A Review of Fatty Liver/NASH and Liver Cirrhosis: Genetics, Prevention, Nutritional, Behavioral Modification, Exercise, Pharmaceutical, Biophysics and Biotech Therapy

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Abstract
This article reviews the current factors concerning obesity, metabolic syndrome or DM2 and the accumulation of fat which can result in fatty liver or steatohepatitis (NAFLD) and its progression to non-alcoholic steatohepatitis (NASH) and cirrhosis. The pathophysiology is discussed as well as the current treatment and nutritional options of steatohepatitis, SAMe, phosphatidylcholine, silymarin with vitamin E and probiotics. Since obesity, metabolic syndrome and NAFLD/NASH are polygenic as well as epigenetic, the current nutritional, pharmaceutical and biotech solutions are fairly limited. Future options may include biophysical such as temperature control, light therapy omeprazole and moderate magnetic field therapy capable of regulating over 2500 genes as well as novel can nabinoxidagents and polyphenols.

Keywords: Mitochondria; NASH; Metabolic-syndrome; Obesity, ATP; Insulin-resistance; DC electromagnets; PPAR; UCP2; TNF-alpha

Background
Obesity, which is growing by leaps and bounds and is virtually epidemic in every country in the world, is now considered one of the leading health problems within increased rates of fatty liver, Non-Alcoholic Steatohepatitis (NASH) and it’s progression to cirrhosis and liver cancer / heptato cellular carcinoma (HCC). Non Alcoholic Fatty Liver Disease (NAFLD) is one of the most common forms of chronic liver diseases and was considered previously to be benign.

By proton MR spectroscopy, the Dallas Heart study which used a population-based cohort study of a community that was ethnically diverse in the USA, reported that one in three adult Americans have hepatic steatosis. The findings indicate that over 70 million Americans have NAFLD or fatty liver [1].

However, there is indirect evidence that supports the progressive nature of NASH in the features of cryptogenic cirrhosis which is closely related to NAFLD [2,3]. Patients with cryptogenic cirrhosis have disproportionately high prevalence of metabolic risk factors (diabetes mellitus type2, obesity and the metabolic syndrome) typical of patients with NAFLD or fatty liver. Additionally, their liver biopsies often show one or more features of NASH. Studies have also demonstrated the loss of histological features of NASH with the development of cirrhosis or extensive fibrosis [2-4].

A pathological study of the natural history of NAFLD was published with a mean of 5.7 years at Brooke Army Hospital. Many of the patients had NASH on initial biopsy. One third had fibrosis progression and one third of these had rapid progression to advanced fibrosis, with the only clinical correlate of histologic progression being a higher serum AST [5].

There is the existing dogma that the liver biopsy is the most credible approach for identifying the presence of steatohepatitis and fibrosis inpatients with NAFLD. However it is acknowledged that biopsy is the most credible approach for identifying the presence of steatohepatitis and fibrosis inpatients with NAFLD. Accordingly, there has been significant interest in discovering non-invasive biomarkers for identifying steatohepatitis inpatients with NAFLD [6].

NAFLD is defined by excluding any evidence of ongoing or recent consumption of significant quantities of alcohol or Alcoholic Steatohepatitis (ASH) as the following derived from NASL clinical studies; significant alcohol consumption as defined as >21 drinks per week in men and >14 drinks per week in women over a 2-year period prior to base line liver histology [7].

Diagnostic Overview
The diagnosis of NAFLD requires four criteria; (a) there is hepatic steatosis by imaging or histology, (b) there is no significant alcohol consumption as listed previously (c) there are no competing etiologies for hepatic steatosis, and (d) there are no co-existing causes for chronic liver disease (Figure 1). Common alternative causes of hepatic steatosis are significant alcohol consumption, hepatitis C, parental nutrition, medications, Wilson’s disease, severe malnutrition and a few others listed below in Table 1 [6].

Since liver biopsy is invasive with some morbidity and mortality, there has been intense interest in non-invasive methods to identify advanced fibrosis in patients with NAFLD with biomarkers; these biomarkers include the NAFLD Fibrosis Score, Enhanced Liver Fibrosis (ELF) panel and transient elastography. The NAFLD Fibrosis Score is based on six readily available variables (age, BMI, hyperglycemia, albumin, platelet count, AST/ALT ratio) and it is calculated using the

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Received November 15, 2013; Accepted December 10, 2013; Published December 17, 2013
Citation: Nichols TW (2013) A Review of Fatty Liver/NASH and Liver Cirrhosis: Genetics, Prevention, Nutritional, Behavioral Modification, Exercise, Pharmaceutical, Biophysics and Biotech Therapy. J Liver 3: 144. doi:10.4172/2167-0889.1000144
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published formula (http://naflds-core.com) [7]. Other biomarker test for fibrosis are Fibro Test, Acti Test, Steato Test and Nash Test which have been used in French patients with severe obesity evaluated in a meta-analysis of individual patient data. The authors demonstrated a significant diagnostic performance of Fibro Test, Steato Test and ActiTest for liver lesions [8,9].

The ELF Panel comprises plasma levels of three matrix turn over proteins (hyaluronic acid, TIMP-1) a tissue metalloproteinase inhibitor and amino terminal propeptide of type III, procollagen (PIIINP). It had an Area under the Curve (AUROC) of 0.90 with 80% sensitivity and 90% specificity for detecting advanced fibrosis [7]. The NAFLD Fibrosis Score has an AUROC of 0.85 for predicting advanced fibrosis (i.e.,bridging fibrosis or cirrhosis) and a score < -1.455 had 90% sensitivity and 60% specificity to exclude advanced fibrosis. The authors of the AASLD Guidelines concluded that the presence of metabolic syndrome and the NAFLD Fibrosis Score may be used for identifying patients who are at risk for steatohepatitis and advanced fibrosis [6].

Pathophysiology

Hepatic steatosis is thought to arise from an imbalance between triglyceride accumulation and removal as outlined in Figure 2 seen below [10].

(Figure reproduced with permission from Cohen JC, Horton JD, and Hobs HH. Science, vol 332, p1519-23, June 24 2011) [11].

Genetic defects have been discovered that prevent the removal of triglycerides from the liver, which cause steatosis. Additionally, defects in the enzymes required for oxidation of free fatty acids in mitochondria (the hydroxy acyl-CoA transferase) also cause hepatic steatosis [11]. Recent insight into the pathophysiology of fatty liver now suggest deficits in oxidative phosphorylation, fatty acid and glucose disposal in various stages of insulin resistance along with impairments in mitochondrial function and pathways [12,13]. Adipose tissue IL-6 expression is increased in obesity and in a mouse model was a strong predictor of abnormalities in adipocyte and systemic metabolism. A study demonstrated that IL-6 impairs insulin signaling in both 3T3-L1 model of adipocyte model system and human adipocytes [14]. The Two Hit Hypotheses for NAFLD was first proposed by Day & James in 1985 [15].

First hit was macro vesicular steatosis with insulin resistance and Peroxisome Proliferators- Activated Receptor (PPAR). Second hit: oxidative stress with tumor necrosis factor (TNF-a).

TNF-a is increased in NAFLD patients and obese (but not normal weight) patients with diabetes according to a review of the NASH by McCullough. He goes onto state, TNF-a cause hepatic insulin resistance via up-regulation of suppressor of cytokines signaling (SOCS 1 and 3) proteins that bind to JAK tyrosine which changes its ability to phosphorylate Signal Transducer and Activator of Transcription (STAT) proteins. This attenuates insulin's ability to activate its signaling pathway. Additionally TNF-a up regulates mitochondrial permeability, impairs mitochondrial respiration, and depletes mitochondrial cytochrome by activation of caspase 3 resulting in apoptosis [16,17]. Human's magnetic resonance spectroscopy studies suggest that a defect in insulin-stimulated glucose transport inhibits insulin-stimulated glucose transport.

![Figure 1: An overview for the diagnosis of NAFLD.](image-url)
tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and IRS-1–associated phosphatidylinositol 3 kinase activity. Metabolic abnormalities may increase fat delivery to muscle and liver secondary to either excess energy in take or defects in adipocyte fat metabolism. This in turn may occur as acquired or inherited defects in mitochondrial fatty acid oxidation [18]. Adiponectin also known as ACRP30 has emerged as an important metabolic cytokine which when increased improves insulin sensitivity via increasing fatty acid oxidation of AMP kinase and suppression of gluconeogenesis [19]. Plasma adiponectin levels are known to be decreased in human fatty liver and when given in animal models of both non alcoholic and alcoholic fatty liver [20]. More importantly, polymorphisms in its gene (ADIPOQ) are known to affect the individual's predisposition to metabolic syndrome and type 2 diabetes and association with gestational diabetes [21]. Recent ly adiponectins rs1501299 and rs266729 gene polymorphisms were found to be associated with NAFLD [22].

Uncoupling Protein-2 (UCP2) is a mitochondrial membrane transporter expressed in white adipose tissue and is associated with energy balance. UCP2 gene expression demonstrated significant association with the obesity parameters waist circumference, insulin and HOMA-IR, the lipid parameter triglyceride and the adipokine adiponectin. UCP2 gene down-regulation was found in obese and diabetic patients and its association with obesity parameters and a clinical measure of insulin resistance (HOMA-IR) supports its role as a candidate gene in the study of obesity and diabetes [23]. Until the last

| Nutritional/Intestinal |
|------------------------|
| Surgical               |
| Jejunoileal bypass     |
| Gastroplasty for morbid obesity |
| Extensive small bowel resection |
| Total parenteral nutrition |
| Rapid weight loss      |
| Starvation and cachexia|
| Protein calorie malnutrition: marasmus and kwashiorkor |
| Inflammatory bowel disease |
| Jejunal diverticulosis with bacterial overgrowth |

| Drugs and toxins |
|------------------|
| Amiodarone       |
| Methorexate      |
| Tamoxifen/synthetic estrogens |
| Glucocorticoids  |
| Nucleoside analogs |
| Calcium channel blockers |
| Perhexiline maleate |
| Phosphorous       |
| Organic solvents  |
| Petrochemicals    |
| Dimethyldimethane |
| Rapeseed oil      |

Table 1: Uncommon causes of NAFLD. (Digestive Diseases and Sciences, Vol. 50, No.1, 2005, 171-180) with permission.
A NASH study by Hideyuki Kojima and associates in 2007 found that the enhanced oxidative stress is associated with hepatic inflammation and the degree of fat infiltration in the liver. They feed Zucker rats and their lean normal littersmates choline-deficient diet and therefore, exposed them to oxidative stress, both developed NASH. Zuckerrats which naturally develop lept in recept or mutations alone were the only type associated with a mitochondrial abnormality. These findings indicate that a mitochondrial abnormality plays a role in the onset and progression of NASH in correlation with oxidative stress [24].

Mitochondrial function of an oxidative metabolism which then generates a proton gradient across the inner membrane and channel this energy towards ATP synthesis to produce energy. Additionally the proton gradient may then be dissipated or uncoupled by a specific mitochondrial protein termed UCP1 (Uncoupling Protein1) at least in brown fat of rodents. UCP 2 is expressed in a variety of tissues including adipose tissue, heart, muscle, liver and pancreatic islets. UCP3 found in rodent brown fat is 73% homologous to UCP2 in humans and predominately expressed in skeletal muscle [25].

mitochondria use free energy obtained through the electron transport chain (ETC) and the Reactive Oxygen Species (ROS). Both the levels and activity of UCP 2 and the rate of ROS production are increased by high fat diet, probably through the direct actions of fatty acids. Patti and Corvera published in 2010 their hypothesis that the normal feedback loop is compromised by a direct activation of UCP2 by Free Fatty Acids (FFA), and the effect of FFA to increase the quantity of UCP 2. They concluded that when uncoupling occurs to an excessive degree, ATP synthesis is compromised enough to impair insulin secretion and (6) beta cell function [26].

See Figure 3 for Model of UCP 2 of the mitochondria in insulin secretion of the beta cell of the pancreas. Elevated plasma glucose leads to increase in the cytoplasmic concentration due to uptake of glucose transporter (GLUT2). This increases the NADH/NAD+ ratio, elevated mitochondrial membrane potential and increase in ATP synthesis. The increase in ATP/ADP ratio causes closure of K ATP channels, leading to depolarization of the plasma membrane potential and influx of Ca, triggering insulin release. UCP2 activity dissipates the proton motive forces, lowering the ATP/ADP ratio and decreases insulin secretion. "(Figure permission: Echtay K. Mitochondrial uncoupling proteins, what is their physiological role? Free Rad Biol Med Sci 2007; 43: 1351-1371)".

Green and associates in 2004 proposed the suggestion that over production of superoxide by them mitochondrial respiratory chain occurs during hyperglycemia. This happens because hyperglycemia increases the flow of electrons to the respiratory chain by maintaining a large mitochondrial NADH/NAD to FADH2/FAD ratio. The mitochondria in many tissues would then spend more time under a state of low respiratory rate and reduced electron carrier, all favoring superoxide formation. They concluded that, increased mitochondrial ROS production during hyperglycemia may be a major factor in the pathology of diabetes [27].

Rolo and collaborators in 2012 demonstrated that the increased generation of Reactive Oxygen Species (ROS) in them itochondria and reactive aldehydeic derivatives causes oxidative stress and cell death, via ATP, NAD, glutathione depletion and DNA, protein and lipid damage. Oxidative stress increases the production of inflammatory cytokines, causing inflammation and a fibrogenic response. This ultimately results in the development of NASH from NAFLD, which can result in end-stage liver disease [28].

Genetics of Obesity and NAFLD

Bertola et al. in 2010 reported the expression of 222 genes quantified by RT-PCR in the livers of patients with morbid obesity with histologically normal liver (n=6) or with severe steatosis without (n=6) or with NASH (n=6), and in lean controls (n=5).

Hepatic expression of 58 out of 222 inflammatory and immune response genes was up-regulated in their NASH patients. Importantly, 47 other genes were already up-regulated in histologically normal liver (e.g. CRP, Toll-like receptor (TLR) pathway) [29].

The metabolic pathway influencing the development of NASH from NAFLD is complex with numerous genetic factors contributing to the variability in the natural history of the disease. Figure 4 demonstrates an overview of these metabolic processes influencing fatty liver to develop and progress into NAFLD.

It is now well established that there are strong environmental and lifestyle influences on the development of hepatic steatosis and that there is also emerging evidence of numerous genetic modifiers. According to Hopper and associates, genetic disorders e.g., Familial Hypo Beta Lipoproteinemia (FHBL) are directly associated with the development of NAFLD, or genetic polymorphisms affects susceptibility in the presence of other pathogenic risk factors. SNPs which are single nucleotide substitutions in DNA which can result in the altered expression of a particular gene or altered function of the expressed protein. Generally, the increased risk of disease related to a single SNP is considered generally small, and it is felt that multiple SNPs
may influence the phenotypic expression of NAFLD as a “polygenic” disease. Genetic variants may therefore predispose to hepatic steatosis by influencing lipid trafficking or indirectly via an effect on insulin resistance [11]. A genome-wide association study for liver fat has been performed in the Dallas Heart Study which was multi-ethnic population based. A Single Nucleotide Polymorphism (SNP) in the PNPLA3 gene, rs 738409 (I148M), was found to be the only variant in the 9,000 subjects that was strongly associated with hepatic fat content [30]. This association has been validated in several other studies, and despite the plethora of published genetic association studies in fatty liver and NASH, it remains the only robust and convincing association between a single nucleotide polymorphism and the presence of hepatic steatosis according to Hooper’s group.

**NAFLD into NASH**

In an experimental mouse model of NASH, Farrell et al. feed a high fat diet to different strains of obese mice to establish whether diabetes or obesity is more closely associated with NASH fibrosis. They found NASH development is linked to strain differences in hyperinsulinemia or obesity is more closely associated with NASH fibrosis. They found that NAFLD into NASH development and/or progression of NAFLD. Variants within genes involved in these processes may impact the development and/or progression of NAFLD.* By permission from Hooper et al. Genetic determinants of hepatic steatosis in man. J. Lipid Res 52(4):593-617.

**Figure 4: Metabolic processes influencing the development of NAFLD and genes involved** [11].

*Overview of the metabolic processes influencing the development of NAFLD. Variants within genes involved in these processes may impact the development and/or progression of NAFLD.*

Hepatic cytochrome P4502E1 and cytochrome P3A4 are increased in the livers of patients with NASH with generation of reactive oxygen species, decreased intracellular glutathione, increasing kupffer cell reactivity and subsequent tellate cell fibrosis [33].

Thus the combination of a high fat and alcohol can increase gut permeability induced liver injury via TLR-4-mediated recognition of LPS. Alcohol and high fat diet lead to changes in bacterial species and overgrowth of gram-negative bacteria in the intestine. These factors contribute to increased gut permeability to LPS and the increased endotoxemia in the portal circulation. LPS is taken up by Kupffer cells via a TLR4-mediated mechanism. In response to LPS, Kupffer cells produce a significant amount of pro inflammatory cytokines, resulting in liver injury and fibrosis [34].

Mechanism of high fat diet and alcohol induced liver injury via TLR-4-mediated recognition of LPS. Alcohol and high fat diet lead to changes in bacterial species and overgrowth of gram-negative bacteria in the intestine. These factors contribute to increased gut permeability to LPS and increased endotoxemia in the portal circulation. LPS is taken up by Kupffer cells via a TLR4-mediated mechanism. In response to LPS, Kupffer cells produce a significant amount of pro inflammatory cytokines, resulting in liver injury [34].

McClain and associates at University of Louis ville School of Medicine also demonstrated that demonstrate that Unsaturated Fat (USF) that is corn oil/ linoleic acid by itself results in dysregulation of intestinal Tight Junctions (TJ) integrity leading to increased gut permeability, and alcohol further exacerbates these alterations. They postulate that elevated blood endotoxin levels in response to USF and alcohol in conjunction with up-regulation of hepatic TLRs combine to cause hepatic injury in Alcoholic Liver Disease (ALD) [35] (Figure 5).

**Nutritional Therapy in NAFLD and NASH**

Low fat, low glycemic, no corn syrup diet; One of the most important mediators of metabolic function in the liver is (SREBP) Sterol Regulatory Element Binding Protein. A high glucose intake in the diet triggers the pancreas to produce increased amounts of insulin and the liver continues to produce glucose despite the high glucose intake. Ultimately, the downstream effects of sustained hyperglycemia leads to type 2 diabetes mellitus and the abnormally elevated SREBP-1c activity leads to increased synthesis of fatty acids and triglyceride in the liver, resulting in a fatty liver [36].

Fatty liver i.e. hepatic steatosis has been shown to be linked to diet; lifestyle choices and altered genetic signaling intertwined inidious cycle that produces abnormalities in lipid and glucose metabolism. Joseph L. Goldstein MD, Nobel laureate, professor of medicine and genetics, South western University said “that although many assume the process requires years of poor diet, inadequate exercise, and less than optimal lifestyle, it could be accelerated enormously”. He cited the 2004 documentary “Super-Size Me” in which Morgan Spur lock monitored his metabolic function while consuming all his meal sat a fast food restaurant over a 30-day period. Morgan Spur lock developed
the metabolic syndrome in less than a month but it took him six months to reverse it. Goldstein pointed out the major physiological change was fatty liver in Spurlock as well as others with metabolic syndrome. He went on to note that (SREBP) can maintain lipids dynamic equilibrium by regulating the expression of fatty acids, triglycerides and phospholipids. Furthermore, a high fructose intake such as corn syrup in soft drinks in the diet triggers the pancreas to produce increased amounts of insulin. The liver continues to produce glucose despite the high fructose intake. Therefore many savvy clinicians then encouraged a low fat, low glycemic (no corn syrup diet) for patients [37].

A study was conducted by Steve Solga MD and Anna Mae Diehl MD of 74 obese patients undergoing bariatric surgery at Johns Hopkins. All patients underwent a preoperative dietary evaluation using a standardized 24-hr food recall. Food intake was evaluated for total calories and macronutrients and compared to liver histopathology from biopsies routinely obtained during surgery. The authors found there were no significant associations between either total caloric intake or protein intake and either steatosis, fibrosis, or inflammation. However, higher carbohydrate intake was associated with significantly higher odds of inflammation, while higher fat intake was associated with significantly lower odds of inflammation [38]. This contradicted previous recommendation of fat restriction and the increase therefore of carbohydrates [38].

Bariatric surgery, i.e. gastric bypass with intestinal diversion; Bariatric surgery has been studied in NASH. Roux-en-Y gastric bypass surgery with intestinal diversion was retrospectively studied in 29 patients undergoing surgery. At that time, patients had achieved a mean weight loss of 116.9 lb., with a significant decrease in body mass index (28.9 ± 5.8 kg/m² vs. 47.8 ± 6.6 kg/m²), relative to pre-surgery mean weight loss of 116.9 lb., with a significant decrease in body mass surgery with intestinal diversion was retrospectively studied in 29 patients at high risk of developing liver cirrhosis [40]. The problem with the article is that it was written by two ER physicians who may not have seen many of the long term complications of the surgery.

Nutrients and supplements

A number of nutrients or natural occurring substances have also been tried for NASH. Historically nutrients like sily marin since Roman times and others like vitamin E since the discovery of antioxidants, have found a place in NASH armamentarium.

PUVA

N-3 polyunsaturated fatty acids (n-3PUVA) enriched diets have been shown in animal studies to reduce hepatic triglyceride content and the development of steato hepatitis [41]. A clinical trial in 2005 studying the role of n-3PUVA in NASH was conducted. ALT and gamma glutamyl transaminase and ultrasound improvement in NASH was shown in the 42 patients who received 1 gm n-3PUVA daily over a period of 12 months. A larger study evaluating 134 patients randomized to a control group (calorie restricted diet and placebo) and the study group who received 2 gm thrice daily of n-3PUVA demonstrated a 53% reduction in fatty liver compared to 35% reduction in those on diet and placebo [42].

Betaine

Betaine which is N-trimethylglycine, a methyl donor initially found in sugar beets, has been used in a number of clinical trials along with folate, and vitamin B12 to reduce homocysteine, a toxic amino acid implicated in cardiovascular and neurodegenerative disease [43]. A Mayo Clinic small clinic trial demonstrated that betaine, a naturally occurring metabolite of choline which had been shown to raise S-adenosyl methionine (SAMe) levels that may decrease hepatic steatosis. Ten adult patients with NASH were enrolled and received betaine in two daily doses for 12 months. A significant improvement in serum levels of AST (p=0.002) and ALT (p=0.007) occurred during treatment. Amino transferase normalized in three of seven patients that completed the year-long trial. A marked improvement in the degree of steatosis, necroinflammatory grade and stage of fibrosis was also noted at 1 year [44].

Phosphatidylcholine

Phosphatidylcholine (PC) is a phosphorylated fatty acid incorporated into cell membranes where it influences membrane movement and the function of Transmembrane proteins. Suboptimal amounts of the nutrient choline have been measured in the U.S. population. A study of 2 month in patient diet of 15 female subjects controlled or choline level was conducted. Using pyro sequencing of 16s ribosomal RNA bacterial genes, the authors’ characterized them microbiotain stool samples collected over the course of the study. They identified bacterial biomarkers of fatty liver that resulted from choline deficiency [45].

When combined with sily bin and vitamin E, known as Realisil (RA) or placebo (P) was administered to 138 patients. Patients receiving RA but not P showed significant improvements in HOMA scores, liver enzyme plasma levels and liver histology. Body mass index normalized in 15% of RA patients (2.1% with P). HCV-positive patients
in the RA but not the P groups showed improvements in fibrogenesis markers. Treatment with RA but not P for 12 months was associated with improvement in AST/ALT, insulin resistance, and liver histology, without increases in body weight. The investigators concluded that the findings warrant further investigation [46].

Silymarin (Mil克 Thistle)

Silymarin or milk thistle has been used in a number of clinical trials of NASH. Silybin is the active component of silymarin that is absorbed when linked with a phytosome. This substance reduces in rats, lipid peroxidation and the activation of hepatic stellate cells. In humans, some non-controlled studies show that silybin is able to reduce insulin resistance, liver steatosis and plasma markers of liver fibrosis [47].

The regulatory action of silymarin (silybin) on cellular and mitochondrial permeability associated with increased membrane stability against xenobiotics injurys supported by a number of studies. In a rat model of ischemia by reperfusion injury, silybin reversed the severity of mitochondrial bioenergetics including increasing ATP levels, decreasing susceptibility to mitochondrial permeability transition (MPT), and improving defects in mitochondrial respiration [48].

Silymarin has direct effects on fibrinogenesis inhibiting collagen accumulation, even when administered late, inhibiting increases in serum aminoterminal propeptide of type III procollagen [49]. Silybin was used in combination with vitamin E and phospholipids to improve its antioxidant activity. Eighty-five patients were divided into 2 groups: those affected by non-alcoholic fatty liver disease (group A) and those with HCV-related chronic hepatitis associated with non-alcoholic fatty liver disease (group B). After treatment, group A showed a significant reduction in ultrasonographic scores for liver steatosis, hyperinsulinemia, AST/ALT levels, and indexes of liver fibrosis showed an improvement in treated individuals. A significant correlation among indexes of fibrosis, body mass index, insulinemia, plasma levels of cytokines, degree of steatosis, and gamma-glutamyltranspeptidase was observed. The author's data suggest that silybin conjugated with vitamin E and phospholipids such as phosphatidyl choline could be used as a complementary approach to the treatment of patients with chronic liver damage [50].

In a recent Italian clinical study, 72 patients affected by NAFLD, main metabolic, hepatic and anti-inflammatory parameters were assayed after 3 months of a restricted diet and before silymarin treatment (twice a day orally). The brightness of liver echography texture (hepatoportal ratio brightness) was also defined at same time. These evaluations were repeated after 6 months of treatment. Serum levels of some metabolic and anti-inflammatory data nonsignificantly lowered after 6 months of silymarin. On the contrary, Steato-test, AST, ALT and gamma-glutamyl transpeptidase were significantly reduced (P<0.001). Instead, the AST/ALT ratio unchanged. Finally, the hepato renal brightness ratio, as an index of hepatic steatosis, significantly (P <0.05) dropped [51].

Probiotics

Beneficial bacteria have been demonstrated by a number of studies to have protective effects exerted directly by specific bacterial species, control of epithelial cell proliferation and differentiation, production of essential mucosal nutrients, such as short-chain fatty acids and aminoacids, prevention of overgrowth of pathogenic organisms, and stimulation of intestinal immunity. Oral probiotics are living micro organisms that upon ingestion in specific numbers exert health benefits beyond those of inherent basic nutrition [52]. The accumulation of fat in hepatocytes with a necro inflammatory component-steatohepatitis-that may or may not have associated fibrosis is becoming a frequent lesion as discussed earlier. Probiotics have therefore attracted attention for their inclusion in the therapeutics for NASH after being used in inflammatory bowel disease and irritable bowel. Although steatohepatitis is currently recognized to be a leading cause of cryptogenic cirrhosis, the pathogenesis has not been fully elucidated. Among the various factors implicated, intestinal bacterial overgrowth may play a role. Infact, various rat models of intestinal bacterial overgrowth have been associated with liver lesions similar to NASH, and bacterial over growth has been observed significantly more often in patients with NASH compared with control subjects. The authors of this paper discuss the relationship among intestinal bacterial overgrowth, steato hepatisits development, and probiotic treatment [53].

One of the problems with probiotics is the FDA classification. Probiotics since they are living organism, beneficial bacteria, are neither classified as a food or a drug and most manufactures classify them as a dietary supplement. This would seem ideal but this classification keeps university medical trials where there are stringent IRBs at arm's length. Hence the lack of some institutions like Johns Hopkins Medical Center pursuing clinical trials in NASH further where the original trial looked promising but misses statistical significance [54].

A study in Hong Kong randomized patients with histology-proven NASH to receive probiotics (n =10) or usual care (n=10) for 6 months. The Lepicol probiotic formula contained Lactobacillus plantarum, Lactobacillus delbrueckii, Lactobacillus acidophilus, Lactobacillus rhamnosus and Bifido bacterium bifidum. The primary end point was change in Intrahepatic Triglyceride content (IHTG), as measured by proton-magnetic resonance spectroscopy, from base line to month 6. Secondary end points included changes in liver biochemistry and metabolic profile. The Triglyceride Content (IHTG) decreased from 22.6 ± 8.2% to 14.9 ± 7.0% in the probiotic group (P=0.034) but remained static in the usual care group (16.9 ± 6.1% to 16.0 ± 6.6%; P=0.55). Six subjects in the probiotic group had IHTG reduced by more than 30% from baseline, compared to 2 subjects in the usual care group (P=0.17). Probiotics reduced the abundance of Bacteroides (92.1% to 89.1%; P=0.00006) but increased the Firmicutes (6.6% to 9.4%; P=0.000006). The probiotic group also had greater reduction in serum aspartate aminotransferase level (P=0.008). However, the use of probiotics was not associated with changes in body mass index, waist circumference, and glucose and lipid levels. They concluded that probiotics treatment may reduce liver fat and AST level in NASH patients. The therapeutic potential of probiotics in NASH should be tested in larger studies [55].

Medications

Metformin (insulin sensitizing)

An open-label trial at the University of Turin consisting of 110 patients with NASH received metformin 2 grams/day (35 patients), vitamin E800IU/day (28 patients) orordinary-induced weight loss (27 patients) for 12 months. Among transferrases improved more with metformin than with vitamin E or diet alone. A control biopsy in 17 metformin-treated cases (14 non responders) showed a significant decrease in liver fat (p =0.0004), necro inflammation, and fibrosis (p =0.012 for both). No side effects were observed during metformin treatment. They concluded that metformin treatment is better than a prescriptive diet or vitamin E in the therapy of NAFLD patients receiving nutritional counseling. Their limited histological data support an association between improved aminotransferases and biopsy findings, and a further double-blind trial with appropriate statistical power based on liver histology was recommended [56].
Haukeland et al. in Oslo reported a similar lack of efficacy in a larger (n=488) Randomized Control Trial (RCT) of metformin vs. placebo with a similar dietary and exercise intervention in both groups. However, beneficial effects of metformin were observed on changes in body-weight, glucose, levels of cholesterol, LDL-cholesterol glucose and on HBAlc [57].

A recent meta-analysis at Fall Church Virginia INOVA Hospital concluded that 6-12 months of metformin plus lifestyle intervention did not improve aminotransferases or liver histology, compared with lifestyle interventional one, independently of metformin dose or the presence of diabetes [4].

**Thiazolidinediones**

Neuschwander-Tetri’s group at Saint Louis reported thirty adults with prior biopsy evidence of NASH were enrolled to receive rosiglitazone, 4 mg twice daily for 48 weeks. All patients were overweight (body mass index [BMI]> 25 kg/m (2)) and 23% were severely obese (BMI >35 kg/m (2)); 50% had impaired glucose tolerance or diabetes.

Liver biopsy specimens were obtained before beginning treatment and at treatment completion. Twenty-six patients had post treatment biopsies. Twenty-two who had initial protocol liver biopsies that met published criteria for NASH on subsequent blinded evaluation. The NASH group's necro inflammatory score significantly improved with treatment and biopsies of 10 patients (45%) no longer met published criteria for NASH after treatment. Significant improvement in hepatocellular ballooning and zone 3 perisinusoidal fibrosis also occurred. The twenty-five patients completing 48 weeks of treatment had significantly improved insulin sensitivity and mean ALT levels (104 initially, 42 U/L at the end of treatment). Adverse side effects caused 3 patients to withdraw. Within 6 months of completing treatment, liver enzyme levels had increased to near pretreatment levels. In conclusion, rosiglitazone by improving insulin sensitivity resulted in improved histologic markers of NASH. They concluded that insulin resistance contributes to its development and that improving insulin sensitivity may be important in treating this liver disease [58].

Ratziu et al. observed that rosiglitazone improved aminotransferases and hepatic steatosis, but not necro inflammation or fibrosis and its two-year open-label extension phase also showed similar results [59]. Belfort in San Antonio in a randomized clinical trial (RCT) found administration of pioglitazone was associated with improvement in histologic findings with regard to steatosis (P=0.003), ballooning necrosis (P=0.02), and inflammation (P=0.008) when compared to placebo. Subjects in the pioglitazone group had a larger reduction in necroinflammation (85% vs. 38%, P=0.001), but the reduction in fibrosis did not differ from that in the placebo group (P=0.08) on statistical analysis [60].

A large RCT (PIVENS) was conducted in 247 adults with NASH and without diabetes to receive pioglitazone at a dose of 30 mg daily (80 subjects), vitamin E at a dose of 800 IU daily (84 subjects), or placebo (83 subjects), for two years. Vitamin E therapy was, compared with placebo and associated with a higher rate of improvement in NASH (43% vs. 19%, P=0.001), but the difference in the rate of improvement with pioglitazone as compared with placebo was not significant. AST and ALT were reduced with vitamin E and with pioglitazone, as compared with placebo and both agents were associated with reductions in hepatic steatosis (P=0.005 for vitamin E and P=0.001 for pioglitazone) and lobular inflammation (P=0.02 for vitamin E and P=0.004 for pioglitazone) but not with improvement in fibrosis scores. They concluded that vitamin E was superior to placebo for the treatment of NASH in adults without diabetes.

Surprisingly, there was no benefit of pioglitazone over placebo for the primary outcome but for significant benefits were seen for secondary outcomes by improving insulin sensitivity [61].

There has been considerable debate about the long-term safety of thiazolidinediones regarding cardiovascular disease, Congestive Heart Failure (CHF), bladder cancer, and bone loss. Recent meta-analysis of 19 trials enrolling a total of 16,390 patients with type 2 diabetes, pioglitazone (Actos) treatment was associated with a significant reduction in the primary outcome of death, myocardial infarction, or stroke. However, there was also a higher rate of CHF with pioglitazone (2.3% vs. 1.8% in the control group); therefore caution must be considered in its use in patients with impaired myocardial function. Due to an increased risk of coronary events, rosiglitazone (Avandia) is no longer marketed in Europe and its use is highly restricted in the United States [6].

**Statin use in NAFLD and NASH**

Statins are an important class of agents to treat dyslipidemia in patients with DM and with coronary heart disease and yet there is continued reluctance to use statins in patients with suspected or established chronic liver disease, including NAFLD and NASH. Although elevated amino transferases are not uncommon in patients receiving statins, serious liver injury from statins is rarely seen in clinical practice. Over the last decade, one randomized clinical trial and several retrospective and prospective studies have been conducted. Statins are safe in patients with liver disease and there is no evidence that patients with chronic liver disease including at NAFLD and NASH are at higher risk for serious liver injury from statins than those without liver disease [6].

The GREACE was a prospective study randomly assigned by a computer-generated list of 1600 patients with coronary heart disease (aged<75 years, with serum concentrations of LDL cholesterol>2.6 mmol/L and triglycerides<4.5 mmol/L) at the Hippokration University Hospital, The saloniki, Greece to receive statin or usual care, which could include statins. They observed that statins significantly improve transaminases and cardio-vascular outcomes in patients with elevated liver enzymes likely due to NAFLD. They concluded that statin treatment is safe and can improve liver tests and reduce cardiovascular morbidity in patients with mild-to-moderately abnormal liver tests that are potentially attributable to non-alcoholic fatty liver disease [62].

**NAFLD and NASH are polygenic**

As we have seen from the above review, NASH is a disease in search of an effective therapy. Many of the current regimens had been tested in open label, uncontrolled trials that have been carried out over a relatively short period of time and most of these studies did not adhere to a strict histologic end point [32]. None have been convincingly effective. NAFLD, NASH and its progression to cirrhosis whose cellular processes which began generally years before hand, presents broader possibilities for treatment interventions and is therefore more of a reason to diagnose disease early. Novel broad spectrum therapeutic modalities are needed since obesity with over 127genes, metabolic syndrome of about 22 genes and fatty liver (NAFLD) with about 55genes are by definition, polygenic! [63-65].

With personalized healthcare coming to the fore-front in the distant future, with gene typing such as “23 and Me,” genetic inheritance will be elucidated and individualized therapy specified.
Environmental factors; Temperature, light and toxins (obesogens)

Temperature control of Adipose cell plasticity: White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT).

Brown adipose tissue (BAT) which until recently was felt to be predominately rodent fat rich in mitochondria burns fat to produce heat when the body is exposed to cold and plays a role in energy metabolism. The developmental relationship between white and brown adipocytes is not clear. It has been shown that inguinal white fat cells arise independently of the brown adipose lineage in mice kept at ambient temperature. It was previously thought that BAT in large mammals was totally converted to WAT in adults whereas in rodents, BAT persist into adulthood [36]. However, recent studies demonstrate acute cold exposure at 19°C for 2 hours increased energy expenditure (EE). Cold-induced increments of energy expenditure (CIT) correlated strongly with brown adipose activity independently of age and fat-free mass. Daily 2-hour cold exposure at 17°C for 6 weeks resulted in a parallel increase in BAT activity and CIT and a concomitant decrease in body fat mass [66].

Light and the regulation of BAT

The availability of artificial light sources, and the excessive light exposure after darkness in modern societies according to Tan and associates should be considered a potential contributory factor to human obesity as light at night dramatically reduces endogenous melatonin production. Melatonin readily regulated the physiology of BAT is which not only increases recruitment of brown adipocytes but also elevates their metabolic activity in mammals. It is hypothesized that the hypertrophic effect and functional activation of BAT induced by melatonin may likely apply to the human. Thus, melatonin, a naturally occurring substance with no reported toxicity, may serve as a novel approach for treatment of obesity [67]. Additionally the addition of bright light treatment to a 6-week moderate exercise program can alter body composition by significantly reducing body fat [68].

Obesogens

Diethyldilbestrol (DES), bisphenolA, phthalates and organotins occur in the environment as toxins which target nuclear hormone receptor signaling pathways: sex steroid, retinoic X receptor peroxisome proliferative activity receptor-gamma (RXR-PPAR) and glucocorticoid receptor (GR) have been identified and are termed obesogens. The ubiquitous presence of bisphenol A in the environment results from its use as the monomer in polycarbonate plastic and epoxy resins used to line food cans. In vitro studies have demonstrated the ability to synergize with insulin to promote tumorigenesis and differentiation of preadipocytes [68].

Plant derived phyto estrogens; genistein and daidzein found in soy products have generally mimicked estrogen action adipogenesis and lipogenesis. Organotins used to increase the production of high value food crops, can inhibit aromatase, the key cytochrome P450 enzyme required for the conversion of androgen to estrogens.

Additionally, estrogen receptor (ERR) has been found to function in PPAR coactivation in mitochondria biogenesis. Phthalates have been used as plasticizing agents to soften polyvinylchloride products. These compounds act as agonist of the nuclear receptor PPARα, δ that control lipid flux, adipocyte differentiation, and proliferation [69].

During the 1960s, DES was used as a growth hormone in the beef and poultry industries. It was later found to cause cancer and was “phased out in the late 1970s”. When DES was discovered to be harmful to humans, it was moved to veterinarian use. DES was found to cause liver failure in cats [70].

Novel NAFLD and NASH Therapy

Cannabinoid receptor agonist and antagonists

The brain has 2 cannabinoid receptors, CB1and CB2. A prominent role for CB1 receptor signaling in obesity is further confirmed by knock out animals, which are leaner and resistant to diet induced obesity [71]. Elevated endocannabinoid levels have been discovered in obese subjects and correlate with increased visceral fat. Hence, pharmacological CB1 receptor antagonists, like rimonabant, are effective at reducing food intake, weight loss and eliciting favorable metabolic parameters, including reduced leptin, insulin, free fatty acids and cholesterol levels, and improving insulin resistance [72-74].

A large number of studies have demonstrated that CB1 receptor antagonists represent an important therapeutic target, owing to beneficial effects on lipid metabolism and in light of its antiinflammatory properties. Unfortunately, the brain-penetrant CB1 antagonist rimonabant, initially approved for the management of overweight and related cardiac metabolic risks, was withdrawn because of an alarming rate of mood adverse effects. However, the efficacy of peripherally-restricted CB1 antagonists with limited brain penetration has now been validated in preclinical models of NAFLD, and beneficial effects on fibrosis and its complications are anticipated. CB2 receptor is currently considered as a promising anti-inflammatory and antiobesity target, although clinical development of CB2 agonists is still awaited. A review of the CB2 agonist has recently been published in 2013 [75].

Muscadine grape (vitis rotundifolia) and polyphenols

Muscadine grape (MGP) or wine phytochemicals (MWP) supplementation reduced plasma free fattyacids, triglycerides, and cholesterol in obese mice. Inflammation was reduced, and activity of GLUT4 and adiponectin was enhanced. Consumption of MGP or MWP improves insulin sensitivity and glucose control in mice. Thus, consumption of muscadine grape and wine phytochemicals in the diet may help to prevent obesity-related metabolic complications [76]. Future clinical trials of (MGP) in NAFLD and NASH are planned by this author.

Moderate magnetic field therapy is polygenic and epigenetic.

Therefore, in an attempt to change metabolism, gene expression and up- regulate mitochondria, down-regulate oxidative stress and inflammation, moderate magnetic field therapy was used. It has been found recently to effective in an animal model of obesity, ob/ob knock mice with no leptin receptor, which develop fatty liver. Static Magnetic Field (0.23-0.28T) was found to up–regulate and down-regulate over 2500 genes in 2 human stem cell lines [77].

DC electromagnetic therapy was instituted in an animal model and a pilot human study by this author and recently published in 2012. The mice when placed in the (DCEMF) were energized to increase their metabolism dramatically and lose weight. We had previously demonstrated that the electromagnets by inducing a weak electric current in the bodies of the mice by Faraday induction adding electrons to the respiratory chain (ETC) of their mitochondria enabling their dramatic change in their (ATP) adenosine triphosphate kinase and subsequent energy production. With more ATP production, the APase, Ca+ transporting, plasmamembrane1 gene (ATP2B1) is down-regulated with a total of 18 genes, 11 down- regulated and 7
Increased gut permeability induces liver injury via TLR-4-mediated recognition of LPS and free radical damage resulting in apoptosis of the mitochondria, hepatocellular inflammation with TNF, and activation of the stellate cell resulting in fibrosis and eventual fibrosis and cirrhosis.

The evidence for polygenics and epigenetics in obesity, metabolic syndrome and fatty liver/NASH is considerable. The one gene, one protein and one drug therapeutic approach is therefore doomed to failure in these complex diseases. Understanding the biophysical approaches such as the effects of light, temperature, exercise and nutrition as well as bioelectromagnetics, will be the solutions now for this obesity epidemic.

Mitochondrial and metabolic bioenergetics as well as gene up and down regulation with these modalities has been demonstrated and the wide spread adoption should begin as soon as the research community becomes aware of these findings.

Current medical approaches are weight reduction with low fat, low refined carbohydrate diet and reduced calories along with exercise and behavior modification. Alcohol is restricted. Pioglitazone can be used to treat steatohepatitis in patients with biopsy-proven NASH and Vitamin E and silymarin (Reasils) have been found to be efficacious.

Gastric bypass with Roux-en-y is now reserved for those who have failed all the above.

For patients who have progressed to cirrhosis, liver transplantation is presently the only solution now available but currently only 1 in 10 will receive a liver and 30 % of those will regain fatty liver post transplantation. Antifibrotic trial of pen toxify line in NASH was recently presented by Hems worth-Peterson of Dalhousie University at 2nd International Conference on Gastroenterology and Urology 2013 [82].

GR-MD-02, a line polysaccharide polymer derived from guar galactomannan, has been presented at DDW2012. In a mouse model, there was a significant reduction in fat accumulation, hepatocyte degeneration and inflammation in the liver histology and improvement using a standard NASH grading system. Additionally, the percent of collagen in the livers (fibrotic tissue as demonstrated by percent Sirius red staining) was reduced by treatment with GR-MD-02 to levels equivalent to normal levels. A phase I clinical trial is currently underway [83,84].

Novel approaches such as CBD, resveratrol and other polyphenols in muscadine grape have future potential. DC EMF therapy capable of regulating over 2500 genes is extremely promising because obesity, metabolic syndrome, DM2, NAFLD, and its progression to NASH/cirrhosis are so polygenetic and epigenetic that the paradigm, one gene, one protein, one drug approach is doomed to fail.

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