World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i18.2223

World J Gastroenterol 2010 May 14; 16(18): 2223-2226
ISSN 1007-9327 (print)

TOPIC HIGHLIGHT

Metin Basaranoglu, PhD, Associate Professor, *Series Editor*

Understanding mechanisms of the pathogenesis of nonalcoholic fatty liver disease

Metin Basaranoglu, Serra Kayacetin, Nevin Yilmaz, Ertugrul Kayacetin, Orhan Tarcin, Abdullah Sonsuz

Metin Basaranoglu, Division of Gastroenterology, Ankara Yüksek Ihtisas Hospital, Ankara 06100, Turkey
Serra Kayacetin, Department of Pathology, Konya Education and Teaching Hospital, Konya 42080, Turkey
Nevin Yilmaz, Mugla University, School of Medicine, GI/Transplant Hepatology, Kötekli, Mugla 48120, Turkey
Ertugrul Kayacetin, Division of Gastroenterology, Meram Medical Faculty, Selcuk University, Konya 42080, Turkey
Orhan Tarcin, Division of Gastroenterology, Yeni Yüzyil University, Istanbul 34000, Turkey
Abdullah Sonsuz, Division of Gastroenterology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul 34000, Turkey
Author contributions: Basaranoglu M contributed extensively to the work, performed literature search and designed and wrote the paper; Kayacetin S, Yilmaz N, Tarcin O, Sonsuz A and Kayacetin E contributed equally to this work; Kayacetin S and Kayacetin E performed literature search; all authors discussed and commented on the manuscript.
Correspondence to: Dr. Metin Basaranoglu, Division of Gastroenterology, Ankara Yüksek Ihtisas Hospital, Ankara 06100, Turkey. metin_basaranoglu@yahoo.com
Telephone: +90-212-5540570 Fax: +90-212-6217580
Received: December 3, 2009 Revised: February 20, 2010
Accepted: February 27, 2010
Published online: May 14, 2010

Abstract

A central issue in the understanding of the pathogenesis of nonalcoholic fatty liver disease is the problem of the underlying mechanisms which are not fully understood. In the setting of excessive central adiposity, insulin resistance is the major underlying cause of fat accumulation in hepatocytes. Because of the difficulties with human trials, several animal models have been developed for this purpose mainly characterized as follows: genetically disturbed or murine fatty liver, methionine-choline deficient diet fed or murine steatohepatitis, and high-fat or sucrose diet fed models. Although these animal models have provided useful information, none of them accurately reflect genetic, metabolic and biochemical characteristics of the human disease.

Key words: Nonalcoholic fatty liver disease; Pathogenesis; Rat; Rodents

Peer reviewers: Dr. MH Ahmed, MD, PhD, Chemical Pathology Department, Southampton University Hospital NHS trust, Mail point 6, Level D, South Academic Block, Southampton SO16 6YD, United Kingdom; Maarten Tushuizen, MD, Department of Gastroenterology & Hepatology, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands; Dr. Mihaela Petrova, MD, PhD, Clinic of Gastroenterology, Medical Institute, Ministry of Interior, Sofia 1606, Bulgaria

Basaranoglu M, Kayacetin S, Yilmaz N, Kayacetin E, Tarcin O, Sonsuz A. Understanding mechanisms of the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2010; 16(18): 2223-2226 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i18/2223.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i18.2223

OBESITY AND NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent forms of chronic liver disease[1-4]. The reported prevalence of NAFLD in developed countries is 30% and 13% in adults and children, respectively. It is likely that in type 2 diabetes mellitus, NAFLD is one of the consequences of obesity. The prevalence of NAFLD in the obese population is nearly 95%. Factors contributing to NAFLD include sedentary life style, and increased consumption of foods with high-fat and high fructose corn syrup content. A cafeteria style diet which includes both high fat and fructose corn syrup is the

leading cause of obesity in the population. Furthermore, in the setting of excessive central adiposity, insulin resistance is the major underlying cause of fat accumulation in the liver[5-11]. NAFLD is characterized by hepatic fat accumulation in hepatocytes (> 5% of liver weight).

NONALCOHOLIC STEATOHEPATITIS

Nonalcoholic steatohepatitis (NASH), as a subgroup of NAFLD, is characterized by chronic, progressive liver pathology which has the ability to lead to advanced fibrosis, cirrhosis, hepatocellular carcinoma, and liver-related death[1,2,4,5]. The prevalence of NASH is 3% among adults. The mechanisms underlying NASH pathogenesis are not fully understood. One of the hypotheses has been termed the "two-hit" theory[5,6]. According to this paradigm, NAFLD is a result of inappropriate fat storage or ectopic fat accumulation and the primary abnormality is most likely insulin resistance which leads to the accumulation of triglycerides (TGs) within the hepatocytes. After the first hit of steatosis, the second hit of oxidative stress leads to hepatocyte injury and inflammation.

Animal models

There are several types of animal model used for NAFLD studies, and these are mainly characterized as follows: genetically disturbed or murine fatty liver, methionine-choline deficient (MCD) diet fed mice or murine steatohepatitis model and feeding high-fat and/or sucrose diets with or without high caloric intake model[12-24]. Manipulation of the mouse genome and production of new animal models, such as leptin-deficient ob/ob mice, leptin-resistant db/db rats or knockout mice regarding a special character, have given us great opportunities for research[12-16]. However, it is not possible to extrapolate all the information gained from these animal models into knowledge on the human species as there are some fundamental differences regarding their genetics, mediators, and the mechanisms of the events. For example, although obese patients have higher circulating leptin levels, ob/ob mice exhibit complete leptin deficiency. Additionally, leptin has some immunologic functions, besides its metabolic regulatory capacity. Secondly, although insulin resistance is a universal feature of patients with NASH, the MCD model is not insulin-resistant and not obese[15]. MCD mice have increased insulin hypersensitivity, and their serum has both insulin and glucose levels lower than mice fed a standard diet.

High caloric intake models

With regard to the problems mentioned above, it is reasonable to try to find a suitable model for investigating the mechanism behind human NASH. Actually, there have been several attempts to mimic human species by feeding mice with high fat and/or sucrose diets with or without high caloric intake in order to produce obesity, insulin resistance, and NASH[16-23]. The type and amount of fat, and total daily caloric intake on these diets are very important and there has been no standardized diet reported by study groups. In this regard, Lieber *et al*[25] fed Sprague-Dawley rats with a high-fat, liquid diet (71% of energy from fat which included corn, olive, and safflower oil) for 3 wk and developed a steatohepatitis model in murines which resembled human NASH. These mice displayed obesity and insulin resistance together with increased hepatic tumor necrosis factor (TNF)-α, TNF-α messenger RNA, cytochrome 2E1, cytochrome 2E1 mRNA, increased oxidative stress and lipid peroxidation, fatty liver histopathology, mononuclear cell infiltration, abnormal mitochondria and increased collagen in the liver. Bruce *et al*[26] used a high fat diet (45% kcal from fat, 20% kcal protein, 35% kcal carbohydrate) and Tetri *et al*[27] fed mice with 45% calories in the chow from fat and 30% of the fat in the form of partially hydrogenated vegetable oil (28% saturated, 57% monounsaturated fatty acids, 13% polyunsaturated fatty acids). Both research groups developed mice displaying obesity, insulin resistance and NASH.

There is substantial diversity within and between mouse strains resembling phenotypic variations in human populations. C57 BL6J mice have usually been chosen by NASH researchers because of their predisposition to develop insulin resistance by means of diet and the availability of genetically manipulated mice[14,27-29]. These features of the strain are explained by a strong influence of the genetic background on the susceptibility to diet-induced obesity and insulin resistance[14].

WHAT WE KNOW ABOUT THE MECHANISMS UNDERLYING NASH PATHOGENESIS?

For NASH pathogenesis, it is a prerequisite firstly to develop fatty liver which is then unusually vulnerable to various second hits or injury[5,6]. Although insulin resistance is a universal finding for both simple steatosis and NASH, only a small group of insulin-resistant patients develop NASH, even in patients with metabolic syndrome (MS). MS is the most severe form of insulin resistance and might instigate more advanced NASH. It is believed that oxidative stress and lipid peroxidation might play a central role in the transition of simple steatosis to NASH[30].

Increased production of ROS and lipid peroxidation of hepatocyte membranes and organelles promote necroinflammation, satellite cell activation and fibrosis in the liver. In this context, elevated free fatty acids (FFAs) in both circulation and the liver, mitochondrial abnormalities (dysfunction and structural abnormalities such as mega mitochondria with true crystalline inclusions), gut-derived endotoxins, ethanol secondary to gut and liver interaction, and disturbed production of adipokines should be major concerns in the development of steatohepatitis in an animal model.

Insulin resistance and peripheral lipolysis cause an

increased FFA pool in the circulation[7,21,30]. This pool is one of the major sources of hepatic TGs. FFAs are also the major source of hepatic mitochondrial, peroxisomal and microsomal ROS production. It has been reported that increased hepatic and serum FFA concentrations promote hepatic and systemic insulin resistance by the activation of PKCθ and serine phosphorylation of insulin receptor substrates. Feldstein *et al*[30] reported that FFAs promote lysosomal permeabilization, release cathepsin B which is a lysosomal protease within hepatocytes, and cause hepatocyte apoptosis and injury. In addition to these aspects, increased serum concentrations of FFAs due to obesity were found to be correlated with the severity of fibrosis in patients with NASH[31]. FFAs play a major role in the transition from simple steatosis to NASH.

High-fat diet-induced obesity and insulin resistance have been reported in Wistar rats[32,33], Sprague-Dawley rats[34,35], F344 rats[36], and in Long-Evans rats[37]. Additionally, prolonged feeding periods with high fat (59% fat), such as a 3 mo duration, promoted a greater degree of insulin resistance in Wistar rats[33]. Borst and Conover induced obesity and developed an insulin-resistant animal model by feeding them for 39 d with a high-fat (50%) inclusive diet[38]. In this model, daily caloric intake was not increased, and it was even slightly less than for the normal rat chow (12.4% fat). These mice developed increased visceral and subcutaneous fat mass, insulin resistance, elevated fasting serum insulin, decreased insulin-stimulated glucose transport in skeletal muscle, increased TNF-α expression on visceral adipose tissue, and an undetectable serum TNF-α concentration. Most importantly, serum FFA concentration was not increased significantly in both mice fed high-fat diet and controls, while liver TNF-α expression was not affected. This observation is interesting, as obesity is strongly associated with increased concentration of FFAs in the circulation.

It was previously reported that a five-fold increase of plasma FFAs caused up to 100-fold increase in plasma insulin concentrations[26,30]. Thus, FFAs are more important than TNF-α for inducing insulin resistance. TNF-α expressed in visceral adipose tissue macrophages, and maybe TNF-α in muscle, appears the major cause of systemic insulin resistance in these animal models, since FFA levels were not increased. Oxidative stress is one of the most popular proposed mechanisms of hepatocellular injury in NASH in both animal experiments and human trials. It has been reported that obesity correlates with systemic oxidative stress. Obese adults with MS have a higher plasma concentration of oxidative stress biomarkers than obese adults without MS[2,6,7]. Increased ROS production has been selectively shown in adipose tissue of obese mice. There is also some indirect evidence as to the benefit of antioxidants such as vitamin E, S-adenosylmethionine and betaine, phlebotomy to remove iron, and N-acetylcysteine for treating NAFLD. However, a causal relationship or a pathogenic link between NASH and oxidative stress has so far not been established.

Hepatocyte mitochondria are the main site of β-oxidation of FFAs and adenosine triphosphate (ATP) production is one of the crucial issues in the understanding of NASH pathogenesis. It was previously reported that mitochondrial structural abnormalities, depletion of mitochondrial DNA and ATP, and mitochondrial dysfunction are characteristics of NASH patients as well as of diet-induced and animal models[39-41]. Increased oxidative stress and lipid peroxidation products are integral components of the pathway progressing to NASH from fatty liver. Specifically, rats fed a high-fat diet have been shown to have reduced electron transport chain capacity and increased oxidative stress in liver mitochondria[39,41].

Excessive fatty acids might be used as an alternative pathway to produce mitochondrial injury rather than the mitochondrial β-oxidation pathway. These possible routes of injury include the peroxisomal and microsomal oxidation systems[42]. Alternative fatty acid oxidation systems produce more hydrogen peroxide and thus may contribute to oxidant stress. Increased cytochrome P450 2E1 expression and induction have been well-established previously in both murine SH and human NASH studies.

In a very recent study, researchers developed a novel model which suggested maternal fat intake contributes toward the NAFLD progression in adult offspring; mediated through impaired hepatic mitochondrial metabolism and up-regulated hepatic lipogenesis[26]. Large-scale gene expression study, particularly a whole genome array analysis, is a very powerful technique which was used to measure the differences between the samples in this model[26]. This technique was able to measure the mRNA expressed in the tissue analyzed, assessing parameters such as relative expression of genes involved in inflammation, oxidative stress, lipogenesis, and beta-oxidation.

CONCLUSION

One of the areas for ongoing research is the understanding of how much calorie intake and composition of the diet affect the development of NASH. In conclusion, although these animal NAFLD models are already in use and further improve our knowledge, the underlying mechanisms in NAFLD pathogenesis need further research.

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