FALK SYMPOSIUM 117

Hepatology 2000
Symposium in Honour of

Gustav Paumgartner

Edited by

A. L Gerbes, U. Beuers, 0. Jungst, G. R. Pape, M. Sackmann
Medizinische Kilnik II
Kimikum Grosshadern der LMU Munchen
Munchen, Germany

T. Sauerbruch
Medizinische Klinik
Allgemeine Innere Medizin
Kilnikum der Universitat
Bonn, Germany

Proceedings of the Falk Symposium 117 held in Munich Germany, May 15, 2000

Non-alcoholic steatohepatitis: potential causes and pathogenic mechanisms

D. PESSAYRE, A. MANSOURI and B. FROMENTY

INTRODUCTION

Hepatologists might be too good at their job. They are actively depleting the pool of their patients. Vaccination may soon eradicate hepatitis B, while better prevention and treatment will control hepatitis C. Severe alcoholism is decreasing in several countries, and general practitioners have been taught to quickly discontinue offending drugs. Although these declining trends might suggest a leaner future for hepatologists, an emerging disease of affluence is providing a different outlook. Western population~, including the youth, are getting fatter and fatter, and the consulting rooms of hepatologists are increasingly filled with patients with steatosis-related liver diseases.

Ironically, although this disease may soon represent a major part of their activity, hepatologists have long been blind to the pathogenic consequences of hepatic steatosis. Older reports of its fibrogenic potential have been mostly overlooked, and steatosis has been considered as a benign liver condition. In recent years, however, it has become increasingly clear that hepatic fat accumulation, whatever its cause, can be the basis for the development of a potentially severe liver condition, called steatohepatitis. Like all other forms of chronic hepatitis, steatohepatitis can progressively develop into cirrhosis.

The purpose of this review is to recall the role of mitochondria in hepatic fat disposition and the formation of reactive oxygen species (ROS), then describe the clinical aspects of steatosis and steatohepatitis, and finally discuss potential mechanisms of steatohepatitis.

ROLE OF MITOCHONDRIA IN HERA TIC FAT DISPOSITION

Fatty acids are directly synthesized within the liver or are transferred to the liver from the intestine and adipose tissue. In the liver, fatty acids either enter mitochondria and undergo mitochondrial β-oxidation or are esterified into triglycerides which accumulate in part within the cytoplasm and, in part, undergo partial de-esterification and then resynthesis in the endoplasmic reticulum, and are then secreted, together with apolipoprotein B, as very low density lipoproteins (VLDL).
The entry of long-chain fatty acids in mitochondria is critically dependent on the activity of carnitine palmitoyl transferase I (CPTI), an outer membrane enzyme whose activity is inhibited by malonyl-CoA. Once within mitochondria, fatty acids are split by β-oxidation cycles into acetyl-CoA sub-units which, together with other fuels, may then be completely degraded to $C0_2$ by the tricarboxylic acid cycle.
The NADH and $FADH_2$ that are generated by β-oxidation and the tricarboxylic acid cycle, are then reoxidized by the mitochondrial respiratory chain attached to the inner mitochondrial membrane. This regenerates the NAD+ and FAD necessary for other cycles of fuel oxidation.
Most of the electrons that are transferred to the first complexes of the respiratory chain by NADH and $FADH_2$ migrate all the way along the respiratory chain, up to cytochrome-c oxidase, where they safely combine with oxygen and protons to form water. During this transfer of electrons along the respiratory chain, protons are extruded from the mitochondrial matrix into the inter-membrane space. This creates a large electrochemical potential across the inner membrane whose potential energy is then used to generate ATP. When energy is needed, protons re-enter the matrix through the F_0 portion of ATP synthase. This causes the rotation of a molecular rotor in the F_1 portion of ATP synthase and ATP synthesis.
Normally, the hepatic handling of fatty acids is regulated by the availability of glucose and insulin. Under conditions of high glucose/insulin levels, such as the normal postprandial state, hepatic malonyl-CoA is high[7]. These high hepatic malonyl-CoA levels cause fatty acid synthesis, because malonyl-CoA is the first committed step in this synthesis[7]. Concomitantly, the high malonyl-CoA levels inhibit CPTI and thus the entry of long-chain fatty acids into mitochondria, and their mitochondrial β-oxidation. This inhibition of β-oxidation directs the fatty acyl-CoA towards triglyceride synthesis, triglyceride deposition in the cytoplasm and VLDL secretion. Conversely, during fasting (with low glucose/insulin levels) opposite regulations occur[7].

ROLE OF MITOCHONDRIA IN ROS FORMATION

A small fraction of the electrons that are transferred to the first complexes of the respiratory chain by NADH and $FADH_2$ directly react with oxygen, forming the superoxide anion radical and other ROS. As a consequence, even in the basal state, mitochondria are by far the main source of ROS in the cell[9].
This high basal ROS formation is further increased whenever the transfer of electrons along the respiratory chain is impaired at some step of the respiratory chain. This impairment may be due to different mechanisms. Drugs, lipid peroxidation products, cytokines or NO may directly inhibit the transfer of electrons along the respiratory chain. Ethanol, copper and some drugs can oxidatively damage mitochondrial DNA which encodes some of the polypeptides of the respiratory chain'[0]. When the flow of electrons is blocked at some point of the respiratory chain, respiratory chain components located upstream become overly reduced and directly transfer their electrons to 02, thus increasing the basal formation of ROS. Increased ROS formation oxidatively damages mtDNA, causing more impairment of respiration and more ROS formation and creating a vicious circle.
Increased ROS formation may also cause opening of the mitochondrial permeability transition pore. Mitochondria integrate most anti-death or pro-death signals by either closure or opening of this inner membrane pore, and play a critical role in most forms of cell death. Increased ROS formation, or exposure to some cytokines, can cause opening of this pore and hepatocyte cell death, through either necrosis (due to severe ATP depletion) or apoptosis (due to caspase activation)" .

CLINICAL ASPECTS

Different types of hepatic steatosis

Hepatic steatosis is characterized by the accumulation of fat (mainly triglycerides) within the cytoplasm of hepatocytes[8]. Two main morphological aspects can he distinguished[8]. In macrovacuolar steatosis, hepatocytes are distended by a single, large vacuole of fat, displacing the nucleus to the periphery of the cells. By contrast, in microvesicular steatosis, numerous tiny lipid vesicles leave the nucleus in the centre of the cell and give the hepatocyte a 'foamy', 'spongiocytic' appearance. In several conditions, however, both types of steatosis are concomitantly observed: some hepatocytes exhibit macrovacuolar steatosis, while other hepatocytes are filled with tiny lipid vesicles. Furthermore, transitional cells are often present, with both small vesicles and larger vacuoles. These associations and transitions indicate that tiny lipid vesicles can coalesce into larger vacuoles.
Nevertheless, for reasons that are unclear, microvesicular steatosis predominates in conditions due to acute impairment of the mitochondrial β-oxidation of fatty acids[8]. This could be due to a difference in the nature of accumulated lipids. When β-oxidation is impaired, free fatty acids increase in the liver, and these amphiphilic compounds might form an emulsifying rim around a core of neutral triglycerides, thus favoring small fat vesicles[8]. However, the major reason for the predominance of microvesicular fat may just be that the acute onset or sudden aggravation of these mitochondrial impairments leave no time for progressive

coalescence of small lipid vesicles into larger vacuoles. Instead, macrovacuolar steatosis tends to predominate in stable, prolonged causes of steatosis due to excess weight; diabetes or regular alcohol abuse for example[8].
Hepatic steatosis can be aetiologically classified into two main subgroups. In a first subgroup, fat accumulation is due to diverse combinations of obesity, hypertriglyceridemia, diabetes and insulin resistance. These factors are intertwined, and this aetiological group should be considered as a whole, for example as 'primary steatosis' or 'thrifty steatosis'. In a second subgroup, steatosis is related to diverse single causes ('secondary steatosis').

Primary steatosis and steatohepatitis

As we live an increasingly virtual life (sitting all day in front of computers and TV), avoid all physical exercise (thanks to cars, lifts and domestic appliances), refrain from smoking (due to public disapproval and government pressure), and indulge in soft drinks, sweets, cookies, brownies, ice cream, hamburgers and French fries, an ever-increasing fraction of the Western population has become overweight or frankly obese. As always, the US has led the race, but Canada, Japan, England and several northern European countries are quickly catching up, while several southern European countries are still lagging behind.
The body mass index (BMI) is the weight in kilograms divided by the square of the height in metres'[3]. A BMI of 25-29.9 indicates overweight; a BMI of 30-39.9 defines obesity and a BMI of 40 or more characterizes morbid obesity'[3]. About 20% of US men and *25%* of US women are already obese'[3]. In affluent socioeconomic status, while the converse is observed in emerging countries[13]. In both men and women the prevalence of overweight and obesity increases with the age until 50-60 years. This age group has adopted affluent lifestyle habits more quickly than their more conservative elders. A worrying observation is that this trend also affects the youth. In the US, 27% of 6-11 year-old children and 22% of 12-18-year-olds are overweight'[4]. Since obese adolescents tend to become obese adults, this suggests an even gloomier forecast in the future. The prevalence of obesity could reach 40% of the US population around the year 2025.
Besides favoring diabetes, hypertension, arteriosclerosis, cardiac failure, sleep apnoea, arthrosis, reflux oesophagitis, cholelithiasis, poor general health and early death'[3], obesity causes liver problems. In several countries, hepatic steatosis has become the main cause of liver test abnormalities in adolescents'[4], and the second or third cause in adults~[5].
Hepatic steatosis may slightly increase serum transaminase and/or gammaglutamyl transferase activity, while ultrasonography may show hyper-reflective, and sometimes enlarged, liver. In mildly overweight subjects the loss of some kilograms can be enough to restore normal liver tests, and solve the problem. Otherwise, a liver biopsy can provide a distinction between simple steatosis and steatohepatitis. In the latter, steatosis is associated with other liver lesions. In mild cases there is some inflammation and mild perisinusoidal fibrosis'[6]. In more severe cases there is also hepatocyte ballooning, Mallory bodies (often poorly developed), and marked fibrosis'[6]. Ultrastructural mitochondrial lesions (with the presence of linear crystalline inclusions in megamitochondria) are found in most (8/10) of these patients'[7], and the activity of respiratory chain complexes is decreased'[8].
The exact prevalence of marked fibrosis and cirrhosis in these patients is unclear, as different criteria for 'steatohepatitis' have been used in different studies, and the fibrogenic potential depends on the severity of the necroinflammatory activity[5,19]. Published figures range from 15% to *50%* . In a recent study of moderately overweight patients with abnormal liver tests, 30% had septal fibrosis, including 11% who had silently progressed to cirrhosis'[9].
Whereas pure steatosis may remain stable for years[9,20], in contrast steatohepatitis (with necroinflammatory activity) may develop into cirrhosis[5~20]. In one long-term study, 30% of patients with initially marked fibrosis bad developed cirrhosis 10 years later[21].
Paradoxically, rapid weight loss is best avoided in severely obese subjects, as this may dramatically increase peripheral lipolysis, thus increasing the delivery of free fatty acids to the liver. Free fatty acids are toxic to mitochondria[8], and excessive dieting may paradoxically aggravate the liver injury. Extensive weight loss due to starvation[4], severe dieting[22], jejunoileal bypass[23] or gastroplasty[24] has been found to paradoxically increase liver inflammation and fibrogenesis, despite a decrease in steatosis.
Instead, the combination of increased physical exercise and a moderately hypocaloric diet (high in green and red vegetables but low in sugar, amidon and fat), with sometimes the help of a hypolipidaemic drug or antidiabetic agent as needed, can improve liver tests, decrease steatosis and stop fibrogenesis~[26]. In a pilot study, ursodeoxycholic acid administration seemed to further improve liver tests[27].

Although it would seem preferable, whenever possible, to obtain weight loss without resorting to drugs, treatments are available to reduce food intake or fat absorption[28]. Sibutramine inhibits the reuptake of noradrenaline and serotonin, and decreases food intake[28]. Its principal side-effects are dry mouth, insomnia and asthenia, with also a small increase in blood pressure and heart rate[28]. Orlistat is a lipase inhibitor that blocks pancreatic lipase, thus decreasing fat digestion and absorption. About 30% of ingested triglycerides are lost in the stools, helping patients to lose weight[28]. Partial fat malabsorption may cause minor side-effects, such as increased defaecation and faecal urgency[28].

After weight loss, regular follow-up is mandatory, to make sure the patient does not put on weight again. Unlike pounds of money, pounds of weight are difficult to lose but easy to gain.

In patients with severe steatohepatitis and cirrhosis, liver transplantation can be performed. Although a new liver is provided, the underlying cause must still be treated since steatohepatitis may develop again in the transplanted liver[29,30].

'Secondary' steatosis and steatohepatitis

In addition to this 'primary/thrifty' form, there are several causes of 'secondary' steatosis and steatohepatitis[8], including alcohol abuse, Wilson's disease, cholestasis, some drugs (amiodarone, perhexiline, tamoxifen), jejunoileal bypass[23], total parenteral nutrition[31], hepatitis C[32], HIV infection[33], and perhaps exposure to petrochemical products[34]. The hepatitis C core protein may affect mitochondria and lipid disposition while the HIV rnRNA[36] and the HIV viral protein R[37] have deleterious effects on mitochondrial function.

Interestingly, two different causes of steatosis in the same patient may exert additive effects on the fibrotic outcome. Overweight-related steatosis may increase the fibrotic Outcome of chronic hepatitis C[32] and alcoholic liver disease[38].

MECHANISM OF PRIMARY[1] STEATOSIS

'Primary/thrifty' steatosis is due to various combinations of excess weight, hypertriglyceridemia and/or overt type 2 diabetes, with some degree of insulin resistance in most cases[26~3941]. Insulin resistance (with high insulin levels) is observed even in non-alcoholic steatohepatitis patients who are lean and do not exhibit glucose intolerance[42]. Severe insulin resistance syndromes also cause steatohepatitis[43].

Insulin resistance probably has a genetic basis, as relatives of subjects with type 2 diabetes often also have insulin resistance. Normally the muscles are the principal site of insulin-stimulated glucose disposal, with less glucose being transported into adipose tissue. The main insulin-responsive glucose transporter is GLUT-4, a transporter which is selectively located in muscle and adipose tissue. The interaction of insulin with its plasma membrane receptor uses the translocation of GLUT-4 from intracellular vesicles to the plasma membrane.

The interplay of insulin resistance, steatosis and hypertriglyceridaemia is poorly understood. A first difficulty is that there seems to be a vicious circle in which insulin resistance causes hyperariglyceridernia, which may decrease fatty acid oxidation in muscle, decrease muscle glucose oxidation, and thus cause further insulin resistance[45], making it difficult to tell which comes first. A second difficulty is that insulin resistance may differently affect different tissues and different metabolisms. Normally, two major short-term effects of insulin are to block postprandial adipose tissue lipolysis and to decrease the hepatic mitochondrial β-oxidation of fatty acids[7]. In the insulin resistance state, peripheral insulin resistance could result in inadequately persistent adipocyte lipolysis in the post-prandial State (thus overloading the liver with free *fatty* acids)[45], while increased glucoselinsulin levels might normally inhibit mitochondrial fatty acid β-oxidation in the liver, thus orienting fatty acids towards triglyceride synthesis, accumulation in the cytoplasm, and secretion as VLDL.

MECHANISM OF STEATOHEPATITIS

Although the mechanisrn. of steatohepatitis is incompletely understood, there is growing evidence that it may be caused by a basal oxidative stress that can be aggravated by several added factors.

Basal oxidative stress

In ruice, acute or chronic steatosis due to 11 different treatments was always associated with lipid peroxidation[48]. After a single dose of tetracycline or ethanol, maximal ethane exhalation (an *in-vivo* index-of lipid peroxidation) occurred at the time of maximal hepatic triglyceride accumulation. Whereas a single dose of doxycycline or glucocorticoids did not increase ethane exhalation (or hepatic triglycerides), repeated doses increased hepatic triglycerides and ethane exhalation[48]. Extensive lipid peroxidation was also observed in rats with steatohepatitis caused by a diet deficient in methionine and choline[49]. These observations suggest that the high basal formation of ROS by mitochondria is enough to oxidize hepatic fat deposits, causing some lipid peroxidation. Obviously, however, any condition that further increases ROS formation ('second hit') will further increase oxidative stress and the development of steatohepatitis.

Increased ROS formation In 'primary' steatohepatitis

Several mechanisms might concur to increase ROS formation in some patients with 'primary' ('thrifty') steatosis.

Lipid peroxidation and mitochondrial ROS formation

The lipid peroxidation product, 4-hydroxynonenal (HNE), reacts with respiratory chain polypeptides, including cytochrome-c oxidase, and inhibits mitochondrial respiration[50]. In 'primary' steatohepatitis, mitochondria exhibit ultrastructural lesions'[7] and the activity of respiratory chain complexes is decreased'[8]. Whenever the transfer of electrons is impaired at some step of the respiratory chain. respiratory chain components located upstream become overly reduced. These overly reduced components then directly transfer their electrons to 02, thus increasing the basal formation of ROS. Thus lipid peroxidation will further

increase mitochondrial ROS formation, causing more peroxidation, more mitochondrial DNA damage and more ROS formation in a vicious circle.

Tumour necrosis factor-α and membrane oxidase

Adipose tissue is an important source of TNF-α. This cytokine causes opening of the mitochondrial permeability transition pore[12] and impairs mitochondrial respiration[52], two effects which increase mitochondrial ROS formation. Second, insulin induces hydrogen peroxide formation in human adipocytes, through stimulation of a membrane-bound NADPH-dependent oxidase[53].

Iron

For reasons that are not yet clear, the insulin resistance steatosis state is associated with increased ferritin concentrations, and several patients with this syndrome also have increased hepatic iron deposits, even when they do not carry the C282Y mutation of the HFE gene[57,58]. Ferrous iron is a powerful generator of the hydroxyl radical, and iron accumulation may thus further increase ROS formation. Increased hepatic iron stores seem to potentiate fibrogenesis in non-alcoholic steatohepatitis or chronic hepatitis C. This may be only a minor added factor, however, and iron was not detected as an independent variable for steatohepatitis in another study[60].
Heterozygous HFE mutations may further increase hepatic iron stores and may represent an added susceptibility factor to steatoheptitis[57~861].

Antioxidant vitamins and glutathione (GSH)

Antioxidant vitamins (e.g. α-tocopherol) and GSH help prevent lipid peroxidation, but both are consumed by lipid peroxidation. When these protective substances become depleted, lipid peroxidation increases.
Despite similar intakes, obese children exhibit a lower α-tocopherol/plasma lipid ratio than non-obese children[62], and supplementation with a-tocopherol (vitamin E) may normalize serum aminotransferase activity in these children[4]. Hepatic steatosis also decreases hepatic glutathione[63], and this depletion may further increase lipid peroxidation.

Antioxidant enzyme's

Although reports are scarce and sometimes discordant, decreases in GSH S-transferase, GSH-peroxidase and GSH-reductase have been reported in fatty liver. Other enzymes involved in the defence against oxidative stress, such as catalase and Cu,Zn-SOD, may also be decreased[66]. The mechanism(s) by which these antioxidant enzymes can be altered in fatty liver has not been elucidated.

Cytochrome P450 (CYP)2E1

CYP2El is a powerful generator of ROS and can cause or aggravate oxidative stress and fibrogenesis in the liver[7]. Different animal models of hepatic steatosis have shown different results with either an increase or a decrease in CYP2El protein and activity[6~71]. In humans, however, several studies suggest increased CYP2El activity and protein in diabetes, obesity and steatohepatitis[72-74]
A genetic polymorphism in the 5'-flanking region of the CYP2El gene is associated with an increased CYP2EI activity in obese individuals only[75].

CYP4A and dicarboxylic acids

The accumulation of fatty acids in the steatotic liver activates the peroxisome proliferator-associated receptor alpha (PPARα)[76]. PPARα and retinoid X receptors (RXRs) form heterodimers that bind to peroxisome proliferator response elements (PPREs) in the promoter of genes involved in fatty acid disposition, and this binding activates gene transcription[77]. In particular, PPARα activation induces CYP4A, which could form ROS and cause lipid peroxidation[78]. In addition, CYP4A ω-hydroxylates fatty acids, initiating their conversion into dicarboxylic acids. In rodents, dicarboxylic acids are degraded through concomitant PPARα-rnediated induction of peroxisomal β-oxidation enzymes[79]. However (for reasons that are not yet clear), peroxisomal induction is deficient in humans. In this species the increased formation of dicarboxylic acids without enhanced degradation could further impair mitochondrial function. Dicarboxylic acids uncouple oxidative phosphorylation and inhibit electron transfer within the mitochondrial respiratory chain[8]. Their toxic role has been demonstrated in a transgenic animal model[79,80]. Mice nullizygous for the peroxisomal fatty acyl-CoA oxidase exhibit high hepatic dicarboxylic acid levels[79,80]. Dicarboxylic acids are elevated due to both decreased degradation caused by the lack of peroxisomal β-oxidation and also increased generation through fatty acids/PPARα-mediated CYP4A induction. These dual effects thus reproduce what may occur in the human liver. These mice develop microvesicular steatosis, increased hepatic H_20_2 levels and steatohepatitis[79,80].

Uncoupling protein 2 (UCP2)

Fatty acids and PPARα also induce the hepatic expression of UCP2 both in animals with fatty liver and in humans with primary or secondary steatohepatitis[81]. UCP2 could allow the re-entry of protons through the inner membrane, thus by-passing ATP synthase[82]. Normally, the flow of electrons along the respiratory chain is blocked when a high membrane potential is achieved. When protons re-enter the matrix (either through ATP synthase or through UCP2-mediated uncoupling), more electrons can flow along the respiratory chain and basal respiration increases[82]. This increased respiration permits the reoxidation of the NADH and $FADH_2$ that are formed by mitochondrial β-oxidation, thus regenerating the NADH and FAD necessary for other β-oxidation cycles. UCP2 induction could thus enhance,fatty acid β-oxidation and this may be an adaptive mechanism favoring lipid disposition.
However, the enhanced respiration that is due to uncoupling occurs in vain to produce heat, instead of ATP (since ATP synthase is by-passed). Reduced ATP resynthesis after a fructose infusion has indeed been documented in humans with fatty live. Impaired ATP generation will enhance the detrimental effects of any cause that tends to decrease mitochondrial ATP generation, such as ischaemia. Furthermore, uncoupling also decreases the membrane potential, a condition which may favor opening of the inner membrane permeability transition pore[85], and may sensitize cells to cytokine-induced hepatocyte apoptosis.

Still higher ROS formation In 'secondary' steatohepatitis

While all the added factors described above will also play a role in 'secondary' steatohepatitis, the situation is even worse, because the causative disease itself increases ROS formation. This added increase in ROS formation may cause both higher lipid peroxidation and cytokine formation[86]. The severity of the 'second hit' in this case may explain the increased prevalence and severity of steatohepatitis in several forms of secondary steatosis, including alcohol abuse, Wilson's disease, administration of perhexiline or amiodarone, jejunoileal bypass or total parenteral nutrition.

Ethanol

Ethanol abuse causes a considerable increase in ROS formation and lipid peroxidation[87]. Ethanol induces CYP2El whose pro-oxidant effects were mentioned above. Furthermore, the metabolism of ethanol increases the NADH/NAD+ ratio, which may cause the reduction of ferric iron to ferrous iron, a potent generator of the hydroxyl radical. In mice a single dose of ethanol (causing plasma concentrations of 4 g/L) causes extensive mtDNA degradation and depletion within 2 h, followed by increased mtDNA synthesis and restoration of mtDNA levels. Although damaged mtDNA molecules are efficiently repaired or resynthesized *de novo* after a single dose, the chronic presence of mtDNA strand breaks during chronic alcoholism may increase the likelihood that some of these strand breaks may cause an mtDNA deletion through slipped mispairing. The prevalence of mtDNA deletions is increased in alcoholics, particularly those with microvesicular steatosis[89].

Copper

Wilson's disease is caused by diverse mutations of a nuclear gene encoding a copper-transporting P-type ATPase[91]. Decreased biliary elimination of copper causes progressive accumulation within hepatocytes. Due to its ability to cycle between the oxidized and the reduced State, copper generates the hydroxyl radical and other ROS. Because copper forms Cu-DNA complexes, these ROS are generated close to DNA, making it an elective target. Because copper selectively accumulates within mitochondria during copper overloads, mtDNA may be particularly affected. Indeed, despite their young age, half of patients with Wilson's disease already had one or several mtDNA deletion(s), whereas only 3% of older controls carried one mtDNA deletion[91].

Amiodarone, perhexiline and diethylaminoethoxyhexestrol

These cationic amphiphilic compounds have a lipophilic moiety and an amine function which can become protonated. The unprotonated, lipophilic form easily crosses the mitochondrial outer membrane and is protonated in the acidic intermembranous space[86,92-95]. This positively charged, protonated form is 'pushed' inside mitochondria by the high electrochemical potential existing across the mitochondrial inner membrane and thus reaches high intramitochondrial concentrations. These high concentrations inhibit both β-oxidation (causing Steatosis) and respiration (increasing the mitochondrial formation of ROS)[86,92-9~.

Jejunolleal bypass and total parenteral nutrition

In these two conditions, bacterial proliferation in the excluded/unused intestine may release endotoxins, cytoltines and NO, which all impair mitochondrial respiration[t0]. Lipid peroxidation may be aggravated by deficiencies in antioxidant vitamins[31].
Thus, in all these circumstances the causative disease itself may either directly increase ROS formation (e.g. alcohol, Wilson) and/or first impair the transfer of electrons along the mitochondrial respiratory chain (e.g. amiodarone, perhexiline, jejunoileal

bypass, total parenteral nutrition), which secondarily increases mitochondrial ROS formation. This may increase both lipid peroxidadon and cytokine production, and may explain the high prevalence of steatohepatitis in these secondary forms of steatosis.

Mechanisms of steatohepatitis lesions

Steatohepatitis lesions could be caused by the combined effects of lipid peroxidation cytokines and the Fas/Fas. ligand system, with the resulting cell death being potentiated by UCP2 induction.

Lipid peroxidation and steatohepatitis lesions

Lipid peroxidation products have the potential to cause the various steatohepatitis lesions. Lipid peroxidation causes cell death, which may explain liver cell necrosis. Peroxidation also releases malondialdehyde and 4-hydroxy-nonenal[96]. Both covalently bind to proteins, and these modified proteins may cause immune reactions and immune hepatitis. Both 4~hydroxynonenal and malondialdehyde are bifunctional agents that cross-link proteins, and might be involved in the formation of Mallory bodies, which contain cross-linked cytokeratin monomers[97,98]. Both 4-hydoxynonenal and malondialdehyde increase collagen synthesis by Ito cells, hence fibrosis. 4-Hydroxynonenal has a chemotactic activity for neutrophils, which may account for the neutraphilic cell infiltrate. Finally, ROS and/or lipid peroxidations may trigger the release of cytokines, which may cause further liver damage.

Increased production of cytokines

Several different mechanisms may increase cytokine production during steatohepatitis. First, as already mentioned, the adipose tissue itself is an important source of TNF-α[51]. Second, the lipid peroxidation product, 4-hydroxynonenal, up-regulates TGF-β1 expression in macrophages, and this could be a further link between oxidative injury and fibrosclerosis"'. Finally, ROS increase the synthesis of several cytokines, possibly through nuclear translocation of NF-κB. Indeed, ethanol-induced oxidative stress causes the release of several cytokines (including TNF-α, TGF-β and IL-8) from both Kupffer cells and hepatocytes themselves'[07]. Like lipid peroxidation products, cytokines may participate in the development of steatohepatitis lesions.

Cytokines and steatohepatitis lesions

TNF-α and TGF-β cause caspase activation and hepatocyte death[108~l09]. TGF-β activates tissue transglutaminase, which cross-links cytoskeletal proteins, in particular intermediate filament proteins[111], into large protein scaffolds, that might be involved in the formation of Mallory bodies. TGF-β also activates collagen synthesis by Ito cells. Finally, IL-8 is a potent chemoattractant for human neutrophils.

Among these diverse cytokines the release of TNF-α by Kupffer cells may play an important role in experimental alcohol-induced steatohepatitis. Ethanol-induced liver lesions can be attenuated by gadolinium chloride (which is selectively toxic to Kupffer cells), by the administration of anti-TNF-α antibodies, or through invalidation of the TNF receptor 1, which signals TNF-α-mediated cell death. In humans a polymorphism in the TNF promoter seems to be partially involved in the susceptibility to develop alcoholic steatohepatitis[116].

Fas and Fas ligand

Fas-mediated fratricidal killing may also be involved in cell death during chronic oxidative stress. Hepatocytes express Fas (a membrane receptor), but do not normally express Fas ligand, preventing them from killing their neighbors. However, the Fas ligand promoter contains NF-κB binding sites . Normally, NF-κB is maintained in the cytoplasm by IκB. However, ROS cause the phosphorylation, ubiquitination and proteasome-mediated degradation of IκB, allowing nuclear translocation of NF-κB. Conditions leading to increased ROS formation may thus cause Fas ligand expression by hepatocytes. At the same time, increased ROS formation may overexpress p53, and increase Fas expression by hepatocytes'[20]. Thus Fas ligand on one hepatocyte may interact with Fas on another hepatocyte, causing opening of the inner membrane permeability transition pore and Fas-mediated fratricidal killing.

Possible potentiation of cell death by UCP2

As already explained above, induction of UCP2 may decrease the mitochondrial membrane potential and could sensitize cells to necrosis or apoptosis caused by lipid peroxidation products, cytokines and Fas ligand.

GENETIC FACTORS

In both 'primary' and 'secondary' hepatic steatosis, the tendency of different subjects to develop steatohepatitis varies considerably. Although the various additional factors described above (iron accumulation or not, deficiency in antioxidants or not) may play some role, genetic polymorphisms probably play the most important role..

Genetic factors may act at two steps. First, some (mostly unknown) genetic polymorphisms may favour the development of obesity and the insulin resistance syndrome. Second, other polymorphisms may favour development of necroinflammation and fibrosis in subjects with hepatic steatosis. Besides the genetic factors which have been discussed above, namely CYP2El inducibility[75], heterozygous HFE mutations[61] and polymorphism of the TNF-α promoter, other genetic factors are probably involved. Hopefully, these other genetic factors will be discovered in the near future.

References

1. Zelman S. The liver in obesity. Arch Intern Med. 1958;90: 141-56.
2. Falchuk KR, Fiske SC, Haggilt RC, Federman M, Trey C. Pericentral hepatic fibrosis and intracellular hyalin in diabetes mellitus. Gastroenterology. l98O;78:53~l.
3. Ise RG. Nonalcoholic~ steatohepatitis: a study of 49 patients. Hum PathoL 1989;20:59~8.
4. Ludwig 1, McGill DB, Lindor KD. Nonalcoholic steatohepatitis. 3 Gastroenterol Hepatol. l997;12:39~03.
5. James 0, Day C. Non-alcoholic steatohepatitis: another disease of affluence. Lancet. 1999;35:l63~.
6. Diebl AM. Nonalcoholic steatohepatitis. Semin Liver Dis. 1999;19:221-9.
7. McGan'y JD, Foster DW. Regulation of hepatic fatty acid oxidation and ketone body production. Annu Rev Biochem. I 980;49:395~2O.
8. Fromenty B, Pessayre D. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. Pharmacol Ther. 1995;67:10l-54.
9. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proc Nail Acad Sci USA. l994;91:1o771~.
10. Promenty B, Berson A, Pessayre D. Microvesicular Steatosis and steatohepatitis: role of mitochondrial dysfunction and lipid l'eroxidation. J Hepatol. 1997;26(Suppl. l):l~22.
11. Pessayre D, Haouzi D. Fau D, Robin MA, Mansouri A, Berson A. Withdrawal of life support, altruistic suicide, fratricidal killing and euthanasia by lymphocytes: different forms of drug-induced hepatic apoptosis. J Hepatol. 1999;3 1:76~70.
12. Pessayre D, Feldmann G, Haouzi D, Fau D, Moreau A, Neuman M. Hepatocyte apoptosis triggered by natural substances (cytokines. other endogenous substances and foreign toxins). in: Cameron RG, Feuer 0, editors. Apoptosis and its Modulation by DrugL Heidelberg: Springer V&lag; Handbook Exp PharmacoL 2000;142:59-log.
13. Kopelman PG. Obesity as a medical problem. Nature. 2000;404:63i~3.
14. Lavine JE. Relative antioxidant deficiency in obese children: a weighty contributor to morbidity? *I* Pediatr. 1999;134:132-3.
15. Byron D, Minuk GY Profile of an urban hospital-based practice. Hepatology. l996;24:8l~l5.
16. Brunt EM, Janney CO, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am I Gastroenterol. 1999;94:2467-74.
17. CaIdwell SH, Swerdlow RH, Khan EM *et al* Mitochondrial abnormalities in non-alcoholic steatohepatitis. I Hepatol. 1999;3 I :430A.
ill. P&ez~arrera M, Del Hoyo P, Martin Met *al* Activity of the mitochondrial respiratory chain enzymes is decreased in the liver of patients with nonalcoholic steatohepatitis. Hepatology.
19. Ratziu V, Giral P, Charlotte F *et aL* Liver fibrosis in overweight patients. Gastroenterology. 2000;tl8:1ll7~23.
20. Teli MR. James OFW~ Buit AD, Bennett ~ Day CP. The natural history of non-alcoholic fatty liver: a follow up study. Hepatology. 1995;22:l7l~19.
21. Younossi ZM, Matteoni CI, Gramlich T et *aL* Patient characteristics predioting cirrhosis and death in non~coho1ic steatohepatitis. Hepatology. 1998;28:303A.
22. Andersen T, Glnud C, Franzmann MB, Christoffersen P. Hepatic effects of dietary weight loss in morbidly obese subjects. J Hepatol. l99];l2:22~9.
23. Hocking Ml, Davis GL, Franzini DA, Woodward ER. Long-term consequences after jejunoileal bypass for morbid obesity. Dig Dis Sd. l998;43:249~9.
24. Luyckx PH, Desaive C, Thiry A el a!. Liver abnormalities in severely obese subjects: effect of
25.' drastic weight loss after gastroplasty. mt J obes. l998;22:222~.
Ueno T, Sugawara H, Sujalru K ci *aL* Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. 3 HepatoL l997;27:l0~7.
26. Knobler H, Schattuer A, zilornicki T ci *aL* Patty liver an additional and treatable feature of the insulin resistance syndrome. QJ Mcd. l999;92:7~9.
27. Laurin 3, Lindor KD, Crippin 35 ci *aL* Ursodeoxycholic acid and clofibrate in the treatment of nonalcoholic steatohepatitis: a pilot study. Hepatology. l996;23:1~7.
28. Bray GA, Tartaglia LA. Medicinal strategies in the treatment of obesity. Nature. 2000;404:672~7.
29. Carson K, Washington MK, Treem WR, Clavier PA, Hunt CM. Recurrence of nonalcoholic steatohepatitis in sliver transplant. Liver Transplant Surg. l997;3:l7~.
30. Molloy RM, Komorowski R, Varma RR. Recurrent nonalcoholic steatohepatitis and cirrhosis after liver transplantation. Liver Transplant Surg. 1997;3:1778.
31. Pironi L, Rugged E, Zolezzi Cci *aL* Lipid peroxidation and antioxidant status in adults receiving lipid-based home parenteral nutrition. Am J Clin Nutr. 199S;68:888-93.
32. Hourigan LF, Macdonald GA, Purdie D *et a!.* Fibrosis in chronic hepatitis C correlate' significantly with body mass index and steatosis. Hepatology. l999;29:l2l~l9.

33. Albisetti M, Braegger Cl, Stallmach T, Willi UV, Nadal D. Hepatic steatosis: a frequent non-specific finding in HIV-infected children. Eur 3 Pediatr. l999;l58:97l~.
34. Cotrim HP, Andrade ZA. Parana R, Portugal M, Lyra LG, Freita, LAR. Nonalcoholic steatohepatitis: a toxic liver disease in industrial workers. Liver. l999;j9:29~304
35. Modys K, Fujie H, Shintani Y ci *al.* The core protein of hepatitis C virus induces hepatocellar carcinoma in transgenic mice. Nature Med. I 998;4: l06~7.
36. Somasundaran M, Zapp ML, Beattie LK ci *aL* Localization of HIV RNA in mitochondria of infected cells: potential role in cytopathogenicity. 3 Cell Biol. l994;I26:l35~0.
37. Jacotot B, Ravagnan L, Loeffier M ci *aL* The R~V viral protein R induces apoptosis via a direct effect on the mitochondrial permeability transition pore. [3]Bxp Med. 2000;l9l:3~5.
38. Navesu S, Giraud V, Borotto B, Aubert A, Capron P Chaput JC. Excess weight risk factor for alcoholic liver disease. Hepatology. l997;25:l0~1 I.
39. Banerji MA, Buckley MC, Chalken RL, Gordon D, Lebowitz HE, Keal JG. Liver fat, serum triglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black n'en with NIDDM. Tnt 3 Obes. l995;l9:84~50.
40. Goto T, Onuma T, Takebe K, Kral IG. The influence of fatty liver on insulin clearance and insulin resistance in nondiabetic Japanese subjects. Int 3 Ole's. 1995;19:841-5.
41. Tankurt B, Biberoglu S, Ellidokus B ci *aL* Hyperinsulinemia and insulin resistance in nonalcoholic steatohepatitis. 3 HepatoL I999;3l:96~8.
42. Marchesini G, Brizi M, Morselli-Labate AM ci aL Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med. 1999; 107:4505.
43. Herion DW~ Ariogln B, Doo B *et aL* Severe insulin resistance syndromes and NASH. Hepatology. [1]999;30:589A.
44. Shepherd PR, Knan BB. Glucose transporters and insulin action. Implications for insulin resistance and diabetes mellitus. N EngI J Med. 1999;34l:248~57.
45. Sparks JD, Sparks CE. Insulin regulation of triacylglycerol~rich lipoprotein synthesis and Secretion. Biochim Biophys Act& 1994;12[5:9-32.
46. Pessayre D. Liver failure and mitochondrial disease. in: Balistreri WP Lindsay K, Stecluer S, editors. American Association for the Study of Liver Diseases 1999 Postgraduate Course:
Hepatology into the next Millennium: Lessons from the Past - Issues for the Future. AASLD Postgraduate Course, Dallas. 1999:147-57,
47. Pessayre D, Fromenty B, Mansouri A. Drug-induced steatosis and steatohepatitis. In:
Lemasters 31, Niemenen N-L, editors. Mitochondria in Pathogenesis. New York: Plenum Press;2000 (In press).
48. Lett6ron P, Fromenty B, Terris B, Degolt C, Pessayre D. Acute and chronic steatosis lead to *in* vivo lipid peroxidation in mice. I Hepatol. 1996;24:20(IS.
49. Pera N, Phung N, Farrell GC. Oxidative stress in hepatic fibrogenesis: implications from a nutritional model of nonalcoholic steatohepatitis. Hepatology. l999;30:493A.
50. Chen 3, Schenker S, Frosto TA, Hensderson GI. Inhibition of cytochrome C oxidase activity by
4~hydroxynonenal (HNE). Role of HNB adduct formation with the enzyme catalytic site.
Biochem Biophys Acta. 1998;1380:33644.
51. Kern PA, Saghizadeb M, Ong ml, Bosch RI, Deem R, Simsolo RB. The expression of tumor necrosis lactor in human adipose tissue. Regulation by obesity. weight loss, and relationship to lipoprotein lipase. I Clin InvesL 1995;95:21 11-19.
52. Lancaster JR, Isaster SM, Gooding IR. Inhibition of target cell mitochondrial electron transfer by tumor necrosis factor. FEBS IetL 1989,248:169-74.
13. Kieger-Btauer H, Kather H. Human cells possess a plasma membrane-hound H_20_2-generating system that is activated by insulin via a mechanism bypassing the receptor kinase. 3 Clin invest. 1992;89:100~l3.
54. Bacon BR, Far'shvash MI, Janney CG, Neuschwander-'Ietri BA. Nonalcoholic steatohepatitis:
an expanded clinical entity. Gastroenterology. 1994;107: I 10~9.
55. Tuomainen Th Nyyssbnen K, Salonen R *et aL* Body iron stores are associated with serum insulin and blood glucose concentrations. Population study in 1,013 Finnish men. Diabetes
56. Care. l997;20:42~.
Fernandez-Real ml Ricart-Engle W, Arroyo e: *aL* Serum ferritin as a component of the insulin
resistance syndrome Diabetes Care. 1998;2l:62~.
57. George DK, Goldwurm S. Macdonald GA Ct al. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology.
1998;114:311-18.
58. Mendler MH, ~I~irlin B, Moirand R *ɛı* aL Insulin resistance-associated hepatic iron overload. Gastroenterology. 1999;1 17:1 l5~3.
59. H6zode C. Cazeneuve C, Cou4 0 *et aL* Liver iron accumulation in patients with chronic active hepatitis C: prevalence and role of hemoebromatmis gene mutations and relationship with hepatic histological lesions. I Hepatol. 1999;3 1:979-84.
60. Angulo P, Keach IC, Batts Ki~ Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology. l999;30:I35~2.
61. Bonkovsky HL, lawald Q, Tortorelli K et *aL* Non-alcoholic steatohepatitis and iron: increased
- prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. I Hepatol. t999;3l:421~.
62. Strauss RS. Comparison of serum concentrations of a-tocopherol anti ~-carotene in a cross-sectional sample of obese and nonobese children (NHANES III). I Pedistr. l999;l34:l6~5.
63. Sastre I, Pallardo Fv, Llopis I, Furukawa T, Vina JR, Vms 3. Glutathione depletion by hyperphagia~1nduced obesity. Life Sci. 1989;45:183-7.
64. Barnett CR, Abbott RA. Bailey CI, Flatt PR, bannides C. Cytochrome P450-dependent mixed-function oxidase and glutathione 5-transferase activities in spontaneous obesity-
- diabetes. Biochem Phartnacol. 1992;43:1868-71.
65. Capel ID, Dorrell HM. Abnormal antioxidant defence in some tissues of congenitally obese mice. Biochem 3. 1984;219:41-9.
66. Watson AM, Poloyac SM, Howard G, Binuin ItA. Effect of leptin on cytochrome P-450, conjugaison, and antiosidant enzymes in the oblob mouse. Drug Metab Dispos. l999;27:69~700.
67. Nieto N, Friedman SL. Greenwel P, Cederbaum Al. CYP 2lt1-mediated oxidative Stress induces collagen type I expression in rat hepatic stellate cells. Hepatology. 1999;30:987-96.
68. Weltman MD, Farrell GC, Liddle C. Increased hepatocyte C~El expression in a rat nutri tional model of hepatic steatosis with inflammation. Gastroenterology. 1996;l 11:1645-53.

69. Leclercq 1, Ho'smans Y. Desager JR Pauwels S, Geubel AP. Dietary restriction of energy and sugar results in a reduction in human cytochrome P450 2E1 activity. Br I Nutr. l999;82:217~2.
70. Leclercq I, Horsmans Y, Desager IF, Delzenne N. Geubel AR Reduction in hepatic cytochrome P-450 is correlated to the degree of liver fat content in animal models of steatosis in the absence of inflammation. I HepatoL l998;28:4l~l6.
71. Enriquez A. Leclercq I, Farrell OC, Robertson G. Altered expression of hepatic CYP2EI and CYP4A in obese, diabetic obIob mice, and falfa Zucker rats. Biochem Biophys Res Commun. l999;255:3o(~~.
72. Lucas D, Farez C, Bardou LG, Vaisse I, Attali JR, Valensi P. Cytoebrome P450 2E1 activity in diabetic and obese patients as assessed by chiorroxaxone hydroxylation. Fund Clin Phamacol. 1998;12:5538.
73. Kotliar M, Carson SW. Effects of obesity on the cytochrome P450 enzyme system. mt I Clin Pharmacol Ther. 1999;37:9-18.
74. Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytocbrome P450 2E1 is increased in patients with nonalcoholic SteatohepatitiL Hepatology. 1998,'27: l2~33.
75. McCarver DG, Byun R, Hines RN, Itichme M, Wegenek WA genetic polymorphism in regulatory sequences of human CY~E1: sssociation with increased chlo'zoxazone hydroxylation in the presence of obesity and ethanol intake. Toxicol AppI Pharmacol. 1998; l52:27~ I.
76. Vamecqi, Latruffe N. Medical significance of peroxisome proliferator-activated receptors. Lancet. 1999;354:1418.
77. Schoonjans K, Staels B, Auwerx I. The peroxisome proliferstor activated receptors OPPARo) and their effects on lipid metabolism and adipocyte differentiation. Biochim Biophys Acta. 1996; 1302:93-109.
78. Lecle~ IA, Field I, Bell DR, Farrell OC. Involvement of C~El and CYP4A in the path~ genesis of experimental nonalcoholic steatohepatitis: insights from studies in C~El knockout mice. Hepatology. 1999;30:424A.
79. It~hirnoto T, lojita T, Usuda *Net aL* Peroxisomal and mitochondrial fatty acid ~oxidation in mice nullizygous for both peroxisome proliferator-activated receptor a and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. I Biol Chem. 1999;274: 19228-36.
80. Fan CY, Pan I, Usuda N. Yeldandi AV, Rao MS, Reddy IK. Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-COA oxidase. Implications for peroxisome proliferator-activated receptor a natural ligand metabolism. I Biol Chem. 1998;273: l563~5.
81. Rashid A, Koteish AK, Cortez-Pinto H *et aL* Subcellular localization of uncoupling protein (UCP)2 and evidence for increased UCP2 expression in nonalcoholic steatohepatitis. Hepatology. 1999;30:405A.
82. Rial E, Gonzalez-Barroso M, lleury C ci *aL* Retinoids activate proton transport by the uncoupling proteins UCPI and UCfl. EMBO I. 1999;18:5827-33.
83. Cortez-Pinto, Chatham I, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver Alp homeostasis in human nonalcoholic steatohepatitis. A Pilot study. I Am Med Assoc. 1999,282:1659-64.
84. Chavin KD, Yang SQ, Lin Hz *et aL* Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver Alp depletion. I Biol Chem. 1999,274.5692-700.
85. Lemasters II, Nieminen AL, Qian T Ct *aL* The mitochondrial permeability transition in cell desth: a common mechanism in necrosis, apoptosis and autophagy. Biochim Biophys Acts. 1998;1998: 177-96.
86. Berson A, Dc Beco V, lectt~on P et *aL* Steatohepatitis-inducing drogs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes. Gastroenterology. 19981 l4:7~74.
87. lett~ron P, Duchalelle V, Berson A *ct aL* Incraased ethane exhalation, an *in vivo* index of lipid peroxidation, in alcohol abusers. CiuL 1993;34:409-[4.
88. Mansouri A, Gao" I, Dc Kerguenec C et aL An alcoholic binge causes massive degradation of hepatic mitochondrial DNA in mice. Gastroenterology. 1999;l 17:181-90.
89. Fromenty B, Grimbert S, Mansouri A er *aL* Hepatic mitochondrial DNA deletion in alcoholics: association with microvesicular StestOSis. Gastroenterology. l995;l08:l9~200.
90. Mansouri A, Fromenty B, Berson A et *aL* Multiple hepatic mitochondrial DNA deletions Suggest premature oxidative aging in alcoholic patients. I Hepatol. I997;27:9~l02.
91. Mansouri A, Gaou I, Fromenty B er *aL* Premature oxidative aging of hepatic mitochondrial DNA in Wilson's disease. Gastroenterology. 1997;l l3:599~05.
92. Fromenty B, Fiech C, Berson A, Lett6ron P, Larrey D, Pessayre D. Dual effect of amiodarone on mitochondrial respiration. Initial protonophoric unco"plin~ effect followed by inhibition ofthe respiratory chain at the levels of complex I and complex II. J Pharmacol Exp Ther. 1990;255: 1377-84.
93. Fromenty B, Fisch C, Labhe G et *aL* Amiodarone inhibits the mitochondrial ~oxidation of fatts acids and produces microvesicular Steatosis of the liver in mice. I Pharmacel Exp Ther. 94. 0;255:l37l~. Fromenty B, Latteron P, Fiach C, Berson A. Deschamps D, Pessayre D. Evaluation of human blootl lymphocytes as a model to study the effects of drugs on human mitochondria. Effects of low cOncentrations of amiodarone on fatty acid oxidation, Alp levels and cell survival. Btochem Pharmacol. 1993;46:421-32.
95 Deschamps D, De Beco V, Fiach C, Fromenty B, Guillouzo A, Pessayre D. Inhibition by perhexiline of oxidative phosphorylation and the ~oxidation of fatty acids; possible role in pseudoalcoholic liver lesions. Hepatologv. 1994; l9:948~l.
96 Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of~hydroxynonet,al, malondialdehyde and related aldehydes. Free Radic Biol Med. 1991:11:81-128.
97 Zatloukal Ic, Bock G, Rainer I, Denk H, Weher H. High molecular weight components are n constituents of Mallory bodies isolated with a fluorescence activated cell sorter. Lab
98 Zhang-Gouillon ZQ, Yuan QX, Hu B £1 *aL* Mallory body formation by ethanol feeding in drug-primed mice. Hepatology. 1998:27:1 l~22.
99 Kamimura S, Gaal K, Brltton RS, Bacon BR, 'lIriadafilopoulos G, Tsukamoto H. Increased ~ ~hydroxynonenal levels in experimental alcoholic liver disease: association of lipid peroxidation with liver fibrogenesis. Hepatology. 1992:1 6:44~53.

100 Bedossa P Houglum 11, Trautwein C, Holstege A, Chojkier, M. Stimulation of collagen s-I (1) gene expression is associateil with lipid peroxidation in liepatocellular injury: a link to tissue fibrosis? Hepatology. 1994; 19:1262-71.
101. Cursio M, lllsterbauer H, Dianzani MU. Chemotactic activity of hydroxyalkenals on rats neutrophils. mt I Tissue R'tact. 1985;7: l37~2.
102. Leonarduxti G, Scavasta A, Biasi *FetaL* The lipid peroxidation end product 4-hydroxy-2,3-nonenal up-regulates trsnsforming growth factor ~l expression in the macrophage lineage: a link lietween oxidative injury and fibroscl"rosis. FASEB 1. 1997; 11:851-7.
103. Albrecht H, Schook LB, Jongeneel CV. Nuclear migration of NF-irB correlates with TNF-a mRNA accumulation. I Inflam. l995:45:~7l.
104. Gressner AM, Wulbrand U. Variation in immunocytochemical expression of transforming growth factor (TGF)-bets in hepatocytes in culture and liver slices. Cell Tissue Res.
1997;287: I 4~52.
105. Dong W, Simeonova Pp Gallucei R *etaL* Cytokine expression in hepatocytes: role of oxidative Stress. linterferon Cytokine Res.
1998:18:629-38.
106. Fang C, Lindrot KO, Badger TM, Ronis MIJ, Ingelmsn-Sundberg M. Zonated expression of cytokines in rat liver: effects of chronic ethanol and the cytochrome P450 2E1 inhibitor, Chlormethiazole. Hepatology. l998;27:l3O~l0.
107. Neuman MG, Shear NH, Bellentani S. Tiribelli C. Role of cytokines in ethanol-induced cytotoxicity in *yitro* in HepG2 cells. Gastroenterology. 1998; 115: l57~6.
108. Higuchi M, Aggarwal BE, Yeh EllH. Activation of CPP32-like protease in tumor necrosis factor-induced apoptosis is dependent on mitochondrial function. I Clin Invest.
1997;99: 175 l~.
109. Insyat-Hussain SH, Couct C, Cohen GM, Cain K. Processingiactivation of CPP32-like proteases is involved in transforming growth factor P1-induced apoptosis in rat hepatocytes. Hepatology. l997;25:l5l~26.
110. Ritter *SI,* Davies PIA. Identilication of a transforming growth factor-pt/bane morphogenic protein 4 (TGF-pl~MP4) response element within tissue transglutaminase gene promoter. 3 Biol Chem. 1998;273: I2798~06.
111. Trejo-Skalli AV, Velasco PT, Murthy SNP Lorand L. Goldman RD. Association of a transglutaminase-related antigen with intermediate filaments. Proc NatI Acad Sci USA.
1995;92:89404.
112. Casini A, Pinzani M, Milan S *et al.* Regulation of extracellular matrix synthesis by transforming growth factor-~1 in human fat storing cells. Gastroenterology.
1993;105:245-53.
113. Adachi Y, Bradford BU, Gao W, Bojes HK, Thurman RG. Inactivation of Kupifer cells prevents early alcohol-induced liver itiurt. Heoatoloav. 19942o45q~o
114. limuro Y; Gallucci RM, Luster MI, Kono H, Thurman RG. Antibodies to factor-a attenuate hepatic necrosis and inflammation due to chronic expost rats. Hepatology. 1997;26:l53~7.
115. Yin M, Wheeler MD, Kono H *et aL* Essential role of tumor necrosis laco induced liver injury in mice. Gsstroenterology. 1999;117:942-52.
116. Grove I, Daly AK, Bsssendine Ml, Day CP. Association of a tumor necrosis polymorphism with susceptibility to alcoholic steatohepatitis. Hepatology. 19'.
117. Takahachi T, Tanaka M, Inazawa I. AbeT, Suda T, Nagata S. Hurean Fss hg ture, chromosomal location and species specificity. Int Immunol. 1994;6:1567
1111. Naumann M, Scheidereil C. Activation of NF~~cB Ln *Wvo* is regulated by multil tions. BMBO I. l994;13:4597~07.
119. Strand S, Hofmann WI, Grambibler A et *aL* Hepatic failure and liver cell Wilson's disease involve CD95 (APC~lIFss) mediated apoptosis. Nature Med.
120. Miller M, Strand S, Hug H Ct *aL* Drug4nduced apoptosis in hepatoma cells is CD95 (APO-lIFas) receptorfligand system and involves activation of wild-t Invest. 1997;99:403-13.
121. Feldmano G, Haouzi D, Moreau A et *aL* Opening of the mitochondrial perrne~ pore causes matrix expansion and outer membrane rupture in Fss-rnediated h in mice. Hepatology. ~000;3l :67~3.
122. Barseb OS, Farooqi S, O'Rahilly S. Genetics of body weight regu
2000;404:64~5 1.