An Update on Further Progression of NAFLD, NASH with Prospective Therapies Like L-Carnitine (LC), Nicotinamide Ribose (NR) Combination, as well as Apical Sodium Dependent Bile Acids Transporter (ASBT) or Volixibat and Silybin as Alternatives

Introduction

Overweight and obesity in adults and children has escalated at an astonishing rate in the past few decades bringing in a lot of the cost burden [1]. Increased Adipose Tissue (AT) as well as ectopic fat collection is typical properties of obesity which results in associated comorbidities like, Type 2 Diabetes Mellitus (T2DM), Hypertension, dyslipidemia. Recently Nonalcoholic Fatty Liver Diseases (NAFLD) has become the commonest chronic liver disease correlated with obesity [2]. Right now the therapy of obesity concentrates on lifestyle changes which include exercise. No reduction in obesity incidence has occurred in spite of lifestyle as well as diet changes over the past 3 decades [1]. We have reviewed multiple ways of treating obesity medically that includes Qsymia, Contrave, Thylakoids, GLP-1 agonists, BAT thermogenesis enhancement utilizing mirabegron, probiotics etc but none have been of help in maintenance of weight reduction for long term other than Bariatric Surgery (BS) [3-9]. Further with escalating obesity liver disorders are increasing remarkably with no effective way of treating them. All over the world work is being done at war footing with the enhanced rates of liver transplants needed in those under 50 yrs [10,11]. Thus we have further tried to present the advances done in working out some medical therapy to prevent propagation of NAFLD and halt it further to not progress till Nonalcoholic Steato Hepatitis (NASH) level, cirrhosis and Hepatocellular Carcinoma (HCC).

Methods

Thus we did an update on the further advances that are being made in treating NAFLD, NASH and carried out a PubMed search using the MeSH Terms NAFLD; NASH; HCC; Preventive or curative therapies.

Results and Discussion

We came across 377 articles out of which we chose 70 articles for updating the earlier information we had reviewed. No meta-analysis was done.

Role of carnitine shuttle and targeting lipid peroxidation

L-Carnitine (LC) has a great part in oxidative metabolism as it is needed for moving Long Chain Fatty Acids (LCFA's) from the cytoplasm into the mitochondrial matrix in which β-oxidation takes place. For getting shifted these LCFA require activation into acyl CoA's and get converted into acyl carnitines for movement across mitochondrial membrane. These acyl carnitines are under C22 in length can get into mitochondrial matrix [12], where get exchanged with free carnitine, they get converted back to acyl CoA's and thus can get utilized for β-oxidation [13]. Also carnitine is needed for shifting the end products of peroxisomal β-oxidation, medium as well as short chain acyl CoAs, out of the peroxisomes for promoting more mitochondrial processing [14]. Decrease in carnitine amounts correlates with Insulin Resistance (IR) as well as Diet Induced Obesity (DIO) and was pointed to be secondary to prolonged lipid excess, impaired energy metabolism, associated with fat oxidation that is not complete [15]. On the other hand, LC administration in obese rats was demonstrated to reinstate carnitine amounts and thereby enhancing metabolic working [15] (Figure 1).

Nicotinamide Ribose (NR) is a molecule present within diet that is supposed to be the molecule that is responsible for synthesis of nicotinamide adenine nucleotide (NAD+) which increases oxidative metabolism and in mice has been demonstrated to protect HFD induced obesity within mice [16]. Reducing equivalents are supplied by NAD+ for oxidative phosphorylation, that is necessary for oxidative metabolism as well as metabolic...
Long-chain fatty acids enter the cell from the bloodstream and enter the mitochondria through the carnitine shuttle, followed by a step-wise degradation involving a series of enzymes of the long-chain fatty acid oxidation machinery resulting in the production of acetyl-CoA. Potential treatments to produce acetyl-CoA independent of the fatty acid oxidation enzymes are indicated. These include medium-chain triglycerides, ketone bodies and triheptanoin. Abbreviations: CI-V, Complex I-V; CACT, Carnitine Acylcarnitine Translocase; CD36, Cluster of Differentiation 36; CoA, Coenzyme A; CPT1, Carnitine Palmitoyl Transferase type 1; CPT2, Carnitine Palmitoyl Transferase type 2; FABP pm, plasma membrane-associated Fatty Acid Binding Protein; FATP, Fatty Acid Transport Protein; LCEH, Long-Chain Enoyl-CoA Hydratase; LCHAD, Long-Chain 3-Hydroxyacyl-CoA-Dehydrogenase; LCKAT, Long-Chain Ketoacyl-CoA Thiolase; MCAD, Medium-Chain Acyl-CoA Dehydrogenase; MCKAT, Medium-Chain 3-Ketoacyl-CoA Thiolase; OCTN2, Organic Cation Transporter 2; SCAD, Short-Chain Acyl-CoA Dehydrogenase; SCHAD, Short-Chain 3-hydroxyacyl-CoA Dehydrogenase; T2, acetoacetyl-CoA thiolase.

Homeostasis. In obesity along with other components of Metabolic Syndrome (Mets) like Type 2 Diabetes Mellitus (T2DM) as well as NAFLD [17,18], pointing that with external supplementation to escalate NAD+ levels might help in reducing these conditions. Further NAD+ helps in avoiding extent of injury secondary to Oxidative Stress (OS) [17]. In obesity as well as NAFLD formation role of Oxidative Stress (OS) has been documented [19]. An escalation of 4-hydroxy nonenal (4-HNE), that represents a marker of Oxidative Stress (OS)-stimulated lipid peroxidation [20] which takes place in NAFLD subjects [21,22]. Thus Salic et al analyzed if giving a combination of l-Carnitine (LC) and Nicotinamide Ribose (NR), both parts of natural substances which might increase transfer of FA's inside mitochondria and thus enhance NAD+ amounts, that are known to be essential for β-oxidation as well as tricarboxylic acid cycle (TCA) respectively (Figure 2). Ldlr-/-Leiden mice get treated with High Fat Diet (HFD) that had administration of LC (0.4% w/w), (NR 0.3% w/w) or both (COMB) for 21 weeks. Levels of LC that were decreased by HFD got to normal after LC administration. Similarly with NR administration increased its plasma amounts of metabolism products suggesting proper administration. Inspite of food orally along with locomotion were relatively same in all groups, COMB therapy markedly ameliorated HFD stimulated enhancement of body weight, fat mass increase which was -17% as well as hepatic steatosis that reduced by 22%. Further, both NR and COMB decreased liver 4-HNE adducts. On checking the upstream regulator gene analysis showed that COMB finished the negative actions of HFD on liver metabolism pathways correlated controllers like ACOX, SCAP, SREBP, PPARGC1B and INSR. Thus a combination therapy of LC and NR confers protection on metabolic pathways and might reflect a prospective therapy for obesity caused by HFD as well as NAFLD [23].

Role of targeting bile acid metabolism

Nonalcoholic Steato Hepatitis (NASH) represents a severe and mostly propagating type of NAFLD [24-26] for which no approved

Figure 1: Courtesy ref number 16 - Schematic representation of mitochondrial fatty acid oxidation in humans

Description: Long-chain fatty acids enter the cell from the bloodstream and enter the mitochondria through the carnitine shuttle, followed by a step-wise degradation involving a series of enzymes of the long-chain fatty acid oxidation machinery resulting in the production of acetyl-CoA. Potential treatments to produce acetyl-CoA independent of the fatty acid oxidation enzymes are indicated. These include medium-chain triglycerides, ketone bodies and triheptanoin. Abbreviations: CI-V, Complex I-V; CACT, Carnitine Acylcarnitine Translocase; CD36, Cluster of Differentiation 36; CoA, Coenzyme A; CPT1, Carnitine Palmitoyl Transferase type 1; CPT2, Carnitine Palmitoyl Transferase type 2; FABP pm, plasma membrane-associated Fatty Acid Binding Protein; FATP, Fatty Acid Transport Protein; LCEH, Long-Chain Enoyl-CoA Hydratase; LCHAD, Long-Chain 3-Hydroxyacyl-CoA-Dehydrogenase; LCKAT, Long-Chain Ketoacyl-CoA Thiolase; MCAD, Medium-Chain Acyl-CoA Dehydrogenase; MCKAT, Medium-Chain 3-Ketoacyl-CoA Thiolase; OCTN2, Organic Cation Transporter 2; SCAD, Short-Chain Acyl-CoA Dehydrogenase; SCHAD, Short-Chain 3-hydroxyacyl-CoA Dehydrogenase; T2, acetoacetyl-CoA thiolase.

Figure 2: Courtesy ref no-13 Schematic representation of the different actions of NR in metabolic homeostasis

Description: The scheme summarizes the hypothesis by which NR supplementation would increase NAD+ content in key metabolic tissues, leading to SIRT1 and SIRT3 activation and the deacetylation and modulation of the activity of key metabolic regulators. This model does not rule out the participation of additional mechanisms of action for NR to achieve its beneficial effects. Abbreviations can be found in the text and enzymes are indicated in italics.
medical therapy exists pointing to severe need for developing one [27]. On histology, hepatic steatosis, kubal inflammation with damage of the liver cells injury or ballooning that causes cell necrosis either with or without fibrosis [28]. Since classically NASH remains silent a liver biopsy is needed to pick it up early [28,29]. It might cause cirrhosis, Hepatocellular Carcinoma (HCC), [30] as well as liver failure [31,32], being the biggest cause for liver transplantation in US adults below 50years [33]. There is complicated pathophysiology of NASH along with multifactorial [reviewed earlier by us unpublished, under review] having multiple factors in combination like genetic, environmental, Gut Microbiota (GM). Additionally dysfunctional metabolism of bile acids, lipids that include cholesterol as well as Insulin Resistance [IR] [34]. Excessive synthesis as well as decreased excretion of cholesterol from liver ends up in the collection of free cholesterol [35] that correlates with hepatic injury, mainly via interfering of mitochondrial function [36] as well as facilitation of Oxidative Stress [OS] [37].

Escalated serum insulin as well as triglycerides associated with increased amounts of Very Low Density Lipoproteins (VLDL) cholesterol and decreased High Density Lipoprotein (HDL) cholesterol [37,38]. In NASH subjects IR as well as hyperlipidemia is frequent just like visceral obesity accompanied with MetS [24]. White Adipose Tissue (WAT) enhancement might aid in disease propagation via synthesis of adipokines, inflammatory cytokines as well as lipids [39].

Commonly serum bile acids are increased in NASHaubjects, with levels 2 times >than normal [40]. With bile acids amounts elevated inflammatory responses might present with Oxidative Stress (OS), cell death path that might lead to liver injury [41,42]. Primary bile acids get manufactured via cholesterol in the liver and shifted to gut via bile where they might be metabolized to secondary bile acids by bacteria [Figure 3] [38,43]. Secondary bile acids like Lithocholic Acid (LCA) and Deoxy Cholic Acid (DCA), have > hydrophobicity [42,43] and might stimulate inflammation, enhance Reactive Oxygen Species (ROS), and stimulate necrotic pathways [42-44].

Although the rates are constantly escalating we don’t have any effective therapy but for diet changes and exercise [25,45]. Lot of pharmacological therapies that hold a promise is being studied [45]. One strategy is to block the enterohepatic recirculation of bile acids back to liver by blocking the Apical Sodium Dependent Bile Acids Transporter (ASBT) [46]. The ASBT is a transmembrane protein present in the luminal surface of enterocytes. Roughly 95% of the bile acids pool gets resorbed in the terminal ileum and then shifted back to liver, and the rest gets excreted via feces [43,47] (Figure 3).

Blocking bile acids recycling via inhibition of ASBT enhances the fecal excretion [46,48] and is believed to induce the liver for more bile acids manufacture from hepatic cholesterol for sustaining the bile acids homeostasis [49]. In mice inhibition of ASBT escalated hepatic expression of cholesterol 7α hydroxylase (Cyp 7a1) [49],,bile acids, lipids, liver enzymes were evaluated. Ultimate examination was liver histology, intra hepatic lipids, mesenteric WAT mass as well as liver gene profiles testing. Volixibat escalated the total amount of fecal bile acids. At the maximum dose, Volixibat significantly ameliorated the HFD stimulated increased hepatocyte hypertrophy, liver triglycerides as well as cholesterol ester levels as non-alcoholic fatty liver disease (NAFLD) getting accumulated. Nonalcoholic fatty liver diseases scores (NAS) were significantly low in Volixibat treated mice act HFD controls. Gene profiling demonstrated that Volixibat finished the inhibitory action of HFD on metabolic controllers like peroxisome proliferator activated peroxisome proliferator activated receptor – γ coactivators (PGC1s), insulin receptor as well as sterol regulatory element binding transcription factor 2. Thus Volixibat might be effective physiologically as well as regarding metabolic part of NASH pathophysiology [53].

Role of Silybin

Globally the incidence of metabolic disorders like Nonalcoholic Fatty Liver Diseases (NAFLD), Type 2 Diabetes Mellitus (T2DM), as well as obesity has presented in an epidemic fashion worldwide. NAFLD incidence is 6-35% (median 20%) worldwide with this number escalating worldwide, in view of the diet and lifestyle patterns [54]. This NAFLD prevalence is markedly related with obesity, T2DM as well as dyslipidemia [55]. The “multiple hit hypothesis” is thought to be responsible for the etiopathogenesis of NAFLD, that manifests IR, Adipose Tissue (AT) liberated hormones, nutritional factors, GM as well as genetic and epigenetic factors [56,57]. As NAFLD progresses simple steatosis might shift to the more severe form labeled Nonalcoholic Steatohepatitis (NASH) that includes inflammation as well as apoptosis, with or without fibrosis as well as cirrhosis. No proper way of avoiding the occurrence and formation of NASH exists other than diet control. Discovery of new drugs for curing NAFLD and NASH will be very important.

For treating these chronic problems Traditional Chinese Medicine (TCM) have been utilized for long in China as well as some Asian countries for centuries. As no medical therapy from West has been shown to be safe and efficacious for treating NAFLD, Traditional Chinese Medicine (TCM) pose a particular superiority. Silybin is extracted from milk thistle (Silybum Marianum) seeds and is used as a hepato protective agent broadly [58]. Different potential biological actions of Silybin have been looked for, that includes cancer therapy, DM treatment and liver diseases therapy [59,60]. Mode of action includes control of lipid metabolism, antioxidant radical scavenger. Cellular membrane getting stabilized and promotion of ribosomal and RNA synthesis [61,62] has also been documented that silybin manages a variety of metabolic pathways in acute liver injury [63]. But no metabolism information of Silybin has been totally given that might give us understanding of its mechanism of action.

Metabolomics gives information in a comprehensive manner that regarding endogenous molecules in the body and is very useful in diagnosing the variety of diseases for decreasing the chances of these conditions [64,65]. Moreover biomarkers are used in an unbiased way to for classification of the stage of disease progression, metabolic control by Silybin of NAFLD C57BL/6J mice that were given HFD/High cholesterol diet for 8wks and treated with Silybin (50 or 100mg/kg/day) by gavage for the last 4 weeks. Blood biochemical markers, like total cholesterol, triglycerides, C peptide and Glucose Tolerance Test (GTT), have been utilized in diagnosing NAFLD for several years. Right now there are more upcoming biomarkers giving important complementary knowledge, like Apo lipoproteins A1, apolipoprotein B lepton, adiponectin, Free Fatty Acids (FFA), ghrelin and Tumor Necrosis Factor Alpha (TNFα) [69]. But a comprehensive and a total view of metabolites that are altered in NAFLD and following Silybin therapy is not available. Hence Sun et al. [70] tried to evaluate the metabolic control by Silybin of NAFLD BALB/c mice that were given HFD/High cholesterol diet for 8wks and treated with Silybin (50 or 100mg/kg/day) as well as sodium taurocholate (TDCA, 50mg/kg/day) by gavage for the last 4 weeks. Blood biochemical indexes and hepatic lipid measurement along with Oil red 0 staining of the liver were carried out for analyzing the model as well as the lipid decreasing effect of Silybin and TDCA. Moreover serum and liver samples were checked utilizing a metabolomics platform on the basis of gas chromatography-mass spectrometry (GC/MS). Multivariate/univariate data analysis and pathway evaluation were utilized for evaluation of different metabolites as well as metabolic pathways. As per the results the mouse NAFLD model got established successfully and Silybin and TDCA decreased both serum as well as hepatic lipid accumulation at significant levels. Metabolomics evaluation of serum and liver demonstrated that HFD/High cholesterol diet led
to the anomalous metabolism of metabolites participating in lipid metabolism, polyol metabolism, amino acid metabolism, the urea cycle and the TCA cycle. Silybin and TUDCA therapy together reversed metabolic disorders produced by HFD feeding. Thus concluding that a HFD/High cholesterol diet resulted in anomalous metabolism in the serum and liver of mice, and Silybin therapy improved hepatic lipid collection and manipulated global metabolic pathways that gave a probable way for the multiple target modes of action [70].

Conclusions

Thus, here we have summed up the role of utilizing LC and NR for affecting lipid metabolism by enhancing LC levels and with use of NR how OS is reduced along with reduction of 4-hydroxy nonenal (4-HNE) levels following increase of nicotine adenine diamide on supplemented LC and NR together that might be one strategy for reducing propagation of NAFLD. The further role of Apical Sodium Dependent Bile Acids Transporter (ASBT) inhibitor volixibat by affecting bile acid metabolism that is undergoing phase 2 trials in humans for NASH and further the role of Silybin a product that has been and rosmarinic acid we have researched from TCM. Already probiotics, work on Vitamin D Metabolism, allylisothiocyanate reviewed along with role of the gut microbota in aetopathogenesis etc. Hopefully we will find some permanent method for prevention of NAFLD, NASH propagating to cirrhosis HCC, liver failure and avoid need of liver transplantation with incidence increasing with epidemic of obesity and Diabetes itself being a causative factor in NAFLD as well as NASH independent of obesity.

References
