Int J Pharm Bio Sci 2012 July; 3(3): (B) 1085 - 1090

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

NON ALCOHOLIC STEATOHEPATITIS (NASH) EXPERIMENTAL MODEL INDUCTION IN RATS

SURAPANENI KRISHNA MOHAN*[1], SARASWATHI. P[2] AND MALLIKA JAINU[3]

*[1]*Assistant Professor, Department of Biochemistry, Saveetha Medical College & Hospital, Faculty of Medicine, Saveetha University, Saveetha Nagar, Thandalam, Chennai – 602 105, Tamilnadu, India.*
[2]*Professor & Head, Department of Anatomy, Saveetha Medical College & Hospital, Faculty of Medicine, Saveetha University, Saveetha Nagar, Thandalam, Chennai – 602 105, Tamilnadu, India.*
3Assistant Professor, Department of Biomedical Engineering, SSN Engineering College, OMR, Kalavakkam, Chennai – 603 110, Tamilnadu, India.

ABSTRACT

Non Alcoholic steatohepatitis (NASH) is a silent asymptomatic disease which progress towards the fibrosis and cirrhosis, an end stage liver disease. Liver biopsy is the only method to confirm the diagnosis of NASH and is considered gold standard to confirm NASH. Liver biopsy is an invasive procedure and associated with many risks and this feature hinders to conduct studies in NASH in human beings. The development of an experimental NASH model in rats, which mimics human NASH is very much needed to conduct the studies related to the diagnosis and treatment strategies. Male Wister rats were divided into three groups as Group A, B & C treated with new experimental dose and for various periods such as 4 weeks, 8 weeks and 12 weeks. A portion of the liver tissue was taken after 4, 8, 12 weeks and its histological features were studied. Rats fed with high fat diet for 8 weeks showed diffused fatty Infiltration of hepatocytes with mono nuclear inflammatory infiltrate, confirming the development of NASH. A high-fat diet (HFD) is used to create a new experimental model of NASH and NASH has been successfully developed in the rats fed with high fat diet for 8 weeks. Thus, this model mimics the most common features of NASH in humans and provides an ideal tool for further research in NASH.

KEY WORDS: ***Non alcoholic steatohepatitis, fibrosis, NASH, high fat diet, experimental model, non alcoholic fatty liver disease***

SURAPANENI KRISHNA MOHAN
Assistant Professor, Department of Biochemistry, Saveetha Medical College & Hospital, Faculty of Medicine, Saveetha University, Saveetha Nagar, Thandalam, Chennai – 602 105, Tamilnadu, India.

INTRODUCTION

Fatty liver, or fatty liver disease (FLD), is characterized histologically by triglyceride accumulation within the cytoplasm of hepatocytes[1] and refers to fat accumulation in the liver exceeding 5%–10% by weight[2]. Accumulation of fat may also be accompanied by a progressive inflammation of the liver, called steatohepatitis[2]. Fatty liver disease may be of two types depending on the involvement of the alcohol viz. alcoholic steatosis or nonalcoholic fatty liver disease (NAFLD), and the more severe forms were termed as alcoholic steatohepatitis (part of alcoholic liver disease) and non alcoholic steatohepatitis (NASH). The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing year by year all over the world and is becoming the major part of the burden of the liver disease[3, 4]. Increased free fatty acid release from adipose tissues (lipolysis), increased *de novo* synthesis of fatty acids (lipogenesis), decreased mitochondrial β-oxidation and decreased very low-density lipoprotein secretion have been considered as the major metabolic alterations that contribute to the pathogenesis of the non alcoholic steatohepatitis[5]. Obesity, diabetes, and hyperlipidemia are important risk factors for NASH. Common conditions associated with fatty liver and NASH includes obesity, type 2 diabetes mellitus, the presence of high levels of triglyceride (fat) in the blood and high blood pressure[6, 7].

NAFLD represents a spectrum of disease ranging from simple hepatic steatosis through steatohepatitis to fibrosis and cirrhosis, which is an irreversible condition and life threatening as well. The prevalence of NAFLD has risen rapidly in parallel with the dramatic rise in obesity and diabetes[8, 9].

NASH is a form of metabolic liver disease in which, steatosis is associated with lobular inflammation, hepatocyte injury and/or hepatic fibrosis. NASH typically causes no symptoms. When present, clinical features such as fatigue, hepatomegaly and aching hepatic discomfort are non-specific[8, 9]. In 20–25% of cases, NASH may progress to advanced stages of hepatic fibrosis, cirrhosis and liver failure then becomes the most common cause of death, and hepatocellular carcinoma (HCC) may occasionally occur. Correction of insulin resistance by dietary measures and increased physical activity is a logical approach to prevent or reverse early NASH, and modest weight reduction can normalize liver test abnormalities[8, 9].

MATERIALS AND METHODS

Male Wistar rats weighing approximately 150 – 200 g were housed in solid-bottomed polypropylene cages under strict veterinary supervision and maintained in control rooms with 12 h light/dark cycle. The animals received commercial rat diet, standard diet, high-fat diet and water *ad libitum* as per the experimental protocol. This study conformed to the guiding principles of Institutional Animal Ethical Committee (IAEC), Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Guide for the care and use of laboratory animals (IAEC Approval Number: 001/006/2010 & 01/007/2011).

Despite the availability of various other techniques like ultrasound, fibroscan, elastography etc., to diagnose the non alcoholic steatohepatitis (NASH)[10], liver biopsy is the only way to confirm the presence or absence of NASH in a person with features of NASH. It also remains the "gold standard" for fibrotic severity, hindering to conduct studies in human beings with NASH.

COMPOSITION OF THE DIET

Experimental NASH was established according to the model of Rivera et al (2006) with slight modifications[11]. Male Wistar rats, which were individually housed and fed either a standard diet with Protein 20%; fat 5%; Carbohydrates 5%; Fiber 5% and a high-fat diet with 20% of energy derived from Protein; 15% from Corn Oil; 50% from Sucrose; 5% from Fiber. The standard diet has the same fat content as the average "normal" diet available commercially. *The overall compositions of the standard and high fat diets are shown in Table 1.*

Table 1

g/kg	Standard diet	High fat diet
Casein	200	200
Sucrose	50	500
Dextrin	50	50
Cornoil	2.5	150
L-methionine	3	3
Choline	2	2
Cyanocobalamine	10	10
NaCl	2.5	2.5
Fibre	5	5
Fructose	50	5
Cholesterol	0	2

EXPERIMENTAL PROTOCOL TO CREATE THE MODEL OF NASH IN RATS

The animals were broadly divided into three major groups (n=18) as shown below to establish the experimental model of NASH in rats:

These 3 major groups were sub-grouped as shown below:

Group A: Dose & Duration = 4 weeks (n=18)

- **Group A1; Control (n=6):** Rats fed with normal rat pellet diet *ad libitum* (available commercially) for 4 weeks.
- **Group A2; Standard Diet (n=6):** Rats fed with standard diet *ad libitum* for 4 weeks.
- **Group A3; High Fat Diet (n=6):** Rats fed with high-fat diet *ad libitum* for 4 weeks.

Group B: Dose & Duration = 8 weeks (n=18)

- **Group B1; Control (n=6):** Rats fed with normal rat pellet diet *ad libitum* (available commercially) for 8 weeks.
- **Group B2; Standard Diet (n=6):** Rats fed with standard diet *ad libitum* for 8 weeks.
- **Group B3; High Fat Diet (n=6):** Rats fed with high-fat diet *ad libitum* for 8 weeks.

Group C: Dose & Duration = 12 weeks (n=18)

- **Group C1; Control (n=6):** Rats fed with normal rat pellet diet *ad libitum* (available commercially) for 12 weeks.
- **Group C2; Standard Diet (n=6):** Rats fed with standard diet *ad libitum* for 8 weeks.
- **Group C3; High Fat Diet (n=6):** Rats fed with high-fat diet *ad libitum* for 12 weeks.

After the experimental period (4^{th}, 8^{th}, 12^{th} week), liver tissues of all the experimental groups were dissected out and fixed in 10% buffered neutral formalin solution for histopathological studies for the assessment of the development of NASH.

RESULTS

The results of the histopathological studies conducted to create experimental model of NASH in rats have been shown in Fig. 1, 2 and 3.

Fig 1 (A-C)
Histopathological Studies in Group A (Week 4)

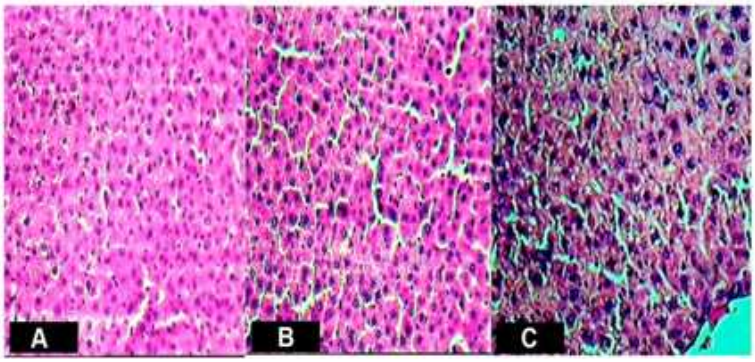

Fig. 1A: Group A1 (Control) - Showed Normal Hepatocytes; Fig. 1B: Group A2 (Standard Diet) - Showed Normal Hepatocytes, No pathological changes observed; Fig. 1C: Group A3 - (High fat diet) - Showed slightly enlarged hepatocytes with feathery degeneration of cytoplasm (early change)

FIG 2 (D-F)
Histopathological Studies in Group B (Week 8)

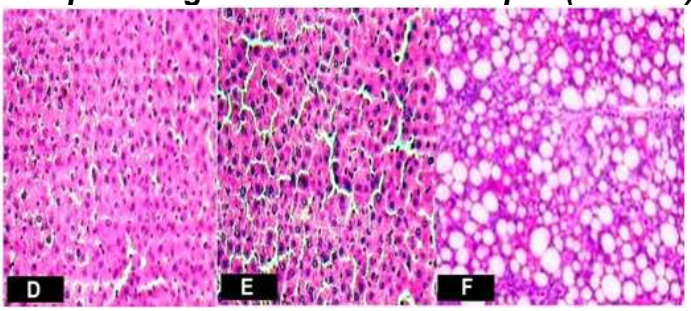

Fig. 2D: Group B1 (Control) - Showed Normal Hepatocytes; Fig. 2E: Group B2 (Standard Diet) - Showed Normal Hepatocytes, No pathological changes observed; Fig. 2F: Group B3 (High Fat Diet) - Showed Diffused Fatty Infiltration of Hepatocytes with Mono Nuclear Inflammatory Infiltrate

Fig. 3 (G-I)
Histopathological Studies in Group C (Week 12)

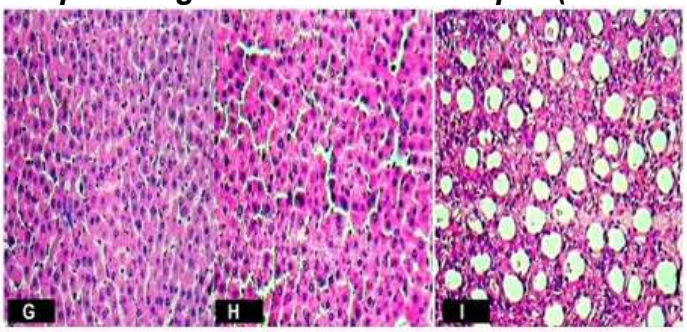

Fig. 3G: Group C1 (Control) - Showed Normal Hepatocytes; Fig. 3H: Group C2 (Standard Diet) - Showed Normal Hepatocytes, No pathological changes observed; Fig. 3I: Group C3 - (High Fat Diet) showed hepatic macrovesicular steatosis, obvious fibrosis, larger lipid accumulation (fatty cysts & larger fatty vacuoles).

DISCUSSION

The goal of diagnostic procedures is to identify the patients with NASH before the onset of advanced fibrosis. To date, the pathophysiological pathways involved in liver damage and in the progression of pure fatty liver to NASH remain largely unknown. Currently, the most accepted theory to explain the pathogenesis of NASH is the so-called "two-hit" hypothesis[12]. According to this model, the development of hepatic steatosis constitutes the first hit, and cellular events leading to hepatic inflammation constitute the second hit[12]. Experimental and clinical data have suggested a role for hepatocyte apoptosis in liver inflammation and tissue damage, regeneration of parenchyma, and fibrosis[13,14]. In this regard, a reduction in hepatocyte apoptosis has been shown to result in decreased liver fibrosis in animal models of cholestasis[15].

Liver Biopsy is required not only to confirm the diagnosis but also provide important prognostic information. Liver biopsy is now considered the "gold standard" for the assessment of liver fibrosis, which is an invasive procedure associated with severe pain and many complications and risks. Moreover, the prognosis of patients with NASH appears to be dictated by the presence and extent of fibrosis present on liver biopsy[16,17,18]. Thus, at present an invasive liver biopsy is the only reliable way to diagnose the presence of NASH and assess the severity of liver damage present[19]. Obtaining liver biopsy of a patient with suspected NASH to confirm the presence or absence of NASH is a very difficult task since liver biopsy is an invasive procedure and since NASH is an asymptomatic disease patients will not give consent to proceed with the liver biopsy and this becomes the major obstacle for the clinicians and researchers to conduct studies in NASH pertaining to its diagnosis and treatment. This emphasizes the importance of the establishment of the experiment model of NASH to conduct the studies related to the diagnosis and treatment of NASH.

But, the Progress in the understanding and treatment of NASH has been hindered by the lack of a practical experimental model that reproduces the key features of the disease in human beings. In the present study, our findings demonstrated that rats fed with high-fat diet *ad libitum* for 8 weeks, showed diffused fatty infiltration of hepatocytes with mono nuclear inflammatory infiltrate, confirming the development of NASH and this observation concurred with an earlier report[20]. This rat model reproduces the key features of human NASH and provides a realistic experimental model for elucidating its treatment. The present study clearly demonstrated that the ingestion of the high-fat diet for 8 weeks produces all the prominent characteristics of NASH and the principal histological features of NASH, including steatosis, inflammation, which mimics the NASH in humans.

CONCLUSION

A high-fat diet (HFD) is used to create a model of NASH and NASH has been successfully developed in the rats fed with high fat diet for 8 weeks. Thus, this model mimics the most common features of NASH in humans and provides an ideal tool to study the role of events involved in the pathogenesis of NASH and to define any future experimental therapy, which ameliorates the degree of liver injury.

REFERENCES

1. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology,* 128: 1898-1906, (2005).
2. Szczepaniak LS, Nurenberg P, Leonard et al., "Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population," *Amer J Phy*, 288: E462–E468, (2005).
3. Kim WR, Brown RS, Terrault NA, El-SeraHH: Burden of liver disease in the united states: summary of a workshop. Hepatology, 36: 227-242, (2002).
4. Suneel B., Balakrishna D., Sridhar M., Aparna RR and Sowjanya B. Zinc, Copper, C-reactive protein and lipid peroxidation in chronic liver diseases, Int J Pharm. Bio. Sci, 3 (2); April-June, (2012).
5. Matsuzaka T, & Shimano H, Molecular mechanisms involved in hepatic steatosis and insulin resistance. *J Diabetes Invest*, 2: 170–175, (2011).
6. Shulman AI and Mangelsdorf DJ., "Retinoid X receptor heterodimers in the metabolic syndrome". *The New England J of Med*, 353: 604–615, (2005).
7. Anitha U and R. Kuppuswamy. Sesame meal administration attenuate the high-fat diet induced lipid abnormalities and improve insulin sensitivity in wistar rats. Int J Phar. Bio. Sci., 2 (4); Oct-Dec: B499-B505, (2011).
8. Charlton M. Nonalcoholic fatty liver disease: a review of current understanding and future impact. Clin Gastroenterol Hepatol, 2:1048–58, (2004).
9. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. Hepatology, 49:306–17, (2009).
10. Satyanand Tyagi and Sachin Kumar. Clinical applications of elastography: an overview. Int J Pharm. Bio. Sci., 1 (3); July-Sep: 1-8, (2010).
11. Rivera CA, Abrams SH, Tcharmtchi MH, Allman M, Ziba TT, Finegold MJ and Smith CW. Feeding a corn oil/sucrose-enriched diet enhances steatohepatitis in sedentary rats. *Am J Physiol Gastrointest Liver Physiol,* 290: G386–G393, (2006)..
12. Day CP, James OF. Steatohepatitis: a tale of two 'hits'? Gastroenterology, 114:842–845 (1998).
13. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. *Toxicol Sci,* 65: 166-176, (2002).
14. Rust C, Gores GJ. Locoregional management of hepatocellular carcinoma. Surgical and ablation therapies. *Clin Liver Dis,* 5: 161-173, (2001).
15. Canbay A, Friedman S, Gores GJ. Apoptosis: the nexus of liver injury and fi brosis. *Hepatology,* 39: 273-278, (2004).
16. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology, 116:1413–1419, (1999).
17. Adams LA, Talwalkar JA: Diagnostic Evaluation of Nonalcoholic Fatty Liver Disease. J Clin Gastroenterol, 40:S34-S38, (2006).
18. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Longterm follow-up of patients with NAFLD and elevated liver enzymes. Hepatology, 44:865– 873, (2006).
19. Wieckowska A, Zein NN, Yerian LM, et al. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholicfatty liver disease. Hepatology, 44:27–33, (2006).
20. Lieber CS, Leo MA, Mak KM, et al.Amodel of non-alcoholic fatty liver disease (NAFLD), including steatohepatitis (NASH), in rats fed a high fat Lieber-DeCarli diet. Hepatology, 36:402A, (2002).