

Mechanisms of Diabetes-Induced Liver Damage

The role of oxidative stress and inflammation

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اليات تلف الكبد التي يسببها مرض السكري دور الأوكسدة والالتهابات

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ABSTRACT: Diabetes mellitus is a non-communicable disease that occurs in both developed and developing countries. This metabolic disease affects all systems in the body, including the liver. Hyperglycaemia, mainly caused by insulin resistance, affects the metabolism of lipids, carbohydrates and proteins and can lead to non-alcoholic fatty liver disease, which can further progress to non-alcoholic steatohepatitis, cirrhosis and, finally, hepatocellular carcinomas. The underlying mechanism of diabetes that contributes to liver damage is the combination of increased oxidative stress and an aberrant inflammatory response; this activates the transcription of pro-apoptotic genes and damages hepatocytes. Significant involvement of pro-inflammatory cytokines—including interleukin (IL)-1 β , IL-6 and tumour necrosis factor- α —exacerbates the accumulation of oxidative damage products in the liver, such as malondialdehyde, fluorescent pigments and conjugated dienes. This review summarises the biochemical, histological and macromolecular changes that contribute to oxidative liver damage among diabetic individuals.

Keywords: Diabetes Mellitus; Liver Diseases; Inflammation; Oxidative Stress.

المخلص: داء السكري هو مرض غير معدي يحدث في كل من البلدان المتقدمة والنامية. يؤثر هذا المرض الأيضي على جميع أجهزة وأعضاء الجسم، بما في ذلك الكبد. يؤثر ارتفاع السكر في الدم، الناجم أساساً عن مقاومة الأنسولين، على عملية التمثيل الغذائي للدهون والكربوهيدرات والبروتينات ويمكن أن يؤدي إلى أمراض الكبد الدهنية غير الكحولية، والتي يمكن أن تتطور لالتهاب الكبد الدهني غير الكحولي وتليف الكبد و سرطان الخلايا الكبدية. إن الآلية الكامنة وراء مرض السكري الذي يساهم في تلف الكبد هي مزيج من الإجهاد التأكسدي والاستجابة الالتهابية الشاذة، مما ينشط النسخ من الجينات الموالية لموت الخلايا المبرمج وتدمير خلايا الكبد. ويؤدي وجود كميات من السيتوكينات، بما في ذلك انترلوكين IL-6، IL-1 β وعامل نخر الورم α -إلى تراكم المنتجات المؤكسدة الضارة في الكبد، مثل مالونديالدهايد، وأصبغ الفلورسنت والداينات المترافقة. يلخص هذا الاستعراض التغيرات البيوكيميائية، النسيجية والجزئية التي تساهم في أكسدة وتلف الكبد بين مرضى السكري.

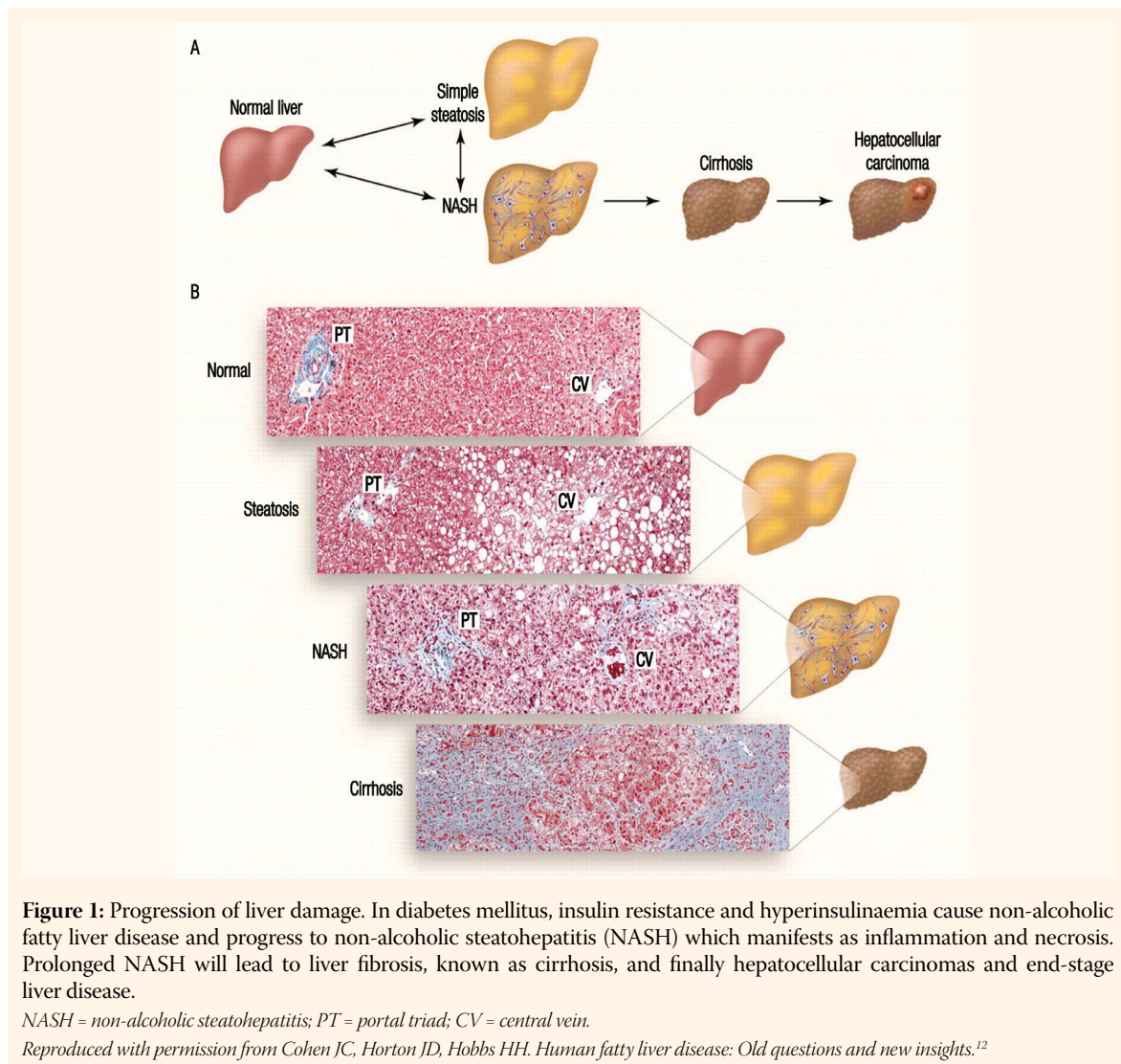
كلمات مفتاحية: داء السكري؛ أمراض الكبد؛ الإلتهاب؛ الإجهاد التأكسدي.

DIABETES MELLITUS (DM) IS A MAJOR global public health problem with an escalating incidence and prevalence, particularly in developing and newly industrialised countries.¹ Concern regarding this chronic disease is focused on serious DM-related complications which can affect multiple vital organ systems, thereby leading to more severe and irreversible pathological conditions such as nephropathy, retinopathy, vasculopathy, neuropathy and cardiovascular diseases, as well as hepatopathy.² Research indicates that DM is associated with a number of liver abnormalities, such as abnormal glycogen deposition, non-alcoholic fatty liver disease (NAFLD), fibrosis, cirrhosis, hepatocellular carcinomas (HCCs), abnormal elevated hepatic enzymes, acute liver disease and viral hepatitis.^{3,4} Additionally, an excessive accumulation of fat in the liver may worsen insulin resistance and lead to severe metabolic

dysfunction. A fatty liver and hyperglycaemia can destroy the hepatocytes and contribute to increased morbidity and mortality among diabetic patients.³ However, this subject requires further exploration and elucidation from various perspectives in order to promote understanding of the entire process involved. This review therefore analyses the various DM mechanisms which induce liver damage through the formation of a fatty liver. In addition, pathways of the histopathological and macromolecular changes that result in this complication are discussed.

Progression of Liver Damage in Diabetes Mellitus

Several critical pathways have been identified as causing liver damage in diabetic patients. Insulin resistance, the main cause of hyperglycaemia and



compensatory hyperinsulinaemia, is the predominant causative factor.⁵⁻⁷ As a collection of insulin-sensitive tissues, the liver is among the primary organs susceptible to the effects of hyperglycaemia-induced oxidative stress, which may lead to liver tissue injury.⁷⁻⁹ This is followed by derangement of protein, carbohydrate and lipid metabolism, thereby leading to increased oxidative stress and further triggering the inflammatory cascade.^{6,8,9} Both oxidative stress and inflammatory responses act as damaging agents in aggravating the pathological condition of DM.^{4,10} In some cases, DM causes excessive accumulation of fat cells in the liver resulting in a fatty liver and, consequently, NAFLD. Subsequently, 2–3% of NAFLD patients experience hepatic inflammation, necrosis and fibrosis, which are symptoms of a condition known as non-alcoholic steatohepatitis (NASH).^{2,10} Injured or fibrotic livers will then become cirrhotic, form HCCs and, eventually, go into liver failure [Figure 1].¹⁰⁻¹²

Diagnosis of Liver Damage

HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES

Studies have revealed the prominent histopathological changes in the livers of patients with DM.^{2,13} During the initiation of steatosis, the accumulation of fat in the vesicles displaces the cytoplasm of the liver. This condition is known as microvesicular steatosis, which then progresses to macrovesicular steatosis when excess lipids distort the nucleus. Besides glycogenated nuclei, other prominent features of steatosis include mild lymphocytic, neutrophilic and other inflammatory infiltrates. Steatosis usually takes place in acinar zone III.^{2,3} These features progress and worsen with steatohepatitis.^{3,7,14,15} Steatohepatitis and NASH can be diagnosed by the presence of hepatocellular steatosis, lobular inflammation and varying degrees of pericellular, perisinusoidal and periportal fibrosis,

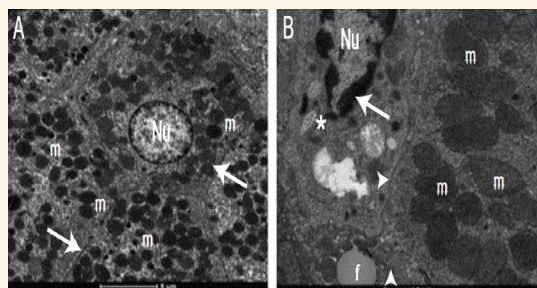


Figure 2A&B: Electron micrographs of a (A) healthy and (B) diabetic liver. Note the difference in appearance between the hepatocyte nuclei (Nu), organelles, mitochondria (m) and Disse's space (arrows). In the diabetic liver, there is accumulation of homogenous substances in the nuclear chromatin (*), reduction in the number of organelles, loss of mitochondrial cristae (▲) and granular degeneration (▶) in the hepatocytes.

Reproduced with permission from Lucchesi, AN, Cassettari LN, Spadella CT. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans.¹⁹

including cirrhosis.^{2,4} Other dominant features of steatohepatitis besides glycogenated nuclei are hepatocyte ballooning, varying degrees of hepatocyte necrosis and the presence of small, sparse and inconspicuous Mallory bodies. Degenerative changes are characterised by the vacuolation of hepatocytes in the centrilobular area of the cell and a discrepancy in the size of the nuclear and dilated sinusoidal spaces.^{2,4} Cirrhosis has three major histological features: (1) the formation of macro- and/or micronodular hepatocytes; (2) bridging of the fibrous septa; and (3) the disruption of the entire liver architecture. Under these conditions, steatosis and necroinflammatory mechanisms are difficult to observe.¹⁵⁻¹⁸

Ultrastructurally, the rough endoplasmic reticulum is significantly reduced in diabetic patients in comparison with the smooth endoplasmic reticulum.⁹ Moreover, DM causes a marked increase in mitochondrial volume fraction with highly diminished densities of mitochondrial cristae, pyknotic nuclei with damaged nuclear membranes and the extension of the perinuclear area [Figure 2].¹⁶⁻¹⁹ In addition, increased lipid accumulation and decreased glycogen content are also seen in the liver cells.¹⁶⁻¹⁹

BIOCHEMICAL CHANGES

The spectrum of biochemical changes that typically occur in DM resemble those of liver diseases, from the secretion of abnormal liver enzymes to the development of HCCs or even end-stage liver failure.^{17,20} The initial and most important indicators in assessing liver injury are levels of plasma alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and γ -glutamyl transpeptidase (GGT).^{13,21,22}

Research has shown an increase of AST and ALT in 50–80% of NAFLD patients; ALT levels were higher than AST levels for NAFLD patients, while patients with alcoholic fatty livers had higher AST than ALT levels.² An increment in ALT levels may indicate fatty changes in the liver regardless of the presence of DM. Furthermore, serum ALT concentrations have been found to correlate with hepatic insulin resistance and later decline in hepatic insulin sensitivity compared with AST and GGT levels, which showed no connection with hepatic insulin.²² However, while ALP and GGT levels may be elevated, serum albumin and bilirubin levels are not.^{2,18,21} Hepatocytes work exclusively to synthesise serum albumin, but are not a good indicator of acute or mild hepatic dysfunction.¹³

Increased plasma triglyceride and low-density lipoprotein levels and decreased high-density lipoprotein levels are common characteristics of dyslipidaemia and can be observed in diabetic and steatosis patients.^{13,15} This is due to insulin resistance-related lipolysis which increases the circulating fatty acids taken up by the liver as an energy source. The accumulation of fatty acids disturbs the β -oxidation system in the hepatic mitochondria and leads to further infiltration of fats in the liver. The strong association between insulin resistance and steatosis makes DM an independent predictor for cirrhosis and HCCs among steatosis patients.^{13,15,17}

OTHER CHANGES

The medical history and physical examination of a patient can reveal other laboratory abnormalities associated with liver damage, including hypoalbuminemia, prolonged prothrombin times and high levels of ferritin.^{13,23} In addition, a radiological assessment may identify fats through increased echogenicity on ultrasonography, hypodense fat on non-contrast computed tomography and fat content on magnetic resonance imaging.²³

Mechanisms of Damage

The liver plays a vital role in regulating glucose levels in physiological and pathological states such as DM. In type 2 DM, insulin resistance in the liver will cause hyperglycaemia and further distortion of glucose metabolism. Even under severe hyperglycaemic conditions, the liver does not detect the availability of glucose and produces more via the combined action of a few enzymes such as glucose-6-phosphate dehydrogenase, fructose-1,6-diphosphate, hexokinase and glucokinase.^{2,13} The liver also regulates glucose homeostasis by modulating the expression of various

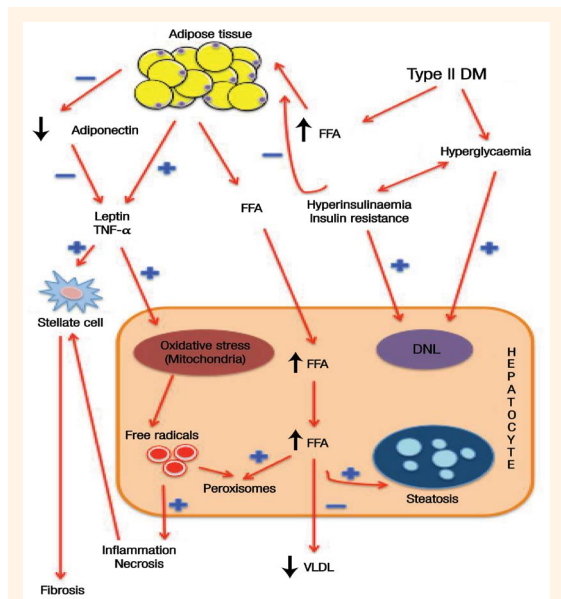


Figure 3: Diagram showing the mechanism of liver damage in diabetes mellitus. Insulin resistance causes peripheral adipocytes to undergo lipolysis. Free fatty acids are then released to the bloodstream and eventually accumulate in the liver. At the same time, adipocytokines release tumour necrosis factor- α and leptin, worsening the hepatocyte damage by increasing oxidative stress in the mitochondria. The combined action of mitochondrial oxidative stress, hyperinsulinaemia and hyperglycaemia produce free radicals which in turn induce inflammation and cellular necrosis. Tissue inflammation stimulates the hepatic stellate cells to produce collagen, leading to fibrosis, cirrhosis and, finally, hepatocellular carcinomas.

DM = diabetes mellitus; FFA = free fatty acids; TNF = tumour necrosis factor; DNL = de novo lipogenesis; VLDL = very-low-density lipoprotein.

kinds of genes encoding secretory proteins (i.e. hepatokines).^{24,25} Different types of hepatokines via different pathways activate either positive or negative feedback mechanisms to control the metabolism process.²⁴ Three dominant hepatokines exist: fetuin-A, betatrophin/angiopoietin-like protein 8 (ANGPTL8) and fibroblast growth factor 21 (FGF21). Fetuin-A results in increased inflammation and insulin resistance by inhibiting the insulin receptor tyrosine kinase in skeletal muscle tissue and hepatocytes.²⁴ In contrast, ANGPTL8 is known for its proliferation action on the beta cells of the pancreas while FGF21 is recognised as an insulin-sensitising hormone.²⁵ These hepatokines significantly exacerbate the diabetic condition, especially in type 2 DM, by regulating subclinical inflammation.²⁴

According to certain studies, the aberrant signal which promotes glucose production in the liver during DM supposedly also enhances fatty acid oxidation due to a lack of fuel demand.^{2,13} However, other research has found that the liver stops oxidising fatty acids and uses them instead to synthesise triglycerides which

then accumulate abnormally in the liver.^{2,13,21} In type 1 DM, insulin deficiency upregulates hormone-sensitive lipase in the adipose tissues, subsequently leading to increased lipolysis and the circulation of free fatty acids, which subsequently accumulate in the liver. These processes enhance the hepatic uptake of very-low-density lipoproteins and synthesis of triglycerides.^{2,13,14} Concurrently, elevated glucagon levels inhibit hepatic triglyceride output. Therefore, accumulation of fat in the liver may be due to an imbalance in the uptake, synthesis, export and oxidation of free fatty acids in the liver.^{12,14,15} Aside from abnormalities in lipoprotein metabolism, an accumulation of hepatic fat in DM may be due to either hyperglycaemia-induced activation of the transcription factor carbohydrate-responsive element-binding protein and sterol regulatory element-binding protein 1c, the upregulation of the glucose transporter 2 protein with subsequent intrahepatic fat synthesis or a combination of these mechanisms.¹²

It is worth noting that an accumulation of adipocytes in the liver will cause hepatic insulin resistance.⁷ Environmental conditions and genetic traits also contribute to the phenotypic expression of insulin resistance; these factors act in combination with hyperinsulinaemia to increase fat mass and lipolysis and elevate the level of free fatty acids. At the same time, insulin signalling is reduced in a dose-dependent manner, eventually increasing hepatic glucose and lipotoxicity.⁷ However, modifications of adipocytokine secretion in hyperinsulinaemia occur by triggering gene expression of pro-inflammatory cytokines, such as leptin and tumour necrosis factor- α (TNF- α); these modifications are not only involved in the development of insulin resistance but also interfere with insulin signalling, thereby worsening DM.¹⁵ An increase in adipocytokines may contribute to mitochondrial oxidative stress and trigger the formation of free radicals and peroxisome.²⁶ An increase in mitochondrial oxidative stress will also cause DNA mutation damage and trigger a series of deleterious effects on the mitochondrial respiratory chain by producing reactive oxygen species (ROS). This vicious cycle will escalate exponentially to an incompatible level, which will be countered by normal physiological feedback and thereby lead to liver damage.^{26,27} Hyperinsulinaemia may downregulate adiponectin, leading to hepatic lipogenesis and further decreasing free fatty acid oxidation.¹⁵ Thus, hyperinsulinaemia may act as a major contributor to the progression of liver damage.⁷ Inflamed and necrotic hepatocytes and adipocytokines will release several types of chemical mediators which will activate the stellate cells to produce connective tissue growth factor and collagen and cause an accumu-

lation in the extracellular matrix, thereby favouring fibrosis [Figure 3].²⁶

In addition to the aforementioned factors, the expression of certain genes also influences the progression and severity of DM. For example, the most important cytochrome P450 member—*cytochrome P450 family 2 subfamily E polypeptide 1 (CYP2E1)*—is expressed at a higher concentration in the liver than in other organs. Indeed, the activity and expression of this protein increases in obese individuals and for those with NAFLD or NASH.²⁸ Under these conditions, excessive metabolism of polyunsaturated fatty acids by *CYP2E1* may yield cytotoxic products harmful to the liver. Furthermore, *CYP2E1* is also involved in mitochondrial respiratory chain-induced ROS generation, which contributes to the increase in oxidative stress, thereby favouring the progression of NAFLD to NASH.²⁸

Oxidative Stress-Mediated Damage

Oxidative stress is defined as an imbalance in the oxidant-to-antioxidant ratio, causing the generation of free radicals.⁹ The liver is the main detoxification organ of the body and plays an important role in controlling normal glucose homeostasis.⁶ The production of oxidants such as ROS-like superoxide anions, hydrogen peroxide and hydroxyl radicals by activated Kupffer cells has been identified as central to hepatic injuries.²⁹ Kupffer cells, also known as hepatic macrophages, are one type of nonparenchymal cell that help maintain the integrity of liver cells. However, these phagocytic cells are also susceptible to the effects of oxidative stress produced by the surrounding cells and its own immune reactions.^{2,13}

Excessive ROS production results in several deleterious events, including an irreversible oxidative modification of lipids, proteins and carbohydrates.^{28,30} In addition, it will induce *apoptosis* in hepatocytes and the release of inflammatory cytokines, thereby increasing the expression of adhesion molecules and the infiltration of leukocytes. A combination of all of these processes causes massive tissue destruction in the liver.^{16,29} However, the liver is equipped with potent antioxidants—such as superoxide dismutase (SOD), catalase (CAT) and the glutathione (GSH) enzyme family, including glutathione-S-transferases (GSTs) and glutathione peroxidases (GPXs)—so as not only to neutralise free radicals but also to protect the liver cells from oxidative damage.^{30,31} Previous research has proven that a decrease in SOD and CAT activities within a hyperglycaemic state leads to an increase

in ROS, which eventually contributes to oxidation-induced liver damage.^{8,30,31}

The liver also plays a pivotal role in the homeostasis of the GSH enzyme family. Different cell components such as the endoplasmic reticulum, mitochondria and nucleus consist of separate pools of GSH. Among these, mitochondrial GSH is more essential than cytoplasmic GSH in maintaining cell viability.³¹ Acting as the first line of defence as an endogenous antioxidant, the oxidation process takes place in the thiol group of GSH.³¹ Reduced levels of GSH in diabetic rat livers have been associated with depletion of decreased GST, GPX and glutathione reductase activity and with the accumulation of oxidative stress products, such as advanced glycation end-products (AGEs), protein oxidation products (POPs) and lipid peroxidation (LPO).^{32–34} However, measuring certain redox couples in cells—such as oxidised nicotinamide adenine dinucleotide (NAD)/reduced NAD and oxidised NAD phosphate (NADP)/reduced NADP—is difficult; as a result, the GSH-to-glutathione disulphide ratio in the liver is considered representative of a redox state.³¹

OXIDATION PRODUCTS

Carbohydrates

AGEs are complex heterogeneous molecules characterised by fluorescence, a brown colour and cross-linking reactions.^{33–35} Several distinct compounds exist such as pyrroline, pentosidine, imidazolone, N-carboxymethyllysine and methylglyoxal. The latter can yield irreversible AGEs which impair insulin signalling by forming a complex with insulin receptor substrate, thereby leading to conformational changes that affect the tyrosine phosphorylation and the docking function of these proteins.³² The formation of AGEs is closely related to the degree and duration of hyperglycaemia, the half-life of the protein and the permeability of the tissue to free glucose.^{32,34} Haemoglobin A1c is a product of glycosylated haemoglobin and has been widely used as an indicator of long-term elevated plasma glucose levels.^{33–35}

Zuwała-Jagiello *et al.* proved that the liver plays a major role in the clearance of plasma AGEs, which accumulate heavily in conditions of chronic liver damage.³⁶ Moreover, AGE levels have been found to correlate positively with the severity of liver damage and decrease markedly after liver transplantation.^{36,37} However, hepatic stellate cells and myofibroblasts responsible for fibrogenesis in chronic liver disease will express the receptor for AGE (RAGE). The binding of AGE with RAGE will injure the structure of the extracellular matrix and induce a toxic effect by activating nuclear factor-kappa B (NF-κB) to stimulate

the transcription of genes for cytokines and growth factors, further increasing expression of adhesive molecules and vascular permeability.^{9,32,34,35,38} Hence, AGE can be used as a clinical marker to evaluate liver function and diabetes severity.^{34–37}

Proteins

Protein oxidation has been defined as “the covalent modification of a protein induced either directly by ROS or indirectly by reaction with secondary by-products of oxidative stress”.³⁸ This means that a massive production of free radicals may also give rise to the formation of protein-protein and protein-lipid cross-links, which in turn change the structure and function of the proteins by causing protein fragmentation and obstruction of the amino acid side chains.^{34,35,38} These changes lead to deleterious consequences such as inhibition of enzymatic and binding activities, increased susceptibility to aggregation, resistance to proteolysis, increased or decreased uptake by cells and altered immunogenicity.^{32,34,35,38} The same mechanism occurs when proteins are exposed to reactive nitrogen species (RNS). Protein nitration may disturb normal signalling pathways (e.g. the insulin signalling pathway), to detrimental effect; nitrated proteins also mediate hepatic injury by promoting the release of cytokines and chemokines through stimulation of Kupffer cells, thereby resulting in neutrophil infiltration and causing inflammatory tissue injury.³⁹ Cysteine and methionine are among the most susceptible amino acids and can be modified by almost all oxidising species.^{36,38,40} Coincidentally, cysteine is an important residue in many metabolic enzymes, kinases, phosphatases and transcription factors; oxidative modification of cysteine may therefore alter the functional properties and activity of proteins.^{36,38}

Much like AGEs, research has shown that POP serum concentrations increase with the progression of chronic diseases and are exacerbated in the presence of DM.^{37,40,41} Therefore, POPs are distinct biomarkers for oxidative stress. Previous studies have reported that the liver plays an important role in the elimination of POPs.^{42,43} Zuwała-Jagiełło *et al.* demonstrated a significant increase of POPs in a group of cirrhotic patients; POP levels were proportional to the severity of the disease.³⁶

Lipids

LPO occurs when polyunsaturated fatty acids interact with oxygen and is often used as a marker of tissue oxidative stress.⁴⁴ Various aldehydes derived from lipid hydroperoxides can be measured to quantify LPO as they reflect the extent of damage associated with oxidative stress at the tissue and cellular levels. These aldehydes have longer half-lives than reactive

free radicals and can diffuse from their site of origin, circulate freely in the blood vessels or incorporate with lipoproteins, thereby reaching and attacking target sites.⁴⁴ Notably, LPO causes a prominent increase in malondialdehyde and thiobarbituric acid-reactive substances in the livers of people with DM.³ The formation of these products alters the transbilayer fluidity gradient of plasma membranes and damages the membrane components. It also affects membrane-bound enzymes and receptor activity and leads to inflammation and cell necrosis.^{31,37} In NAFLD patients, LPO triggers the release of inflammatory mediators such as TNF- α via the activation of adipocytokine and other pathways.^{44–46} This vicious cycle activates hepatic stellate cells and further leads to the proliferation and synthesis of collagenous substances, which favours the formation of fibrosis; this is an essential step in all types of chronic liver damage processes.⁴⁵

Inflammation-Mediated Damage

Inflammation plays a vital role in host defence by releasing pro-inflammatory cytokines to protect the host from injury, including TNF- α , interleukin (IL)-1 β and IL-6. Chronic inflammatory conditions cause tissue damage, fibrosis and loss of cellular function. Growing evidence links a low-grade chronic inflammatory state to DM complications, particularly liver-related complications.⁴³ These conditions are further exacerbated by the combined action of a complex regulatory network of cells and mediators designed to resolve inflammatory responses, mainly macrophages. The resulting increase in circulatory cytokines will then aggravate insulin resistance; however, anti-inflammatory drugs reverse these complex reactions.^{14,47,48} Thus, inflammation might constitute a major causal factor for non-communicable diseases such as DM, rather than an associated risk factor. For example, IL-1 β together with TNF- α is responsible for the development of DM types 1 and 2 via inhibition of glucose-induced insulin secretion and impairment of β -cell function, thereby decreasing the biosynthesis of insulin and inducing apoptotic cell death.⁴⁸ In addition, IL-1 β may also stimulate proliferation of T and B cells and induce the release of adhesion molecules, in addition to triggering the production of other cytokines and pro-inflammatory mediators, thereby having a detrimental effect on the liver.^{29,49,50} The biological response to IL-1 β is mostly shown through the activation of the c-Jun N-terminal kinase (JNK) pathway and the nuclear translocation of NF- κ B.⁴⁹

Table 1: Comparative analysis of previous studies investigating the effectiveness of antioxidant supplements in preventing diabetes-induced liver damage

Author and year of study	Study design	Antioxidant source	Dose	Duration	Protective effect
Welt <i>et al.</i> ¹⁶ 2004	<i>In vivo</i>	<i>Ginkgo biloba</i> extract	100 mg/kg/day	4 months	Antioxidative
Palsamy <i>et al.</i> ⁹ 2010	<i>In vivo</i>	Resveratrol	5 mg/kg/day	30 days	Anti-inflammatory, antiglycative and antioxidative
Parveen <i>et al.</i> ³⁰ 2010	<i>In vivo</i>	<i>Pinus pinaster</i> bark	10 mg/kg/day	4 weeks	Anti-diabetic and antioxidative
Hamden <i>et al.</i> ¹⁸ 2008	<i>In vivo</i>	17 β -estradiol	1 μ g/kg/day	4 weeks	Anti-apoptotic, anti-diabetic and antioxidative
Manna <i>et al.</i> ⁸ 2010	<i>In vitro</i> and <i>in vivo</i>	Arjunolic acid	20 mg/kg/day	4 weeks	Anti-inflammatory, anti-apoptotic, anti-diabetic, antiglycative and antioxidative
Kim <i>et al.</i> ⁵⁵ 2009	<i>In vivo</i>	Kangen-karyu extract	50, 100 and 200 mg/kg/day	20 days	Anti-inflammatory, anti-diabetic, antiglycative and antioxidative
Maritim <i>et al.</i> ⁵⁶ 2003	<i>In vivo</i>	α -lipoic acid	10 mg/kg/day	14 days	Antioxidative
Jain <i>et al.</i> ⁵⁷ 2009	<i>In vivo</i>	L-cysteine	1 mg/kg/day	8 weeks	Anti-inflammatory, anti-diabetic, antiglycative and antioxidative
Guyen <i>et al.</i> ⁴ 2006	<i>In vivo</i>	Melatonin	200 μ g/kg/day	9 weeks	Antioxidative

Signalling Mechanisms Contributing to Damage

NUCLEAR FACTOR-KAPPA B

First described as a nuclear factor specific to B cells bound to the B site of the κ -light chain gene enhancer, NF- κ B was then found to be expressed in other cell types, including the liver epithelium, where it regulates the proliferation and survival of hepatocytes during regeneration and development.^{31,51} This protein complex may be activated by the release of pro-inflammatory molecules such as IL-1 β , IL-6, TNF- α and C-reactive protein.^{8,50} Other non-inflammatory states and mediators may also trigger NF- κ B activation, such as free fatty acids, hyperglycaemia, obesity, protein kinase C and oxidative stress.^{8,50,52} The activation of NF- κ B may trigger different types of responses depending on stimuli received, such as pro-apoptotic gene activation or cell regeneration. Hence, the activation of NF- κ B in DM may initiate a series of deleterious events via the further elevation of pro-inflammatory cytokines.^{31,50,51} Moreover, NF- κ B may also directly and indirectly promote the production of ROS and RNS and increase LPO, AGEs and POPs, thereby increasing liver tissue damage.¹⁴ These products are thought to be generated through the phosphorylation-induced ubiquitination and subsequent proteolysis of inhibitor of NF- κ B kinase subunit- α and through TNF- α - or lymphotoxin- β -mediated pathways.^{51,52} It has been postulated that inappropriate activation of NF- κ B increases the

likelihood of hepatic steatosis progression to cirrhosis, thereby causing further liver damage.⁵¹ Therefore, NF- κ B is not only an appropriate target for DM treatment but also for the treatment of hepatocellular injuries.⁹

MITOGEN-ACTIVATED PROTEIN KINASE

Mitogen-activated protein kinases (MAPKs) are signalling transducers which transmit external stimuli to the nucleus by dual phosphorylation of the Thr183 and Tyr185 antibodies. The MAPK families consist of three different signalling cascades converging on extracellular signal-regulated kinases, JNKs and p38 MAPKs, which exert different types of reactions upon stimulation.⁵² Activated by cytokines and growth factors, extracellular signal-regulated kinases play a central role in regulating cell growth and differentiation. Meanwhile, JNK and p38 MAPKs, also known as stress-activated MAPKs, are triggered by pro-inflammatory cytokines such as TNF- α and IL-1 β as well as environmental and genotoxic stressors.⁵² Stimulation of stress-activated MAPKs will exert various cellular functions, such as *apoptosis*, necrosis, survival, proliferation, migration and inflammation.¹⁰ In DM, MAPK cascades are activated by osmotic perturbation caused by hyperglycaemia, oxidative stress and AGEs.⁸ Studies have shown that prolonged exposure to oxidative stress because of high levels of liver TNF- α will activate the pro-apoptotic function of JNKs; detrimentally affecting the hepatocytes.⁵² Thus, JNKs are a promising therapeutic target for individuals with liver injuries.

Clinical Implications, Management and Treatment

The treatment of DM remains a challenging issue. Researchers are exploring safe and effective medications to overcome the detrimental effects of insulin resistance-related metabolic derangement, including hyperglycaemia, hyperinsulinaemia, hyperlipidaemia, oxidative stress, inflammation, atherosclerosis and other complications.⁵³ For patients with NAFLD and NASH, no specific treatments yet exist apart from diet and lifestyle modifications to reduce body weight and prevent further injury.^{23,54} However, combined pharmacological therapy is recommended to improve insulin sensitivity in the liver (metformin and pioglitazone) and its periphery (thiazolidinediones), together with other drugs such as betaine, atorvastatin, losartan and orlistate.^{7,13,53} However, the clinical value of these treatments are very subjective. Patients taking these drugs should be closely monitored due to possible contraindications with DM medications and the vulnerable condition of the liver during the drug detoxification process.⁵⁴

Antioxidant therapy is a potential future therapeutic strategy; increasing antioxidant levels in patients with DM-induced liver damage may hopefully counter the effects of oxidative stress and inflammation, thereby reducing the severity of diabetic complications. A few plant-based products and vitamins have been investigated as ways of protecting against and possibly reversing liver damage believed to be caused by oxidative stress and inflammation.⁴⁸ Vitamin E and betaine are just a few of the antioxidants which have shown good clinical implications in the reduction of liver disease severity and the protection of the liver from DM-induced damage [Table 1].^{4,8,9,16,18,30,55-57}

Conclusion

Liver damage is a serious complication among patients with DM. Insulin resistance, which is aggravated by oxidative stress and aberrant inflammatory signals, has become one of the main factors contributing to liver damage. Insulin resistance subsequently leads to more chronic and potentially fatal conditions such as cirrhosis and end-stage liver failure. Antioxidants from plant-based and natural products with strong anti-diabetic, anti-inflammatory and antiglycative properties are emerging as a future therapy. As opposed to conventional medications, antioxidants may be an alternative and beneficial way to prevent and treat this life-threatening disease.

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