CHAPTER 1

LITERATURE REVIEW

1.1 NON ALCOHOLIC FATTY LIVER DISEASE

1.1.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized as one of the commonest causes of chronic liver disease worldwide that may progress to end stage liver disease. The pathological picture resembles that of alcohol induced liver disease even though alcohol is not abused.\textsuperscript{1,2} Risk factors for NAFLD include obesity, type 2 diabetes, hyperlipidaemia, total parenteral nutrition, jejuno-ileal bypass surgery and the use of certain drugs.\textsuperscript{3} A variety of other terms have been used to describe this entity such as fatty liver disease, diabetic hepatitis, alcohol like liver disease, and hepatic steatosis, a more serious subset of NAFLD.\textsuperscript{3,4}

1.1.2 Definition

NAFLD comprises a spectrum of liver disease, ranging from simple steatosis to steatohepatitis, advanced fibrosis and the potential to
progress to cirrhosis with its inherent complications of hepatic failure and hepatocellular carcinoma.\textsuperscript{3, 4} Non-alcoholic steatohepatitis or NASH represents a stage within the spectrum of NAFLD and is defined pathologically by the presence of steatosis together with necro-inflammatory activity.\textsuperscript{5}

\subsection*{1.1.3 Epidemiology}
NAFLD affects 10\% to 24\% of the general population in various countries.\textsuperscript{4} This prevalence increases to 57.5\%\textsuperscript{6} to 74\%\textsuperscript{7, 8} in obese persons. It is a common explanation for abnormal liver function tests in blood donors and a cause of asymptomatic elevation of liver enzymes in up to 90\% of cases once other causes of liver diseases are excluded.\textsuperscript{9} With the increasing prevalence of obesity, it is estimated that at least about a quarter of the American adult population currently has NAFLD. Approximately 20\% to 30\% of patients with NAFLD will develop fibrosing steatohepatitis that may progress to cirrhosis, end-stage liver disease, and hepatocellular carcinoma.\textsuperscript{10} The prevalence of NAFLD in the different population groups of South Africa is unknown.
1.1.4 Characteristics

a) Clinical features

Patients with NAFLD are generally asymptomatic.\textsuperscript{3, 4} It is often diagnosed after abnormalities are noted during “routine” laboratory testing.\textsuperscript{11} Patients may report fatigue or malaise and a sensation of fullness or discomfort on the right side of the upper abdomen.\textsuperscript{3, 4} Hepatomegaly may be the only physical sign.\textsuperscript{3, 4} Acanthosis nigricans may be found in children with NAFLD.\textsuperscript{12, 13} Other signs may include signs of chronic liver disease such gynaecomastia, spider naevi, testicular atrophy and palmar erythema.

b) Laboratory Abnormalities

Elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) or both are a common laboratory finding in patients with NAFLD. The ratio of AST to ALT in subjects with NAFLD is usually less than 1, but this ratio increases as fibrosis advances, leading to a loss of its diagnostic accuracy in patients with cirrhotic NAFLD.\textsuperscript{14}
Serum alkaline phosphatase, gamma glutamyltransferase or both are often above the normal range in many patients. Other laboratory abnormalities include hypoalbuminaemia, a prolonged prothrombin time, and hyperbilirubinemia, but this is usually only found in end stage liver disease.

c) Imaging Studies

Fatty infiltration of the liver produces a diffuse increase in echogenicity as compared with that of the kidneys on ultrasonography. Regardless of the cause, cirrhosis has a similar appearance on ultrasonography. Ultrasonography has a sensitivity of 89%, and a specificity of 93% in detecting steatosis. It has a sensitivity of 77% and a specificity of 89% in detecting fibrosis. Fatty infiltration of the liver produces a low density hepatic parenchyma on computed tomographic scanning. Steatosis is generally diffuse however, occasionally it may be focal, and hence may be misinterpreted as showing malignant liver masses. In such cases, magnetic resonance imaging can help distinguish the two.
d) Histology

The histologic features of NAFLD are indistinguishable from those of alcohol-induced liver disease.\(^4\) This includes steatosis (fatty liver), which is predominantly macrovesicular and generally diffusely distributed throughout the liver lobule. Microvesicular steatosis has been reported occasionally. It can progress to a hepatitis (parenchymal inflammation with or without accompanying focal necrosis) where there is lobular infiltration with both acute and chronic inflammatory cells and Mallory body formation, and it may result in variable degrees of perivenular and sinusoidal fibrosis progressing to cirrhosis.\(^1, 3, 16, 21-24\)

### 1.1.5 Pathogenesis of NAFLD

The pathogenesis of NAFLD has remained poorly understood since the earliest description of the disease. A growing body of literature have documented the close relationship of NAFLD with the metabolic syndrome, which is characterized by abdominal obesity, insulin resistance with or without frank hyperglycaemia, dyslipidaemia, and hypertension.\(^10\)
However, much of the current thinking remains hypothetical, since the mechanism or mechanisms remain uncertain. A current concept in the pathogenesis of NAFLD involves a “two-hit” hypothesis in which an initial metabolic disturbance, namely insulin resistance causes steatosis, and a second pathogenic stimulus causes oxidative stress, reactive oxygen species generation, lipid peroxidation, and resultant steatohepatitis.

Another theory put forward is that NAFLD is almost certainly a polygenic disease affected by environmental factors and stressors, with disease pathogenesis related to “multiple hits.” Although the metabolic syndrome and insulin resistance are important risk factors for the development of NAFLD, our current knowledge of the genetic, biochemical, metabolic, inflammatory, endocrine, and environmental mechanisms responsible for the pathogenesis of NAFLD are poorly understood.

It is also not fully understood why simple steatosis develops in some patients, whereas steatohepatitis and progressive disease develop in others.

Differences in Body Mass Index (BMI) or antioxidant systems in the context of genetic predisposition may be an explanation. Retention of
lipids within hepatocytes, mostly in the form of triglycerides is a prerequisite for the development of NAFLD.\textsuperscript{4} The primary metabolic abnormality leading to this, is not well understood. It is thought that it may be as a result of alterations in the pathways of uptake, synthesis, degradation or secretion in hepatic lipid metabolism resulting in insulin resistance which is a reproducible factor in the development of NAFLD.\textsuperscript{25}

The molecular pathogenesis of insulin resistance is multifactorial, and several molecular targets involved in the inhibition of insulin action have been identified (Figure 1). These include Rad (Ras associated with diabetes, an enzyme, which is a member of the Rad-guanosine triphosphatase superfamily),\textsuperscript{26} which interferes with several essential cell functions, such as growth, differentiation, vesicular transport and signal transduction; PC-1, a membrane glycoprotein that has a role in insulin resistance,\textsuperscript{27} reduces insulin-stimulated tyrosine kinase activity; leptin,\textsuperscript{28} which induces dephosphorylation of insulin-receptor substrate-1; fatty acids,\textsuperscript{29} which inhibit insulin-stimulated peripheral glucose uptake; and tumour necrosis factor $\alpha$,\textsuperscript{30} which down regulates insulin-
induced phosphorylation of insulin receptor substrate-1 and reduces the expression of the insulin-dependent glucose-transport molecule Glut 4. Insulin resistance leads to fat accumulation in hepatocytes by two mechanisms: lipolysis and hyperinsulinemia. Significant amounts of dicarboxylic acids, which are potentially cytotoxic, can be formed by microsomal \( \omega \)-oxidation. This pathway of fatty acid metabolism is closely related to mitochondrial \( \beta \)-oxidation and peroxisomal \( \beta \)-oxidation. Deficiency of the enzymes of peroxisomal \( \beta \)-oxidation has been recognised as an important cause of microvesicular steatosis and steatohepatitis.\textsuperscript{31} Deficiency of acyl-coenzyme A oxidase disrupts the oxidation of very-long-chain fatty acids and dicarboxylic acids, leading to extensive microvesicular steatosis and steatohepatitis. Loss of this enzyme also causes sustained hyperactivation of peroxisome-proliferator-activated receptor-\( \alpha \) (PPAR-\( \alpha \)), leading to transcriptional up-regulation of PPAR-\( \alpha \) regulated genes.\textsuperscript{31} PPAR-\( \alpha \) has been implicated in promoting hepatic synthesis of uncoupling protein-2, which is expressed in the liver of patients with NAFLD.\textsuperscript{32} Increased intrahepatic levels of fatty acids provide a source of oxidative
stress, which may in large part be responsible for the progression from steatosis to steatohepatitis to cirrhosis. Mitochondria are the main cellular source of reactive oxygen species, which may trigger steatohepatitis and fibrosis by three main mechanisms, a) lipid peroxidation, b) cytokine induction and c) induction of Fas ligand. Patients with steatohepatitis have ultrastructural mitochondrial lesions, that is absent in patients with simple steatosis and in healthy subjects. They also slowly resynthesize ATP in vivo after a fructose challenge, which usually causes acute hepatic ATP depletion. This impaired ATP recovery may reflect the mitochondrial injury found in patients with steatohepatitis. Although, symptoms of liver disease rarely develop in patients with the metabolic syndrome, the steatotic may be vulnerable to further injury when challenged by additional insults. This has lead to the presumption that progression from simple steatosis to steatohepatitis and advanced fibrosis results from two distinct events. Firstly, insulin resistance leads to fat accumulation within hepatocytes and secondly, mitochondrial reactive oxygen species cause lipid peroxidation, cytokine oxidation and
the induction of Fas ligand (Figure 2). Lipid peroxidation results in end-products, malondialdehyde and 4-hydroxynonenal, which have proinflammatory and profibrogenic properties, may account for the typical histological features observed in NASH. The correlation between the degree of lipid peroxidation and the amount of fat in the liver also provides an explanation for the association between the severity of steatosis and the risk of NASH.

In summary, the two metabolic abnormalities most strongly associated with NAFLD are insulin resistance and an increased supply of fatty acids to the liver. These advances in the understanding of the pathogenesis of NAFLD have lead to the rational design of therapy for this disorder.
Figure 1: The First Hit for NAFLD: [Adapted from Ref 4]
Figure 2: The Second Hit for NAFLD: [Adapted from Ref 4]
1.1.6 Diagnosis of NAFLD

NAFLD is usually suspected in persons with asymptomatic elevation of aminotranferase levels, radiologic findings of fatty liver, or unexplained persistent hepatomegaly.\textsuperscript{4} Histology of a liver biopsy specimen is generally the gold standard. Whilst the typical features on liver biopsy are well described, given the anticipated high numbers of patients having NAFLD in the context of a high prevalence of both obesity and diabetes, it could be argued that the number of patients likely to warrant a biopsy would be enormous.\textsuperscript{40} However, a liver biopsy may be considered when other hepatic pathologies need to be excluded and it may also be helpful in ascertaining the extent of inflammation so as to separate simple steatosis from NASH, which may have important prognostic implications. A biopsy is also expensive and invasive, and it is associated with the risk of complications. It has therefore been argued that although there is promise of new treatments, the only effective therapy at present for the obese patient is weight loss, and why subject these patients to a liver biopsy?\textsuperscript{40} There is also the opportunity for sampling errors. Histologic lesions of NASH are unevenly distributed throughout the liver parenchyma, therefore, sampling error of liver biopsy
can result in substantial misdiagnosis and staging inaccuracies. Therefore, each case has to be assessed individually. Recently, measurements of serum concentration of adipocytokines have been used as an assessment for separating patients with bland steatosis from NASH, suggesting that liver biopsies may become unnecessary in the majority of patients with suspected NAFLD. Recent studies have found that serum levels of leptin and resistin were significantly higher in patients with NASH as compared to those with simple steatosis, whereas serum levels of adiponectin were significantly lower. This suggests that these adipocytokines may play a role in the pathogenesis of NAFLD. Leptin is a mediator of hepatic inflammation and fibrosis in NAFLD, and resistin and adiponectin are important modulators of insulin resistance, a central factor in the pathogenesis of NAFLD.

1.1.7 Role of Obesity and Alcohol

Obesity increases the risk of both fatty liver and cirrhosis in patients who consume alcohol, the mechanism of which remains obscure. The pathological picture of NASH resembles that of alcohol induced liver injury, but it occurs in patients who do not abuse alcohol as stated earlier.
Obesity increases hepatic exposure to alcohol; this is because ingested alcohol accumulates in the excessive adipose tissue of obese individuals due to its lipid solubility, prolonging the alcohol exposure from social drinking.\cite{45} Long-term alcohol intake can also alter enteric flora, resulting in the overgrowth of gram negative bacteria. Increased permeability produces endotoxaemia, which activates Kupffer cells to up-regulate lipopolysaccharide receptors and mediators such as macrophage inflammatory protein 2, prostaglandin E\textsubscript{2}, tumour necrosis factor-\alpha, and \alpha-hydroxyethyl. This can result in steatohepatitis. Subsequently, fibrogenesis results from the activation of hepatic stellate cells, which are responsible for the overproduction of the extracellular matrix that produces the fibrous scar.\cite{47}

It is important to note that even small increases in gut derived ethanol might increase portal blood alcohol levels enough to induce hepatic steatosis, given evidence that alcohol produces obligatory redox changes that promote the accumulation of triglycerides in hepatocytes.\cite{45} Evidence has also suggested that obesity is associated with alterations in gut motility, because of decrease in sensitivity to neuropeptides such as cholecystokinin and bombesin.\cite{48} This may favour bacterial overgrowth.
Therefore, there is a possibility that obese individuals, who do not consume alcohol, are capable of producing and metabolising alcohol endogenously, and this possibility may play a role in the pathogenesis of obesity related fatty liver disease and merits further consideration.45

A recent study showed that breath collected from genetically obese mice, ob/ob mice showed a higher alcohol content than their lean littermates. Hence, even in the absence of alcohol ingestion, alcohol could be detected in exhaled breath, reflecting an increase production of alcohol by intestinal bacteria and hence probably contributing to the pathogenesis of obesity related fatty liver disease.45

Another study in human subjects found that the breath alcohol levels in obese individuals where higher as compared to lean controls.46

Of note, because of efficient hepatic metabolism of alcohol, this eliminates most of the toxin before it reaches the systemic circulation, resulting in blood alcohol determinations which underestimate the actual production of alcohol in the gut.45 Hence it is important to look at urine and breath levels of alcohol, apart from just blood levels.
1.1.8 Alcohol Metabolism and the Liver

Normal liver function is essential for life. Alcohol-induced liver damage disrupts the body’s metabolism, eventually impairing the function of other organs. Most of the alcohol that a person imbibes is eventually broken down by the liver. Alcohol is readily absorbed from the gut. The metabolism of ethanol occurs in the cytosol via hepatic alcohol dehydrogenase (ADH) and certain microsomal enzymes. Alcohol cannot be stored and must be metabolised, which produces acetaldehyde, this is then rapidly biotransformed by aldehyde dehydrogenase (ALDH) in the cytosol and mitochondria to acetoacetate.

These by-products are toxic to the liver and other organs. Methanol is metabolized primarily in the liver through similar sequential oxidative steps to formaldehyde, formic acid and carbon dioxide. The initial step involves oxidation to formaldehyde by hepatic ALDH. Formaldehyde is then oxidized by formaldehyde dehydrogenase to formic acid/or formate depending on the pH. In the final step, formic acid is detoxified to carbon dioxide by folate-dependent reactions.

If methanol is co-ingested with a significant amount of alcohol, the
methanol conversion is temporarily blocked since ethanol has nine times greater affinity for alcohol dehydrogenase than does methanol. This allows elimination of methanol from the blood via the urine and exhaled air before conversion to formaldehyde.\textsuperscript{49} The by-products of alcohol are more toxic than alcohol itself. These metabolites accumulate within cells and interfere with normal cellular function, sometimes without ever reaching levels that are detectable in body fluids or tissues, and affecting virtually every system in the body, especially the liver.\textsuperscript{49}

\textbf{1.2 METABOLIC SYNDROME}

\textbf{1.2.1 Introduction}

The metabolic syndrome is a metabolic disorder that is becoming more common with the increased prevalence of obesity.\textsuperscript{50} The disorder is defined in various ways, and a new definition was recently proposed by the International Diabetes Federation (IDF).\textsuperscript{51} In my study, the latest National Cholesterol Education Program’s Adult Treatment Panel III (NCEP: ATP III) criteria \textsuperscript{53} were used.
The NCEP: ATP III identified the metabolic syndrome as a multiplex risk factor for cardiovascular disease (CVD). However, according to the new stricter IDF definition, for a person to be defined as having the metabolic syndrome they must have central obesity plus any two other factors as listed in figure 4.

The concept of the metabolic syndrome has existed for at least 80 years. In 1988, Reaven resurrected the syndrome and noted that several risk factors, in particular dyslipidaemia, hypertension, hyperglycaemia, commonly cluster together in what he called Syndrome X.54

Even though CVD is a primary clinical outcome of the metabolic syndrome, most people with this syndrome have insulin resistance, which increases the risk for type 2 diabetes.52 Other associations of the metabolic syndrome include hyperuricaemia, gout, gallbladder disease, polycystic ovary syndrome, hypertension, sleep disturbances, asthma, some forms of cancer as well as NAFLD.52

1.2.2 Components of the Metabolic Syndrome

Six components of the metabolic syndrome that relate to CVD, have been identified by the ATP III:52,53
a) Abdominal obesity as measured by waist circumference

b) Atherogenic dyslipidaemia, manifested by raised concentrations of serum triglycerides and low concentrations of HDL cholesterol.

c) Hypertension, which is strongly associated with obesity and insulin resistance.

d) Glucose intolerance, that may later evolve into frank diabetes mellitus, constituting a major independent risk factor for CVD.

e) A proinflammatory state, manifests clinically by a raised hs C-reactive protein (hs CRP)

f) A prothrombotic state, recognized by an elevation in the levels of plasminogen activator inhibitor-1 (PAI-1) and fibrinogen.

1.2.3 Pathogenesis of the Metabolic Syndrome

There are several proposed aetiological factors. These include the following:

a) Obesity and Abdominal Body Fat Distribution

Obesity is a chronic condition characterised by an excess of body fat. It is most often defined by the Body Mass Index (BMI), a mathematical formula
that is highly correlated with body fat. BMI is the weight in kilograms divided by the height in metres squared (kg/m²). The World Health Organization recognises the following classification system for health consequences of obesity using the BMI. A BMI of more than or equal to 25kg/m² is defined as overweight, which is further classified as pre-obese with index between 25.0 and 29.9 kg/m², and a BMI above 30 kg/m² is categorised as obese.55 Those with a BMI of more than and equal 40.0 kg/m² are morbidly obese.56 Obesity has reached epidemic proportions in America, effecting 25% to 30% of the population.10 The epidemic however, is no longer confined to the USA. In South Africa, the prevalence of obesity is about 19%.57 These figures parallel the rise in insulin-resistant states such as the metabolic syndrome and type 2 diabetes.10 Obesity contributes to hypertension, high serum cholesterol, low HDL cholesterol and hyperglycaemia and is associated with a higher CVD risk. Abdominal obesity is strongly associated with the other metabolic risk factors. Excess adipose tissue releases several by-products which aggravate the risk. These include nonesterified fatty acids (NEFA), which overload muscle with lipids that are later diverted to the liver enhancing insulin resistance and promoting fatty liver. Other products include, high interleukin 6, resistin, TNF α and CRP
levels signifying a proinflammatory state and an elevated PAI-1 contributing to a prothrombotic state. Low adiponectin levels on the other hand correlate inversely with the degree of obesity.

b) Insulin Resistance:

It is thought that insulin resistance plays a greater role than obesity in the pathogenesis of the metabolic syndrome, where it directly causes other metabolic risk factors.\textsuperscript{54, 58} However, trying to assign a unique role for insulin resistance is complicated by the fact that it is linked to obesity making the identification of the cause and effects difficult. Insulin resistance rises with an increasing BMI, yet a broad range of insulin sensitivities exist at any given BMI.\textsuperscript{59} Insulin resistance may exist even with BMIs of less than 25 kg/m\textsuperscript{2} such as in the South Asians. In fact, it may contribute to a high prevalence of type 2 diabetes and premature CVD in this population group. They can be said to have primary insulin resistance. However, even with primary insulin resistance, weight gain may worsen insulin resistance and the metabolic syndrome. Thus, the two appear to be closely linked and are not easily dissociated.\textsuperscript{52}
c) Other Independent and Contributing Factors that mediate specific components of the Metabolic syndrome:

Each risk factor of the metabolic syndrome is subject to its own regulation by both genetic and acquired factors and, as a result, there is a variation in their expression. For example, lipoprotein metabolism is modulated by genetic variation, hence the expression of dyslipidaemias in response to obesity and/or insulin resistance varies considerably. Glucose levels depend on insulin secretory capacity as well as insulin sensitivity. Endocrine factors, advancing age and proinflammatory states have also been implicated.52, 60

1.2.4 Diagnosis of the Metabolic Syndrome

Using the ATP III criteria for the diagnosis of the metabolic syndrome, the clinical signs of the metabolic syndrome can be easily detected (Figure 3). It is critical to ensure proper diagnosis, dissemination of information to patients regarding their risks and appropriate therapies, and initiation of appropriate therapies. The evaluation of patients who present features suggestive of metabolic syndrome should include the following:

a) Measurement of vital signs including blood pressure, and body weight;

b) Measurement of waist circumference;
c) Measurement of fasting blood sugar; and

d) A fasting lipid profile (total cholesterol, HDL-C, TG, and a calculated LDL-C)

According to the ATP III definition, a subject has the metabolic syndrome if 3 or more of the criteria listed in Figure 3 are present. These are similar to the Guidelines for Type 2 Diabetes at Primary Care in 2003 published by the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA).  

Figure 3. The ATP III Diagnostic Criteria for the Metabolic Syndrome [53]

1. Abdominal obesity * (waist circumference > 102 cm [40 in] in men, > 88 cm [35 in] in women)
2. Hypertriglyceridaemia (>=1.7mmol/L [>= 150 mg/dL] ) or drug treatment for elevated TG).
3. Low HDL-C (<1.03mmol/L [< 40 mg/dL] in men, <1.3mmol/L [< 50 mg/dL] in women or drug treatment for reduced HDL-C).
4. High blood pressure (>= 130/85 mm Hg or drug treatment for hypertension).
5. High fasting glucose (>=5.6mmol/L [>= 100 mg/dL ] or drug treatment for elevated glucose)

*These cut offs are lower for Europids, >= 94 cm in men and >= 80 cm in women, and even lower for South Asians >= 90 cm in men and >= 80 cms
in women. For South Africa (Sub-Saharan Africans), the Europid cut offs should probably be used, and for Asians, the South Asian cut offs.\textsuperscript{51}

**Figure 4. The IDF Diagnostic Criteria for the Metabolic Syndrome\textsuperscript{[51]}**

According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have:

- Central obesity (waist circumference $\geq 94$ cm in men, $\geq 80$ cm in women)*
- **Plus** any two of the following four factors:
  1. Hypertriglyceridaemia ($\geq 1.7$ mmol/L [$\geq 150$ mg/dL]), or specific treatment for this lipid abnormality
  2. Low HDL-C ($< 1.03$ mmol/L [$< 40$ mg/dL] in men, $< 1.3$ mmol/L [$< 50$ mg/dL] in women), or specific treatment for this lipid abnormality
  3. High blood pressure (systolic BP $\geq 130$ or diastolic BP $\geq 85$ mm Hg), or treatment of previously diagnosed hypertension
  4. High fasting glucose ($\geq 5.6$ mmol/L [$\geq 100$ mg/dL]), or previously diagnosed type 2 diabetes

* Using the Europid cutoffs