Chapter I

Introduction

The consumption of fructose, primarily from high-fructose corn syrup (HFCS), has increased considerably in the United States during the past several decades. The increase in HFCS consumption exceeds the increase intake of any other food or food group. high-fructose corn syrup is now used extensively in sugar sweetened carbonated beverage (SSCB) and other sweetened drinks, baked goods, candies, canned fruits, jams, jellies and dairy products (Alan and Gaby 2005). Some nutritionists believe fructose is a safer form of sugar than sucrose, particularly for people with diabetes mellitus, because it does not adversely affect blood-glucose regulation, at least in the short-term.

However, fructose has potentially harmful effects on other aspects of metabolism. Rats feeding with fructose is used as an animal model of insulin resistance and is considered parallel to non- alcoholic fatty liver disease (NAFLD) observed in humans (Ackerman *et al*. 2005). Excessive fructose consumption may be responsible in part for the increasing prevalence of obesity, diabetes mellitus and non-alcoholic fatty liver disease (Alan and Gaby 2005).

It has been proposed that dietary habits especially fast-foods may promote NAFLD directly by modulating hepatic triglyceride accumulation and antioxidant activity and indirectly by affecting insulin sensitivity and postprandial triglyceride metabolism (Musso *et al*. 2003).

High dosage of fructose in the diet (HFD) has been documented to induce insulin resistance accompanied by deleterious metabolic consequences including hyperinsulinaemia, hyperglycaemia, glucose intolerance, hypertriglyceridaemia and hypertension in rodents (Thoroburn *et al.* 1989). The condition presents profound alterations in the metabolic pathways regulated by insulin. It has been shown that reactive oxygen species (ROS) are elevated in HFD and can interfere with nitric oxide (NO) production, which maintains vascular relaxation in resistance arteries (Taniyama and Griendling 2002).

Nitric oxide synthase (NOS) activity itself is also reduced in HFD (Miatello *et al*. 2001). Thus, a reduction of NOS activity and an increase of ROS production in HFD together could lead to decrease NO bioavailability, resulting in the increased vascular contraction and cardiovascular risk could be seen in this model. Moreover, in rats, fructose administration increases hepatic lipid peroxidation (LPO) and activation of inflammatory pathways (Cline *et al.* 1999).

Several compounds have the ability to enhance the cell utilization of fatty acids and optimizing the production of ATP by mitochondria including carnitine (CAR). Carnitine (L-3-hydroxy-4-N,N,N-trimethylaminobutyrate) is an essential metabolic mediator, which has a number of indispensable roles in intermediary metabolism (Evans and Fornasini 2003).

In humans, the carnitine supply is derived in part from food and in part by endogenous synthesis from lysine and methionine (Koumantakis *et al.* 2003). Exogenous L-carnitine is used clinically for the treatment of carnitine deficiency disorders and a range of other conditions (Vaz and Wanders 2002). Carnitine concentration is increased in the muscle and liver of obese women (Harper and Wadstrom 1995), and also in the plasma of starved rats (Sandor *et al.* 1990) and

humans (Hoppel and Genuth 1980). Carnitine functions to transport long-chain fatty acids across the inner mitochondrial membrane into the matrix for β-oxidation and has effects on oxidative metabolism of glucose in tissues (Broderick *et al.* 1992).

Supplementation studies show that exogenous carnitine reduces the levels of plasma very low density lipoprotein cholesterol (VLDL-C) and very low density lipoprotein triglycerides (VLDL-TG) in hyperlipidemic rabbits (Leighton *et al.* 1995) and plasma lipoprotein(a) levels in type 2 diabetic patients with hypercholesterolemia (Derosa *et al.* 2003).

Chapter II

Review of Literatures

2.1.Metabolic Syndrome

Metabolic Syndrome (MS) is multiple set of risk factors that commonly appear together, in most people with glucose intolerance or type 2 diabetes, which has previously been termed 'Syndrome X' (Reaven 1988), the 'Deadly Quartet' (Kaplan 1989) and more recently, the 'Insulin Resistance Syndrome' (Haffner and Cassells 2003). Metabolic syndrome was first described Reaven in (1988) as a clustering of cardiovascular disease (CVD) risk factors. It is characterized by a multiple of pathologies that include glucose intolerance, insulin resistance, obesity, dyslipidemia and hypertension. Insulin resistance generally develops as the first indicator of type 2 diabetes and manifests as a decreased biological response to normal levels of circulating plasma insulin (Rita, Heather and Khosrow 2006).

The most current definition of metabolic syndrome according to the World Health Organization (WHO) includes insulin resistance as defined by glucose intolerance, impaired fasting glucose or type 2 diabetes accompanied by at least two of the- above mentioned - pathologies. Recent consideration of the various aspects of the metabolic syndrome has led the National Cholesterol Education Program's (NCEP). Figure 2.1 shows some of the key components of the metabolic syndrome that lead to increased risk of type 2 diabetes (T2D) and cardiovascular disease (Rita, Heather and Khosrow 2006).

The metabolic syndrome is a cluster of the most dangerous heart attack risk factors: diabetes and pre-diabetes, abdominal obesity, high cholesterol and high blood pressure. It is estimated that around a quarter of the world's adult population have metabolic syndrome

(Dunstan, Zimmet and Welborn 2002). It is estimated that around 20-25 percent of the world's adult population have the metabolic syndrome and they are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome. In addition, people with metabolic syndrome have a fivefold greater risk of developing type 2 diabetes (Stern, Williams and Gonzalez-Villalpando 2004). The prevalence of metabolic syndrome in the United States is increasing and now affects almost one-third of the U.S. adult population (Ford, Giles and Mokdad 2004), an estimated 47 million U.S. residents have the metabolic syndrome. The age-adjusted prevalence of the metabolic syndrome for adults is 23.7 percent. The prevalence ranges from 6.7 percent among people ages 20–29 to 43.5 percent for ages 60–69 and 42.0 percent for those age 70 and older (Ford 2002).

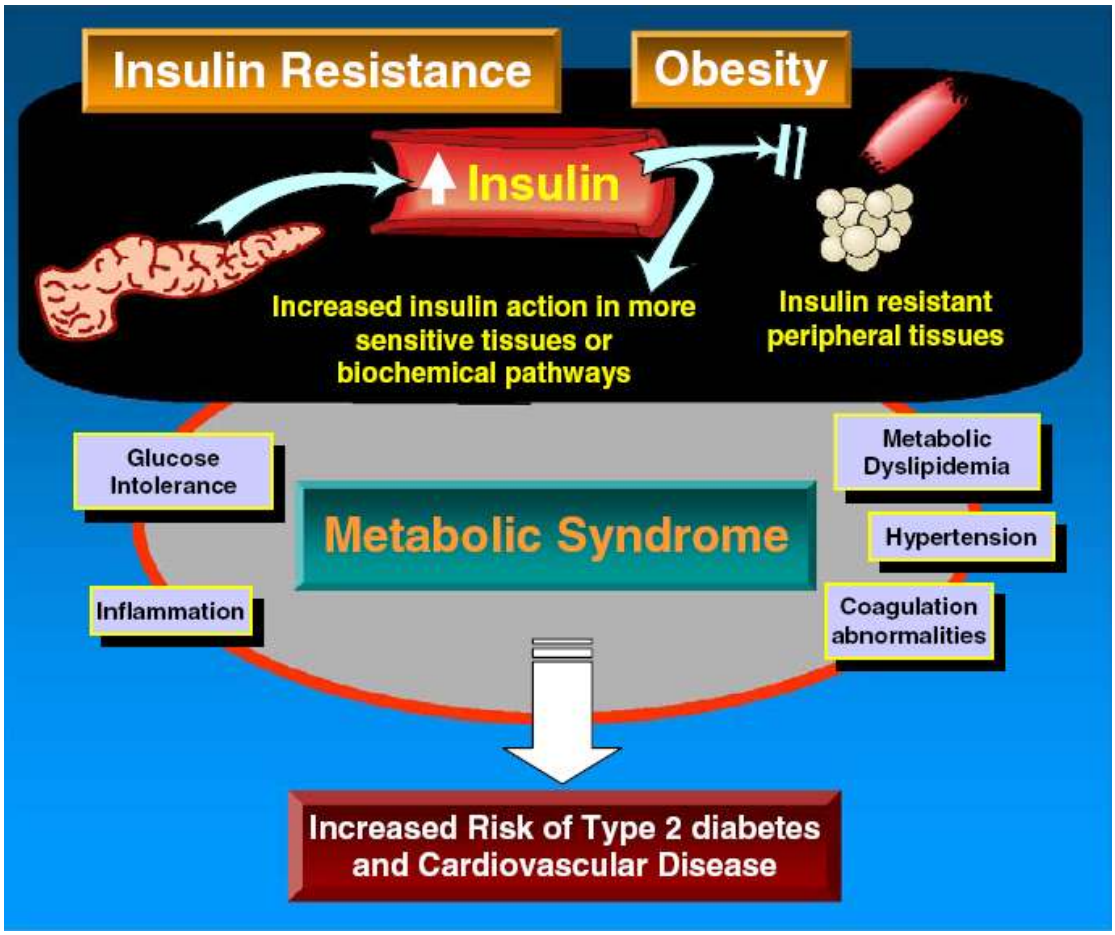

Fig. 2.1 Insulin resistance, the metabolic syndrome, and cardiovascular disease risk. Obesity and insulin resistance are closely associated and appear to be important underlying factors in the development of the metabolic syndrome. This multi-component disorder is associated with several other conditions such as hypertension, dyslipidemia, and coagulation abnormalities. The metabolic syndrome significantly increases the risk of the development of both type 2 diabetes and atherosclerotic cardiovascular disease (Rita, Heather and Khosrow 2006).

Many factors affect the prevalence of metabolic syndrome like age, gender, ethnicity, diet and physical activity, birth weight, genetic, endocrine factors, chronic subclinical inflammation

and alcohol. The pathogenesis of metabolic syndrome is poorly understood, although insulin resistance and central obesity are fundamental (Bird 2007).

The major health consequences of metabolic syndrome are the complications associated with diabetes relating to the heart, vessels, eyes, kidneys and the nervous system. Even where complications are not fatal, they can cause severe morbidity such as blindness, renal failure and the loss of limbs (Bird 2007).

2.2.Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) has recently been recognized as one of the leading causes of chronic liver disease and as the hepatic manifestation of obesity and the metabolic syndrome (MS) (Bedogni, Miglioli and Masutti 2005). Nonalcoholic fatty liver disease represents a spectrum of disease ranging from fat accumulation in the liver (steatosis) to non-alcoholic steatohepatitis (NASH) (Angulo 2002). The non-alcoholic steatohepatitis (NASH) is an aggressive form of NAFLD. Liver with steatosis and inflammation develop hepatic insulin resistance، lipotoxicity, oxidative stress and mitochondrial abnormalities, which lead to hepatic fibrosis or cirrhosis. Fifty percent of NASH patients develop liver fibrosis, 15% develop cirrhosis and 3% experience terminal liver failure (Farrell and Larter 2006). The relation between liver steatosis and cardiovascular diseases has been confirmed (Fan 2008).

Obesity is the primary cause of NAFLD (Clark and Diehl 2003), although obesity is a strong risk factor, the condition of obesity alone is not sufficient to produce NAFLD (Schwimmer *et al.* 2006). In a community-based cohort study، patients with NAFLD had a higher mortality rate compared to the general population. Liver disease was the third most common cause of death in

those with NAFLD while it was the thirteenth leading cause of death in the general population (Adams *et al.* 2005).

Nonalcoholic fatty liver disease may be categorized as primary or secondary depending on the underlying pathogenesis. Primary NAFLD is associated with insulin resistance and metabolic syndrome. Other conditions associated with NAFLD are total parenteral nutrition, acute starvation, abdominal surgery e.g. extensive small bowel resection, use of several drugs (e.g. tamoxifen, glucocorticoids, synthetic estrogens, aspirin). It is also associated with hepatitis C, HIV and metabolic disorders i.e. lipodystrophy, hypopituitarism, hypothalamic obesity and acute fatty liver of pregnancy (Adams and Lindor 2007; Duvnjak, Lerotic et al. 2007).

Nonalcoholic fatty liver disease (NAFLD) affects approximately 15% to 25% of the general population worldwide (Farrell and Larter 2006). Data from clinical studies indicate that NAFLD is an independent risk factor for the development of atherosclerosis and cardiovascular disease (Targher *et al.* 2007). The prevalence of NAFLD is much higher in obese patients (60-95%) (De Moura *et al.* 2008). Many studies suggest that NAFLD often represents the hepatic component of the metabolic syndrome, which is characterized by obesity، hyper-insulinemia, peripheral insulin resistance, diabete، and hypertension (Marchesini *et al.* 1999). The feeding of a high fructose diet (HFD) induces metabolic derangements such as hyper-insulinemia with glucose and insulin intolerance, dyslipidemia, hypertension and endothelial dysfunction in humans and rats (Braciano, Lisa and Adeli 2005). The reported prevalence of NAFLD in patients with diabetes is 40-80%, and it is frequently associated with obesity- mainly abdominal- hyper-triglyceridemia and high normal levels of alanine aminotransferase (ALT) (Angelico *et al.* 2005). Ninety percent of individuals with NAFLD have at least one risk factor of MS and 33% have all the

features of MS. One study concluded that liver fat content is significantly increased in subjects with the MS as compared with those without the syndrome (Kotronen *et al.* 2007).

Recent studies emphasize the role of insulin resistance, oxidative stress and subsequent lipid peroxidation, proinflammatory cytokines, adipokines and mitochondrial dysfunction in the development and progression of NAFLD (Paschos and Paletas 2009).

2.2.1. Pathogenesis of NAFLD

The pathogenesis of NAFLD is not well understood, but several mechanisms are thought to be important. Factors involve in the development of NAFLD include insulin resistance, oxidative stress, apoptosis and cytokine (McCullough 2007).

In patients with metabolic syndrome and diabetes, several molecular mechanisms and inflammatory mediators are involved in the development of steatosis, steatohepatitis and fibrosis. Among them, insulin resistance may play a major role in the blockade of hepatic insulin-receptor signaling through activation of different molecules, such as protein kinase C, an inhibitor of kappa B kinase (Mendez, Regev and Molina 2003).

2.2.2 NAFLD: risk factors and mechanisms of the disease

2.2.2.1. Body Fat

Overweight and obesity are clearly associated with NAFLD with the likelihood of developing NASH increasing with the degree of obesity (Ratziu *et al.* 2000).

However, recent data have shown that many individuals defined as “non-obese” NAFLD on the basis of body mass index have central obesity, identifying visceral fat as the key factor associated with NAFLD (Thomas *et al.* 2005). Cheung (2007), showed that visceral fat assessed by magnetic resonance, independently of insulin resistance (IR), was closely linked to severity of NAFLD. All these data are confirmed that visceral fat is not only a storage organ for free fatty acid (FFA), but also seems to participate directly in NAFLD pathogenesis in different ways, dependently and independently of IR, therefore, interfering with both liver fat accumulation and progression from fatty liver to NASH. In fact, visceral fat also acts as an endocrine organ, able to interfere with IR and the cytokine/adipokine network, secreting different molecular mediators, such as FFA, adiponectin, leptin, TNF and IL-6 (Ronti, Lupattelli and Mandarino 2006). According to these data, visceral fat likely plays a pivotal role in the pathogenesis of IR and NAFLD. However, liver fat, via enhanced basal insulin secretion, decreased suppression of hepatic glucose output, supra-normal plasma glucose and fatty acid delivery, and decreased glucose-responsive insulin secretion, could also amplify IR, generating a vicious circle that can ultimately lead to diabetes (Taylor 2008).

2.2.2.2. Insulin resistance (IR)

Insulin resistance is a common feature present in a number of physiological and pathological conditions in humans. It plays a leading role in diseases related to adipose-tissue dysfunction such as abdominal obesity and the metabolic syndrome, which are characterized by increased amounts of abdominal fat that lead to insulin resistance and have repercussions on metabolic parameters such as altered glycemia، dyslipidemia with decreased circulating HDL and increased low density lipoprotein (LDL) and raised blood pressure. Insulin resistance is also

central to diseases that associated to adipose-tissue dysfunction and endocrine pancreas deficiency such as type 2 diabetes. Severe insulin resistance is observed in patients with less common diseases such as lipodystrophy, with decreases in some fat depots such as subcutaneous adipose tissue and, sometimes, with increases in other depots such as visceral fat. This abnormal fat repartitioning results in severe metabolic alterations with dyslipidemia and insulin resistant diabetes together with NAFLD and a frequent evolution towards NASH (Capeau *et al.* 2006).

In the liver, insulin is involved in a number of actions responsible for glucose control and lipid metabolism. In case of insulin resistance, insulin levels are raised to overcome this resistance. The resulting effects depend on the metabolic pathway: a deficient insulin response for glucose metabolism leads to increase glucose production in the fasting state, while elevated insulin leads to activation of the lipid biosynthetic pathway, resulting in increased VLDL production and dyslipidemia. Given the central role played by the liver in lipid metabolism, any imbalance between the entry and export of lipid derivatives results in steatosis (Capeau 2008).

Increase oxidative stress and stress of the endoplasmic reticulum are probably some of the altered mechanisms in this setting. Insulin resistance at the adipose-tissue level plays an important role in hepatic insulin resistance. Increased free fatty acid (FFA) production favors lipid deposition in the liver. The inflammatory signals released in adipose-tissue diseases also play a leading role with increased proinflammatory and decreased adiponectin signalling in the liver. Inflammatory signals within the liver have also to be considered. Activation of the immune system results in the local release of proinflammatory cytokines (Capeau 2008).

2.2.2.3. Oxidative stress

Many experimental models and human studies have found a strong association between severity of NASH and degree of oxidative stress (Yang *et al.* 2000). Oxidative stress in NAFLD arises from the excess of FFA that have undergone oxidative processes within the cells (Sanyal *et al.* 2001).

Oxidative stress is considered to play a central role in the pathogenesis of NAFLD because the increased production of ROS is known to cause lipid peroxidation, followed by activation of the inflammatory response. Lipid peroxidation usually leads to the formation of peroxyl radicals, which are the central species of the peroxidation chain reaction (Yang *et al.* 2000).

Fat accumulation correlated with systemic oxidative stress in humans and mice. Experimental and clinical observations indicate oxidative stress as an important mechanism in hypertension, diabetes and obesity-associated metabolic syndrome and its complications (Furukawa *et al.* 2004).

Oxidative stress plays critical roles in the pathogenesis of various diseases (Brownlee 2001). In the diabetic condition, oxidative stress impairs glucose uptake in muscle and fat (Maddux 2001).

2.2.2.4. Cytokine/adipokine interplay

Adipokines and cytokines are molecular mediators critically involved in the physiology and in the pathophysiology of many diseases, in which it is possible to observe an imbalance of proinflammatory mediators with respect to anti-inflammatory molecules and role of adiponectin,

leptin, TNF-α and IL-6. In fact, these molecular mediators, whose expression is also strongly associated with visceral obesity, seem to play critical role in the modulation of insulin signaling and of inflammatory cascade, two conditions that appear to be central not only to liver fat accumulation, but also to disease progression. Adiponectin is hypoexpressed in NAFLD (Kaser *et al.* 2005). Serum IL-6 levels have been found to be elevated in animal and human models of IR and NAFLD (Howard and Flier 2006) and human studies have found a linear relationship between both serum and hepatic IL-6 and severity of steatosis, necroinflammation and fibrosis (Van der Poorten and Milner 2008).

2.3. Complication of metabolic syndrome

2.3.1. Cardiovascular Disease

They would add to the 230 million people worldwide who already have diabetes (Diabetes Atlas 2006). One of the most common chronic diseases worldwide and the fourth or fifth leading cause of death in the developed world. The clustering of cardiovascular disease (CVD) risk factors that typifies the metabolic syndrome is now considered to be the driving force for a new CVD epidemic (Sir George *et al.* 2006).

The idea of a syndrome linking diabetes and cardiovascular disease has been around since the 1920s. In the 1940s, attention was drawn to the association between central body fat deposits and other cardiovascular disease risk factors. However, not until 1988 Reaven's pointed to a strong association between insulin resistance, hypertension, glucose intolerance, low high-density lipoprotein (HDL)-cholesterol and raised very low density lipoprotein (VLDL)-triglycerides. He called this syndrome X (Bird 2007).

People with metabolic syndrome are at risk for cardiovascular disease, including coronary heart disease (CHD) and stroke (Lakka *et al.* 2002). The importance of NAFLD and its relationship with MS is now increasingly recognized as recent data suggest that NAFLD is linked to increased cardiovascular risk independently of the broad spectrum of risk factors of MS (Targher *et al.* 2005). Indeed, it is hypothesized that NAFLD is not just a marker of cardiovascular disease but may also be involved in its pathogenesis (Targher, Bertolini and Rodella 2007).

People with the syndrome are about twice as likely to develop cerebrovascular disease and over four times as likely to develop type 2 diabetes compared with subjects who do not have metabolic syndrome (Meigs 2002).

2.3.2. Chronic Kidney Disease

Chronic kidney disease (CKD) is a complex disease effect more than twenty million individuals in the United States (Thomas, Kanso and Sedor 2008). The worldwide prevalence of metabolic syndrome is increasing and has been associated with chronic kidney disease. To date, kidney pathological characteristics in individuals with metabolic syndrome have not been described in detail (Alexander *et al.* 2009).

The cardiovascular risk associated with renal impairment increases earlier in the course of kidney disease progression than was initially hypothesized. Several cardiovascular risk factors associated with CKD are unique to patients with this disease (non-traditional risk factors). A complete fasting lipid profile with assessment of total, LDL and HDL cholesterol and

triglyceride levels should be included in the evaluation of patients with CKD and hyperlipidemia (Thomas, Kanso and Sedor 2008).

Progression of CKD is associated with a number of serious health complications, including increased incidence of cardiovascular disease (figure 2.2) (Thomas, Kanso and Sedor 2008).