes mellitus was first reported by Naunyn in 1898, who coined the term “hepatogenous diabetes”\textsuperscript{108, 109}. Further evidence followed that initial report supporting the strong association between liver cirrhosis and impaired glucose tolerance (IGT) and/or diabetes\textsuperscript{110-114}. Many studies have focused on patients with established liver cirrhosis but it is increasingly recognised that for some aetiologies of chronic liver disease, the propensity for IGT and diabetes is present prior to the onset of end-stage liver disease. In particular, patients with NAFLD\textsuperscript{115}, ALD\textsuperscript{116} and HCV have an increased prevalence of diabetes\textsuperscript{117}.

Diabetes in patients with liver cirrhosis may share the same pathophysiology as type 2 diabetes mellitus although the association remains to be clarified\textsuperscript{118}. In both cirrhosis and type 2 diabetes mellitus, glucose intolerance results from failure of pancreatic $\beta$-cell compensation for the underlying reduction in insulin sensitivity\textsuperscript{25, 26, 113, 119}. Cirrhotic and non-cirrhotic patients with IGT primarily have a reduction in peripheral insulin sensitivity and mild (if any) reduction in hepatic insulin sensitivity\textsuperscript{120, 121} but following the onset of diabetes, hepatic insulin sensitivity worsens\textsuperscript{26, 122}. Oxidative disposal of glucose is also reduced in both conditions following the onset of diabetes\textsuperscript{122, 123}.

Briefly, glucose tolerance is determined by the balance between insulin sensitivity and $\beta$-cell function\textsuperscript{26}. Both these concepts are pivotal to this thesis and are defined in Table 2-2. Common methods for measurement of these parameters are also summarised in the table as an introduction and a more detailed discussion will follow in the later sections of this chapter.
<table>
<thead>
<tr>
<th>Determinant of glucose tolerance</th>
<th>Definition</th>
<th>Method of assessment</th>
<th>Indices</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin sensitivity</strong></td>
<td>The response of cells to the action of endogenous or exogenous insulin</td>
<td>Hyperinsulinaemic euglycaemic clamp</td>
<td>M value</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frequently sampled IVGTT</td>
<td>SI-clamp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OGTT</td>
<td>Minimal model assessment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oral glucose insulin sensitivity index</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Matsuda composite index</td>
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<tr>
<td></td>
<td></td>
<td>Plasma insulin and/or plasma glucose</td>
<td>Homeostasis model assessment of insulin resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Quantitative insulin sensitivity check index</td>
</tr>
<tr>
<td><strong>β-cell function</strong></td>
<td>The secretion of insulin by pancreatic β-cells in response to glucose and other stimuli</td>
<td>Hyperglycaemic clamp</td>
<td>First phase insulin secretion</td>
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<tr>
<td></td>
<td></td>
<td>IVGTT</td>
<td>Second phase insulin secretion</td>
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<td></td>
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<td></td>
<td>Acute insulin response</td>
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<td>C-peptide minimal model assessment</td>
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<td>OGTT</td>
<td>Mari model</td>
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<tr>
<td></td>
<td></td>
<td>Composite</td>
<td>Disposition index</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma insulin, C-peptide and/or plasma glucose</td>
<td>Homeostasis model assessment of β-cell function</td>
</tr>
</tbody>
</table>

Abbreviations: IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test
2.3.1. Guidelines for the diagnosis of diabetes have changed over time

The diagnosis and definition of diabetes has changed over time as new evidence informs the threshold above which complications of diabetes arise\(^\text{124}\). In 1979, both the method for diagnosis and the definition of diabetes were standardised by consensus of the National Diabetes Data Group\(^\text{125}\). Diabetes was diagnosed by using either a fasting glucose sample or a 75g 2 hour OGTT and patients with a fasting glucose level of 7.8mmol/L or a 2-hour glucose level above 11.1mmol/L were classified as diabetic. In 1995, the American Diabetes Association followed by the World Health Organisation in 1997 lowered the fasting glucose level required for diagnosis of diabetes to 7.0mmol/L\(^\text{126, 127}\). A new classification for impaired fasting glucose was also introduced. Subsequently, the American Diabetes Association lowered the threshold for diagnosis of impaired fasting glucose from 6.1mmol/L to 5.6mmol/L in 2005\(^\text{128}\). The glucose level required for diagnosis of IGT has lowered in parallel with the definition of diabetes with each revision of the guidelines since 1979. Recently, the HbA\(_1C\) assay has been proposed as a new method for diagnosing diabetes\(^\text{129}\) but it remains to be seen if this is translatable to patients with liver cirrhosis because values in cirrhotic patients are often falsely lowered due to anaemia and a high turnover of red blood cells\(^\text{130, 131}\).

2.3.2. Diabetes is prevalent in patients with liver cirrhosis

The overall prevalence of diabetes in patients with liver cirrhosis is generally believed to be high but the prevalence has not been systematically summarised. A prevalence of up to 30% is commonly quoted but with wide variation (10% - 40%) in the reported estimates for the prevalence of diabetes\(^\text{110, 113, 132}\). The strong association between certain aetiologies of chronic liver disease and diabetes persists with the onset of cirrhosis and the prevalence of diabetes is higher in these patients.

Patients with cirrhosis secondary to chronic HCV infection have a high prevalence of diabetes (20% - 35%)\(^\text{133-136}\) due to a direct role of HCV genotypes 1, 3b and 4 in the reduction of insulin sensitivity\(^\text{137, 138}\). The association was first reported in a retrospective study of 100 cirrhotic patients with HCV undergoing assessment for liver transplantation\(^\text{139}\). Chronic HCV infection appears to exacerbate hepatic steatosis, inflammation and oxidative stress, and the HCV core protein may directly inhibit the insulin signalling cascade\(^\text{140}\).

The estimated prevalence of diabetes in patients with NAFLD is not as well reported because the importance of NAFLD as a driver for chronic liver disease has only recent been recognised. Many
earlier studies estimated the prevalence of diabetes in cryptogenic cirrhosis. Cryptogenic cirrhosis was an all-encompassing diagnosis for chronic liver disease of unknown aetiology after known causes for liver disease were excluded. While patients with NAFLD are likely to comprise a major proportion of patients with cryptogenic cirrhosis, there may be a significant proportion of patients with burnt-out autoimmune hepatitis, occult alcohol abuse and uncommon (non-A, non-B and non-C) viral hepatitis. Studies of cryptogenic cirrhosis suggest a prevalence of diabetes between 40% - 50%, which is similar to that of patients with NAFLD and cirrhosis. The study by Caldwell et al also compared the prevalence of diabetes between NAFLD/cryptogenic cirrhosis and HCV cirrhosis, and found that the prevalence was higher in NAFLD and cryptogenic cirrhosis. Autoimmune hepatitis had the lowest prevalence of diabetes (15%).

The third aetiology of liver disease with a strong association for diabetes is alcoholic cirrhosis. Estimates published over the last 20 years suggest a prevalence of between 25% - 35%. ALD (and haemochromatosis) uniquely reduces pancreatic exocrine function including for insulin secretion from repeated alcohol toxicity in addition to reducing insulin sensitivity in cirrhotic patients. A combination of steatosis and inflammation from a high alcohol intake results in decreased insulin binding to cell receptors in a rat model of chronic ALD. Despite the reduction in both β-cell function and insulin sensitivity, the prevalence of diabetes in patients with alcoholic cirrhosis may be lower than in cryptogenic cirrhosis.

The severity of liver disease is also an important determinant for the prevalence of diabetes in cirrhotic patients. The largest study reporting this association to date recruited 1151 cirrhotic patients with chronic HCV and 181 cirrhotic patients with chronic HBV and showed a strong association with an odds ratio of 3.83 for developing diabetes with increasing Child-Pugh score. Another study of 461 cirrhotic patients (of undefined aetiology) reported that almost 60% of patients with Childs C cirrhosis were diabetic compared to 20% of Childs B patients and 10% of Childs A patients. Other studies however have shown no difference in the risk of diabetes with more severe cirrhosis although one of these studies also failed to show an association between HCV cirrhosis and diabetes. The study by Del Vecchio Blanco also reported that older cirrhotic patients with Childs B and C disease and those with a family history of diabetes had a higher prevalence of diabetes compared to those who were younger, or who did not have a family history.
2.4. Patients with liver cirrhosis have reduced insulin sensitivity

Glucose tolerance is determined by the balance between insulin sensitivity and β-cell function as briefly discussed previously\(^\text{26}\). Reduced insulin sensitivity (otherwise known as insulin resistance) is defined as the failure of normal concentrations of the hormone (insulin) to produce a normal biological response\(^\text{152}\). The activity of insulin can be measured in skeletal muscle, adipose tissue or hepatocytes and the biological response to insulin may involve substrates like glucose, insulin or amino acids. In practice the commonly measured metabolic end-points are the insulin-mediated non-oxidative glucose disposal (primarily a measure of peripheral insulin sensitivity) and the inhibition of hepatic glucose production (HGP) (a measure of hepatic insulin sensitivity).

The measurement of insulin sensitivity from different studies should be compared with caution. Many tests of insulin sensitivity rely on measuring plasma insulin either at the fasting state, or in response to a challenge (usually glucose). However insulin assays have not been standardised and there are significant differences in the types of insulin standard used, laboratory protocols and different approaches for deriving units of measurement\(^\text{153}\). Not all commercially available assays were accurate enough to satisfy the desired allowable measurement bias of ± 15.5%, imprecision of 10.6% CV and total analytical error of 32.0% for any result\(^\text{154}\). Standardising the insulin assay will simplify comparison of findings between studies and may improve the intra- and inter-individual co-efficient of variation of insulin sensitivity measurement\(^\text{155}\).

2.4.1. Peripheral insulin sensitivity

Peripheral insulin sensitivity appears to be reduced prior to the onset of liver cirrhosis for some aetiologies of liver disease. As seen in glucose tolerance of patients with chronic liver disease, patients with NAFLD\(^\text{156, 157}\), ALD\(^\text{147}\) and HCV\(^\text{137}\) appear to be predisposed to a reduction of peripheral insulin sensitivity as part of the underlying disease process.

With the onset of liver cirrhosis, peripheral insulin sensitivity is reduced in most patients. Several studies measured insulin sensitivity with the gold standard hyperinsulinaemic euglycaemic clamp (see Section 2.5.1) and they show that the reduction of insulin sensitivity is in the skeletal muscle (peripheral insulin resistance)\(^\text{27, 158-161}\). One study did not show a difference in insulin sensitivity between cirrhotic patients and healthy controls but the clamp was performed at supra-physiological
hyperinsulinaemia\textsuperscript{162}. Such high insulin levels may overcome a modest reduction in insulin sensitivity and misleadingly show comparable glucose uptake between cirrhotic patients and healthy controls.

Nevertheless, insulin sensitivity may not be reduced in all cirrhotic patients. Patients with cholestatic cirrhosis (for example primary biliary cirrhosis or primary sclerosing cholangitis) have similar insulin sensitivity to healthy controls when measured using the hyperinsulinaemic euglycaemic clamp\textsuperscript{162}. Patients with cryptogenic cirrhosis in that study were insulin resistant. Insulin sensitivity measured by the homeostasis model assessment ([HOMA), see Section 2.5.2] also showed no difference between patients with primary biliary cirrhosis and healthy controls\textsuperscript{61}. In contrast, a study by Muller \textit{et al} reported a similar reduction of insulin sensitivity in patients with alcoholic, post-necrotic (post-hepatitis) and primary biliary cirrhosis\textsuperscript{163}. This suggests that the reduction of insulin sensitivity varies among cirrhotic patients of different aetiology and that patients with cholestatic cirrhosis are likely to have normal insulin sensitivity or a mild impairment at most.

The severity of insulin resistance does not appear to be associated with the severity of liver disease. Two studies examined this issue using the hyperinsulinaemic euglycaemic clamp and the hyperglycaemic clamp in one study\textsuperscript{164}, and the hyperinsulinaemic euglycaemic clamp alone in another\textsuperscript{163}. Both studies showed no association between the severity of insulin resistance and the Child-Pugh score of patients\textsuperscript{150}. Two other studies utilising HOMA also found no association\textsuperscript{61, 165}. Only a single study\textsuperscript{166} of 67 cirrhotic patients measuring insulin sensitivity using HOMA showed an inverse correlation between insulin sensitivity and the model for end-stage liver disease (MELD) score\textsuperscript{167}. The MELD score is a measure of the severity of liver disease and predicts mortality in this group of patients.

2.4.2. Hepatic insulin sensitivity

In contrast to peripheral insulin sensitivity, it is still unclear if liver cirrhosis is also associated with a reduction of hepatic insulin sensitivity. Several studies have shown that cirrhotic patients prior to the onset of diabetes have preserved fasting hepatic insulin sensitivity compared to healthy controls. HGP was measured using glucose tracers and the hyperinsulinaemic euglycaemic clamp in these studies\textsuperscript{168, 169}. HGP was also suppressed normally when insulin was infused at physiological levels (around 1mU/kg/min) during a hyperinsulinaemic euglycaemic clamp\textsuperscript{170, 171}. A comprehensive study by Petrides \textit{et al} measured the difference in hepatic insulin sensitivity in patients with liver cirrhosis prior to and following the onset of diabetes, and compared the results with healthy controls\textsuperscript{26}. In contrast to patients with normal glucose tolerance, diabetic patients did not have normal hepatic
insulin sensitivity. Fasting HGP was higher than in healthy controls and was not completely suppressed (only by 78%) in response to hyperinsulinaemia\textsuperscript{26, 122}.

Other studies have measured endogenous glucose production by calculating the exchange of glucose across the splanchnic circulation. Endogenous glucose production is an estimate of HGP and includes glucose uptake and production by the kidneys and other splanchnic organs. Nevertheless, endogenous glucose production was suppressed normally in non-diabetic patients with cirrhosis\textsuperscript{170, 172-174}.

2.4.3. Oxidative glucose disposal

The onset of diabetes in cirrhotic patients leads to a reduction in oxidative glucose disposal in addition to the reduction in non-oxidative glucose disposal by skeletal muscle (peripheral insulin sensitivity). Glucose oxidation is also stimulated by insulin\textsuperscript{175} but is preserved in patients with IGT\textsuperscript{26, 176} and normal glucose tolerance\textsuperscript{177}. With diabetes, cirrhotic patients develop impaired oxidative glucose disposal\textsuperscript{26, 122}. However, data from in vitro studies using liver biopsy specimens from cirrhotic patients did not show a significant change in oxidative glucose metabolism with worsening glucose tolerance\textsuperscript{178}. Further studies are required to confirm the reduction in oxidative glucose disposal in diabetes.
2.5. Methods for measurement of insulin sensitivity in liver cirrhosis

Insulin sensitivity can be measured in many ways and a comprehensive discussion of this subject is beyond the scope of this thesis. An understanding of the measurement of insulin sensitivity is important when appraising studies. This section summarises the commonly used methods and their strengths and weaknesses and there are several reviews available that may be referred to for more detail\textsuperscript{179-182}.

Insulin sensitivity can be measured by direct or indirect methods, or estimated using surrogate indices of insulin sensitivity based on fasting (steady-state) or dynamic tests\textsuperscript{181}. Insulin sensitivity is directly measured with the hyperinsulinaemic euglycaemic clamp and the insulin suppression test. Indirect methods include the minimal model approach using the frequently sampled IVGTT and the continuous infusion of glucose with model assessment approach. HOMA and the quantitative insulin sensitivity check index (QUICKI) are examples of fasting surrogate indices of insulin sensitivity while dynamic surrogate indices of insulin sensitivity are generally derived from the OGTT.

2.5.1. Hyperinsulinaemic euglycaemic clamp

The hyperinsulinaemic euglycaemic clamp is accepted as the gold standard measure of insulin sensitivity. The clamp is discussed in detail in Section 5.2. Briefly, plasma insulin is elevated by a primed, constant infusion of insulin while a variable infusion of glucose is used to maintain or ‘clamp’ the blood glucose level at a pre-determined set point. The amount of glucose required to clamp the blood glucose is a direct measure of insulin sensitivity. The hyperinsulinaemic euglycaemic clamp and the related hyperglycaemic clamp (see Section 2.7.1) are not used in large studies and clinical practice due to the technical expertise required and the invasive, expensive and time-consuming nature of the process. Furthermore, insulin is infused peripherally during the clamp rather than into the portal circulation and the sustained hyperinsulinaemia does not reproduce normal physiological conditions\textsuperscript{183}. A hyperglycaemic clamp provides a composite measure of insulin-mediated glucose disposal, glucose-mediated glucose disposal and renal glucose excretion but results are still closely correlated with the hyperinsulinaemic euglycaemic clamp\textsuperscript{184}.
2.5.2. Homeostasis model assessment of insulin sensitivity

Insulin sensitivity is now most commonly measured by fasting surrogate indices, and the most common index in use is HOMA. HOMA was proposed by Matthews et al and is modelled on the premise of a closed feedback loop between the liver and pancreatic β-cell in the fasted state. Non-linear empirical equations describing this loop predict glucose, insulin and C-peptide concentrations for any combination of insulin sensitivity (or resistance). This in turn allows prediction of insulin sensitivity (HOMA-IS, or the reciprocal HOMA-IR) from pairs of fasting glucose and insulin samples collected from an individual using the equation:

\[
\text{HOMA = fasting serum insulin (\(\mu U/mL\)) x fasting plasma glucose (mmol/L) / 22.5}
\]

The updated HOMA2 model was derived after the physiological basis of the first model was elucidated. The HOMA2 model is computer-based and is the recommended method for calculating HOMA-IS because it allows assessment of subjects with glucose levels ≤ 25mmol/L while accounting for renal glucose losses and the use of total or specific insulin assays. The model also assumes reduced suppression of hepatic glucose production and increased insulin secretion in response to glucose levels greater than 10mmol/L.

A prime limitation of HOMA-IS is that results are not easily reproducible with co-efficients of variation greater than 10% especially when three basal measurements of glucose and insulin are not used. The model also assumes that insulin sensitivity in the liver and peripheral tissues are equivalent when they are not. Furthermore, HOMA-IS is primarily a test of hepatic insulin sensitivity because glucose homeostasis while fasting is primarily determined by the regulation of hepatic glucose production by basal insulin and uptake by mostly insulin-independent tissues like the brain and liver. HOMA-IR (the reciprocal of HOMA-IS) correlates with hepatic insulin sensitivity but not peripheral insulin sensitivity determined by the frequently sampled IVGTT. Therefore, HOMA-IS (and other fasting surrogate indices of insulin sensitivity) should be used cautiously in situations where hepatic and peripheral insulin sensitivity may not necessarily be related (as in non-diabetic patients with liver cirrhosis). Lastly, the lack of a standard insulin assay precludes comparison between studies from different laboratories and limits the use of this index in wider clinical practice. Despite these concerns, HOMA-IS has been shown to correlate well with the hyperinsulinaemic euglycaemic clamp.
2.5.3. Quantitative insulin sensitivity check index

HOMA and QUICKI share similar variables but have been derived from different conceptual standpoints. QUICKI was developed by sensitivity analysis of data from the hyperinsulinaemic euglycaemic clamp and the first 20min of a frequently sampled IVGTT. Fasting steady-state glucose and insulin levels were log transformed to improve linear correlation with the clamp. QUICKI is defined as:

$$\text{QUICKI} = \frac{1}{\log(\text{fasting insulin}) + \log(\text{fasting glucose})}$$  \hspace{1cm} (1.7)

QUICKI is proportional to $1/\log(\text{HOMA-IR})$ and may have an improved reproducibility compared to HOMA but whether or not QUICKI is a better index of insulin sensitivity compared to HOMA remains to be confirmed.

2.5.4. Measurement of hepatic insulin sensitivity

The methods discussed above are measures of either peripheral or total body insulin sensitivity. Total body insulin sensitivity is a composite measure of both peripheral and hepatic insulin sensitivity. Hepatic insulin sensitivity can be independently measured and the most common method is by measuring HGP. There are 3 direct techniques for measurement of HGP, the most common being the isotope dilution method. The other techniques are the arteriovenous-difference (or splanchnic balance) method and labelled nuclear magnetic resonance spectroscopy. The isotope dilution method is often paired with the hyperinsulinaemic euglycaemic clamp to assess HGP at fasting and also during hyperinsulinaemia. However, the amount of insulin required to suppress HGP by half ($\frac{1}{2} V_{\text{max}}$) is significantly less than the amount of insulin required to suppress skeletal muscle glucose uptake and it is necessary to choose an appropriate level of hyperinsulinaemia. Practically, this is often achieved by performing a multi-step hyperinsulinaemic euglycaemic clamp where several different levels of steady-state hyperinsulinaemia are established. A multi-step clamp is rarely performed because it significantly increases the cost and time required for performing the clamp. In addition, a single tracer study allows estimation of HGP but using dual- or triple-tracer methods allows gluconeogenesis to be estimated, and thus its contribution to HGP. Further discussion on techniques for measurement of hepatic insulin sensitivity can be found in the thorough review by Choukem et al.
2.6. Mechanisms for the reduction of insulin sensitivity and glucose tolerance in liver cirrhosis

2.6.1. The role of insulin

Insulin plays a critical role in glucose metabolism. Insulin stimulates the production of adenosine triphosphate (ATP) by glucose oxidation (oxidative glucose disposal) and/or glycogen synthesis and storage (non-oxidative glucose disposal) after a meal or glucose challenge. In addition, insulin stimulates the uptake of glucose by muscle (and adipose) tissue. Insulin also inhibits HGP (mostly a combination of gluconeogenesis and glycogenolysis) following a meal in order to maintain normal glucose tolerance. Insulin works by binding to the insulin receptor and thus activating tyrosine kinase. Tyrosine kinase phosphorylates multiple downstream signalling substrates leading to the many actions of insulin.

2.6.2. Skeletal muscle is the site of reduced insulin sensitivity in liver cirrhosis

As discussed in Section 2.4.1, studies of non-diabetic cirrhotic patients using the hyperinsulinaemic euglycaemic clamp in addition to glucose tracers suggest that the site of reduced insulin sensitivity is in the skeletal muscle. Skeletal muscle accounts for 80-90% of whole body glucose disposal following intravenous glucose, while adipose tissue only accounts for 1-2%. Following uptake into skeletal muscle cells glucose is primarily metabolised by non-oxidative glucose disposal. Therefore, a reduction in non-oxidative glucose disposal in patients with liver cirrhosis during a hyperinsulinaemic euglycaemic clamp strongly suggests that the site of reduced insulin sensitivity is the skeletal muscle.

Positron-emission tomography was elegantly used to confirm that the site of reduced insulin sensitivity is the skeletal muscle. The uptake of $^{18}$F-fluorodeoxyglucose into the thigh muscle of cirrhotic and healthy volunteers in response to hyperinsulinaemia was compared and this confirmed that insulin-dependent transport of glucose into skeletal muscle was reduced compared to healthy volunteers in vivo. Muscle biopsies from the thigh of cirrhotic patients during the steady-state period of the hyperinsulinaemic euglycaemic clamp also confirm that cirrhotic patients are unable to increase their glycogen stores in response to hyperinsulinaemia. However, glycogen synthetic ability was adequate to maintain fasting muscle glycogen levels even in severe cirrhosis.
2.6.3. Glycogen synthesis and the role of the insulin receptor

Glycogen synthesis can be impaired at various levels along the synthetic pathway. The possible mechanisms for impaired glycogen synthesis can be clustered into 3 broad levels - upstream to the insulin receptor (pre-receptor), at the level of the insulin receptor or downstream to the receptor (post-receptor). The likelihood of a pre-receptor defect is low because exogenous insulin does not improve insulin sensitivity\(^{208}\) and other actions of insulin are also preserved in cirrhotic patients\(^{160}\).

Similarly, the evidence for a defect of the insulin receptor is not conclusive. The affinity of the insulin receptor for insulin is reduced in monocytes\(^{209, 210}\) and erythrocytes\(^{211}\) of cirrhotic patients although there are contradictory studies that suggest insulin binding may be preserved\(^{212-215}\). However, glucose transport into isolated adipocytes was reduced in cirrhotic patients when directly measured using 3-ortho-methyl-1-\(^{14}\)C]-glucose (labelled glucose that is not metabolised by cells)\(^{208}\). Scatchard analysis\(^{216}\) of insulin binding has not elucidated the reason for reduced insulin binding in cirrhotic patients with some studies reporting a reduction in the affinity of receptors for insulin\(^{217}\) while others report a reduction in the number of insulin receptors\(^{218-220}\).

Erythrocytes, monocytes and adipocytes are not the primary target cells for insulin-dependent glucose metabolism. Insulin binding by these cells only provides surrogate measures of insulin binding by the actual target cells (hepatocytes and myocytes) and the above findings should be confirmed by studies using skeletal muscle cells and hepatocytes. There is evidence to suggest that insulin binding on adipocytes may not be directly associated with insulin binding on monocytes\(^{221}\). However, monocytes may be preferred to erythrocytes for studies of insulin binding because insulin binding of erythrocytes is inversely proportional to cell age and monocytes have a more uniform population of cells capable of the same receptor-mediated endocytosis exhibited by hepatocytes\(^{222}\). The type of Scatchard analysis (two-class vs. one-class model) and the subjective nature of the manual technique may lead to poor reproducibility and differing findings\(^{223}\). Petrides et al attempted to overcome this by using computerised nonlinear least squares analysis of a one-class receptor model and by using highly purified tracers\(^{215}\). They showed unchanged insulin binding by erythrocytes of patients with haemochromatosis and cirrhosis.

The possible reduction of insulin binding by target cells is unlikely to be the only defect in the glycogen synthetic pathway\(^{158}\). Dose-response curves for insulin-dependent uptake of glucose during the hyperinsulinaemic euglycaemic clamp have been constructed using different levels of hyperinsulinaemia (multi-step clamp). The dose-response curves are shifted to the right in cirrhotic patients compared to healthy volunteers, and the maximal effect of insulin is blunted despite very high
levels of insulinaemia suggesting that there is a post-receptor defect\(^{208, 218}\). Furthermore, other actions of insulin including suppression of lipolysis\(^{158, 169, 170, 213, 224}\), hepatic gluconeogenesis\(^{123, 158, 170, 225, 226}\), vascular tone\(^{202, 227, 228}\) and glucose oxidation\(^{121-123, 176, 177, 229}\) are preserved in non-diabetic cirrhotic patients.

Changes to the phospholipid membrane surrounding cells may also contribute to the reduction in insulin sensitivity. All human cells have a lipid bilayer of constant fluidity. Membrane fluidity allows the movement of molecules (proteins and lipids) across the cell membrane\(^{230, 231}\) and a reduction in membrane fluidity may impair the activity of the insulin receptor\(^{232}\) and the glucose transporter\(^{233, 234}\) in patients with liver cirrhosis. Membrane fluidity in these patients was reduced due to an increase in the cholesterol and phosphatidylcholine concentration of the cell membrane\(^{235, 236}\). The increase was associated with a higher phosphatidylcholine/sphingomyelin molar ratio, a higher cholesterol/phospholipid molar ratio but a lower phosphatidylethanolamine/sphingomyelin ratio.

Membrane fluidity improved (but did not normalise) after the cell membrane lipid composition was modified by intravenous administration of phosphatidylcholine over 3 days. The cell membrane of erythrocytes showed an increase in phospholipid content leading to a decrease in the cholesterol/phospholipid molar ratio\(^{237}\). The change in phospholipid content was associated with a progressive improvement of the insulin receptor activity. It is important to note that while erythrocyte insulin receptor binding and internalisation is comparable to liver, muscle and adipose cells\(^{238-240}\), it has been assumed that abnormalities in membrane composition and receptor processing in erythrocytes translate to these other cells, and that treatment with phosphatidylcholine improves the function of the insulin receptor. Further studies are required to confirm this.

### 2.6.4. Patients with liver cirrhosis have a defect of the insulin signalling pathway

The signalling pathways downstream to the insulin receptor have also been tested using a rodent model of liver cirrhosis\(^{241}\). Figure 2-1 is a schema of the insulin signalling pathway that provides a background for the discussion to follow. Insulin receptor substrate-1 (IRS-1) activity was normal following activation of the insulin receptor but the activity of phosphatidylinositol 3-kinase (PI3-kinase) was increased by 85% compared to rodent controls. Similarly, phosphorylation of the downstream Akt/Protein kinase B (Akt/PKB) was increased by 85% and this was associated with a 22% increase of Akt/PKB protein expression after correcting for the increase in phosphorylation (compared to rodent controls). These findings suggest that the defect in the insulin signalling pathway is downstream to Akt/PKB.
Further evidence to support this hypothesis comes from a small study of compensated cirrhotic patients who underwent a hyperinsulinaemic euglycaemic clamp with measurement of muscle glycogen synthase (UDP-glucose-glycogen glucosyltransferase) activity at baseline and during the steady-state of the clamp. As expected, basal glycogen synthase activity was preserved but the activity of glycogen synthase during physiological hyperinsulinaemia did not increase as much as for healthy volunteers. Reduced activation of glycogen synthase was confirmed in cirrhotic patients when muscle glycogen stores did not increase to the same extent as for the healthy volunteers by the end of the clamp. The change in muscle glycogen stores was also associated with the severity of insulin resistance. However when the muscle biopsy weight was extrapolated to include the whole skeletal muscle mass, muscle glycogen synthesis and storage would only account for 62% of glucose disposal during the hyperinsulinaemic euglycaemic clamp in the cirrhotic patients. This suggests that the reduction in glycogen synthesis cannot completely account for the reduction in insulin sensitivity.

The signalling pathway for translocation of glucose transporter 4 (GLUT4) to the surface of insulin-sensitive cells is also promoted by insulin. GLUT4 is the predominant insulin-responsive glucose transporter and is expressed in skeletal muscle, adipose tissue and cardiac muscle. GLUT4 facilitates the entry of glucose into the cell and is the rate-limiting step for glycogen synthesis. Loss of GLUT4 reduces insulin sensitivity and is associated with type 2 diabetes. The expression of GLUT4 mRNA in cirrhotic patients is reduced by 56% compared to healthy volunteers but total GLUT4 protein content in skeletal muscle is not reduced. GLUT4 mRNA levels were inversely related to fasting plasma insulin and hyperinsulinaemia may have a role in down-regulation of GLUT4 gene expression as seen in other models of diabetes. However, the role of GLUT4 in the reduction of insulin sensitivity in cirrhotic patients has not been clarified. A recent study did not show a reduction in GLUT4 protein or mRNA expression in cirrhotic patients. Total GLUT4 protein expression was also normal in a rodent model of liver cirrhosis induced by common bile duct ligation.
Insulin (Ins) binds to its cell surface receptor (IR) activating its tyrosine kinase activity leading to phosphorylation of multiple substrates. Activation of the Ras (a guanosine triphosphatase) pathway via the growth factor receptor-binding protein (Grb2) and son of sevenless (Sos) signalling proteins mediates the effect of Ins on gene expression and cell proliferation, whereas the activation of the phosphatidylinositol 3-kinase (PI3K) and phosphoinositide-dependent protein kinase (PDK) signalling pathway results in activation of multiple other effector molecules, leading to its effects on protein, lipid and glycogen synthesis, and the inhibition of lipolysis.
Figure 2-2 Rate-limiting steps of insulin induced glycogen synthesis in a muscle cell. Glucose\textsubscript{ex} and glucose\textsubscript{in} denote the extracellular and intracellular glucose concentrations; $V_{GT}$ and $V_{\text{GT}}^{-}$ the velocity of glucose transport into and out the muscle cell; $V_{HK}$ the velocity of glucose phosphorylation by hexokinase; G6P glucose-6-phosphate; $V_{\text{glycolysis}}$ the net velocity of the glycolytic flux of G6P.

2.6.5. Hyperinsulinaemia in liver cirrhosis may play a role in the reduction of insulin sensitivity

Lastly, elevated insulin levels may also reduce the action of insulin directly. Hyperinsulinaemia induced by an infusion of insulin for 40 hours resulted in a reduction of insulin sensitivity in healthy volunteers\textsuperscript{250}. The reduction of insulin sensitivity was in the skeletal muscle because HGP was suppressed normally (although the level of hyperinsulinaemia may have been too high to detect a small reduction in hepatic insulin sensitivity). Insulin binding to monocytes and erythrocytes were not affected suggesting a post-receptor defect. Insulin infused over a longer period (72 – 96 hours) also reduced insulin sensitivity in cirrhotic patients\textsuperscript{251}.

Persistent elevation of insulin levels is prevalent in liver cirrhosis\textsuperscript{158, 252, 253} and may contribute to the reduction of insulin sensitivity. Reversal of the persistent hyperinsulinaemia for 96 hours using octreotide normalised insulin sensitivity in cirrhotic patients\textsuperscript{121}. The finding persisted after other potential confounding factors like the change in plasma free fatty acid, growth hormone and glucagon.
levels in response to octreotide were taken into account. In another study, hyperinsulinaemia was suppressed for 1 week but octreotide was stopped 20 hours prior to undergoing a hyperglycaemic clamp. It is possible that the return of hyperinsulinaemia prior to testing may have reduced insulin sensitivity to pre-treatment levels.

Fasting hyperinsulinaemia in patients with liver cirrhosis partly arises from decreased hepatic degradation secondary to porto-systemic shunting. Intra-hepatic shunts are present around hepatic nodules and play a significant role in diverting portal blood flow from functional hepatocytes. This reduces the ability of the liver to metabolise insulin. Varices which are present in around 30% - 60% of cirrhotic patients and the use of surgical or radiological shunts also result in further shunting of splanchnic blood away from hepatocytes. Increased secretion of insulin by the pancreatic β-cell probably contributes to fasting hyperinsulinaemia because fasting C-peptide (which is secreted equimolar with insulin) is also increased in patients with liver cirrhosis. Serum C-peptide is an appropriate but crude measure of insulin secretion during steady-state conditions like fasting. More sophisticated modelling of β-cell function using deconvolution of C-peptide secretion also suggest that there is an increased secretion of insulin while the clearance of insulin by Childs B cirrhotic patients was also increased by 10%. Homeostasis model assessment of β-cell function in fasted cirrhotic patients also confirms an increase in insulin secretion.

In summary, the exact mechanism for the reduction of insulin sensitivity in patients with liver cirrhosis has not been clarified. Several studies have shown that there is reduced insulin binding to the insulin receptor and that the activity of the receptor may be impaired by the increase in cholesterol/phospholipid molar ratio of the cell membrane. There may also be reduced expression of GLUT4 mRNA that may impair the movement of glucose into the cell, and following entry into the cell, the activity of glycogen synthase to form glycogen may also be impaired. Underlying all these changes may be hyperinsulinaemia that is prevalent in patients with liver cirrhosis. The reduction in insulin sensitivity is likely to be multi-factorial and the above findings need to be confirmed, especially in vivo and in the actual target cells of insulin for glucose metabolism.

A severe reduction in insulin sensitivity is not enough to induce diabetes in patients with liver cirrhosis. Computer modelling suggests that even an 80% reduction in insulin sensitivity is not sufficient for the onset of diabetes. Diabetes requires an accompanying failure of the pancreatic β-cell which is then unable to compensate for severely reduced insulin sensitivity. This has been observed in patients with liver cirrhosis and diabetes who are unable to mount an insulin secretory response to glucose to the same magnitude as patients with IGT.
2.6.6. Cirrhotic patients with diabetes have further derangements of glucose metabolism

Glucose metabolism is further deranged following the onset of diabetes in patients with liver cirrhosis. Glucose effectiveness is reduced in these patients and this reduction has been termed glucose resistance by Petrides et al\textsuperscript{23}. Glucose effectiveness can be derived from a minimal model following a frequently sampled IVGTT\textsuperscript{275} or by extrapolation of data from the hyperglycaemic clamp\textsuperscript{276}. It refers to the ability of glucose to promote its own uptake due to the law of mass action and is independent of insulin stimulation. The reduction in glucose effectiveness was only present following the onset of diabetes when measured using the hyperglycaemic clamp. Measurement of glucose effectiveness using the minimal model showed that the reduction in glucose effectiveness was already present in cirrhotic patients prior to the onset of diabetes\textsuperscript{277, 278}. It is possible that the reduction glucose effectiveness is related to the known loss of muscle mass in cirrhosis\textsuperscript{8}. In one of the above studies, the urinary creatinine to height ratio (an indirect marker of muscle mass) of cirrhotic patients was associated with glucose effectiveness\textsuperscript{278}.\markparagraph
2.7. β-cell function in liver cirrhosis and methods for measurement

A reduction in β-cell function is necessary for diabetes to manifest in patients with liver cirrhosis and other conditions associated with reduced insulin sensitivity. The assessment of insulin sensitivity is well-established and several well-validated tools for the measurement of insulin sensitivity have been developed (see Section 2.5). The assessment of β-cell function however, lacks an accepted reference method and the complexity of measurement has hampered investigation of β-cell function in patients with liver cirrhosis.

β-cell function is difficult to assess because of the complexity of the β-cell response to various stimuli (including glucose and amino acids) and the time-dependent nature of the response. For example, the β-cell response following an IVGTT is characterised by a first- and second-phase insulin response while the response to an OGTT is influenced by the incretin effect. Most indices for measurement of β-cell function essentially measure certain aspects of the β-cell process, and there is as yet no index for the measurement of “overall” β-cell function. Therefore any discussion of β-cell function requires some familiarity with each index and its appropriate application.

The assessment of β-cell function is based on measurement of either plasma insulin or C-peptide concentration (which is secreted in equimolar amounts with insulin). The use of C-peptide measurements requires modelling (deconvolution) in order to determine pre-hepatic insulin secretion (see Section 2.7.3). Measurement of C-peptide is advantageous because it is not metabolised by the liver following secretion into the portal circulation unlike insulin. Insulin assays have also not been standardised making comparisons between studies difficult (see Section 2.5). Lastly, patients with liver cirrhosis have unpredictable extraction of insulin due to hepatocellular failure and fibrosis which again makes the use of C-peptide measurements preferable.

2.7.1. Hyperglycaemic clamp

The hyperglycaemic clamp is likely to be the closest to a reference standard available for the measurement of β-cell function. The hyperglycaemic clamp differs from the hyperinsulinaemic euglycaemic clamp because a bolus of glucose is given intravenously (instead of insulin) to effect a pre-determined level of hyperglycaemia followed by a variable infusion of glucose titrated to maintain that level of hyperglycaemia. Samples of glucose, insulin and C-peptide are taken throughout the clamp (rather than just at baseline and steady-state) in order to show the biphasic response of insulin.
(or true insulin secretion using C-peptide deconvolution – see Section 2.7.3) to intravenous glucose. The biphasic response to insulin includes the first-phase and second-phase insulin secretion, and both are indices of β-cell function. The complexity and cost of the hyperglycaemic clamp limits its widespread use. There remains methodological uncertainty about the exact level and duration of hyperglycaemia required for the hyperglycaemic clamp, and whether or not to include subjects with markedly different baseline blood glucose levels.

Figure 2-3 Plasma insulin response to an intravenous glucose infusion producing a square wave of hyperglycaemia. The response to the intravenous glucose challenge shows a biphasic profile, with distinct first (0-10min) and second (10-30min) phase insulin secretory responses.

Normal glucose tolerant cirrhotic patients respond to the hyperglycaemic clamp with a biphasic insulin response similar to the response seen in healthy controls (Figure 2-3). The magnitude of response in the cirrhotic patients however was several times higher than for the healthy controls. Diabetic cirrhotic patients have a reduced first-phase insulin response to glucose and do not mount a second-phase insulin response appropriate for the level of hyperglycaemia or may lose the first-phase response completely. A further study that measured β-cell function of cirrhotic patients with the hyperglycaemic clamp did not fully characterise the glucose tolerance of the study cohort (only reporting that patients with overt diabetes were excluded). In that cohort of 6 patients with liver cirrhosis awaiting liver transplantation, both the first-phase and second-phase insulin secretion in response to a square wave of hyperglycaemia were increased compared to healthy controls. This finding suggests that β-cell function may be increased prior to the onset of diabetes.
2.7.2. Intravenous glucose tolerance test

The IVGTT is a more common test of β-cell function owing to the simple protocol that is readily applied in larger studies. A pre-specified dose of intravenous glucose (usually 300mg/kg) is given within 30 seconds and blood is collected at various intervals for measurement of plasma glucose, insulin and often C-peptide. The glucose peak following an IVGTT may differ between individuals of different glucose tolerance whereas the advantage of the hyperglycaemic clamp is that all subjects are exposed to the same glucose increment. The second-phase insulin secretion is also best determined from the hyperglycaemic clamp because of the prolonged elevation of glucose concentration.

The first-phase insulin secretion (or acute insulin response (AIR)) can be defined in several ways. Commonly, it is defined as the incremental trapezoidal area under the insulin concentration curve over the first 10 min following an intravenous glucose bolus. The acute insulin response is important because it suppresses endogenous glucose production and also primes insulin sensitive tissues. Insulin requires time to equilibrate with its extravascular site of action and the AIR rapidly increases the concentration of insulin in the tissues to a new steady-state within 10 min. Loss of the AIR delays the increment of insulin and prolongs the time required for glucose disposal. The AIR is blunted or lost in patients with IGT and type 2 diabetes mellitus, and may predict patients with IGT who will eventually develop diabetes. However, the precision of the AIR may be poor with a reported within subject variation of 22% and between subject variation of 58% in one study. Also, the AIR is dependent on hepatic insulin extraction unless C-peptide deconvolution (see Section 2.7.3) is used to calculate the true insulin secretion. Lastly, the AIR does not correlate well with OGTT-derived indices of β-cell function in patients with diabetes and should be used with caution in that patient population.

Normal glucose tolerant patients with liver cirrhosis respond to intravenous glucose with a normal first-phase insulin response although other studies have shown an increased first-phase insulin response compared to healthy controls. Insulin secretion can also be calculated by deconvolution of C-peptide levels (see below). The first-phase insulin secretion rate in response to intravenous glucose estimated by C-peptide deconvolution was higher in normal glucose tolerant cirrhotic patients compared to healthy controls. Cirrhotic patients with IGT may have an exaggerated response to glucose but with progression to diabetes, the first-phase insulin response is reduced and may be lost. The second-phase insulin response appears to be preserved but is not adequate to maintain glucose tolerance. These changes in insulin response parallel the changes seen in patients progressing to type 2 diabetes mellitus from IGT.
2.7.3. Oral glucose tolerance test

The OGTT is gaining interest as a simple test for the measurement of \( \beta \)-cell function which conveniently allows concurrent measurement of insulin sensitivity. Oral administration of glucose is more physiological than by the intravenous route and provides a direct test of \( \beta \)-cell function. The OGTT is also simple enough to be used in large epidemiological studies. However, the secretion of insulin after ingesting oral glucose is an integrated response of several complex factors. For example, the rate of glucose absorption is not predictable and oral glucose ingestion also stimulates other drivers of insulin secretion like the incretin hormones. Separating the impact of these individual factors on \( \beta \)-cell function can be difficult.

The complexity of the underlying mechanism of \( \beta \)-cell function requires mathematical modelling in order to account for the changing nature of the stimulus (glucose concentration over time) and the various drivers for insulin secretion. The historical studies that underpin our current understanding of the mathematical modelling of \( \beta \)-cell function have been described in detail by several narrative reviews.

Mathematical modelling for estimation of \( \beta \)-cell function generally incorporates a model of insulin secretion and a \( \beta \)-cell model. The reference methods for estimation of absolute insulin secretion are usually based on C-peptide deconvolution data due to the factors previously discussed in the introduction to Section 2.7. C-peptide deconvolution is the use of advanced mathematical algorithms to construct a mathematical model in order to estimate the true insulin secretion. A commonly used mathematical model is the model proposed by Van Cauter et al. This model estimates insulin secretion rates using a two compartment mathematical model to account for C-peptide distribution and degradation kinetics. C-peptide degradation kinetics is estimated from individually derived measurements of 200 participants with varying degrees of insulin sensitivity and glucose tolerance but preserved renal function. Therefore the model should be applied with caution in patients with renal impairment due to the differences in C-peptide kinetics.

A \( \beta \)-cell model describes the relationship between insulin secretion and glucose concentration. All models include a dose-response function (the static component) that describes the relationship between insulin secretion and glucose concentration. More complex models couple the static component with a dynamic component that accounts for the increased stimulation of insulin secretion in response to rapid increases in glucose concentration. A further parameter is required to explain the
persistent elevation of insulin secretion following a glucose load of up to several hours. Some models describe the persistent elevation by applying a first-order delay to the glucose concentration (originally proposed by Licko et al\textsuperscript{303}). A first-order delay would predict a symmetrical onset and offset of second-phase insulin secretion in response to a square wave of glucose concentration but in practice, a slow onset and rapid offset is found in the perfused pancreas model\textsuperscript{304}.

Mari et al introduced a parameter called the potentiation factor as an alternative explanation for the persistent elevation of insulin secretion in their model for β-cell function\textsuperscript{305}. The potentiation factor is hypothesised to represent the incretin effect\textsuperscript{306}, the Staub-Traugott effect\textsuperscript{307}, non-glucose secretagogues and other characteristics of insulin secretion like circadian rhythms or pulsability that are not otherwise explicitly represented in the model. The potentiation factor is reduced and delayed in diabetic patients and it may explain the blunted and delayed potentiation previously reported in patients with Type 2 diabetes mellitus and gestational diabetes\textsuperscript{308-310}. However, the potentiation factor was compared to the incretin effect in a study using an OGTT followed by an IVGTT that matched the glucose concentration produced by the OGTT\textsuperscript{311}. The potentiation factor was not associated with the incretin effect in that study casting doubt on the validity of the potentiation factor from a physiological standpoint.

The β-cell function of patients with cirrhosis secondary to HCV infection prior to the onset of diabetes has been estimated using the Mari model in physiological, free living conditions\textsuperscript{270}. Nine patients with moderate severity of liver disease (Child’s B) were studied over 24 hours within a calorimetric chamber and were found to have an increase in the basal and total insulin secretion rate. The sensitivity of the β-cell response to the rate of increase in glucose concentration following a mixed meal (the dynamic component of the model) was also increased in this insulin-resistant cohort. However, the increase in β-cell response to the magnitude of increase in glucose concentration \textit{per se} (the static component of the model) was not statistically significant despite a 2.6 fold increase. No other reported studies to date have employed the Mari model in cirrhotic patients. A more detailed discussion of the Mari model for assessment of β-cell function can be found in Section 5.2.6.
2.8. The clinical significance of deranged glucose metabolism in liver cirrhosis

2.8.1. Survival may be reduced

The impact of diabetes on the outcome of patients with liver cirrhosis is not conclusive. Survival in cirrhotic patients with diabetes is probably reduced although there are several studies that did not show a reduction in mortality. Interestingly, poorer survival relates to the complications of cirrhosis and not diabetes in these patients. The severity of both hepatic encephalopathy and refractory ascites appear to be worse with the presence of diabetes. Complications of diabetes generally require many years to manifest and the shorter duration of diabetic status in conjunction with the reduced survival of patients with liver cirrhosis probably preclude such complications from developing.

The prognostic significance of a reduction of insulin sensitivity in patients with liver cirrhosis is even less conclusive. The first study to test for the association of reduced insulin sensitivity (measured by HOMA-IS) and survival did not show an association using a Cox regression model. However, a subsequent study reported in 2010 with a much larger sample size (n=248) showed an increased risk for death, liver transplantation and the occurrence of hepatocellular carcinoma. The implication of an isolated reduction in $\beta$-cell function on the outcome of patients with liver cirrhosis has not been investigated.

2.8.2. The risk of hepatocellular carcinoma is increased

In addition to the increased likelihood of death, several longitudinal studies have confirmed that diabetes is an independent risk factor for the development of hepatocellular carcinoma in patients with liver cirrhosis. Twice as many HCV cirrhotic patients with diabetes developed hepatocellular carcinoma compared to non-diabetic patients over 4 years of follow-up. In that study, diabetes was a stronger risk factor for the development of hepatocellular carcinoma than male sex and age. The exact mechanism by which diabetes induces hepatocellular carcinogenesis is currently unclear. Reduced insulin sensitivity resulting in compensatory hyperinsulinaemia is implicated because insulin promotes the proliferation of human cancer cells. The secretion of insulin-like growth factor 1 (a promoter of carcinogenesis) can also be induced by hyperinsulinaemia. Using the biguanide, metformin, was associated with a reduction of the incidence of hepatocellular carcinoma and
improvement of transplant-free survival in patients with HCV cirrhosis. Metformin activates AMP-activated protein kinase which is an essential mediator of the tumour suppressor gene LKB1. Activation of AMP-activated protein kinase also re-programmes cellular metabolism and enforces metabolic checkpoints. The use of sulphonylureas or exogenous insulin for the treatment of diabetes increased the risk of hepatocellular carcinoma in patients with type 2 diabetes mellitus without cirrhosis of the liver.

2.8.3. The outcome following surgery and liver transplantation is worse

Co-morbid diabetes predicts the outcome of surgery and liver transplantation in patients with liver cirrhosis. Patients with concurrent liver cirrhosis and diabetes had a higher rate of recurrence and a reduction in recurrence-free survival following curative resection for hepatocellular carcinoma. A reduction in cancer-specific survival, liver disease-specific survival and overall survival following curative resection or local ablation of hepatocellular carcinoma was reported by another study although the study cohort included non-cirrhotic patients with chronic liver disease in their cohort. When surgical treatment was compared with non-surgical treatment in a further cohort of cirrhotic and non-cirrhotic patients, a difference in survival following surgical treatment (but not non-surgical treatment) for hepatocellular carcinoma was evident. The risk for infectious complications and death following orthotopic liver transplantation was higher in cirrhotic patients with diabetes. A single contradictory study did not show a difference in survival between patients with and without diabetes following liver resection for hepatocellular carcinoma.

In conclusion, diabetes (and to a lesser extent reduced insulin sensitivity and β-cell function) is associated with a poorer outcome in patients with liver cirrhosis largely from decompensation of their liver disease. Patients with concurrent liver cirrhosis and diabetes are also more likely to develop hepatocellular carcinoma and have a lower cancer-specific survival following treatment. The risk of infectious complications and death following transplantation are also higher in patients with diabetes compared to those without diabetes.
2.9. The effect of β-blockade on glucose tolerance

β-blockers were first developed by Sir James Black in 1962. First generation β-blockers have equal affinity for β₁ and β₂ receptors and are also known as non-selective β-blockers. Examples of first generation non-selective β-blockers include propranolol and nadolol. Cardio-selective β-blockers have a greater affinity for β₁ receptors although the selectivity is lost at higher drug doses. Such second generation β-blockers include atenolol and metoprolol, while third generation β-blockers combine non-selective β-blockade with α₁ receptor blockade (for example carvedilol and nebivolol).

Non-selective β-blockers are commonly used in patients with liver cirrhosis and have proven efficacy for the primary and secondary prevention of variceal haemorrhage. Nadolol has a longer half-life than propranolol and is administered once daily, and is the current standard of care for cirrhotic patients requiring prophylaxis against variceal haemorrhage. However, β-blockade reduces insulin sensitivity and has been demonstrated to increase the rate of new-onset diabetes in certain conditions predisposed to reduced insulin sensitivity and glucose tolerance such as hypertension, type 2 diabetes mellitus, heart failure and obesity.

2.9.1. β-blockade reduces peripheral but not hepatic insulin sensitivity

Most of the studies investigating the effect of β-blockade on insulin sensitivity have been in cohorts of hypertensive patients. Estimates vary between studies but the reduction of insulin sensitivity measured by the hyperinsulinaemic euglycaemic clamp is between 15 – 35% . Other studies reporting different measures of insulin sensitivity show a similar reduction in patients taking certain β-blockers.

Peripheral insulin sensitivity is reduced by a primed intravenous infusion of β-blockers. In this study of 12 healthy volunteers, propranolol was infused for an hour prior to a hyperinsulinaemic euglycaemic clamp. A significant reduction of insulin sensitivity was reported in contrast to the administration of an α-blocker that did not affect insulin sensitivity in the same group of patients. On the other hand, hepatic insulin sensitivity is probably not affected by non-selective β-blockade. Hyperinsulinaemic euglycaemic clamps with glucose tracers were performed in a study of healthy volunteers and the addition of propranolol during steady-state hyperinsulinaemia did not affect HGP.
2.9.2. Different β-blockers affect insulin sensitivity differently

Different β-blockers may have a differential effect on insulin sensitivity, even within the same generation of β-blockers. In a well-designed randomised, double-blind, double-placebo-controlled, cross-over trial of hypertensive patients, treatment with propranolol resulted in a larger reduction of insulin sensitivity compared to pindolol\textsuperscript{23}. It was postulated that the β\textsubscript{2}-agonist properties of pindolol may promote vasodilation, which is necessary for insulin-mediated uptake of glucose (see below).

β-blockade with a cardio-selective β-blocker and a third generation β-blocker has also been compared. Treatment with metoprolol resulted in a reduction of insulin sensitivity in non-diabetic hypertensive patients\textsuperscript{338}. Treatment with carvedilol (which has α\textsubscript{1}-receptor blocking properties combined with non-selective β-receptor blockade) showed an improvement of insulin sensitivity by 13% although the improvement was not statistically significant. Several subsequent studies have shown an improvement of insulin sensitivity following treatment with carvedilol\textsuperscript{339}, including the large GEMINI trial comparing treatment with carvedilol and treatment with metoprolol\textsuperscript{335}.

2.9.3. Mechanisms for the reduction of insulin sensitivity following β-blockade

The reduction of insulin sensitivity following treatment with metoprolol (but not carvedilol) suggests that α\textsubscript{1}-receptor blockade may have a role in determining insulin sensitivity. α\textsubscript{1}-receptor blockade mediates peripheral vasodilation\textsuperscript{340}. Insulin plays a similar role under physiological conditions by capillary recruitment. The vasodilation that follows promotes blood flow to skeletal muscle\textsuperscript{341}. The increase in blood flow is strongly correlated with insulin-mediated glucose disposal by skeletal muscle\textsuperscript{342}. Treatment with non-selective and cardio-selective β-blockers increases total peripheral resistance and this increase may drive the reduction of insulin sensitivity associated with their use\textsuperscript{343}.

β-blockade is also associated with weight gain. Studies that measured weight gain following β-blockade were summarised and the average weight gain has been estimated to be around 1.2kg\textsuperscript{344}. The weight gain results in part from a decrease in total energy expenditure due to lethargy and a decrease in exercise tolerance. Furthermore, weight loss in obese patients has been shown to improve both total peripheral resistance and insulin sensitivity\textsuperscript{345}. Despite this, weight gain following β-blockade is unlikely to be the primary cause for the reduction of insulin sensitivity because patients
who do not gain weight still show a reduction of insulin sensitivity measured by the hyperinsulinaemic euglycaemic clamp\textsuperscript{346}.

\section*{2.9.4. Effect of $\beta$-blockade on insulin sensitivity in liver cirrhosis}

To date, whether or not patients with liver cirrhosis taking $\beta$-blockers show a reduction of insulin sensitivity has not been examined. Liver cirrhosis shares similar derangements of glucose metabolism with hypertension. Insulin sensitivity is reduced and the reduction of insulin sensitivity is primarily in the skeletal muscle\textsuperscript{347}. It remains to be seen if the observed reduction of insulin sensitivity following $\beta$-blockade observed in hypertensive patients may be generalised to patients with liver cirrhosis.

\section*{2.9.5. $\beta$-blockade may reduce $\beta$-cell function in hypertensive patients}

The effect of $\beta$-blockade on insulin secretion and $\beta$-cell function has not been well described. No studies have evaluated $\beta$-cell function using the mathematical models previously described (Section 2.7). The studies to date have recruited mostly patients with hypertension and the findings from these studies may not be generalised to other patient populations.

Only a single series of papers by Lithell and Pollare have reported a reduction of $\beta$-cell function following $\beta$-blockade\textsuperscript{21, 23, 34}. IVGTTs were performed in patients treated with several different $\beta$-blockers and the first-phase insulin secretion (or AIR) was compared with the AIR of patients treated with placebo and other anti-hypertensive medications. There was a relative reduction in AIR following treatment with $\beta$-blockers. In these patients, the secretion of insulin was slightly higher while on $\beta$-blockers but the increase in AIR was not enough and patients still experienced a reduction of insulin sensitivity measured by the hyperinsulinaemic euglycaemic clamp. These findings were supported by subsequent data showing a 40\% reduction of AIR in healthy volunteers taking $\beta$-blockers\textsuperscript{348}. 

\textit{In vitro} studies have observed a similar reduction of $\beta$-cell function and insulin secretion following $\beta$-blockade. Insulin secretion is enhanced when $\beta_2$-adrenergic receptors on isolated pancreatic $\beta$-cells are stimulated. Conversely insulin secretion is reduced when $\beta_2$-receptor blockers are administered \textsuperscript{349-351}. \textit{In vivo} insulin secretion was similarly stimulated by isoproterenol (non-selective $\beta$-adrenergic agonist) and the effect negated by the non-selective $\beta$-adrenergic antagonist propranolol\textsuperscript{352-354}. 

\hrule
There are no published data on the effect of $\beta$-blockade on the $\beta$-cell function of patients with liver cirrhosis.

2.9.6. $\beta$-blockade increases the incidence of new-onset diabetes mellitus

The incidence of new-onset diabetes mellitus is a common end point in studies investigating the effect on the glucose tolerance of patients taking $\beta$-blockade. Diabetes mellitus is defined by the level of hyperglycaemia associated with an increased risk of microvascular complications (retinopathy, nephropathy and neuropathy)\textsuperscript{124, 355} and is a more relevant measure of outcome for clinicians compared to changes in indices of insulin sensitivity (or $\beta$-cell function). Furthermore, the measurement of insulin sensitivity or $\beta$-cell function and the standardisation of normal thresholds for either of these measurements have not filtered into routine clinical practice.

The strongest evidence to date for the increase in the incidence of new-onset diabetes following $\beta$-blockade derives from a network meta-analysis by Elliott et al\textsuperscript{20}. Network meta-analysis is a relatively new statistical technique for meta-analysis that allows direct and indirect comparisons even when two of the strategies have not been directly compared previously\textsuperscript{356}. The systematic review and subsequent analysis requires carefully developed and rigorous methodology\textsuperscript{357} but may overcome the significant heterogeneity that arises in traditional meta-analyses of several treatment classes compared to all other treatments. Essentially, network meta-analysis can attribute risk of diabetes across all classes of antihypertensive agents rather than be restricted to comparing one class of agents against all others. However the findings of these meta-analyses should be interpreted with caution because of the relative infancy of the technique and the lack of methodological research into the validity of the findings.

Nevertheless, the network meta-analysis suggests that $\beta$-blockade increases the incidence of new-onset diabetes mellitus in patients with reduced insulin sensitivity. Twenty two trials were included with a total of 143 153 participants. Most trials enrolled patients with hypertension, three enrolled patients with a high risk for diabetes and one enrolled patients with heart failure. Patients treated with $\beta$-blockers had a higher incidence of new-onset diabetes than patients treated with placebo. The incidence for patients treated with $\beta$-blockers and diuretics were similar while patients treated with other anti-hypertensive agents did not have an increased incidence of new-onset diabetes compared to placebo.
The effect of non-selective β-blockade on new-onset diabetes is more relevant to the present thesis because non-selective β-blockers are most commonly used in patients with liver cirrhosis. A Scandinavian study followed patients for 15 years and found that the relative risk for developing diabetes was significantly higher in patients treated with propranolol than those treated with thiazide diuretics\textsuperscript{358}. In contrast, a recent meta-analysis by Bangalore et al in 2007 found that treatment with propranolol did not increase the incidence of new-onset diabetes when compared to placebo although data were derived from a single study only\textsuperscript{359}. Three other studies in the meta-analysis compared treatment with propranolol and treatment with diuretics\textsuperscript{360-362}. Propranolol was found not to have an increased incidence of new-onset diabetes but this finding should be interpreted with caution because treatment with diuretics also increases the incidence of new-onset diabetes\textsuperscript{363}.

The findings of the meta-analysis by Bangalore et al were surprising considering the known reduction of insulin sensitivity following β-blockade discussed previously in Section 2.9.1. Significantly, the meta-analysis highlighted that many of these studies may lack validity because findings were primarily derived from post hoc analysis of data collected for other pre-defined endpoints\textsuperscript{359}. Therefore screening for diabetes at entry in many of these trials may not have been uniformly rigorous and the data may have been subject to unreported bias.

Further insight may be derived from older studies that measured changes in blood glucose concentration. These studies suggest that cardio-selective β-blockade may not worsen glucose tolerance to the same extent as non-selective β-blockade. Hypertensive patients with diabetes treated with propranolol had an increased fasting blood glucose and mean blood glucose concentration following an IVGTT\textsuperscript{364}. In contrast, treatment with metoprolol did not worsen either of these parameters. Fasting blood glucose was lower in another small cohort of 16 diabetic hypertensive patients when they were switched from a non-selective β-blocker to metoprolol although the reduction in fasting blood glucose was only significant after treatment for 1 month but not 6 months. Hypertensive patients without diabetes treated for 3 months with metoprolol also did not show any changes to blood glucose concentration (compared to placebo) when fasting and following an IVGTT and an OGTT\textsuperscript{365}. In contrast, a randomised controlled cross-over trial of hypertensive patients with diabetes reported that the blood glucose concentration of patients taking propranolol and metoprolol both increased to a similar degree\textsuperscript{366}.

Not all studies have shown a similar reduction in glucose tolerance in patients treated with β-blockade. A cohort of hypertensive patients underwent a 100g OGTT after being treated for 6 months with either atenolol or propranolol\textsuperscript{367}. Plasma glucose concentration at baseline and at the end of the
OGTT did not change with β-blockade although patients treated with propranolol had a lower peak plasma glucose concentration. However, the findings of the study should be interpreted with caution because over a third of the patients were excluded from analysis. Similarly, hypertensive patients treated with the non-selective β-blocker alprenolol for 2 years did not have a higher incidence of new-onset diabetes.

2.9.7. Patients with hypertension and diabetes mellitus have a poorer outcome

Diabetes mellitus is associated with a poorer outcome in patients with hypertension. It is intuitive to extrapolate this to patients with diabetes induced by β-blockade but the prognostic implication of new-onset diabetes following β-blockade remains contentious. Indirect evidence suggests that a blood glucose level greater than 7.7 mmol/L is associated with an increased risk for cardiovascular events in patients treated for hypertension over 9 years. Similarly, a rise in blood glucose was shown to be an independent risk factor for myocardial infarction in men treated for hypertension using either β-blockers alone or in combination with thiazide diuretics in a study where participants were followed for 27 years. In a separate analysis of the same data, increased plasma proinsulin (a predictor for the development of type 2 diabetes) predicted a higher mortality with separation of the Kaplan-Meier survival curves after 7 years of follow-up. Lastly, the “Multiple Risk Factor Intervention Trial” followed 11600 patients over 18 years and showed that hypertensive patients who developed diabetes had an increased risk for total mortality, cardiovascular mortality and coronary heart disease mortality.

However retrospective analysis of the “Systolic Hypertension in the Elderly Program” trial suggested that patients who developed diabetes following treatment with thiazide diuretics did not share an increased risk for cardiovascular events as opposed to patients who had diabetes at study entry. The analysis included extended follow-up of the patients for another 10 years. However, the retrospective nature of the follow-up study meant patients who developed diabetes after the primary study (follow-up of 4 years) were included in the analysis as ‘non-diabetic’ and may have biased the findings. Furthermore the blood pressure of patients at the end of the follow-up study and details of the management of diabetes in both groups of patients were not reported. Lastly, in another study, patients treated with atenolol instead of captopril for 9 years did not have a higher incidence of cardiovascular events.

There is a lack of data on the implications of β-blocker use on the glucose tolerance of cirrhotic patients. The potentially adverse consequence on glucose tolerance is pertinent with the advent of combined non-selective β-receptor and α1-receptor blockers like carvedilol that improve the glycaemic
profile of hypertensive patients. Long-term treatment with carvedilol may also improve survival in patients with moderate to severe congestive heart failure compared to metoprolol. The impact of carvedilol (and other β-blockers that result in vasodilation) on insulin sensitivity, glucose tolerance or mortality in patients with liver cirrhosis is not known.

2.10. Summary

Chapter 2 summarises the relevant published literature and underlying methodology that underpin the work to follow in this thesis. In summary, patients with liver cirrhosis develop complex metabolic changes that can be difficult to measure, and these changes worsen with increasing severity of liver disease.

Malnutrition and hypermetabolism are associated with liver cirrhosis but the assessment of these conditions is complicated by the physiological sequelae of liver cirrhosis. Sophisticated body composition techniques are required in these patients in order to reduce the variation due to different degrees of over-hydration common in liver cirrhosis. Studies utilising such methodology suggest that protein depletion and loss of total body fat are common in patients with liver cirrhosis. The prevalence of hypermetabolism in patients with liver cirrhosis is also significantly higher than previously thought when predicted REE is estimated using more precise measurement of FFM. However, malnutrition and hypermetabolism have not been shown to be associated in patients with liver cirrhosis although both malnutrition and hypermetabolism independently predict a poorer outcome in these patients. Surprisingly, increments of REE over the predicted REE that are within the normal range (2 standard deviations) may also result in a poorer outcome.

In addition, patients with liver cirrhosis are intolerant of glucose. Glucose tolerance is determined by the interaction between insulin sensitivity and β-cell function. Peripheral insulin sensitivity is reduced in patients with liver cirrhosis but hepatic insulin sensitivity also deteriorates following the onset of diabetes. Peripheral insulin sensitivity is dependent on glycogen synthesis and in patients with liver cirrhosis; it is impaired in the skeletal muscle due to defects in the insulin signalling pathway (glycogen synthase) and possibly in the insulin-mediated transport of glucose into cells. Persistent hyperinsulinaemia may also play a role in the reduction of insulin sensitivity.

An isolated reduction in insulin sensitivity is not enough to induce glucose intolerance. A synchronous defect in β-cell function is necessary thereby limiting the ability of the pancreas to compensate for the
reduction in insulin sensitivity. However the assessment of β-cell function has not been standardised and results are not easily compared between studies. There is also no accepted gold standard measure of β-cell function. The few studies that have assessed β-cell function in patients with liver cirrhosis report an increase in insulin secretion prior to the onset of diabetes but insulin secretion is reduced following progression to diabetes.

Understanding the pathophysiology of insulin sensitivity, β-cell function and glucose tolerance in patients with liver cirrhosis is important because a reduction of insulin sensitivity or glucose tolerance is associated with a poorer outcome. However, the excess mortality in these patients results from complications of liver disease and not diabetes. In addition, these patients have a higher likelihood of developing hepatocellular carcinoma and the outcome of diabetic patients with liver cirrhosis following surgery and liver transplantation is worse.

Lastly, β-blockade may play a role in managing the metabolic derangements of patients with liver cirrhosis that has not been previously recognised. β-blockers reduce REE and improve survival in hypermetabolic patients with severe burns and there is some evidence that β-blockers may similarly reduce REE in patients with liver cirrhosis. In contrast, non-selective β-blockers (commonly used in patients with liver cirrhosis for the reduction of portal hypertension) reduce insulin sensitivity and β-cell function, and increase the incidence of new-onset diabetes in other patient cohorts that have a pre-existing impairment of insulin sensitivity (like hypertensive patients). However third generation β-blockers that combine non-selective β-blockade with α₁-receptor blocking properties may have a beneficial effect on insulin sensitivity and these medications may have a role in patients with liver cirrhosis if a detrimental effect is shown in these patients following the use of conventional non-selective β-blockers. The aim of this thesis is to attempt to address some of these hypotheses and to report preliminary data that may inform the design of more definitive studies in the future.
Chapter 3. Prevalence of diabetes in liver cirrhosis—a systematic review

3.1. Introduction

Chronic liver disease is associated with diabetes mellitus and was first described as hepatogenous diabetes in 1898 by Naunyn\(^\text{108}\). Diabetes complicates the medical management of patients with chronic liver disease and is associated with worse survival\(^2\), \(^3\), partly due to the increased risk of developing hepatocellular carcinoma\(^1\), \(^2\), \(^3\). However, the association between liver cirrhosis and diabetes is not well quantified with wide variation in the reported estimates of the prevalence of diabetes in cirrhotic patients\(^1\), \(^2\), \(^3\).

It is unclear how much of the variation in the prevalence of diabetes in cirrhosis is explained by variations in aetiology of liver disease or severity of cirrhosis. Evidence is emerging that certain aetiologies of cirrhosis particularly HCV and NAFLD\(^1\), \(^2\) may be more closely associated with diabetes than others, and may explain the variation in the reported estimates of diabetes prevalence in liver cirrhosis. Similarly the association between decompensated cirrhotic disease and the prevalence of diabetes has not been clarified\(^2\), \(^3\), \(^4\).

This chapter summarises the results of a systematic review to determine, as accurately as possible, the prevalence of diabetes in patients with liver cirrhosis according to aetiology and severity of disease. We also sought to examine the relative importance of other sources of variation on the risk of diabetes in patients with cirrhosis. We examined the study design, criteria used to classify diabetes status and other risk factors such as country of origin, family history of diabetes and BMI. In addition to providing a greater understanding of the association between cirrhosis and the risk of developing diabetes, the findings of this study may help update clinical practice guidelines and suggest gaps in current knowledge that may be important to address in future research. Furthermore, it also contextualises the significance of the research discussed in subsequent chapters.
3.2. Methods

3.2.1. Search strategy

The Medline and EMBASE libraries (OVID Technologies) were searched for studies published in English. The terms “cirrhosis” and “diabetes” or “glucose tolerance” were used both as subject heading (MeSH or Emtree) and truncated keyword searches. Keywords grouped under subject headings were searched for individually (Appendix 1). Bibliographies were cross-referenced manually for further studies. Studies from January 1979 to December 2010 were accepted. In 1979, the dose for OGTT was standardised at 75g of glucose\textsuperscript{125}.

3.2.2. Eligibility criteria

Studies were included if glucose tolerance was measured and reported in adults (defined as age ≥ 16 years) with liver cirrhosis and grouped by aetiology or severity of cirrhosis. Only studies reporting the prevalence of diabetes as a primary or secondary outcome were considered for inclusion. The most recent or complete study was included if patients appeared to be represented in multiple studies, as identified by author names, institution, year of publication, sample demographics and outcomes. Review articles, case reports and non peer-reviewed publications were excluded. Studies that included patients with cirrhosis of mixed aetiology (for example HCV and HBV co-infection) and studies with data that were ambiguous or not interpretable were also excluded.

3.2.3. Data extraction

Studies were initially screened by the title and abstract. Screened studies were then read in full. The number of studies screened and excluded are summarised in Figure 3-1. Where there was uncertainty about inclusion or interpretation, adjudication was carried out by all supervisors. A pro-forma was developed and piloted on 6 randomly selected included studies and revised prior to full data extraction.
The information extracted included the type of study, number of participants and selection criteria, the aetiology and severity of cirrhosis (as measured by Child-Pugh score or grade), the prevalence of diabetes and the criteria used to define diabetes. We abstracted other risk factors associated with diabetes such as country of origin, family history of diabetes, age and BMI when reported. All data were entered into an Excel spreadsheet (Microsoft Corp., Redmond, WA) for analysis. Corresponding authors were not contacted for clarification due to the large number of studies reviewed.
3.2.4. Definitions

Diabetes was defined based on diagnostic classifications published by the American Diabetes Association or World Health Organisation from 1979 till 2005 (Table 3-1) and/or diagnosis based on treatment with insulin, oral hypoglycaemic agents or specific dietary management.

3.2.5. Statistical analysis

Studies that contributed a sample of less than 25 patients to the pooled prevalence of any variable were not included in the pooled estimates. Logit transformation was applied to the prevalence data as outlined by Lipsey and Wilson and weighted by inverse variance of logit transformed prevalence to ensure a normal distribution of the data\(^{377}\).

Pooled prevalence estimates were computed by the DerSimonian-Laird method assuming a random effects model\(^ {378}\). The final logit results and 95% confidence intervals were back-transformed to percentages for easier interpretation.

Between-study heterogeneity was examined using the \(I^2\) statistic\(^ {379}\). The \(I^2\) statistic describes the percentage of total variation across studies that is due to heterogeneity rather than chance. It allows comparisons across meta-analyses of different sizes and types of studies, and using different types of outcome data. Interpretation is intuitive and an \(I^2 = 50\%\) suggests that half of the variation between studies is from heterogeneity rather than chance. The \(I^2\) statistic is preferable to the Cochran’s \(Q\) statistic\(^ {380}\) which only describes the presence or absence of heterogeneity, although both tests lack power when only a small number of studies are included in the meta-analysis\(^ {381}\). Analysis was performed using Comprehensive Meta-Analysis (v.2.2.064, Biostat, Englewood, NJ).
Table 3-1 Summary of diagnostic criteria for diabetes and intermediate hyperglycaemia

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<th>NDDG 1979&lt;sup&gt;125&lt;/sup&gt;</th>
<th>WHO 1980&lt;sup&gt;382&lt;/sup&gt;</th>
<th>WHO 1985&lt;sup&gt;383&lt;/sup&gt;</th>
<th>WHO2006&lt;sup&gt;126&lt;/sup&gt; &amp; 1999&lt;sup&gt;127&lt;/sup&gt; ADA1997-2004&lt;sup&gt;126, 384-390&lt;/sup&gt;</th>
<th>ADA 2005&lt;sup&gt;128&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>&lt;6.4* or &lt;115&lt;sup&gt;†&lt;/sup&gt;</td>
<td>&lt;6 or &lt;100</td>
<td>Not defined</td>
<td>&lt;6.1 or &lt;110</td>
<td>&lt;5.6 or &lt;100</td>
</tr>
<tr>
<td>Random</td>
<td>Not defined</td>
<td>&lt;8 or &lt;140</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
</tr>
<tr>
<td>2h-glucose</td>
<td>&lt;7.8 or &lt;140</td>
<td>&lt;8 or &lt;140</td>
<td>&lt;7.8 or &lt;140</td>
<td>&lt;7.8 or &lt;140</td>
<td>&lt;7.8 or &lt;140</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>≥7.8 or ≥140</td>
<td>≥8.0 or ≥140</td>
<td>≥7.8 or ≥140</td>
<td>≥7.0 or ≥126</td>
<td>≥7.0 or ≥126</td>
</tr>
<tr>
<td>or</td>
<td>or</td>
<td>≥8.0 or ≥140</td>
<td>≥7.8 or ≥140</td>
<td>≥7.0 or ≥126</td>
<td>≥7.0 or ≥126</td>
</tr>
<tr>
<td>Random</td>
<td>Not defined</td>
<td>≥11.0 or ≥200</td>
<td>≥11.1 or ≥200</td>
<td>≥11.1 or ≥200 (for ADA 1997 only)</td>
<td>≥11.1 or ≥200</td>
</tr>
<tr>
<td>2h-glucose</td>
<td>≥11.1 or ≥200</td>
<td>≥11.0 or ≥200</td>
<td>≥11.1 or ≥200</td>
<td>≥11.1 or ≥200</td>
<td>≥11.1 or ≥200</td>
</tr>
<tr>
<td><strong>IGT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>&lt;7.8 or &lt;140</td>
<td>&lt;8 or &lt;140</td>
<td>&lt;7.8 or &lt;140</td>
<td>&lt;7 or &lt;126</td>
<td>&lt;7 or &lt;126</td>
</tr>
<tr>
<td>and</td>
<td>and</td>
<td>&lt;8 or &lt;140</td>
<td>&lt;7.8 or &lt;140</td>
<td>&lt;7 or &lt;126</td>
<td>&lt;7 or &lt;126</td>
</tr>
<tr>
<td>2h-glucose</td>
<td>≥7.8 or ≥140</td>
<td>≥8 or ≥140</td>
<td>≥7.8 or ≥140</td>
<td>≥7.8 or ≥140</td>
<td>≥7.8 or ≥140</td>
</tr>
<tr>
<td>and</td>
<td>and</td>
<td>≥8 or ≥140</td>
<td>≥7.8 or ≥140</td>
<td>≥7.8 or ≥140</td>
<td>≥7.8 or ≥140</td>
</tr>
<tr>
<td>&lt;11.1 or &lt;200</td>
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<td>&lt;11.1 or &lt;200</td>
<td>&lt;11.1 or &lt;200</td>
<td>&lt;11.1 or &lt;200</td>
<td>&lt;11.1 or &lt;200</td>
</tr>
<tr>
<td><strong>IFG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
<td>≥6.1 or ≥110</td>
<td>≥5.6 or ≥100</td>
</tr>
<tr>
<td>and</td>
<td>and</td>
<td>&lt;7 or &lt;126</td>
<td>&lt;7 or &lt;126</td>
<td></td>
<td>&lt;7 or &lt;126</td>
</tr>
<tr>
<td>2h-glucose</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
<td>&lt;7.8 or &lt;140 (if measured)</td>
<td>&lt;7.8 or &lt;140 (if measured)</td>
</tr>
</tbody>
</table>

IGT, impaired glucose tolerance; IFG, impaired fasting glucose; NDDG, National Diabetes Data Group; WHO, World Health Organisation; ADA, American Diabetes Association.

All values are for venous plasma samples.

* mmol/L.

† mg/dL.
3.3. Results

3.3.1. Description of studies

The results of the search strategy and data extraction phase are detailed in Figure 3-1 and Table 3-2 respectively. Initial searches yielded 4399 titles for studies that examined diabetes and cirrhosis. By reviewing titles and abstracts, we excluded articles with no original data, or involving children. The remaining 277 manuscripts were read in full and 144 articles that did not intend to examine prevalence of diabetes were excluded. The vast majority of these articles investigated mechanisms of diabetes in cirrhosis such as insulin sensitivity or β-cell function. Overall, 41 studies met inclusion criteria (Table 3-2). Included studies were only published from 1990 to 2010 despite the study period extending back to 1979.

The majority of studies were from the United States of America, France and Italy (41%). Fifty one percent were cross-sectional, 27% were longitudinal and 22% were case-control studies but all had the prevalence of diabetes as either a primary or secondary endpoint. Almost 60% of included studies were conducted prospectively. The stringent detection of diabetes by universal biochemical screening of all undiagnosed cases was used in 90% of studies. The remaining studies reported that the diagnosis was made by either laboratory testing or current treatment for diabetes 142, 144, 315, 391, suggesting that some cases of diabetes may have been missed.

3.3.2. Aetiology of cirrhosis and prevalence of diabetes

The estimated pooled prevalence of diabetes by aetiology of liver cirrhosis are summarised in Table 3-3. Patients with HCV, cryptogenic and ALD had the highest pooled estimated prevalence of diabetes in liver cirrhosis ranging from 29.1% to 39.0% from a pooled sample size of between 325 and 3636 patients. Patients with cholestatic liver disease had the lowest prevalence of diabetes (7.9%) whereas HBV cirrhosis was associated with an intermediate prevalence of diabetes (19.2%). Only a single study reported the prevalence of diabetes in NAFLD 142 and another in haemochromatosis 391 (45.7% and 39.5% respectively). Studies reporting the prevalence of diabetes in autoimmune liver cirrhosis were of insufficient size to be included in the meta-analysis 109, 139.
<table>
<thead>
<tr>
<th>Ref</th>
<th>Name</th>
<th>Year</th>
<th>Country</th>
<th>Setting</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prospective studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alavian</td>
<td>2004 Iran</td>
<td>Clinic</td>
<td>No HCC</td>
<td>Pancreatitis, NASH, autoimmune, haemochromatosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amarapurkar</td>
<td>2008 India</td>
<td>NS</td>
<td>HCV or HBV</td>
<td>T1DM, HIV co-infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bugianesi</td>
<td>2002 Italy</td>
<td>NS</td>
<td>Pre-transplant for HCC</td>
<td>Mixed aetiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Del Vecchio</td>
<td>1990 Italy</td>
<td>NS</td>
<td>HCC</td>
<td>Chronic pancreatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanco</td>
<td>2004 India</td>
<td>NS</td>
<td>Cryptogenic cirrhosis</td>
<td>Nil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>El-Zayadi</td>
<td>1998 Egypt</td>
<td>NS</td>
<td>HCV</td>
<td>HIV, complications of cirrhosis, pancreatic dysfunction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gao</td>
<td>2010 China</td>
<td>Hospital</td>
<td>HBV with HCC</td>
<td>DM ≤1 year before diagnosis of HCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grimbert</td>
<td>1996 France</td>
<td>Hospital</td>
<td>HCV</td>
<td>HBV or HIV co-infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huo</td>
<td>2004 Taiwan</td>
<td>Clinic</td>
<td>Unresectable HCC</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecube</td>
<td>2004 Spain</td>
<td>Clinic</td>
<td>HCV</td>
<td>T1DM, pancreatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mangia</td>
<td>1998 Italy</td>
<td>Hospital</td>
<td>Consecutive</td>
<td>Nil</td>
<td></td>
<td></td>
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<tr>
<td>Moreau</td>
<td>2004 France</td>
<td>Hospital</td>
<td>Refractory ascites</td>
<td>Nil</td>
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<td></td>
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<tr>
<td>Moucari</td>
<td>2008 France</td>
<td>Clinic</td>
<td>HCV</td>
<td>Decompensated cirrhosis, mixed aetiology</td>
<td></td>
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</tr>
<tr>
<td>N’Kontchou</td>
<td>2006 France</td>
<td>Both</td>
<td>Age ≥ 40, alcoholic/HCV</td>
<td>HCC, HIV, complications of cirrhosis, Childs C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papatheodoridis</td>
<td>2006 Greece</td>
<td>Clinic</td>
<td>HCV or HBV</td>
<td>Other viral co-infection, decompensated cirrhosis, malignancy</td>
<td></td>
<td></td>
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<tr>
<td>Parolin</td>
<td>2004 Brazil</td>
<td>Clinic</td>
<td>Pre-transplant, HCV</td>
<td>Treatment with steroids or interferon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petit</td>
<td>2001 France</td>
<td>Hospital</td>
<td>HCV</td>
<td>Treatment with interferon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rouabhia</td>
<td>2010 Algeria</td>
<td>Hospital</td>
<td>HCV or HBV</td>
<td>Childs C cirrhosis, pancreatitis, cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ryu</td>
<td>2001 Korea</td>
<td>Hospital</td>
<td>Liver cirrhosis</td>
<td>Mixed aetiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigal</td>
<td>2006 USA</td>
<td>Clinic</td>
<td>Pre-transplant, HCV</td>
<td>GI bleed, infection, renal failure, alcohol use, TIPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorrentino</td>
<td>2004 Italy</td>
<td>Hospital</td>
<td>Cryptogenic cirrhosis</td>
<td>Mixed aetiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yokoyama</td>
<td>1994 Japan</td>
<td>Hospital</td>
<td>Alcohol rehabilitation</td>
<td>Non-alcoholic cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zein</td>
<td>2005 USA</td>
<td>Clinic</td>
<td>HCV</td>
<td>Decompensated cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ziol</td>
<td>2010 France</td>
<td>Clinic</td>
<td>HCV, Childs A cirrhosis</td>
<td>HCC, cleared HCV during study period, mixed aetiology</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Retrospective studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Setting</th>
<th>Diagnosis</th>
<th>Cause of Cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akbar</td>
<td>2002</td>
<td>Saudi Arabia</td>
<td>Hospital</td>
<td>HCV or HBV</td>
<td>Unconfirmed diagnosis of DM</td>
</tr>
<tr>
<td>Allison</td>
<td>1994</td>
<td>UK</td>
<td>NS</td>
<td>Pre-transplant</td>
<td>Nil</td>
</tr>
<tr>
<td>Bigam</td>
<td>2000</td>
<td>Canada</td>
<td>NS</td>
<td>HCV or cholestatic</td>
<td>Nil</td>
</tr>
<tr>
<td>Caldwell</td>
<td>1999</td>
<td>USA</td>
<td>Both</td>
<td>Complete data</td>
<td>Inappropriate diagnosis of cryptogenic cirrhosis</td>
</tr>
<tr>
<td>Caronia</td>
<td>1999</td>
<td>Sicily</td>
<td>Both</td>
<td>HCV or HBV</td>
<td>Mixed aetiology, pancreatitis, pancreatic tumour</td>
</tr>
<tr>
<td>Cimino</td>
<td>2001</td>
<td>Italy</td>
<td>Both</td>
<td>HCV</td>
<td>Mixed aetiology</td>
</tr>
<tr>
<td>Fraser</td>
<td>1996</td>
<td>Israel</td>
<td>Clinic</td>
<td>HCV</td>
<td>HBV co-infection</td>
</tr>
<tr>
<td>Kuriyama</td>
<td>2007</td>
<td>Japan</td>
<td>Both</td>
<td>HCV or HBV</td>
<td>Non-viral hepatitis</td>
</tr>
<tr>
<td>Kwon</td>
<td>2005</td>
<td>Korea</td>
<td>Hospital</td>
<td>HCV or HBV</td>
<td>Patients who died during the index admission</td>
</tr>
<tr>
<td>Milman</td>
<td>2001</td>
<td>Denmark</td>
<td>Both</td>
<td>Insulin treated DM</td>
<td>Non-hereditary haemochromatosis</td>
</tr>
<tr>
<td>Navasa</td>
<td>1996</td>
<td>Spain</td>
<td>Both</td>
<td>Pre-transplant</td>
<td>Survived &lt;1 year post-op</td>
</tr>
<tr>
<td>Qureshi</td>
<td>2002</td>
<td>Pakistan</td>
<td>Clinic</td>
<td>HCV or HBV</td>
<td>Chronic pancreatitis, pancreatic tumour, steroids</td>
</tr>
<tr>
<td>Tellez-Avila</td>
<td>2008</td>
<td>Mexico</td>
<td>Both</td>
<td>Cryptogenic cirrhosis</td>
<td>Incomplete/wrong data</td>
</tr>
<tr>
<td>Thuluvath</td>
<td>2003</td>
<td>USA</td>
<td>Hospital</td>
<td>Pre-transplant, HCV</td>
<td>Nil</td>
</tr>
<tr>
<td>Torisu</td>
<td>2007</td>
<td>Japan</td>
<td>Hospital</td>
<td>Alcoholic</td>
<td>Childs C, HCC, mixed aetiology</td>
</tr>
<tr>
<td>Younossi</td>
<td>2004</td>
<td>USA</td>
<td>NS</td>
<td>NASH</td>
<td>Other aetiology</td>
</tr>
<tr>
<td>Zein</td>
<td>2000</td>
<td>USA</td>
<td>Clinic</td>
<td>Pre-transplant</td>
<td>HBV, T1DM</td>
</tr>
</tbody>
</table>

HCV, hepatitis C cirrhosis; HBV, hepatitis B cirrhosis; DM, diabetes mellitus; HCC, hepatocellular carcinoma; UK, United Kingdom; NASH, non-alcoholic steatohepatitis; NS, not specified; T1DM, type 1 diabetes mellitus; HIV, human immunodeficiency virus; USA, United States of America; GI, gastrointestinal; TIPS, transjugular intrahepatic portosystemic shunt
The estimated pooled prevalence of diabetes showed significant heterogeneity for all aetiologies of liver cirrhosis (Figure 3-2, Figure 3-3 and Figure 3-4) except for alcoholic cirrhosis (Figure 3-4). The heterogeneity between studies arose with respect to patient selection and study design and these differences are summarised in Table 3-2. A number of studies excluded cirrhotic patients with decompensated disease\textsuperscript{135-137, 316, 324, 392-394} while other studies recruited patients undergoing evaluation for orthotopic liver transplantation who are more likely to have advanced disease\textsuperscript{139, 316, 376, 395-398}. Individual data points were often not available and precluded the assessment of confounding from other risk factors for diabetes including age, BMI, ethnicity and family history of diabetes (sub-group analysis).

Table 3-3 Summary data for prevalence of diabetes mellitus in liver cirrhosis

<table>
<thead>
<tr>
<th>Category of cirrhosis</th>
<th>Total n</th>
<th>n</th>
<th>%</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV</td>
<td>3636</td>
<td>1111</td>
<td>32.2</td>
<td>28.7</td>
</tr>
<tr>
<td>HBV</td>
<td>943</td>
<td>176</td>
<td>19.2</td>
<td>14.2</td>
</tr>
<tr>
<td>Alcoholic</td>
<td>770</td>
<td>223</td>
<td>29.1</td>
<td>26.0</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>325</td>
<td>127</td>
<td>39.0</td>
<td>26.7</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>281</td>
<td>15</td>
<td>7.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Haemochromatosis</td>
<td>152</td>
<td>60</td>
<td>39.5</td>
<td>N/A</td>
</tr>
<tr>
<td>NAFLD</td>
<td>50</td>
<td>21</td>
<td>45.7</td>
<td>N/A</td>
</tr>
</tbody>
</table>

DM, diabetes mellitus; CI, confidence interval; HCV, hepatitis C virus; HBV, hepatitis B virus; NAFLD, non-alcoholic fatty liver disease; NR, not reported; HCC, hepatocellular carcinoma; N/A, not applicable.
Despite significant heterogeneity, the forest plots generally show a similar prevalence between studies. A single outlier reported a prevalence of diabetes of 67.4% in HCV patients (Figure 3-3)\(^\text{394}\). The manuscript was critically appraised but the variance in prevalence could not be explained. The prevalence of diabetes in HBV cirrhosis in that study was much lower than the pooled estimate from the present study (5% vs. 19.2%) which may represent an unreported selection bias in the study. Bugianesi et al. reported that 10.9% of patients with cryptogenic cirrhosis had diabetes\(^\text{376}\) compared to an estimated pooled prevalence of 39%. Again, selection bias may have arisen as the study was retrospective and determining the prevalence of diabetes was a secondary aim of the study.

**Hepatitis B cirrhosis**

<table>
<thead>
<tr>
<th>First Author and Year</th>
<th>n/N</th>
<th>Prevalence (95% CI)</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amarapurkar(^\text{399})</td>
<td>15 / 52</td>
<td>28.8% (18.2, 42.5)</td>
<td>13.25</td>
</tr>
<tr>
<td>Caronia(^\text{133})</td>
<td>17 / 181</td>
<td>9.4% (5.9, 14.6)</td>
<td>14.85</td>
</tr>
<tr>
<td>Gao(^\text{408})</td>
<td>19 / 122</td>
<td>15.6% (10.2, 23.1)</td>
<td>15.02</td>
</tr>
<tr>
<td>Kwon(^\text{403})</td>
<td>68 / 346</td>
<td>19.7% (15.8, 24.2)</td>
<td>18.47</td>
</tr>
<tr>
<td>Mangia(^\text{145})</td>
<td>12 / 38</td>
<td>31.6% (18.9, 47.8)</td>
<td>11.99</td>
</tr>
<tr>
<td>Papatheodoridis(^\text{393})</td>
<td>13 / 46</td>
<td>28.3% (17.2, 42.8)</td>
<td>12.61</td>
</tr>
<tr>
<td>Ryu(^\text{406})</td>
<td>14 / 102</td>
<td>13.7% (8.3, 21.9)</td>
<td>13.81</td>
</tr>
</tbody>
</table>

Overall: 19.2% (14.2, 25.6) \((Q = 22.53, \text{df} = 6, \ p=0.001. \ I^2 = 73.4)\)

![Figure 3-2 Forest plot of the prevalence of diabetes in patients with hepatitis B cirrhosis](image)
### Forest plot of the prevalence of diabetes in patients with hepatitis C cirrhosis

<table>
<thead>
<tr>
<th>First Author and Year</th>
<th>n/N</th>
<th>Prevalence (95% CI)</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amarapurkar399</td>
<td>28 / 88</td>
<td>31.8% (23.0, 42.2)</td>
<td>4.52</td>
</tr>
<tr>
<td>Bigam400</td>
<td>32 / 110</td>
<td>29.1% (21.4, 38.2)</td>
<td>4.77</td>
</tr>
<tr>
<td>Caronia133</td>
<td>272 / 1151</td>
<td>23.6% (21.3, 26.2)</td>
<td>6.42</td>
</tr>
<tr>
<td>Cimino134</td>
<td>226 / 631</td>
<td>35.8% (32.2, 39.6)</td>
<td>6.31</td>
</tr>
<tr>
<td>El-Zayadi392</td>
<td>24 / 53</td>
<td>45.3% (32.5, 58.7)</td>
<td>3.94</td>
</tr>
<tr>
<td>Grimbert401</td>
<td>33 / 88</td>
<td>37.5% (28.0, 48.0)</td>
<td>4.63</td>
</tr>
<tr>
<td>Kuriyama402</td>
<td>24 / 84</td>
<td>28.6% (19.9, 39.1)</td>
<td>4.36</td>
</tr>
<tr>
<td>Kwon403</td>
<td>38 / 88</td>
<td>43.2% (33.3, 53.7)</td>
<td>4.70</td>
</tr>
<tr>
<td>Lecube404</td>
<td>47 / 118</td>
<td>39.8% (31.4, 48.9)</td>
<td>5.06</td>
</tr>
<tr>
<td>Mangia145</td>
<td>54 / 157</td>
<td>34.4% (27.4, 42.2)</td>
<td>5.32</td>
</tr>
<tr>
<td>Moucar137</td>
<td>11 / 46</td>
<td>23.9% (13.8, 38.2)</td>
<td>3.19</td>
</tr>
<tr>
<td>N’Kontchou136</td>
<td>69 / 220</td>
<td>31.4% (25.6, 37.8)</td>
<td>5.61</td>
</tr>
<tr>
<td>Papatheodoridis393</td>
<td>15 / 50</td>
<td>30.0% (19.0, 44.0)</td>
<td>3.57</td>
</tr>
<tr>
<td>Parolin396</td>
<td>13 / 36</td>
<td>36.1% (22.3, 52.7)</td>
<td>3.18</td>
</tr>
<tr>
<td>Qureshi 405</td>
<td>36 / 108</td>
<td>33.3% (25.1, 42.7)</td>
<td>4.84</td>
</tr>
<tr>
<td>Rouabhia394</td>
<td>29 / 43</td>
<td>67.4% (52.3, 70.7)</td>
<td>3.39</td>
</tr>
<tr>
<td>Ryu406</td>
<td>7 / 28</td>
<td>25.0% (12.4, 43.9)</td>
<td>2.43</td>
</tr>
<tr>
<td>Sigal316</td>
<td>20 / 65</td>
<td>30.8% (20.8, 42.9)</td>
<td>4.02</td>
</tr>
<tr>
<td>Tellez-Avila109</td>
<td>17 / 81</td>
<td>21.0% (13.5, 31.2)</td>
<td>3.98</td>
</tr>
<tr>
<td>Thuluvath397</td>
<td>19 / 97</td>
<td>19.6% (12.9, 28.7)</td>
<td>4.18</td>
</tr>
<tr>
<td>Zein396</td>
<td>16 / 64</td>
<td>25.0% (15.9, 37.0)</td>
<td>3.79</td>
</tr>
<tr>
<td>Zein135</td>
<td>7 / 32</td>
<td>21.9% (10.8, 39.3)</td>
<td>2.49</td>
</tr>
<tr>
<td>Ziol407</td>
<td>53 / 150</td>
<td>35.3% (28.1, 43.3)</td>
<td>5.29</td>
</tr>
</tbody>
</table>

Overall 32.2% (28.7, 36.0)

(Q = 91.1, df = 22, p<0.0001. I² = 75.9)

Figure 3-3 Forest plot of the prevalence of diabetes in patients with hepatitis C cirrhosis
### Alcoholic Cirrhosis

<table>
<thead>
<tr>
<th>First Author and Year</th>
<th>N</th>
<th>Prevalence (95% CI)</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mangia145</td>
<td>14</td>
<td>28.6% (17.7, 42.6)</td>
<td>6.35</td>
</tr>
<tr>
<td>N’Kontchou136</td>
<td>142</td>
<td>29.7% (25.8, 34.0)</td>
<td>63.38</td>
</tr>
<tr>
<td>Ryu406</td>
<td>13</td>
<td>30.2% (18.4, 45.4)</td>
<td>5.76</td>
</tr>
<tr>
<td>Tellez-Avila109</td>
<td>10</td>
<td>30.3% (17.1, 47.7)</td>
<td>4.43</td>
</tr>
<tr>
<td>Torisu224</td>
<td>11</td>
<td>23.4% (13.5, 37.5)</td>
<td>5.35</td>
</tr>
<tr>
<td>Yokoyama116</td>
<td>23</td>
<td>34.3% (24.0, 46.4)</td>
<td>9.59</td>
</tr>
<tr>
<td>Zein396</td>
<td>10</td>
<td>18.9% (26.0, 32.4)</td>
<td>5.15</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td>29.1% (26.0, 32.4)</td>
<td></td>
</tr>
</tbody>
</table>

(Q=4.38, df=6, p=0.63. $I^2 = 0.00$)

### Cryptogenic Cirrhosis

<table>
<thead>
<tr>
<th>First Author and Year</th>
<th>N</th>
<th>Prevalence (95% CI)</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bugianesi376</td>
<td>5</td>
<td>10.9% (4.6, 23.6)</td>
<td>15.56</td>
</tr>
<tr>
<td>Caldwell142</td>
<td>37</td>
<td>52.9% (41.2, 64.2)</td>
<td>22.66</td>
</tr>
<tr>
<td>Duseja109</td>
<td>11</td>
<td>44.0% (26.3, 63.4)</td>
<td>17.61</td>
</tr>
<tr>
<td>Sorrentino143</td>
<td>18</td>
<td>50.0% (34.2, 65.8)</td>
<td>19.76</td>
</tr>
<tr>
<td>Tellez-Avila109</td>
<td>53</td>
<td>39.6% (31.6, 48.1)</td>
<td>24.40</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td>39.0% (26.7, 52.9)</td>
<td></td>
</tr>
</tbody>
</table>

(Q=18.78, df=4, p=0.001. $I^2 = 78.8$)

### Cholestatic Cirrhosis

<table>
<thead>
<tr>
<th>First Author and Year</th>
<th>N</th>
<th>Prevalence (95% CI)</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allison139</td>
<td>4</td>
<td>12.5% (4.8, 28.9)</td>
<td>25.90</td>
</tr>
<tr>
<td>Bigam406</td>
<td>5</td>
<td>4.3% (1.8, 10.0)</td>
<td>35.39</td>
</tr>
<tr>
<td>Caldwell142</td>
<td>5</td>
<td>15.2% (6.5, 31.6)</td>
<td>31.40</td>
</tr>
<tr>
<td>Zein396</td>
<td>1</td>
<td>1.3% (0.2, 8.5)</td>
<td>7.31</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td>7.9% (4.8, 12.8)</td>
<td></td>
</tr>
</tbody>
</table>

(Q=8.64, df=3, p=0.035. $I^2 = 65.3$)

Figure 3-4 Forest plot of the prevalence of diabetes in patients with alcoholic, cryptogenic and cholestatic cirrhosis
3.3.3. Severity of cirrhosis and prevalence of diabetes

Data on the association between severity of liver disease and prevalence of diabetes were only available from 7 studies\(^{133, 148, 151, 315, 332, 395, 407}\). The severity of liver cirrhosis was measured by the Child-Pugh score in all studies\(^{150}\). The sampling cohorts were comprised mainly of patients with HCV and HBV cirrhosis while alcoholic, cryptogenic, NAFLD and cholestatic cirrhosis comprised a minority of patients. As shown earlier the prevalence of diabetes is dependent on the aetiology of cirrhosis. Meta-analysis was not performed because confounding from the difference in aetiology of cirrhosis could not be controlled in most studies.

Only one study (n=1332) elegantly examined the association between severity of liver disease and prevalence of diabetes for a specific aetiology of liver cirrhosis\(^{133}\). In this study, patients with HCV and HBV cirrhosis with a higher severity of liver disease had a higher prevalence of diabetes. The association between severity of liver cirrhosis and the prevalence of diabetes was stronger in patients with HCV than in HBV (p < 0.0001).

3.3.4. Temporal trends in diagnosis of diabetes

The diagnostic classification for diabetes was changed in 1997 when the fasting plasma glucose level was lowered from 7.8mmol/L to 7.0mmol/L. The change was reflected in our findings and studies using the newer classifications consistently reported either the same or a higher prevalence of diabetes than older studies (data not shown).
3.4. Discussion

This systematic review found that the prevalence of diabetes in patients with liver cirrhosis was different for different aetiologies of liver cirrhosis. Patients with HCV, cryptogenic cirrhosis and ALD were found to have a higher prevalence of diabetes (ranging from 29.1% to 39%). The prevalence of diabetes in cirrhosis that is often quoted in the literature is similar to the present findings, ranging from 10% to 30%\textsuperscript{113, 118, 410, 411}. This compares to the estimated prevalence of diabetes in the adult population derived from 2005-2006 US national survey data of 7.7%, ranging from 6.6% in non-Hispanic whites to 12.8% in non-Hispanic blacks\textsuperscript{412}. Surprisingly, patients with cholestatic cirrhosis do not have an increased prevalence of diabetes compared to the general population (7.9%). The present study is the first systematic review to examine this question and the first to specifically evaluate the influence of aetiology and severity of liver disease.

Limitations of the data meant that the association between severity of liver disease and the prevalence of diabetes was not able to be summarised. There are conflicting individual reports in the literature. Large cross-sectional studies including the only study included in this review have highlighted an association between Child-Pugh score and the prevalence of diabetes, predominantly in viral cirrhosis\textsuperscript{133, 148, 151}. However, several recent studies recruiting mixed aetiology cohorts of cirrhotic patients failed to confirm a similar association but they would have been confounded by the differences in aetiology of liver disease within the cohort\textsuperscript{29, 145, 315}.

Controversy also exists for the association of some aetiologies of liver disease and the prevalence of diabetes. Many\textsuperscript{133, 392, 400, 401} but not all\textsuperscript{145, 413} recent reports have pointed to an association in patients with HCV, and the increased prevalence of diabetes may be present prior to the onset of liver cirrhosis\textsuperscript{134, 413}. Further, many patients historically categorised as having cryptogenic cirrhosis are likely to have had underlying NAFLD\textsuperscript{141, 142} which is strongly associated with type 2 diabetes and the metabolic syndrome\textsuperscript{157, 414, 415}.

The high prevalence of diabetes in many patients with liver cirrhosis results from altered glucose metabolism but the relative roles of insulin sensitivity, impaired insulin secretion and overproduction of glucose by the liver (reduced hepatic insulin sensitivity) in causing diabetes in liver cirrhosis remain unclear. However, reduced peripheral insulin sensitivity is thought to play the most important role\textsuperscript{25, 119, 158, 170} (see Section 2.4). Type 2 diabetes manifests only when insulin secretion is no longer sufficient to compensate for the resistance to actions of insulin\textsuperscript{119} (see Section 2.7). Evidence from studies using the hyperinsulinaemic euglycaemic clamp points to peripheral skeletal muscle as the primary site of reduced insulin sensitivity rather than the liver\textsuperscript{60, 159, 170, 198, 202, 207, 212}. Glucose disposal is
primarily via glycogen synthesis and storage in peripheral skeletal muscle (non-oxidative glucose disposal) during the hyperinsulinaemic euglycaemic clamp. In healthy volunteers this accounts for around 85% of total glucose infused\(^{175}\). Storage of glycogen quantified on muscle biopsy has been shown to be deficient during the hyperinsulinaemic euglycaemic clamp, even in compensated cirrhosis\(^{206}\). This suggests that in cirrhosis there is a defect with insulin action on skeletal muscle to enhance glucose uptake and subsequent storage as glycogen (see Section 2.6.4).

The effect of diabetes on prognosis in patients with liver cirrhosis is another important question that has not been well studied. Although our review does not address this question, there is evidence that diabetes may impact adversely on survival\(^{28,29,315}\), although it is uncertain how this relates to specific aetiologies such as viral\(^{313,403}\) and ALD\(^{312}\). Patients with IGT may also share a similar prognostic course to those with diabetes\(^{29,312}\). Mortality appears to result from the complications of cirrhosis rather than diabetes, possibly because patients with cirrhosis do not survive long enough to develop the long-term life threatening complications of diabetes\(^{274,318-320,416}\).

Several reports have suggested that diabetes is associated with an increased risk of hepatocellular carcinoma in patients with cirrhosis\(^{136,272,322-324,330}\). Fasting and post-OGTT hyperinsulinaemia was associated with increased risk for hepatocellular carcinoma in a large male cohort\(^{417}\). Hyperinsulinaemia may increase insulin-like growth factor 1 (IGF-1) levels\(^{418,419}\) and potentially drive tumour growth. Receptors for IGF-1 are over-expressed in hepatocellular carcinoma cells\(^{420,421}\) and were associated with increased mean tumour size\(^{420}\). The association with poorer outcome highlights the need for better conducted studies into the association and sequelae of diabetes in patients with liver cirrhosis.

There are several limitations to this review. Firstly, it draws on information taken from a heterogeneous group of studies that may not be representative of all cirrhotic patients. A population-based prevalence study of patients with liver cirrhosis may therefore give a different result. The risk of selection bias was reduced by including only peer-reviewed studies with a stated aim of measuring the prevalence of diabetes in their respective cohort. Secondly, we cannot be sure if liver cirrhosis preceded the development of diabetes in many of the studies. Both cirrhosis and diabetes are insidious diseases which make this distinction difficult and our analysis simply documents the association between these conditions. Finally, limitations in the available data precluded modelling of the relationship between diabetes and aetiology of cirrhosis and other potential risk factors such as the severity of liver disease, age, ethnicity, family history and BMI. This limitation highlights a deficiency in the literature.
In conclusion, this systematic review provides a pooled estimate of the prevalence of diabetes for the main aetiologies of liver cirrhosis. The prevalence may vary significantly but are significantly higher when compared to the general adult population except for patients with cholestatic cirrhosis. Diabetes was most common in NAFLD, cryptogenic cirrhosis, HCV and alcoholic cirrhosis. Clinical implications of these findings may include the need to screen for diabetes in all patients with cirrhosis at the time of diagnosis and periodically thereafter.
Chapter 4. Oral β-blockade may reduce energy expenditure in patients with liver cirrhosis: a double-blind, randomised cross-over trial

4.1. Introduction

Hypermetabolism occurs when REE is elevated above normal levels and is seen in up to one-third of patients with liver cirrhosis\(^6, 8, 13, 16\) where it is associated with reduced overall and transplant-free survival\(^6, 12, 17, 422\). Earlier work from our group showed that lower REE was associated with improved survival even for patients within the normal range of REE\(^6\). In addition, patients taking β-blockers to prevent variceal bleeding were three times less likely to be hypermetabolic than those not receiving β-blockers\(^9\). Oral β-blockade has been shown to reduce hypermetabolism in burns patients\(^19\) and short-term (12-hr) continuous infusion of propranolol reduced REE by about 5% in cirrhotic patients\(^13\). This reduction was greatest (6%) in cirrhotic patients with elevated baseline REE but was also seen in normometabolic patients (3-4%).

Taken together, these observations suggest that oral β-blockade may reduce REE in patients with liver cirrhosis and thereby confer a clinical benefit by reducing the detrimental metabolic and nutritional consequences of the disease. The primary aim of this pilot study was to determine whether oral β-blockade reduces REE in stable patients with liver cirrhosis. Since REE may contribute to the protein-calorie malnutrition that accompanies progressive liver disease a secondary aim was to examine the effect of β-blockade on TBP. We also measured plasma catecholamine concentrations since higher than normal levels have been associated with hypermetabolism in cirrhosis\(^13\).
4.2. Methods

4.2.1. Patients

Patients (aged ≥ 18 y) with liver cirrhosis (confirmed by histology or radiology/biochemistry) were recruited from the hepatology clinics of the Auckland City and Middlemore Hospitals. Eligible patients were clinically stable (no major bleeding, encephalopathic or septic complication within the preceding month), did not have hepatocellular carcinoma and were not listed for liver transplantation. Patients requiring either primary or secondary β-blocker prophylaxis (severe portal hypertensive gastropathy with chronic bleeding, Grade 3-4 oesophageal or large ectopic varices) or with contraindications to its use were not eligible. Contraindications included a history of bronchospasm, severe peripheral vascular disease, complete heart block, previous intolerance to β-blockade and poorly-controlled diabetes. The trial was approved by the Northern X Ethics Committee (Auckland, New Zealand) and all study patients gave written informed consent.

4.2.2. Study protocol

Patients were randomised to receive nadolol (Group 1) or placebo (Group 2) for 3 months and, following a 4-week wash-out period, switched to placebo or nadolol for a further 3 months. The Auckland City Hospital pharmacy prepared the nadolol and visually-identical placebo in identically-labelled packaging and dispensed to patients according to the block-randomisation (block size 6) schedule determined by the pharmacy. Both patients and investigators were blinded to the treatment allocation. Daily nadolol dose (and placebo ‘dose’) was commenced at 40mg and increased to 80mg after 1 week. Patients were assessed at 2 weeks into each 3-month period and, if necessary, the dose was adjusted to achieve a target resting pulse rate of 60 beats per minute or a 20% reduction in baseline resting pulse rate. This assessment was carried out by one of the blinded investigators on the assumption that the placebo effect and random variation would preclude identification of the group allocation. Furthermore, lack of reduction in heart rate may be observed in almost one-third of patients receiving nadolol423. Blinded dose reduction was permitted for side effects possibly related to the study medication. Compliance was assessed by recall and inspection of drug packaging and quantified as the percentage of prescribed tablets actually taken. At the beginning and end of each 3-month period patients were asked to report to the Body Composition Laboratory of the Department of Surgery after an overnight fast (≥ 8 h). Clinical assessment, anthropometry, REE, body composition analysis and blood sampling were performed by a single observer (WGL). Child-Pugh and MELD scores3 were re-calculated at each time-point.
4.2.3. Anthropometry

Body weight was measured to the nearest 0.1 kg by beam balance and adjusted for the estimated weight of clothing. Height (± 0.5cm) was measured using a stadiometer. Body mass index (BMI) was calculated as weight/height$^2$.

4.2.4. Resting energy expenditure

REE can be measured by direct or indirect calorimetry. Direct calorimetry measures the total heat loss from the body but its application is limited by expense and the inaccessibility of specialised equipment. In most clinical and experimental applications REE is determined by indirect calorimetry which determines the energy expenditure by measuring the oxygen consumption (V\(_{O2}\)) and carbon dioxide production (V\(_{CO2}\)) based on the principle that carbon-based nutrients are converted into CO\(_2\), water and heat in the presence of O\(_2\)$^{424}$. In other words indirect calorimetry measures the production of energy in vivo and relates that to the expenditure of energy.

In the present study V\(_{O2}\) and V\(_{CO2}\) was measured using a ventilated hood in a thermo-neutral environment (22-24°C) over a period of at least 10 min at steady-state and after patients had been resting in a supine position for at least 30 min consistent with the guidelines of a recent systematic review of best practice methods for the measurement of REE$^{425}$. Calibration of the calorimeter was performed prior to each measurement using a reference gas mixture (95% O\(_2\), 5% CO\(_2\)) after a 30 min warm-up of the machine$^{426}$.

The measurement of V\(_{O2}\) and V\(_{CO2}\) is then entered into a modification of the Weir equation$^{427}$ used by the Deltatrac Metabolic Monitor model MBM-100 (Datex Instruments, Helsinki, Finland):

\[
\text{REE (kcal/day)} = [5.5 \times V_{O2} \text{ (mL/min)}] + [1.76 \times V_{CO2} \text{ [mL/min]}]
\]

The modification of the Weir equation ignores the contribution of protein substrates to energy expenditure by omitting urinary nitrogen excretion. The modification only introduces an error of 1% – 2% in the measurement of true energy expenditure$^{427}$. Indirect calorimetry also assumes that all the inspired oxygen is used to oxidise nutrients and that all the carbon dioxide evolved is recovered by
the calorimeter. Energy production by alternative sources like gluconeogenesis is ignored and may introduce a small systematic error in measured REE of patients with increased gluconeogenesis like cirrhosis\textsuperscript{168}. The error is smaller than that of calculated substrate oxidation\textsuperscript{424}.

The predicted REE (REE\textsubscript{p}) was calculated for each patient based on TBP using equations derived from measurements of 80 healthy volunteers in our laboratory\textsuperscript{6}:

\[
\text{REEp (kcal/day)} = 68.21 \times \text{TBP (kg)} + 854
\]

\[ (\text{SEE} = 171 \text{ kcal/d}; r^2 = 0.44) \]

where SEE is the standard error of the estimate. Hypermetabolism was defined as a ratio of REE to REE\textsubscript{p} > 1.22, which represents 2 standard deviations above the distribution mean of the healthy volunteers.

The ratio of carbon dioxide expired to the amount of oxygen inspired (\(V_{CO2}/V_{O2}\)) is termed the respiratory quotient and reflects the type of substrate used and was used as a guide to the quality of the measurement of REE.

\textbf{4.2.5. Body composition}

Total body nitrogen was measured by prompt-\(\gamma\) in vivo neutron activation analysis. The facility at the Department of Surgery, University of Auckland was established in 1983\textsuperscript{428} using the prompt-\(\gamma\) analysis method described by Biggin \textit{et al}\textsuperscript{429}. This method utilises the \(^{14}\text{N}(n, \gamma)^{15}\text{N}\) reaction for measurement of nitrogen. Briefly, patients lie supine on a movable gantry and are slowly passed through a neutron beam emanating from two 7.6 Ci plutonium/beryllium sources above and below the gantry. The release of 10.83 MeV prompt-\(\gamma\) photons as the nitrogen nuclei de-excite are recorded by sodium iodide \(\gamma\)-ray detectors positioned to the sides. Patients underwent partial body scans (neck to knees) twice and the signals analysed for nitrogen and hydrogen counts. The ratio of nitrogen to hydrogen counts over the region scanned is assumed to be representative of the whole-body ratio\textsuperscript{430}. Hydrogen (2.2 MeV photon signature) is used as an internal standard and reduces the measurement error of nitrogen by three-fold\textsuperscript{431}.
Total body nitrogen is calculated from the ratio of nitrogen to hydrogen corrected for body habitus and background counts. TBP was calculated as 6.25 times total body nitrogen (assuming that nitrogen comprises 16% of the protein in the body)\(^5\). Precision of TBP measurement is 2.7% with an accuracy <4% when compared to chemical analysis of anthropomorphic phantoms\(^4\). For each patient, a pre-illness (normal) TBP was estimated based on regression equations developed in our laboratory from measurements by neutron activation analysis of 223 female and 163 male healthy volunteers\(^6\). Significant protein depletion was defined as TBP <82% of pre-illness TBP\(^6\).

### 4.2.6. Biochemistry

Plasma was stored at \(-80^\circ\text{C}\) until analysis for adrenaline and noradrenaline, using high-pressure liquid chromatography and electrochemical detection, and C-reactive protein (CRP), creatinine, bilirubin and liver function, using Hitachi Modular enzymatic assays (Roche Diagnostics, Mannheim, Germany). Full blood count and international normalised ratio were obtained on freshly drawn samples. Laboratory normal range for adrenaline is <570 pmol/L and for noradrenaline is 470 - 3800 pmol/L.

### 4.2.7. Statistical analysis

The primary endpoint for this study was REE at the end of 3 months treatment with nadolol compared with placebo. Ten evaluable patients per group were required to detect a 5% reduction in REE (at \(\beta=0.1\) and \(\alpha=0.05\)) based on the findings of Müller et al\(^13\). The treatment code was not broken until all patients had completed the study and the integrity of the data file was verified. Analysis was performed on an intention-to-treat basis using a mixed model approach with group, treatment and period as fixed factors and patient (nested in group) as a random factor \(^4\). Missing data were imputed using multiple imputation based on 10 iterations\(^4\). Bivariate associations were assessed using the Pearson correlation coefficient. McNemar’s test was used for categorical data. P values <0.05 were considered significant. Statistical analyses were performed using SAS (version 9.1, SAS Institute, Cary, NC). Results are expressed as means ± SEMs unless otherwise stated.
4.3. Results

4.3.1. Patient disposition and characteristics

Twenty-three patients were randomised to nadolol (group 1, n=12) or placebo (group 2, n=11) for the first 3-month period and 21 patients completed both periods (Figure 4-1). Two patients (group 1) withdrew due to increased work commitments, one prior to completing the first period (excluded from further analysis) and one after completing the first period. Characteristics of the 22 patients who were included in the intention-to-treat analysis are summarised in Table 4-1. These stable cirrhotic patients were relatively well-nourished with one only showing evidence of significant protein depletion.

Figure 4-1 Flow of patients through the trial
Table 4-1 Characteristics of patients at randomisation

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=11)</th>
<th>Group 2 (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>56 (45 – 64)</td>
<td>55 (45 – 75)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/4</td>
<td>9/2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.3 ± 1.9</td>
<td>28.9 ± 2.0</td>
</tr>
<tr>
<td>Child-Pugh score</td>
<td>5 (5 – 7)</td>
<td>5 (5 – 11)</td>
</tr>
<tr>
<td>Child-Pugh class (A/B/C)</td>
<td>9/2/0</td>
<td>10/0/1</td>
</tr>
<tr>
<td>MELD score</td>
<td>8 (6 – 12)</td>
<td>8 (6 – 13)</td>
</tr>
<tr>
<td><strong>Aetiology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>HBV</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ALD</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Autoimmune</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>NASH</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Resting energy expenditure (kcal/d)</td>
<td>1619 ± 94</td>
<td>1606 ± 60</td>
</tr>
<tr>
<td>Measured REE/predicted REE</td>
<td>1.01 ± 0.06</td>
<td>0.99 ± 0.03</td>
</tr>
<tr>
<td>Hypermetabolic</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total body protein (% of normal)</td>
<td>96 ± 3</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>3.7 ± 2.3</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Adrenaline (pmol/L)</td>
<td>81.3 ± 29.0</td>
<td>74.9 ± 23.6</td>
</tr>
<tr>
<td>Noradrenaline (pmol/L)</td>
<td>2102 ± 491</td>
<td>1828 ± 574</td>
</tr>
</tbody>
</table>

MELD indicates model for end-stage liver disease; HCV, hepatitis C virus; HBV, hepatitis B virus; ALD, alcoholic liver disease; NASH, non-alcoholic steatohepatitis.

Data expressed as median (range), mean ± SEM or number of patients.
4.3.2. Resting energy expenditure

For the available data on all 22 patients, analysis of REE measured at the end of each period showed that nadolol was associated with a reduction of 31 ± 16 kcal/d or 2.0% compared to placebo (p=0.076, Table 4-2). For the 21 patients with complete data this p value was 0.071.

Table 4-2 Resting energy expenditure, body composition and biochemistry following 3-months treatment with nadolol and placebo in 22 patients with liver cirrhosis

<table>
<thead>
<tr>
<th></th>
<th>Nadolol</th>
<th>Placebo</th>
<th>Difference</th>
<th>p valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting energy expenditure (kcal/d)</td>
<td>1476 ± 40</td>
<td>1506 ± 40</td>
<td>−31 ± 16</td>
<td>0.076</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.6 ± 4.2</td>
<td>86.4 ± 4.2</td>
<td>0.2 ± 0.5</td>
<td>0.77</td>
</tr>
<tr>
<td>Total body protein (kg)</td>
<td>10.63 ± 0.45</td>
<td>10.76 ± 0.45</td>
<td>−0.12 ± 0.15</td>
<td>0.42</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>1.70 ± 0.67</td>
<td>2.47 ± 0.68</td>
<td>−0.77 ± 0.70</td>
<td>0.29</td>
</tr>
<tr>
<td>Adrenaline (pmol/L)</td>
<td>255 ± 43</td>
<td>164 ± 44</td>
<td>90 ± 56</td>
<td>0.12</td>
</tr>
<tr>
<td>Noradrenaline (pmol/L)</td>
<td>2231 ± 259</td>
<td>1918 ± 263</td>
<td>312 ± 224</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM.

aLinear mixed model analysis of variance.

Individual results for these 21 patients are shown in Figure 4-2 where it can be seen that REE was lower on nadolol than placebo for the majority of patients. No patient was hypermetabolic after 3 months on nadolol or placebo. The effect of nadolol was statistically significant (37 ± 17 kcal/d, p=0.042) after multiple imputation for the missing period 2 data. REE measured at the beginning of each period was not used for the primary analysis. However, these data provided further indication that nadolol caused reduction in REE. This is seen from the combined baseline and 3-month data for nadolol and placebo from the two periods. REE for patients on nadolol was 1600 ± 54 kcal/d at baseline and 1476 ± 36 kcal/d at 3 months, a reduction of 124 ± 36 kcal/d or 7.8% (p=0.002). The corresponding data for patients on placebo were 1545 ± 43, 1501 ± 43 and a reduction of 43 ± 21 kcal/d or 2.7% (p=0.052). For patients on nadolol, the reduction in REE was significantly correlated with baseline REE (r=0.75, p<0.0001) and with REE/REEp at baseline (r = 0.85, p<0.0001). When the influence of the single hypermetabolic patient was removed these respective correlation coefficients were 0.64 (p=0.002) and 0.71 (p=0.0004). The relationships between these reductions in REE and REE/REEp for patients on nadolol or placebo are shown in Figure 4-3. For the 11 patients on nadolol
in period 1 these correlation coefficients were 0.89 (p=0.0002) and 0.93 (p<0.0001), respectively, and, removing the hypermetabolic patient, 0.83 (p=0.003) and 0.91 (p=0.0003). In contrast, for patients on placebo in period 1, the respective correlations were 0.17 (p=0.61) and 0.42 (p=0.22).

Figure 4-2 Resting energy expenditure for 21 patients with liver cirrhosis following 3-months treatment with nadolol and with placebo. The line of identity is shown. Patients below the line had a reduction of resting energy expenditure following β-blockade.

Figure 4-3 (next page) Reduction in resting energy expenditure over 3 months treatment from the beginning of period 1 (solid symbols) or period 2 (open symbols) as a function of the ratio of measured to predicted REE at the beginning of each period, expressed as a percentage, for patients on (A) nadolol (r = 0.85, p<0.0001) and (B) placebo (r = 0.21, p=0.38).
A

\[ Y = 9.5 \times -820.8 \]

Percent predicted REE at baseline

REE reduction on nadolol, kcal/d

-400

-200

0

200

400

600

800

B

Percent predicted REE at baseline

REE reduction on placebo, kcal/d

-400

-200

0

200

400

600

800
4.3.3. Body composition

TBP measurements were obtained for 21 of the 22 patients with one patient being too large for the scanning machine. Analysis of the available data showed that TBP measured at the end of each period did not change significantly with nadolol treatment compared to placebo (p=0.42, Table 4-2).

4.3.4. Biochemistry

Measurements of CRP and plasma catecholamines were obtained for all 22 patients at the end of each period and no statistically significant changes were seen with nadolol compared to placebo (Table 4-2). Compared to the other patients, CRP (27 mg/L) and noradrenaline (5844 pmol/L) were elevated in the single patient with hypermetabolism at study entry (Group 1). Adrenaline (73 pmol/L) was not elevated. After 3 months on nadolol, REE dropped from 52% to 7% above predicted values in this patient. CRP remained elevated (10 mg/L), adrenaline was unchanged (83 pmol/L) and noradrenaline remained relatively high (3067 pmol/L) although now within the normal range.

4.3.5. Adverse events

Six adverse events were reported for patients on nadolol and 2 for placebo (p=0.29). Postural hypotension in one patient required a reduction of the nadolol dose to 40mg daily and in another, severe headaches required stopping the treatment. All other adverse events related to tiredness except for one patient (on nadolol) who experienced mild chest tightness with no changes in peak-flow measurement and was tolerated without changes in nadolol dose.

4.3.6. Per protocol analysis

An analysis according to protocol was carried out after excluding period 2 data for 2 patients because of protocol violations. One patient (group 1) bled from oesophageal varices during the washout period and was started on nadolol by her physician. Another patient (group 2) had to stop nadolol halfway through period 2 due to severe headaches. Compliance information was missing for the second period in two patients but was excellent in all other patients. Assessment of returned drug packaging indicated that except for 2 patients (88% compliance) all others took over 90% of their prescribed...
tablets and compliance overall averaged 98%. Reduction in REE due to nadolol was 36.5 ± 17.6 kcal/d (2.4%, p=0.054).

4.4. Discussion

The primary aim of this pilot study was to provide evidence for a REE-lowering effect of oral β-blockade provided in standard dosage over a 3-month period to cirrhotic patients. The effect we observed was smaller (2%) than the anticipated 5% based on the published intravenous infusion data and not statistically significant. There were no measurable effects on nutritional status or catecholamine levels with nadolol treatment. Drug compliance among patients was high and adverse events were not significantly increased when on treatment with nadolol.

The smaller than expected effect was not surprising given that only one hypermetabolic patient was recruited and in the single published study of the effect of β-blockade on REE in cirrhotic patients, greater reduction was seen in hypermetabolic compared to normometabolic patients following intravenous propranolol treatment. In that study, where 5 of 19 patients were hypermetabolic, overall reduction in energy expenditure was close to 5% but in normometabolic patients, REE was only reduced by around 2.5%, consistent with our findings. Our results suggest, that had more of our patients been hypermetabolic at study entry, a greater reduction in REE would have resulted from oral nadolol treatment. Firstly, the reduction in REE with nadolol was proportional to baseline REE and, importantly, to REE indexed to predicted REE (Figure 4-3) and this was also observed when the single hypermetabolic patient was excluded. For patients on placebo, such relationships were not seen. Secondly, at the threshold for hypermetabolism, where measured REE is 122% of that predicted, the regression relationship developed for patients on nadolol (Figure 4-3) predicts a 338 (95% CI: 273 – 403) kcal/d reduction in REE. This equates to a 17% (95% CI: 12 – 22%) reduction from the baseline REE (data not shown). Excluding the hypermetabolic patient, the predicted reduction from the linear regression is also 338 (95% CI: 212 – 464) kcal/d or 19% (95% CI: 11 – 28%).

While the lack of patients with more advanced liver disease and established protein-calorie malnutrition may have limited the impact of oral β-blockade on REE in the present study, this reflects the fact that many patients with more advanced disease are already taking β-blockers for prevention of variceal haemorrhage or are wait-listed for liver transplantation. It would be unethical to interrupt β-blockers or postpone transplantation for participation in the 7-month trial. Had more patients with advanced liver disease and a higher incidence of hypermetabolism been available to participate in the
study, the considerations in the previous paragraph imply that a larger effect may have been observed. Nevertheless, within the limitations of patient selection we believe this study represents the best evidence to date that oral β-blockers reduce REE in cirrhotic patients.

We did not see a difference in TBP stores (used as a measure of malnutrition) after 3 months of treatment with nadolol compared with placebo. Reduction in REE may lead to improved retention of body protein stores. This lack of improvement may be attributed to the inclusion of compensated patients without significant protein-calorie malnutrition, reflected by normal TBP on study entry. The efficacy of β-blockade to ameliorate protein catabolism has to date only been shown in severely catabolic (and hypermetabolic) patients suffering from burn injuries. In addition to the examination of changes in nutritional status, TBP was also used as a measure of the energy-producing tissues of the body not confounded by water retention commonly seen in cirrhotic patients. For that reason, it is a useful predictor for REE and changes in TBP in an individual will result in changes in REE independent of the possible effects of treatments. No adjustment was made to the REE changes seen in the present work since the TBP changes were minor.

Indirect calorimetry provides a measure of respiratory quotient (RQ) which, being a marker of carbohydrate and lipid metabolism, indicates the degree of metabolic adaptation in cirrhotic patients, with lower than normal RQ associated with reduced glycogen storage capacity of the cirrhotic liver and an accelerated starvation state. Treatment with β-blockers may alleviate this catabolic state. However, the small sample size and generally normometabolic state of the patients precluded an informative analysis of the effects of β-blockade on RQ and substrate oxidation.

The REE of cirrhotic patients is partly determined by the metabolic and haemodynamic derangements of portal hypertension and liver cirrhosis. In a small study of 10 patients with moderate to severe ascites (which is the result of portal hypertension), REE was reduced after the ascites was completely drained. Furthermore, a hyperdynamic circulatory state is present in patients with liver cirrhosis across both the splanchnic and systemic circulatory systems. Vasodilation (induced by endogenous mediators such as nitric oxide) in addition to an expanded plasma volume (from sodium and water retention) combine to drive reduced total peripheral vascular resistance and increased cardiac output and whole body oxygen consumption. β-blockade may reduce REE by ameliorating these haemodynamic changes and has been shown to partially reverse the hyperdynamic circulation in cirrhosis. Further studies directly investigating the effects of β-blockade on cardiac output and total peripheral vascular resistance in conjunction with REE measurements may provide mechanistic insights into the effects of β-blockade on reducing REE.
Over-activity of the sympathetic nervous system (in response to portal hypertension and porto-systemic shunting) is a potential key driver of the hyperdynamic circulation in cirrhosis\textsuperscript{440}. In particular, plasma noradrenaline levels are elevated in hypermetabolic and decompensated cirrhotic patients\textsuperscript{13}, \textsuperscript{441, 442} and increase with severity of liver disease\textsuperscript{443}. However, as the present study included a primarily normometabolic sample of patients with compensated disease we were unable to shed further light on the pathophysiology underlying raised REE in liver cirrhosis. We did not observe a significant increase in catecholamine concentrations with nadolol treatment in contrast to the results of Bendtsen et al\textsuperscript{443} where such increases were seen in all Child’s classes 90 min after a single dose of propranolol. C-reactive protein (an inflammatory marker) was raised in the single hypermetabolic patient in the present study.

The key limitation of this study was the cohort of primarily normometabolic patients with compensated cirrhosis that was recruited by random selection. We believe there is merit for a larger study to confirm the findings above but with modified eligibility criteria informed by the shortcomings of the present study. Cirrhotic patients should be assessed for hypermetabolism at entry into the trial and a larger cohort of patients recruited. A larger cohort may reduce the risk of recruiting primarily stable patients with compensated disease. Lastly, a longer duration of treatment with nadolol and placebo may allow changes in TBP and body composition to be captured within the trial period. The placebo arm with a large cohort of hypermetabolic patients may also provide further insight into the association between hypermetabolism and progressive protein catabolism and malnutrition. No studies in cirrhotic patients have been able to show that hypermetabolism leads to loss of TBP.

In conclusion, this pilot study provides proof-of-principle for the REE-lowering effect of oral β-blockade in cirrhotic patients. While three months of treatment with oral β-blockers resulted in a clinically insignificant reduction in REE in well-compensated, normometabolic, cirrhotic patients, a greater effect may be expected in patients that are hypermetabolic or have more advanced liver disease. A larger study with different eligibility criteria would be required to confirm this, and a much longer duration study would be required to demonstrate any clinical benefits. Given the limited treatment options for patients with advanced liver disease these potential benefits warrant further investigation.
Chapter 5. Nadolol reduces insulin sensitivity in liver cirrhosis: a double-blind, randomised cross-over trial

5.1. Introduction

Liver cirrhosis is characterised by hyperinsulinaemia with many patients also resistant to the actions of insulin\(^{158, 160}\). As discussed in Section 2.4.1, the primary site of reduced insulin sensitivity in liver cirrhosis is the skeletal muscle rather than the liver\(^ {202}\). Reduced insulin sensitivity represents a defect predisposing to diabetes that appears to occur early in the course of cirrhosis\(^ {163}\).

Impaired glucose tolerance and diabetes develop when insulin secretion is unable to compensate for reduced insulin sensitivity and maintain normoglycaemia\(^ {26, 122}\). The relationship between insulin sensitivity and β-cell function is generally hyperbolic in individuals that share the same glucose tolerance and the product of insulin sensitivity and β-cell function is constant. This constant is known as the disposition index and worsening glucose tolerance is reflected by a reduction in the disposition index\(^ {275, 444}\).

Non-selective β-blockers are commonly prescribed for prophylaxis against variceal bleeding in cirrhotic patients\(^ {445}\). In other conditions, most notably hypertension, cardioselective β-blockers such as atenolol and metoprolol\(^ {23, 335, 339, 359, 446}\) and, to a greater extent, non-selective β-blockers such as propranolol\(^ {447}\) impair insulin sensitivity, while vasodilatory β-blockers such as carvedilol and nebivolol do not\(^ {335, 448}\). It has not yet been established whether these detrimental effects also occur with non-cardioselective β-blockade in liver cirrhosis. Thus, the aim of the present study was to determine whether non-selective β-blockade with nadolol reduced insulin sensitivity, glucose tolerance and the disposition index in cirrhotic patients. Diabetes associated with liver cirrhosis is concerning as it complicates the medical management of the cirrhotic patient and is associated with increased risk of liver failure\(^ {29}\) and development of hepatocellular carcinoma\(^ {323}\).
5.2. Methods

5.2.1. Patients

Patients (aged ≥ 18 y) with liver cirrhosis (confirmed by histology or radiology/biochemistry) recruited for the primary study, as discussed in Chapter 4 were invited to participate. For the current study, patients requiring insulin or medications that affect insulin sensitivity other than oral hypoglycaemic agents were excluded. All participants gave written informed consent. This secondary study was approved by the Northern X Regional Ethics Committee (Auckland, New Zealand) and conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

5.2.2. Study protocol

As previously discussed patients in this double-blind, prospective, randomised cross-over trial received either nadolol (Group 1) or placebo (Group 2) for 3 months and, following a 4-week wash-out period, switched to the other intervention for a further 3 months. The Auckland City Hospital pharmacy prepared the nadolol and matching placebo and dispensed to patients according to the block-randomisation (block size 6) schedule determined by the pharmacy. Patients and investigators were blinded to the treatment allocation. Daily nadolol dose was commenced at 40mg and increased to 80mg after 1 week. Patients were assessed at 2 weeks into each 3-month period and the dose was adjusted to achieve a target resting pulse rate of 60 beats per minute or a 20% reduction in baseline resting pulse rate, whichever was higher. This assessment was carried out by one of the blinded investigators on the assumption that the placebo effect and random variation would preclude identification of the group allocation. Furthermore, lack of reduction in heart rate may be observed in almost one-third of patients receiving nadolol423. Blinded dose reduction was permitted for side effects possibly related to the study medication. Compliance was assessed by recall as well as inspection of drug packaging, and quantified as the percentage of prescribed tablets actually taken. At the beginning and end of each 3-month period patients reported to the Body Composition Laboratory after an overnight fast (≥ 8 h) where they underwent clinical assessment, anthropometry, and blood sampling performed by a single observer (WGL). At the end of each 3-month period insulin sensitivity was measured by a hyperinsulinaemic euglycaemic clamp which was preceded by an IVGTT449 (Figure 5-1). In addition, glucose tolerance was measured by OGTT, performed at least 3 days apart and patients were requested to refrain from strenuous exercise for 3 days before each visit to the laboratory. Medications that may alter insulin sensitivity (including oral anti-diabetic medications) were stopped for ≥ 48 hours prior to testing. Immediately prior to the OGTT, body composition analysis was carried out.
Figure 5-1 Study protocol detailing blood sampling intervals during the intravenous glucose tolerance test, hyperinsulinaemic euglycaemic clamp and oral glucose tolerance test.

**IVGTT**
- 5-minute glucose sampling on HemoCue™ for titration of glucose 20% infusion to maintain blood glucose at 5.0mmol/L
- 2-min sampling
- Baseline 5-min sampling

**Hyperinsulinaemic Euglycaemic Clamp**
- 10-minute sampling
- Clamp steady-state
- Key
- Glucose, insulin, C-peptide, glucagon, free fatty acids, glycerol

**Oral glucose tolerance test**
- Incretins, glucose, insulin, C-peptide, glucagon
- Key
- Baseline 5-min sampling
- 10-minute sampling
5.2.3. Anthropometry and body composition

Body weight was measured to the nearest 0.1 kg by beam balance and adjusted for the estimated weight of clothing. Height (± 0.5cm) was measured using a stadiometer. Body mass index (BMI) was calculated as weight/height$^2$ and body surface area by the equation derived by Gehan and George:\footnote{450}

\[
\text{BSA (m}^2\) = 0.0235 \times \text{height (cm)}^{0.42246} \times \text{weight (kg)}^{0.51456}
\]

Total body fat was measured by whole-body dual-energy X-ray absorptiometry (DEXA, model DPX+, software version 3.6y, extended research analysis mode; GE-Lunar, Madison, WI). Body mass is partitioned by the software into three compartments: total body fat, bone mineral content and fat-free soft tissue by measuring the differential absorption of x-rays at 38 and 70kV for each field (pixel) of the scanned image. The precision for measuring fat mass is 1.3\%\footnote{451} and the accuracy better than 5\% when determined using anthropomorphic phantoms of known fat content and known degrees of over-hydration\footnote{432}. A single DEXA scan delivers a radiation dose <1\% of that received during a chest x-ray.

Percent fat mass was calculated as 100 x total fat mass/body weight while FFM was calculated as body weight minus total body fat.

5.2.4. Intravenous glucose tolerance test and hyperinsulinaemic euglycaemic clamp

Participants were studied in the basal state after an overnight fast and after bed rest for at least 30min. Participants remained in a semi-recumbent position in a thermoneutral quiet room for the duration of the IVGTT and the hyperinsulinaemic euglycaemic clamp. Performed sequentially these tests allow independent measurement of β-cell function and insulin sensitivity simultaneously. The hyperinsulinaemic euglycaemic clamp is the gold standard measure of insulin sensitivity. First developed in 1966\footnote{452} and refined subsequently\footnote{453}, \footnote{454}, the seminal paper describing the hyperinsulinaemic euglycaemic clamp was published in 1979\footnote{184}. Insulin sensitivity is measured by elevating the plasma insulin of a subject to a state of hyperinsulinaemia for a length of time to allow equilibration between plasma and tissue insulin levels or ‘steady-state’. At the same time a variable glucose infusion is used to maintain or ‘clamp’ the blood glucose level at a pre-determined set point. The amount of glucose required to clamp the blood glucose level is a direct measure of the insulin sensitivity of the subject tested.
In preparation for the IVGTT and hyperinsulinaemic euglycaemic clamp an intravenous catheter was inserted retrograde into a wrist vein for blood sampling and the hand placed in a heated box at a constant temperature ($55^\circ$C) to arterialise venous blood. Arterialised blood sampling was first described by McGuire et al in 1976 to overcome the limitations of venous blood sampling for determination of glucose kinetics\textsuperscript{455}. Venous blood sampled from the antecubital fossa has undergone metabolism by the intervening tissues drained and may show a 3 – 5% difference in glucose concentration compared to arterial or arterialised blood\textsuperscript{456}. The practice of arterialised blood sampling for blood glucose has been validated\textsuperscript{457} but only in young males, and several measurements should be obtained at a particular time point to reduce variability\textsuperscript{458}. A second catheter in an antecubital vein was placed in the standard antegrade fashion for infusion of glucose (25g/100mL) and insulin (Actrapid 100U/mL; Novo Nordisk, Bagsvaerd, Denmark).

A 0.3g/kg intravenous bolus of glucose (50g/100mL) was given (time 0) after baseline blood samples had been obtained at -10, -5 and 0 minutes (Figure 5-1). Further blood samples for plasma glucose and insulin were obtained at 2, 4, 6, 8, 10, 30 and 60 min. At 60 minutes, a priming dose of insulin followed by an infusion (45 mU/m$^2$/min) was begun and continued for 120 min. Blood glucose concentration was maintained at 5mmol/L by a variable glucose (250mg/mL) infusion rate that was adjusted manually according to blood glucose determinations (Glucose 201+, HemoCue AB, Angelholm, Sweden) performed every 5 min. Titrating the glucose infusion rate manually reduces the coefficient of variation of the blood glucose concentration\textsuperscript{459}. Determination of glucose with the HemoCue Glucose 201+ analyser is closely correlated with the more commonly used Yellow Springs Instrument (YSI 2300 STAT; Yellow Springs Instruments, Yellow Springs, OH)\textsuperscript{460} and share a similar measurement precision\textsuperscript{461, 462}. At 160, 170 and 180min further blood samples were collected. All blood samples were collected into pre-chilled vacutainers, centrifuged within 15min of collection and stored at -80°C until analysis for glucose and insulin\textsuperscript{463}.

Measurement of insulin sensitivity with a hyperinsulinaemic euglycaemic clamp performed following an IVGTT correlated strongly with a clamp without preceding intravenous glucose injection in 10 patients ($r = 0.94; p = 0.0001$)\textsuperscript{464}. This finding was validated in a further 9 patients of differing glucose tolerance ($r = 0.953; p < 0.005$)\textsuperscript{449}. Despite the strong correlation, measurement of insulin sensitivity by IVGTT and hyperinsulinaemic euglycaemic clamp performed sequentially may be confounded by the Staub-Traugott effect. The Staub-Traugott effect describes how consecutive loads of intravenous and oral glucose improve the disposal of each subsequent load of glucose in healthy individuals. Recent findings suggest that stronger suppression of endogenous glucose production and the potentiation of β-cell function are implicated in the effect\textsuperscript{307, 465}. However, the Staub-Traugott effect may be lost in patients with diabetes\textsuperscript{525}. Unexpectedly, patients recruited in the study validating the
combined IVGTT and hyperinsulinaemic euglycaemic clamp showed poorer (not improved) insulin sensitivity by an average of 7% when compared to the standalone clamp\textsuperscript{464}. This suggests that the Staub-Traugott effect may not play a significant role during the combined IVGTT and hyperinsulinaemic euglycaemic clamp.

### 5.2.5. Insulin sensitivity

The definition of steady-state is vital to allow accurate comparison of insulin sensitivity at a particular level of hyperinsulinaemia. The clamp steady-state period for the present study was defined as the last 20min of the clamp (160 – 180min) as plasma insulin takes >80min to equilibrate with its extravascular site of action\textsuperscript{453, 454}. Plasma insulin tends to plateau 30 min following the start of the insulin infusion but the glucose infusion rate may not reach steady-state for up to 7 hours\textsuperscript{193, 466}. The difference between the glucose infusion rate at steady-state compared to the rate at 2 hours is between 18 – 30% higher\textsuperscript{466, 467} and it takes longer to reach steady-state in patients with worse insulin sensitivity\textsuperscript{468}. Despite the aforementioned limitations, a 2 hour clamp was used in this study to encourage patient compliance.

Several different indices of insulin sensitivity can be derived from the hyperinsulinaemic euglycaemic clamp. The most common index is the M value or the glucose infusion rate during the clamp steady-state period (mg/kgFFM/min). M value is normalised to FFM to minimise over-estimation of reduced insulin sensitivity in obese patients\textsuperscript{469, 470}. Despite widespread use, the M value can vary from factors other than the insulin sensitivity of the patient. Firstly, identical insulin infusion rates do not result in comparable levels of hyperinsulinaemia even in healthy individuals and normalising the M value by the prevailing steady-state insulin level (M/I) worsens the variability\textsuperscript{184}. This complicates comparison with other patients. Secondly, glucose uptake is partly dependent on the ambient blood glucose concentration during steady-state and the M value does not take this into account\textsuperscript{471, 472}. Thirdly, the decrease in M value can be masked by an increased contribution of insulin-independent glucose disposal in patients with reduced insulin sensitivity that is often unimpaired\textsuperscript{473}.

To avoid the recognised disadvantages of the M value, Ader \textit{et al} proposed the insulin sensitivity index (ISI\textsubscript{FFM}), as a measure of the sensitivity of tissues to insulin\textsuperscript{179}. The ISI\textsubscript{FFM} is defined as:

\[
\text{ISI}_{\text{FFM}} = \frac{M}{(\Delta I \times G)}
\]
where $M$ is the $M$ value normalised to FFM (mg/kg FFM/min), $\Delta I$ the difference between steady-state and basal plasma insulin concentrations (mU/L) and $G$ the steady-state plasma glucose concentration (mmol/L). The ISI_{FFM} measures the increase in glucose disposal as a result of the increment in plasma insulin during clamp steady-state. This index allows comparison between euglycaemic and hyperglycaemic clamps, and comparison between different steady-state insulin levels\textsuperscript{179}.

Insulin sensitivity during the basal state was not measured (by the isotope dilution method\textsuperscript{474}) as glucose tracers were not employed during the hyperinsulinaemic euglycaemic clamp. While important for a complete assessment of insulin sensitivity and basal glucose production, measurements were not repeated in this study due to the published evidence showing no difference between normoglycaemic cirrhotic patients and healthy controls\textsuperscript{177, 475, 476}. We were unlikely to have the necessary power to detect a change following $\beta$-blockade in this cohort (even if there was one) with a significant number of normoglycaemic patients (41%). Likewise HGP during clamp steady-state was not measured as previous studies have shown production was completely suppressed at comparable insulin infusion rates\textsuperscript{171, 226, 477, 478}.

5.2.6. $\beta$-cell function

The AIR is one of the most common empirical indices of insulin secretion and was discussed in detail in Section 2.7.2. Loss of the AIR is an early marker of impaired $\beta$-cell function\textsuperscript{479}. However, the AIR may over-estimate the degree of impaired $\beta$-cell function\textsuperscript{480}.

The AIR was determined by calculating the incremental trapezoidal area under the insulin concentration curve relative to basal insulin concentration during the first 10 min after the intravenous glucose bolus and normalising to body surface area\textsuperscript{481}. Insulin secretion was calculated over the first 10 minutes to reduce the between- and within-subject variation associated with this index\textsuperscript{299, 482}.

5.2.7. The disposition index

The pancreatic $\beta$-cell generally adapts to a change in insulin sensitivity\textsuperscript{33}. The nature of this feedback loop suggests that insulin sensitivity and $\beta$-cell function should not be assessed in isolation as different insulin sensitivity between patients may mask a defect in $\beta$-cell function and vice versa. The concept of a hyperbolic inverse relationship between insulin sensitivity and $\beta$-cell function was first
reported in 1979\textsuperscript{483} and it was recognised this relationship explained how insulin sensitivity and \(\beta\)-cell function may determine glucose tolerance in 1981\textsuperscript{275}. These findings have been validated in healthy volunteers\textsuperscript{298, 484, 485} and in patients at risk of developing diabetes\textsuperscript{486-488}.

The product of insulin sensitivity and \(\beta\)-cell function is a constant (due to the hyperbolic relationship) and is known as the disposition index\textsuperscript{275}. The disposition index measures the ability of the \(\beta\)-cell to compensate for a reduction of insulin sensitivity and may detect an early \(\beta\)-cell defect in otherwise glucose-tolerant individuals\textsuperscript{486, 489, 490}. The disposition index has several limitations which have been discussed in detail elsewhere\textsuperscript{491}. Briefly, the hyperbolic relationship must be established for each comparison between an index of insulin sensitivity and a different index of \(\beta\)-cell function\textsuperscript{298}. The hyperbolic relationship is only true for independent tests of insulin sensitivity and \(\beta\)-cell function, and not indices that are intrinsically inter-dependent and derived from the same or related variables like the HOMA of insulin resistance and HOMA of \(\beta\)-cell function\textsuperscript{279}.

In this study, the disposition index was calculated as \(\text{AIR} \times \text{ISI}_{\text{FFM}}\textsuperscript{484}\). The relationship between the AIR and the clamp-derived insulin sensitivity is known to be hyperbolic and has previously been used to calculate the disposition index in other studies\textsuperscript{298, 449}.

5.2.8. Glucose tolerance

The OGTT is the method recommended by the World Health Organisation for the diagnosis of diabetes. Despite wide acceptance of the OGTT, there are concerns that results of the OGTT are not reproducible and use of the OGTT for the diagnosis of diabetes (compared to fasting plasma glucose) is not justified when the increased cost and inconvenience are taken into account\textsuperscript{482, 492}. However using only the fasting plasma glucose for the diagnosis of diabetes may miss the diagnosis in up to 40\% of patients\textsuperscript{126, 493}.

For this study, a standard 75g OGTT was performed with blood taken at baseline and every 30 min for 2 hours after the glucose load for glucose and insulin determination (Figure 5-1). All blood samples were taken <5 min from the scheduled time point to reduce between-test variation\textsuperscript{355}. The samples were then centrifuged within 15 min of collection and plasma and serum were stored at -80\(^\circ\)C until analysis. Blood samples were arterialised and estimated venous values were calculated with a conversion factor of 5\%\textsuperscript{456}. Glucose tolerance status was determined from the 2-h plasma glucose concentration as defined by the 2006 World Health Organisation guidelines\textsuperscript{355}.
5.2.9. Biochemistry

Plasma adrenaline and noradrenaline levels were measured using high-pressure liquid chromatography and electrochemical detection, and creatinine and lipid profile using Hitachi Modular enzymatic assays (Roche Diagnostics, Mannheim, Germany). Plasma glucose level was measured by a spectrophotometric enzymatic assay (Gluco-quant Glucose, Roche Diagnostics, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation were 0.5% and 2.6%, respectively. Insulin was measured by chemiluminescent immunometric assay (Architect Insulin, Abbott Diagnostics, USA) with an intra-assay CV of 2.5% and inter-assay CV of 2.4%.

5.2.10. Statistical Analysis

No a priori sample size estimation was carried out for this secondary study. The treatment code was not broken until all patients had completed the study and the integrity of the data file was verified. Analysis was performed on an intention-to-treat basis using a mixed model approach with group, treatment and period as fixed factors and patient (nested in group) as a random factor. The Bhapkar marginal homogeneity test for matched-pair data was used to examine changes in glucose tolerance status following nadolol treatment. Differences were considered statistically significant if p<0.05. Analyses were performed using SAS 9.1 (SAS Institute, Cary NC). Data are presented as means ± SEM unless stated otherwise.

5.3. Results

5.3.1. Patient disposition and characteristics

Of the 20 patients from the primary study who were eligible for the present study, 2 refused to participate and one withdrew after completing the first period because of work commitments. The remaining 17 patients completed an OGTT at both time points (Figure 5-2). Clinical characteristics of these 17 patients are presented in Table 5-1 along with the 16 patients (n=7, Group 1; n=9, Group 2) who underwent hyperinsulinaemic euglycaemic clamps with one patient (from Group 1) only contributing data from the first period. Daily carbohydrate intake was ≥ 150g per day for all
patients\textsuperscript{355}. One patient with diabetes required oral hypoglycaemic medication for control of their diabetes, while all others were managed by dietary restriction.

Figure 5-2 Flow of patients through the trial
Table 5-1 Clinical characteristics at randomisation of patients undergoing hyperinsulinaemic euglycaemic clamp and oral glucose tolerance tests

<table>
<thead>
<tr>
<th></th>
<th>Clamp (n = 16)</th>
<th>OGGT (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55 (45–61)</td>
<td>55 (45–61)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>11/5</td>
<td>12/5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.1±5.0</td>
<td>82.5±4.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.1±1.5</td>
<td>27.6±1.4</td>
</tr>
<tr>
<td>Child-Pugh grade (A/B/C)</td>
<td>14/1/1</td>
<td>15/1/1</td>
</tr>
<tr>
<td>Aetiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>HBV</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>ALD</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Autoimmune</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes mellitus type II</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Plasma creatinine (µmol/L)</td>
<td>68.5±4.1</td>
<td>73.2 ± 3.7</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.6±0.2</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.7±0.2</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
</tr>
</tbody>
</table>

Data are median (range), mean ± SEM or number of patients.
Abbreviations: OGTT, oral glucose tolerance test; BMI, body mass index; HCV, hepatitis C virus; HBV, hepatitis B virus; ALD, alcoholic liver disease.
5.3.2. Insulin sensitivity

The mean co-efficient of variation for blood glucose concentration during the clamp steady-state period was 3.2±1.7 (SD) %. The glucose concentrations were similar between placebo and nadolol treatments (Table 5-2). During this period plasma insulin concentrations were significantly higher with nadolol treatment than with placebo (p=0.019) while the glucose infusion rates (M values) did not differ (Table 5-2).

Table 5-2 Glucose and insulin metabolism after nadolol or placebo treatment in 16 patients with liver cirrhosis

<table>
<thead>
<tr>
<th></th>
<th>Nadolol</th>
<th>Placebo</th>
<th>Difference</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose during clamp steady state (mmol/L)</td>
<td>5.5±0.1</td>
<td>5.6±0.1</td>
<td>–0.1±0.1</td>
<td>0.22</td>
</tr>
<tr>
<td>Serum insulin during clamp steady state (mU/L)</td>
<td>114±8</td>
<td>101±8</td>
<td>13±5</td>
<td>0.019</td>
</tr>
<tr>
<td>Glucose infusion rate (mg/kg/kgFM/min)</td>
<td>7.3±0.7</td>
<td>7.9±0.7</td>
<td>–0.6±0.7</td>
<td>0.42</td>
</tr>
<tr>
<td>Insulin sensitivity index [µL/kg/kgFM/min/(mU/L)]</td>
<td>79.7±10.1</td>
<td>99.6±10.3</td>
<td>–20.0±5.8</td>
<td>0.0045</td>
</tr>
<tr>
<td>Acute insulin response (mU/m²/min)</td>
<td>67±14</td>
<td>76±14</td>
<td>–9±7</td>
<td>0.24</td>
</tr>
<tr>
<td>Disposition index (x 10⁻³)</td>
<td>6.1±2.0</td>
<td>8.7±2.0</td>
<td>–2.6±1.2</td>
<td>0.0499</td>
</tr>
</tbody>
</table>

Data are mean ± SEM

Linear mixed model analysis of variance

The ISI/kgFM decreased from 99.6±10.3 µL/kg/kgFM/min/(mU/L) with placebo to 79.7±10.1 µL/kg/kgFM/min/(mU/L) with nadolol, a reduction of 20% (p=0.0045). Nadolol similarly reduced the ISI/kgFM in the three diabetic patients who underwent clamps on both occasions (from 64.4±12.2
µL/kg\textsubscript{FFM}/min/(mU/L) on placebo to 49.1±10.8 µL/kg\textsubscript{FFM}/min/(mU/L) on nadolol (p=0.04)). Individual results for the 15 patients with complete data are plotted in Figure 5-3 which shows that treatment with nadolol reduced insulin sensitivity in 12 of the 15 patients.

![Figure 5-3 Comparison of insulin sensitivity after treatment with nadolol or placebo. Insulin sensitivity index in µL/kg/min/(mU/L) for 15 cirrhotic patients with complete data following 3 months of treatment with nadolol or placebo. The line of identity is shown.](image)

5.3.3. Insulin secretion and disposition index

Glucose and insulin concentrations while fasting and following an intravenous glucose load were similar in patients in the nadolol group and those in the placebo group (Figure 5-4). The AIR (determined from C-peptide deconvolution) was also similar in both groups (76±14 mU/m\textsuperscript{2}/min on placebo and 67±14 mU/m\textsuperscript{2}/min on nadolol, p=0.24, Table 5-2). However, the disposition index fell from 8692±2036 to 6083±2007 (p=0.0499) with nadolol treatment suggesting that the AIR was inadequate for the prevailing insulin sensitivity.
Figure 5-4 Results from intravenous glucose tolerance tests. Plasma glucose (above) and serum insulin (next page) concentration during intravenous glucose tolerance tests in 15 cirrhotic patients with complete data following 3 months treatment with nadolol (open circles) and with placebo (closed circles).
Table 5-3 Glucose tolerance status of 17 patients with liver cirrhosis as determined by oral glucose tolerance test following 3-months treatment with nadolol or placebo

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Nadolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Normal glucose tolerance</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>
5.3.4. Glucose tolerance

Glucose tolerance became impaired after nadolol in four normal glucose tolerant patients and another patient progressed to diabetes from IGT. Glucose tolerance improved in one patient with diabetes where the 2-h glucose concentration decreased from 11.2 mmol/L on placebo to 10.4 mmol/L on nadolol (p=0.073 for changes in glucose tolerance; Table 5-3).

5.3.5. Body composition

Body weight, total body fat and percent fat mass did not differ after treatment with nadolol or placebo (Table 5-4).

Table 5-4 Body composition and plasma catecholamine concentrations following 3-months treatment with nadolol or placebo in 16 patients with liver cirrhosis who underwent an intravenous glucose tolerance test and hyperinsulinaemic euglycaemic clamp

<table>
<thead>
<tr>
<th></th>
<th>Nadolol</th>
<th>Placebo</th>
<th>Difference</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>83.3±5.1</td>
<td>82.7±5.1</td>
<td>0.6±0.7</td>
<td>0.41</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.2±3.9</td>
<td>28.5±3.9</td>
<td>0.7±0.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Fat mass (% body weight)</td>
<td>33.8±2.4</td>
<td>33.0±2.4</td>
<td>0.7±0.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Epinephrine (pmol/L)</td>
<td>254±58</td>
<td>172±61</td>
<td>82±78</td>
<td>0.31</td>
</tr>
<tr>
<td>Norepinephrine (pmol/L)</td>
<td>2006±332</td>
<td>1817±341</td>
<td>189±294</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

* Linear mixed model analysis of variance
5.3.6. Catecholamines

Plasma concentrations of adrenaline and noradrenaline did not differ after treatment with nadolol or placebo (Table 5-4).

5.3.7. Adverse events

The incidence of adverse events did not differ between nadolol and placebo treatments for the patients who underwent the hyperinsulinaemic euglycaemic clamp (5 nadolol vs 2 placebo, P=0.45) nor for those who underwent the OGTT (4 nadolol vs 2 placebo, P=0.69). In the OGTT group one patient (Group 2) suffered severe headaches requiring treatment cessation part way through the second period. All other adverse events related to lethargy except for a patient (on nadolol) who experienced mild chest tightness with no changes in peak-flow measurement and was tolerated without changes in dosage. A further patient in the hyperinsulinaemic euglycaemic clamp group also had postural hypotension and her dose was reduced to 40 mg daily.

5.3.8. Compliance with treatment

Compliance information was missing for the second period in two patients but was excellent in all other patients. Assessment of returned drug packaging indicated that except for 2 patients (88% compliance) all others took over 90% of their prescribed tablets and compliance overall averaged 97.8%.
5.4. Discussion

The present study is the first to investigate the effect of non-selective β-blockade on insulin sensitivity and glucose tolerance in patients with liver cirrhosis. The results showed that 3 months treatment with nadolol was associated with a 20% reduction in insulin sensitivity. The reduction in insulin sensitivity could not be ascribed to weight gain or increased percent body fat. However we did not show a statistically significant deterioration in glucose tolerance despite a subtle impairment in β-cell function resulting in a reduced disposition index.

The observed reduction in ISI_{FFM} was not accompanied by a reduction in the M value. The increase in the insulin concentration while on nadolol (which is reflected in the ISI_{FFM} but not the M value) suggests a possible reduction of insulin clearance by nadolol, which warrants further investigation. Nevertheless, the unchanged glucose infusion rate in the presence of a higher concentration of insulin at the very least indicates a relative reduction of insulin sensitivity following treatment with nadolol.

An IVGTT immediately preceded the hyperinsulinaemic euglycaemic clamp, which has the advantage of providing reliable and independent measures of both insulin sensitivity and insulin secretion during the same test. This protocol has been validated in healthy volunteers and type 2 diabetes and showed close correlation with the results obtained from the hyperinsulinaemic euglycaemic clamp without a preceding glucose bolus.449, 464

With nadolol treatment, insulin secretion was not increased in proportion to the reduction in insulin sensitivity observed in cirrhotic patients, indicating some inhibition of β-cell function. The amount of insulin secreted by the β-cell is dependent on the ambient insulin sensitivity and accounting for differences in insulin sensitivity is critically important when evaluating β-cell function. Thus, when we adjusted insulin secretion for the level of insulin sensitivity using the disposition index (AIR x ISI_{FFM}) we found this to be lower on nadolol. This suggests nadolol may impair β-cell function, as observed in hypertensive patients following β-blockade.23, 34

Detrimental effects on insulin sensitivity and glucose tolerance following non-selective and cardio-selective β-blockers is well documented in other conditions such as hypertension, type 2 diabetes and obesity.20, 23, 339 The mechanism of action remains unclear but increased total peripheral vascular resistance following β-blockade may play a role.343 Glucose disposal in peripheral skeletal muscle is dependent on vasodilation and capillary recruitment under the influence of hyperinsulinaemia.
Unopposed α-receptor activity following β-blockade may increase peripheral vasoconstriction resulting in impaired glucose disposal.

Basal plasma catecholamines were not elevated following nadolol and were not associated with the fall in insulin sensitivity. Infusion of adrenaline in healthy volunteers has been demonstrated to reduce insulin sensitivity. Elevated plasma catecholamines have been reported following propranolol therapy in hypertensive and cirrhotic patients. Activation of the sympathetic nervous system in response to a fall in cardiac output following β-blockade and reduced catecholamine clearance mediated through β-adrenergic receptors are possible mechanisms by which certain β-blockers may increase catecholamines. Our results suggest that nadolol therapy in cirrhotic patients does not impair insulin sensitivity through a rise in catecholamines.

A limitation of this study was its small size and potential lack of representativeness of the cirrhotic populations. Almost all the patients had compensated viral cirrhosis, predominantly due to HCV and, compared to other aetiologies, patients with HCV-related cirrhosis are reportedly more likely to develop diabetes. The effects we have observed may not, therefore, be as pronounced in non-HCV cirrhotic patients and further work is required to confirm this. It should be noted however that HCV infection is a major cause of chronic liver disease worldwide. A significant strength of the current work was the use of a cross-over design in which all participants were exposed to nadolol and placebo, reducing inter-participant variation and enhancing the ability to detect the metabolic effect of nadolol. Despite this we were unable to show a deterioration in glucose tolerance and a larger study may be required to demonstrate this. A further strength of the study was concurrent gold standard body composition measurements which allowed us to exclude changes in adiposity as an explanation for decreased insulin sensitivity.

In conclusion, the results of this study demonstrate that the non-selective β-blocker nadolol significantly worsens insulin sensitivity and the disposition index in patients with liver cirrhosis. The fall in glucose tolerance was not statistically significant despite the observed deterioration in four patients after nadolol treatment. The reduction in insulin sensitivity was not associated with rise in plasma catecholamine levels and could not be explained by increased body adiposity. These adverse effects of nadolol highlight the importance of screening for diabetes or monitoring glycaemic control in patients with liver cirrhosis treated with nadolol. Non-selective β-blockers are used widely in patients with cirrhosis, to prevent index and recurrent variceal haemorrhage. Therefore, further studies of the effects of non-selective β-blockers on glucose metabolism are needed to elucidate the true risk-benefit of such drugs in patients with cirrhosis.
Chapter 6. Nadolol impairs pancreatic glucose sensitivity in patients with liver cirrhosis

6.1. Introduction

In Chapter 5, patients with liver cirrhosis were shown to have a reduction of insulin sensitivity and a lower disposition index following treatment with nadolol for 3 months. A significant reduction in glucose tolerance was not detected, possibly due to the small sample size of the sub-study.

The reduction in β-cell function following nadolol warranted further investigation. The AIR in the first 10 minutes of an IVGTT is a widely used measure of β-cell function but was unchanged in the cirrhotic patients following nadolol. A reduction in β-cell function following nadolol only become evident when the AIR was adjusted for the prevailing level of insulin sensitivity by calculating the disposition index.

In order to better understand the effect of nadolol on β-cell function in patients with liver cirrhosis, complete patient data from the sub-study were entered into a model of β-cell function proposed by Mari et al. The model has the benefit of deriving several parameters of β-cell function that are independently associated with glucose tolerance and may provide further insights into the effect of nadolol on β-cell function.
6.2. Methods

6.2.1. Patients

Patients who completed the IVGTT and hyperinsulinaemia euglycaemic clamp in addition to the OGTT over both phases of the study discussed in Section 5.2.1 were included. This secondary study was approved by the Northern X Regional Ethics Committee (Auckland, New Zealand).

6.2.2. Study Protocol

The study protocol has been described in Section 5.2.2. In addition, healthy volunteers were enrolled from university staff. Each volunteer underwent an OGTT and hyperinsulinaemic euglycaemic clamp preceded by an IVGTT but did not participate in the cross-over trial. These tests were performed at least 3 days apart and participants were requested to refrain from strenuous exercise for 3 days before each visit to the laboratory.

6.2.3. Anthropometry

Similar to Section 5.2.3, body weight was measured to the nearest 0.1 kg by beam balance and adjusted for the estimated weight of clothing. Height (± 0.5cm) was measured using a stadiometer. BMI was calculated as weight/height$^2$ and body surface area by the equation derived by Gehan and George$^{450}$ (see Section 5.2.3).

6.2.4. β-cell function

Further to the discussion in Section 2.7, β-cell function in this study was derived from the OGTT using a mathematical model describing the relationship between insulin secretion (by C-peptide deconvolution) and glucose concentration. This model was proposed by Mari et al in 2001$^{500}$ and further refined in 2002$^{305}$. The Mari model has 3 subunits: a single-compartment model of glucose kinetics, a model for C-peptide kinetics and an insulin secretion model. The model for C-peptide kinetics is the two-compartment model proposed by Van Cauter et al for C-peptide deconvolution$^{501}$. 
The insulin secretion model has a static insulin secretion component and a dynamic insulin secretion component. The static component of the model is expressed as $\beta$-cell sensitivity to the glucose concentration in the blood (glucose sensitivity) and the dynamic component of the model is expressed as $\beta$-cell sensitivity to the rate of change of glucose concentration (rate sensitivity). Glucose sensitivity describes a direct dose-relationship between glucose concentration and insulin secretion while rate sensitivity accounts for the initial, rapid rise in insulin secretion following glucose ingestion. Another parameter, the potentiation factor, was introduced in 2002 as a time-dependent factor to correct for the inability for the static and dynamic components to account for C-peptide measurements. In the model, the potentiation factor modulates the static component. The potentiation factor was postulated to represent the incretin effect and other stimuli like hyperglycaemia, non-glucose substrates and neurotransmitters. However, a recent study has cast doubt on the validity of this parameter from a physiological standpoint\textsuperscript{311}.

Two further parameters can be derived from the model: total insulin secretion is the sum of both insulin secretion components and is equivalent to insulin secretion as calculated by C-peptide deconvolution. Basal secretory tone is the parameter that quantifies the insulin secretion rate derived from the static insulin secretion component at a chosen plasma glucose concentration close to the fasting value for the participants in a study. The basal secretory tone is different to the basal insulin secretion because basal insulin secretion does not correspond to a fixed glucose concentration.

Reproducibility of the parameters of $\beta$-cell function derived from the Mari model can vary. Glucose sensitivity and basal secretory tone have a co-efficient of variation between 15-20\% but greater variability is seen for rate sensitivity and potentiation (30-50\%)\textsuperscript{502,503}. Furthermore, the validity of the Mari model continues to be questioned because the model was not derived using standard modelling methodology of standard non-linear least squares and maximum likelihood methods\textsuperscript{311}.

Despite the ongoing concerns regarding validity, the model appears to reproduce known characteristics of $\beta$-cell function and has been validated in several large groups of patients with glucose tolerance ranging from normal glucose tolerance to diabetes mellitus\textsuperscript{504}. The different parameters of $\beta$-cell function derived from this model are useful to better describe the changes following $\beta$-blockade in the present study. An OGTT-derived measure of $\beta$-cell function was also convenient due to the already significant number and duration of tests participants in this study have been subjected to.
6.2.5. Insulin sensitivity and glucose tolerance

Insulin sensitivity and glucose tolerance for both patients and healthy volunteers were measured using the methodology discussed in Section 5.2.

6.2.6. Biochemistry and statistical analysis

Details of the biochemistry analysis and statistical analysis were similarly discussed in Section 5.2.9.
6.3. Results

6.3.1. Patient disposition and characteristics

Of the 20 patients eligible for the present study, 2 patients declined to participate while another withdrew after completing the first phase due to work commitments (Figure 6-1). Fifteen patients consented to and completed both the hyperinsulinaemic euglycaemic clamp and OGTT and their clinical characteristics are shown in Table 6-1. Daily carbohydrate intake was ≥ 150g per day for all patients. One patient with diabetes required oral hypoglycaemic medication for control of their diabetes, while the other 3 were managed by dietary restriction. Plasma creatinine was within the normal range for all subjects.

---

Figure 6-1 Flow of patients through the trial
Table 6-1 Clinical characteristics of patients at randomisation

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 6)</th>
<th>Group 2 (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>53.5 (47 – 58)</td>
<td>55 (45 – 61)</td>
</tr>
<tr>
<td><strong>Sex (M/F)</strong></td>
<td>3/3</td>
<td>7/2</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>75.3 ± 5.6</td>
<td>85.2 ± 7.2</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>26.5 ± 0.8</td>
<td>29.4 ± 2.6</td>
</tr>
<tr>
<td><strong>Child-Pugh grade (A/B/C)</strong></td>
<td>8/1/0</td>
<td>8/0/1</td>
</tr>
<tr>
<td><strong>Aetiology</strong></td>
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<tr>
<td>HCV</td>
<td>3</td>
<td>7</td>
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<tr>
<td>HBV</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>ALD</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Autoimmune</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Plasma creatinine (µmol/L)</strong></td>
<td>69.8 ± 4.9</td>
<td>75.1 ± 5.4</td>
</tr>
<tr>
<td><strong>Cholesterol (mmol/L)</strong></td>
<td>4.4 ± 0.3</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td><strong>LDL (mmol/L)</strong></td>
<td>2.5 ± 0.3</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td><strong>HDL (mmol/L)</strong></td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>1.4 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

Data are median (range), mean ± SEM or number of patients. Abbreviations: BMI, body mass index; HCV, hepatitis C virus; HBV, hepatitis B virus; ALD, alcoholic liver disease.

6.3.2. β-cell function and insulin sensitivity

Pancreatic glucose sensitivity in the 15 cirrhotic patients reduced by 17% following 3 months of treatment with nadolol compared to the same period on placebo (96.7 ± 15.6 to 80.5 ± 13.1 pmol min⁻¹ m⁻² mM⁻¹, p = 0.03). Over the same period there were no changes in the pancreatic rate sensitivity or potentiation and the changes in glucose sensitivity did not translate to changes in the basal secretory tone or total insulin secretion during the OGTT (Table 6-2). Nadolol was also associated with a
reduction of peripheral insulin sensitivity in the cirrhotic patients of $12.7 \pm 3.5 \mu\text{Lkg}^{-1}\text{min}^{-1}(\text{mU/L})^{-1}$ or 19% ($p < 0.005$).

Table 6-2 Insulin sensitivity and β-cell function parameters after 3-months treatment with nadolol and placebo in patients with liver cirrhosis

<table>
<thead>
<tr>
<th>n = 15</th>
<th>Nadolol</th>
<th>Placebo</th>
<th>Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity index</td>
<td>55.4 ± 5.5</td>
<td>68.1 ± 6.9</td>
<td>-12.7 ± 3.5</td>
<td>0.005</td>
</tr>
<tr>
<td>[µL/kg/min/(mU/L)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose sensitivity</td>
<td>80.5 ± 13.1</td>
<td>96.7 ± 15.6</td>
<td>-16.2 ± 6.9</td>
<td>0.03</td>
</tr>
<tr>
<td>(pmol min$^{-1}$ m$^{-2}$ mM$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate sensitivity</td>
<td>1099 ± 195</td>
<td>1142 ± 272</td>
<td>-43.2 ± 174</td>
<td>0.89</td>
</tr>
<tr>
<td>(pmol m$^{-2}$ mM$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potentiation</td>
<td>1.17 ± 0.08</td>
<td>1.16 ± 0.08</td>
<td>0.01 ± 0.08</td>
<td>0.85</td>
</tr>
<tr>
<td>(no unit)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal secretory tone</td>
<td>127 ± 15</td>
<td>129 ± 20</td>
<td>-1.8 ± 7.7</td>
<td>0.81</td>
</tr>
<tr>
<td>(pmol min$^{-1}$ m$^{-2}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total insulin secretion</td>
<td>60.9 ± 6.8</td>
<td>61.7 ± 8.3</td>
<td>-0.8 ± 4.0</td>
<td>0.63</td>
</tr>
<tr>
<td>(nmol m$^{-2}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SEM

* Linear mixed model analysis of variance

6.3.3. Glucose tolerance

One patient (patient 19) from Group 1 was not known to be diabetic but was subsequently found to have diabetes following the OGTT in the first period (while on placebo). Glucose tolerance worsened in 4 patients following treatment with nadolol for 3 months (Table 6-3). Three cirrhotic patients progressed from normal glucose tolerance to impaired glucose tolerance while another progressed from impaired glucose tolerance to diabetes mellitus. However the same patient that was
unexpectedly found to have diabetes (patient 19) improved from diabetes to impaired glucose tolerance on OGTT in the second period although her 120min glucose only decreased by 7% from 11.2 mmol/L to 10.4 mmol/L. Two patients (patients 21 and 22) were not properly fasted prior to their OGTT and this may have influenced their glucose tolerance while patient 19 may also have not been fully compliant with fasting. The changes in glucose tolerance were not statistically significant (p=0.15) despite the significant changes in β-cell function and insulin sensitivity.

Table 6-3 Glucose tolerance of 15 patients with liver cirrhosis as determined by oral glucose tolerance test following 3-months treatment with nadolol and placebo

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Nadolol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Normal glucose tolerance</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

* p = 0.15

6.3.4. Body composition

After treatment with nadolol for 3 months there were no changes in body weight, fat mass and percent fat mass in the cirrhotic patients (Table 6-4).

Table 6-4 Body composition following 3-months treatment with nadolol and placebo in patients with liver cirrhosis

<table>
<thead>
<tr>
<th></th>
<th>Nadolol</th>
<th>Placebo</th>
<th>Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net weight (kg)</td>
<td>81.8 ± 4.9</td>
<td>81.2 ± 4.9</td>
<td>0.6 ± 0.6</td>
<td>0.42</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>33.2 ± 2.2</td>
<td>32.4 ± 2.5</td>
<td>0.8 ± 0.6</td>
<td>0.27</td>
</tr>
<tr>
<td>Body fat (% body weight)</td>
<td>41.1 ± 2.4</td>
<td>40.4 ± 2.6</td>
<td>0.7 ± 0.6</td>
<td>0.33</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3 ± 1.6</td>
<td>28.1 ± 1.6</td>
<td>0.2 ± 0.2</td>
<td>0.45</td>
</tr>
</tbody>
</table>
6.3.5. Normoglycaemic cirrhotic patients

Six healthy volunteers were enrolled and their clinical characteristics compared to normoglycaemic cirrhotic patients while not on β-blockade in Table 6-5. All cirrhotic patients had compensated cirrhosis resulting from viral hepatitis.

Table 6-5 Clinical characteristics of normal glucose tolerant cirrhotic patients compared to healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Cirrhotics (n = 5)</th>
<th>Controls (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>52 (45 – 56)</td>
<td>45 (35 – 72)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>4/1</td>
<td>4/2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.8 ± 11.1</td>
<td>79.4 ± 4.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.9 ± 4.1</td>
<td>25.9 ± 1.0</td>
</tr>
<tr>
<td>Child-Pugh grade (A/B/C)</td>
<td>5/0/0</td>
<td>N/A</td>
</tr>
<tr>
<td>Aetiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>3</td>
<td>N/A</td>
</tr>
<tr>
<td>HBV</td>
<td>2</td>
<td>N/A</td>
</tr>
<tr>
<td>Plasma creatinine (µmol/L)</td>
<td>59.4 ± 5.6</td>
<td>75.7 ± 5.4</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.8 ± 0.2</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.1 ± 0.3</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.0 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.5 ± 0.5</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Data are median (range), mean ± SEM or number of patients. Abbreviations: BMI, body mass index; HCV, hepatits C virus; HBV, hepatitis B virus.
The sensitivity of pancreatic insulin secretion to glucose concentration was higher in patients with liver cirrhosis compared to healthy controls despite similar peripheral insulin sensitivity (Table 6-6). Basal secretory tone and fasting plasma insulin were also higher although this did not translate to an increased total insulin secretion over the course of the OGTT.

Table 6-6 Comparison of insulin sensitivity and β-cell function parameters between normoglycaemic cirrhotic patients and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Cirrhotics (n=5)</th>
<th>Controls (n=6)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin (mmol/L)</td>
<td>79.0 ± 15.3</td>
<td>34.3 ± 4.7</td>
<td>0.014</td>
</tr>
<tr>
<td>Glucose sensitivity (pmol min⁻¹ m⁻² mM⁻¹)</td>
<td>146.0 ± 21.6</td>
<td>86.8 ± 8.8</td>
<td>0.024</td>
</tr>
<tr>
<td>Rate sensitivity (pmol m⁻² mM⁻¹)</td>
<td>1562 ± 735</td>
<td>1253 ± 358</td>
<td>0.698</td>
</tr>
<tr>
<td>Potentiation (no unit)</td>
<td>1.00 ± 0.19</td>
<td>1.07 ± 0.08</td>
<td>0.716</td>
</tr>
<tr>
<td>Basal secretory tone (pmol min⁻¹ m⁻²)</td>
<td>115 ± 21</td>
<td>61 ± 8</td>
<td>0.03</td>
</tr>
<tr>
<td>Total insulin secretion (nmol m⁻²)</td>
<td>50.1 ± 4.97</td>
<td>40.9 ± 4.5</td>
<td>0.204</td>
</tr>
<tr>
<td>Insulin sensitivity index [µL/kgFFM/min/(mU/L)]</td>
<td>86.0 ± 8.4</td>
<td>117.9 ± 22.9</td>
<td>0.258</td>
</tr>
</tbody>
</table>
6.3.6. Adverse events

The incidence of adverse events for patients with liver cirrhosis did not differ when treated with nadolol or placebo (5 nadolol vs 2 placebo, p=0.45). One patient (Group 2) suffered severe headaches requiring treatment cessation part way through the second period. Postural hypotension in a further patient required a reduction of the nadolol dose to 40mg daily. All other adverse events were related to tiredness except for one patient (on nadolol) who experienced mild chest tightness with no changes in peak-flow measurement and was tolerated without changes in dosage.

6.3.7. Compliance with treatment

Compliance information was missing for the second period in two patients but was excellent in all other patients. Assessment of returned drug packaging indicated that except for 2 patients (88% and 89% compliance) all others took over 90% of their prescribed tablets and compliance overall averaged 97.4% for all patients.
6.4. Discussion

The impetus for this sub-study arose from the observation that β-blockade in patients with liver cirrhosis may lead to a reduction of β-cell function. The reduction of β-cell function was subtle and only apparent by calculating the disposition index. However as previously discussed in Section 5.2.7, the disposition index should be interpreted with caution. To confirm those findings, β-cell function in the same cohort of patients was re-examined using a mathematical model derived from the OGTT. The model confirmed that pancreatic glucose sensitivity was reduced following β-blockade, in addition to a reduction of insulin sensitivity. Other parameters of β-cell function including the basal secretory tone and total insulin secretion rate were unchanged.

These observations are consistent with the reported findings of previous studies. Insulin was secreted when β2-adrenergic receptors on isolated pancreatic β-cells were activated. Conversely insulin secretion was antagonised when β2-receptor blockers were administered349-351. In vivo insulin secretion was similarly stimulated by isoproterenol (non-selective β-adrenergic agonist) and the effect negated by the non-selective β-adrenergic antagonist propranolol352-354. Measurement of insulin secretion by IVGTT showed a reduced AIR after healthy participants were treated with atenolol for 10 days when compared to placebo348. However, insulin secretion in the above studies was measured by plasma insulin levels, a poor measure of insulin secretion due to the saturable kinetics of insulin clearance505 and the lack of a universal insulin assay154, 506. In addition, the clearance of insulin is unpredictably reduced in patients with liver cirrhosis507, 508.

β-cell function was measured in this study using a mathematical model proposed by Mari et al305. The model expresses β-cell function as the sum of two components (static and dynamic) and is coupled with a model of C-peptide kinetics501. Glucose sensitivity (the static component) is the ability of the β-cell to respond to changes of glucose concentration in a dose-response relationship and is increased by potentiation factors including incretin hormones, non-glucose substrates and the Staub-Traugott effect. The Staub-Traugott effect describes how consecutive loads of glucose improve the disposal of each subsequent load of glucose (discussed in Section 5.2.4). Rate sensitivity on the other hand represents the response of insulin secretion to the rate of change of glucose concentration and is the dynamic component of β-cell function.

An isolated reduction in glucose sensitivity suggests that nadolol may impair the glucose sensing apparatus of the pancreatic β-cell. The pancreas is innervated by the autonomic nervous system509 and blockade of the β2-adrenergic receptor has been associated with reduced expression of
peroxisome proliferator-activated receptor (PPAR) \( \gamma \). Consequently, \( \beta_{2} \)-adrenergic receptor knockout mice had lower fasting serum insulin and a reduction in early phase insulin secretion by 50\%. PPAR\( \gamma \) is a nuclear hormone receptor that regulates the expression of genes associated with the glucose sensing apparatus of the \( \beta \)-cell and fatty acid storage. When the expression of PPAR\( \gamma \) is reduced by \( \beta_{2} \)-receptor blockade the expression of the genes encoding for glucose transporter 2 (GLUT2), glucokinase, pancreatic and duodenal homeobox 1 (Pdx-1) and ATP-binding cassette transporter subfamily A member 1 (Abca1) are down-regulated. GLUT2 transports glucose into pancreatic \( \beta \)-cells which is then phosphorylated by glucokinase. Phosphorylation is the rate-limiting step of the insulin secretion cascade and glucokinase is also known as the pancreatic glucose sensor. Pdx-1 is a transcription factor necessary for pancreatic development and \( \beta \)-cell maturation while Abca-1 is a cellular cholesterol efflux transporter that prevents intracellular cholesterol accumulation and lipotoxicity. Thus down-regulation of PPAR\( \gamma \) directly impairs \( \beta \)-cell maturity and survival, as well as the uptake and “sensing” of glucose by the pancreatic \( \beta \)-cell.

When fasting normoglycaemic Child’s A patients with liver cirrhosis were compared with healthy controls, pancreatic glucose sensitivity and basal secretory tone was higher and associated with fasting hyperinsulinaemia despite the cirrhotic patients sharing the same insulin sensitivity with controls. An earlier study comparing Child’s B patients with liver cirrhosis and reduced insulin sensitivity with healthy volunteers observed an increase in pancreatic \( \beta \)-cell rate sensitivity and the basal secretory tone and total insulin secretion rate. The increase in glucose sensitivity was not statistically significant despite a 2.6 fold increase. Comparison of both studies suggests that changes in pancreatic glucose sensitivity and associated hyperinsulinaemia may precede and possibly predispose to changes in insulin sensitivity and other \( \beta \)-cell function parameters in patients with liver cirrhosis. Hyperinsulinaemia per se reduces peripheral insulin sensitivity in healthy volunteers and a similar reduction in insulin sensitivity in association with hyperinsulinaemia follows transjugular intrahepatic portosystemic shunt insertion in patients with liver cirrhosis but no diabetes. Vice versa, prolonged reduction of hyperinsulinaemia in patients with cirrhosis normalised their insulin sensitivity.

The present study did not explore other measures of \( \beta \)-cell function in patients with liver cirrhosis because results of different measures of \( \beta \)-cell function are not directly comparable and may test for different aspects of \( \beta \)-cell function. Treatment with nadolol resulted in a reduction of insulin sensitivity and \( \beta \)-cell function but not glucose tolerance. This may have been due to a Type 2 statistical error as 4 out of 15 patients had worse glucose tolerance. The single diabetic patient who improved her glucose tolerance had a borderline glucose level for diagnosis of diabetes on entry into the study (120 min glucose of 11.2 mmol/L) and may have been misclassified in view of the variability and poor reproducibility of the OGTT.
In conclusion, treatment with nadolol for 3 months leads to reduced pancreatic glucose sensitivity and peripheral insulin sensitivity. Compensated non-insulin resistant cirrhotic patients have an elevated pancreatic glucose sensitivity and basal secretory tone. The resulting hyperinsulinaemia may predispose to the subsequent reduction of insulin sensitivity in these patients but further longitudinal studies are required to confirm this finding.
Chapter 7. Conclusions

Several novel insights into the present understanding of energy metabolism in patients with liver cirrhosis and the strong association between liver cirrhosis and impaired metabolism of glucose have been described in this thesis. The utility of β-blockade for improving energy metabolism in patients with liver cirrhosis in contrast to the detrimental implications of β-blockade on the insulin sensitivity, β-cell function and glucose tolerance of the patients were also explored in some detail. Although the statistical power of the research was limited by the sample size of the study, this thesis contributes several important and novel observations to the literature.

We initially sought to determine the underlying reason for the wide variation in the reported prevalence of diabetes in patients with liver cirrhosis. Specifically, an association between prevalence of diabetes and aetiology or severity of liver cirrhosis has been postulated but not clarified. The systematic review in Chapter 3 confirmed that patients with liver cirrhosis have a prevalence of diabetes between 8% and 50% depending on the aetiology of liver disease. Patients with HCV, alcoholic, cryptogenic and NAFLD cirrhosis had the highest prevalence of diabetes whereas those with cholestatic cirrhosis did not have an increased prevalence of diabetes compared to the normal population. The true prevalence of liver cirrhosis is not known but has been estimated to be between 0.5% and 1.1%\(^2\). This suggests that up to 40 000 New Zealanders may have liver cirrhosis, and of those, up to 20 000 may have synchronous diabetes. The association of diabetes with a poorer outcome highlights the importance of formal screening for diabetes in cirrhotic patients, especially in those at higher risk due to the aetiology of their liver disease. Lastly, despite the large number of studies published on this subject, the review identified a dearth of well-designed studies investigating the prevalence of diabetes in cirrhotic patients with appropriate control for selection bias and potential confounding factors.

Hypermetabolism (elevated REE) has previously been shown to be associated with a worse prognosis in liver cirrhosis, even within the normal range of REE. In addition, patients taking β-blockers for prophylaxis against variceal haemorrhage were less likely to have an elevated REE. We therefore hypothesised that by reducing REE β-blockers could potentially ameliorate the excess mortality associated with an elevated REE in liver cirrhosis. To investigate this we undertook a randomised, double blind, placebo controlled trial of a non-cardioselective oral β-blocker (nadolol) in patients with liver cirrhosis (Chapter 4). Patients already taking β-blockers for prophylaxis against variceal haemorrhage were not eligible, so our study population comprised mainly compensated patients with normal REE at baseline. REE was lower on nadolol than placebo but the difference was
not statistically significant. Nevertheless, REE on nadolol was reduced compared to baseline and the reduction correlated with baseline REE and REE/REEp. A larger study including patients with hypermetabolism would be needed to confirm these findings. Furthermore, a trial demonstrating improved outcomes would then be needed to justify a clinical role for β-blockers in this context.

The use of β-blockers is strongly associated with reduced insulin sensitivity and the development of new-onset diabetes in patients with an existing predisposition such as hypertension and type 2 diabetes mellitus. We utilised the opportunity created by the randomised controlled trial to measure changes in insulin sensitivity and glucose tolerance in the patients with liver cirrhosis following treatment with nadolol or placebo (Chapter 5). These were secondary endpoints and the sample size may not have been sufficient to enable full characterisation of the effect of β-blockers on glucose homeostasis. Nevertheless, insulin sensitivity was significantly reduced following treatment with β-blockers whereas the reduction in glucose tolerance was not statistically significant. Given the frequent use of β-blockers for prophylaxis against variceal haemorrhage, these patients should be screened for diabetes prior to treatment and periodically thereafter, in addition to on-going monitoring of glycaemic control in those found to have diabetes. Furthermore carvedilol (a non-selective β-blocker combined with α-blocking properties) may have some advantages over pure β-blockade in liver cirrhosis since carvedilol does not worsen, and may even improve insulin sensitivity in hypertensive patients. Further clinical trials are needed to evaluate this hypothesis in patients with liver cirrhosis.

β-cell function (in addition to insulin sensitivity) is an important determinant of glucose tolerance and a small series of reports observed a subtle reduction of β-cell function in patients with hypertension following treatment with β-blockers. We applied a similar test of β-cell function formalised as the disposition index to our cohort of patients with liver cirrhosis and found a similar reduction of β-cell function (Chapter 5). More sophisticated mathematical modelling showed that the sensitivity of the pancreatic β-cell to the prevailing blood glucose concentration (pancreatic glucose sensitivity) was reduced in these patients in addition to a reduction of insulin sensitivity. This pancreatic β-cell defect following treatment with nadolol may be mediated by glucokinase (the β-cell glucose sensing enzyme) and the role of glucokinase in mediating β-cell function following treatment with nadolol warrants further investigation. β-blockade may also inhibit the secretion of incretin hormones\textsuperscript{523, 524} and contribution of incretin hormones to the reduction of β-cell function should be clarified. Furthermore, the use of β-blockers in other disease settings such as hypertension will need to be re-assessed for possible detrimental changes in β-cell function.

Lastly, mathematical modelling of the 5 time point OGTT glucose and C-peptide data in Chapter 6 unexpectedly found that β-cell function was up-regulated in otherwise compensated patients with viral
cirrhosis and no evidence of reduced insulin sensitivity. These patients selectively showed an increase in pancreatic glucose sensitivity which has not been previously described. If confirmed, this finding may overturn the current paradigm for the pathogenesis of IGT and diabetes mellitus in patients with liver cirrhosis. At present, IGT and diabetes are thought to result from failure of the pancreatic β-cell (due to a relative reduction of β-cell function) to compensate for an already established reduction of insulin sensitivity, and not vice versa. Alternatively, chronic hyperinsulinaemia as a result of increased pancreatic glucose sensitivity can independently precipitate a reduction of insulin sensitivity. A further study would be needed to confirm these findings and to determine if it may be generalised to other patients with different aetiologies of liver cirrhosis. The proposed mechanism for the up-regulation of β-cell function also needs to be tested. In particular, the incretin effect generally plays an important role in the up-regulation of β-cell function but the incretin effect has not been quantified in cirrhotic patients to date.

The work presented in this thesis has helped characterise the prevalence of diabetes among patients with liver cirrhosis and highlighted the need for more well-designed studies. The randomised controlled trial of β-blockade in patients with liver cirrhosis has shown a lack of efficacy for the reduction of REE in stable patients without hypermetabolism but suggests a possible role in hypermetabolic patients that remains to be confirmed. Lastly, detailed assessment of the glucose metabolism of this cohort of patients with liver cirrhosis using the OGTT, IVGTT and the hyperinsulinaemic euglycaemic clamp provided insights into the mechanism by which nadolol influences glucose metabolism and also a previously unreported early defect in the β-cell function of normoglycaemic patients with liver cirrhosis. Ongoing work following this thesis will focus on defining the role of nadolol in hypermetabolic patients with liver cirrhosis and clarifying the mechanisms underlying the changes in glucose metabolism highlighted by this thesis.
Appendix

7.1. Appendix 1 - Example of Search Strategy: Medline (OVID)

1. Fibrosis/
2. fibrosis.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
3. fibroses.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
4. cirrhosis.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
5. 4 or 1 or 3 or 2
6. diabetes mellitus/ or diabetes mellitus, type 2/ or prediabetic state/ or hyperglycemia/ or glucose intolerance/ or hyperinsulinism/
7. diabetes mellitus, type 2.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
8. diabetes mellitus, ketosis-resistant.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
9. diabetes mellitus, ketosis resistant.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
10. ketosis-resistant diabetes mellitus.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
11. diabetes mellitus, maturity-onset.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
12. diabetes mellitus, maturity onset.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
13. diabetes mellitus, non insulin dependent.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
14. diabetes mellitus, non-insulin-dependent.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
15. non-insulin-dependent diabetes mellitus.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
16. type 2 diabetes mellitus.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
17. diabetes mellitus, slow-onset.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
18. diabetes mellitus, slow onset.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
19. slow-onset diabetes mellitus.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
20. diabetes mellitus, stable.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
21. stable diabetes mellitus.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
22. diabetes mellitus, type ii.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
23. maturity-onset diabetes mellitus.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
24. maturity onset diabetes mellitus.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
25. mody.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
26. niddm.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
27. diabetes mellitus, adult-onset.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
28. adult-onset diabetes mellitus.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
29. diabetes mellitus, adult onset.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
30. diabetes mellitus, noninsulin dependent.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
31. prediabetic state.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

32. prediabetic states.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

33. state, prediabetic.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

34. states, prediabetic.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

35. prediabetes.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

36. hyperinsulinism.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

37. hyperinsulinaemia.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

38. exogenous hyperinsulinism.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

39. hyperinsulinism, exogenous.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

40. compensatory hyperinsulinemia.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

41. hyperinsulinaemia, compensatory.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

42. endogenous hyperinsulinism.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

43. hyperinsulinism, endogenous.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

44. hyperglycemia.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

45. hyperglycemias.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

46. hyperglycemia, postprandial.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
47. hyperglycemias, postprandial.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

48. postprandial hyperglycemias.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

49. postprandial hyperglycemia.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

50. glucose intolerance.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

51. glucose intolerances.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

52. intolerance, glucose.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

53. intolerances, glucose.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

54. 31 or 30 or 19 or 6 or 24 or 15 or 16 or 28 or 14 or 42 or 53 or 25 or 23 or 26 or 38 or 12 or 18 or 47 or 22 or 8 or 29 or 33 or 9 or 51 or 46 or 40 or 20 or 44 or 11 or 21 or 27 or 48 or 37 or 34 or 49 or 7 or 39 or 10 or 45 or 13 or 50 or 36 or 32 or 43 or 35 or 41 or 17 or 52

55. 54 and 5
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