Original Article
Thyroid hormone attenuate nonalcoholic fatty liver disease via inducing autophagy in rats

Yunfei Feng[1], Chengfu Xu[2], Chaohui Yu[2], Jianguo Shen[3], Youming Li[2]

Departments of [1]Endocrinology, [2]Gastroenterology, [3]Endocrine, The First Affiliated Hospital of Medical College, Zhejiang University, Hangzhou, China

Abstract: Background and objective: To investigate whether thyroid hormone could influence the occurrence and/or reversal of steatosis and steatohepatitis induced by a MCD diet and elucidate its molecular mechanism. Materials and Methods: Male Sprague-Dawley (SD) rats at 8 weeks of age were administered either PBS or TH intraperitoneally (0.1 g/kg per day) via stomach lavage for 6 weeks. Changes in weight gain and energy intake were regularly monitored. Blood and liver tissue were harvested after overnight fasting at the end of study. Histological assessment was performed in liver tissue. The concentrations of AST, ALT in blood and GSH, SOD and MDA in liver tissues were measured. Protein abundance involved in autophagy was analyzed in the liver. Quantification of autophagic vacuoles by transmission electron microscopy (TEM) was used to count the autophagic vacuoles. Results: After administration for 28 days, the ALT and AST content of model group rats were obvious lower compared with the control group. Results indicate that low dose chitosan can reduce hepatocellular damage and improve the liver in HF rats. TE treatment with or without exendin resulted in a similar number of autophagosomes, however, TH treatment significantly increased the number of ALs (Figure 6). While visualizing cells for AVs we observed that some large sized lipid droplets had 'shriveled' margins with distinct absence of autophagic vacuoles around them. Conclusion: The present study demonstrates that thyroid hormone might attenuate nonalcoholic fatty liver disease via inducing autophagy.

Keywords: Autophagy, thyroid hormone, nonalcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of chronic diseases including fatty liver, or bland steatosis, as well as more aggressive lesions including steatohepatitis, lobular necroinflammation with fibrosis, or cirrhosis [1-4]. NAFLD-related cirrhosis can lead to end-stage liver disease and hepatocellular carcinoma (HCC). Two broad categories of fatty liver disease have been recognized: alcoholic fatty liver disease (AFLD) and nonalcoholic fatty liver disease (NAFLD). Both categories are characterized by an initial phase consisting of hepatic steatosis that can progress to alcoholic or non-alcoholic steatohepatitis (ASH or NASH), a state characterized by necroinflammatory changes consisting of ballooning degeneration and apoptosis of hepatocytes, eliciting inflammatory and eventually fibrogenic responses, which can lead to development of cirrhosis and hepa-

tocellular carcinoma (HCC) [5-13]. NAFLD prevalence rate has reached about 15%-20% in recent years, and that in the United States and other developed countries is as high as 30%. It has become a public health problem in the world [14].

We choose MCD model for the high-fat methionine-choline deficient diet (MCD) diet, known as a very useful one among the experimental models in fatty liver and NASH. It can cause in rodents fatty change, hepatocyte injury, fibrosis, cirrhosis, oxidative DNA damage via 8-hydroxydeoxyguanosine generation, and HCC.

Autophagy is crucial for development, differentiation, survival, and homeostasis, and has been shown to play important roles in the progression of many diseases including NAFLD [8]. Researchers have demonstrated that autophagy can inhibit NAFLD development by degrading

the intracellular hepatocyte lipid. Increased autophagy breaks more stored lipids, thereby facilitating oxidation or other uses of fatty acids [15-17].

The role of endocrine and metabolic disorders became more important in NAFLD. Thyroid hormone (TH) is an important hormone to regulate the metabolism of the body. In our previous study, the clinical study found that serum free thyroid hormone levels were significantly lower in NAFLD patients, and serum free thyroid hormone levels were negatively correlated with the prevalence of NAFLD in patients [18, 19]. The report of this phenomenon immediately got the attention of the international counterparts. In the same time, the American and Korean peer wrote a corresponding commentary. It was thought that our research provided a new point of control for NAFLD prevention and control.

Thus, the aim of this study was to investigate whether thyroid hormone could influence the occurrence and/or reversal of steatosis and steatohepatitis induced by a MCD diet and elucidate its molecular mechanism.

Materials and methods

Reagents

Thyroid hormone was purchased from Merk Inc. (German). Methionine- and choline-deficient diet was purchased from Research Diets (New Brunswick, NJ, USA). Xylene, alcohol, chloralhydrate and formaldehyde were purchased from Beijing Chemical Factory (Beijing, China), ALT, AST commercial assay kits were purchased from Pointe Scientific inc. (Lincoln Park, MI, USA). Anti-β-actin antibody, anti-α-SMA, SIRT1, HIF-1, p38 MAPK antibody, and second antibody were purchased from Sigma-Aldrich (St. Louis, MO, USA). Poly-L-Lysine, RIPA Lysis Buffer were purchased from DGCS-Biology Technology Inc. (Nanjing, China). MDA and SOD commercial kit were purchased from Nanjing Jiancheng bio-engineering institute (Nanjing, China).

Animals

Male Sprague-Dawley (SD) rats at 8 weeks of age, weight 190±10 g, were purchased from the Laboratory Research Center of Zhejiang University (Certificated No.8000-078, Hangzhou, China). All animals were housed under standard laboratory conditions. They were feed by commercial food and tap water and lived on sawdust in plastic-bottomed cages in groups. They were bred in a temperature-controlled room (at 25±2°C) with lighting from 6 a.m. to 6 p.m.

TH was dissolved in phosphate-buffered saline (PBS). Rats were administered with PBS, 0.1 g/kg TH intraperitoneally once daily. Sixty rats were randomly divided into four groups and treated as follows: (1) Control (n=15), (2) MCD diet + PBS (n=15), (3) MCD diet + 0.1 g/kg TH (n=15), (4) MCD diet + 1 g/kg Lipitor (n=15). After six weeks, animals were sacrificed for the collection of blood and liver samples.

Measurement of organ injury markers

Serum levels of AST and ALT were measured using commercial assay kits according to the manufacturer's instructions.

Measurement of GSH, SOD and MDA in liver tissues

Hepatic homogenates were used for the determination of GSH, MDA and SOD levels by using a commercial kit. The results were corrected for their protein content.
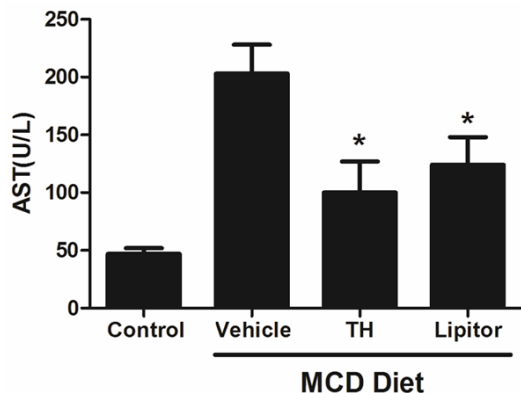
Morphometric analysis

Livers were fixed in 10% neutral buffered formalin and embedded in paraffin. This was followed by the dehydration of fixed tissue in various grades of alcohol (100%, 90%, 80%, 70% v/v) and then cleared in benzene. And 5 µm thick sections were cut using a microtome from the paraffin blocks for hematoxylin eosin (H&E) and Masson stain to evaluate liver injury. Liver biopsies were blindly evaluated using the NASH Clinical Research Network Histologic Scoring System [20]. NAFLD activity score is a sum of three histologic scores, including steatosis (0-3), lobular inflammation (0-2), hepatocellular ballooning (0-2). 0= absent; 1= mild; 2= moderate; 3= severe. OCT-embedded frozen sections were stained with Masson. The images were visualized by microscopy (IX73, Olympus, Japan).
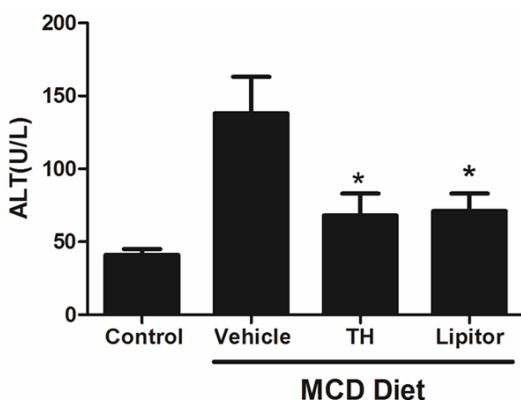
Quantification of autophagic vacuoles by transmission electron microscopy (TEM)

After sections were cut and loaded on grid, they were observed under a Hitachi H-7500 Trans-
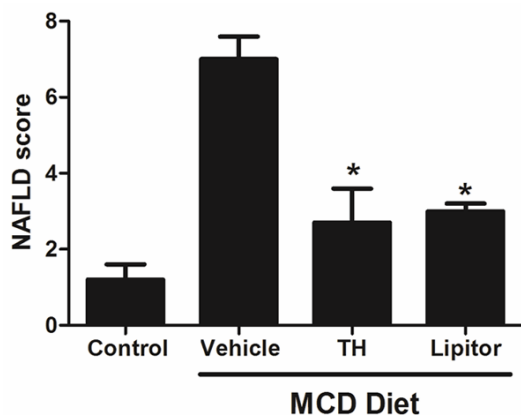
Figure 1. Effects of TH on the level of AST in hepatic fibrosis rats.

Figure 2. Effects of TH on the level of ALT in hepatic fibrosis rats.

Figure 3. Effects of TH on histological features of in hepatic fibrosis rats.

mission Electron Microscope fitted with a Gatan BioScan 1 K CCD camera, under various magnifications. Autophagic vacuoles were co-

unted following the defined protocols. Briefly, the grids were loaded on the microscope and scanned systematically starting from the left edge at the bottom of the grid with surveillance of the specimen to score for AVs and ALs ascertaining that systematic sampling is achieved Separate records of autophagosomes and autophagolysosomes were made to assess the total autophagic response.

Statistical analysis

All data are presented as the means ± SD of at least 3 independent experiment. All statistical analyses were performed by analysis of variance (ANOVA) by Tukey trend test using GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Results were reported as significant only when P<0.05.

Results

Effects of TH on the level of ALT, AST in hepatic fibrosis rats

After administration for 28 days, the ALT and AST content of model group rats were obvious lower compared with the control group (As shown in **Figures 1**, **2**). The low dose group and the middle dose group had a better treatment effect. In addition, the content of AST and ALT in serum of the rats in the low dose group decreased rapidly compared with others, the results show that administration of low dose chitosan had the best effect and there was concentration dependent.

Effects of TH on histological features of in hepatic fibrosis rats

The section from each group was HE stained after chitosan treatment for 7 days, 14 days, 21 days and 28 days (**Figure 3**). Liver tissue of rats in model group seriously injury, there were a large number of macrosteatosis, ballooning cell and transparent cytoplasm, cytoplasm nucleus was pushed to the side. Pseudolobuli formation, which is the characteristic structure of HF, the injury degree depends on days of administration. On day 7, 14 glycyrrhizinate group still has alveolar inflammatory infiltration phenomenon, obvious pseudolobules; 21 days inflammatory infiltration weakened; 28 day, pseudolobule distribution is not obvious. Inflammation was significantly inhibit-
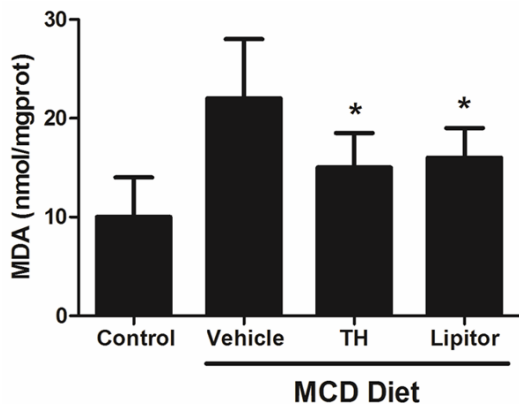
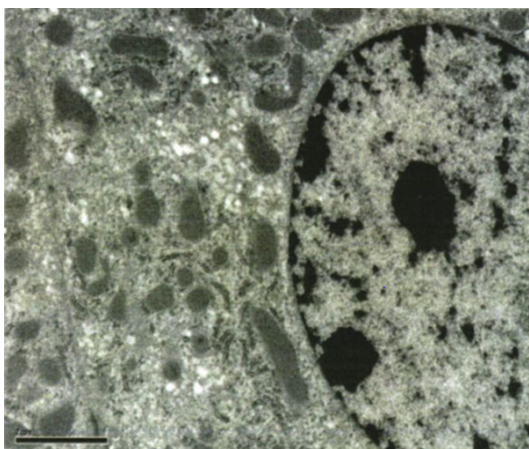Figure 4. TH treatment increased the number of AVs.

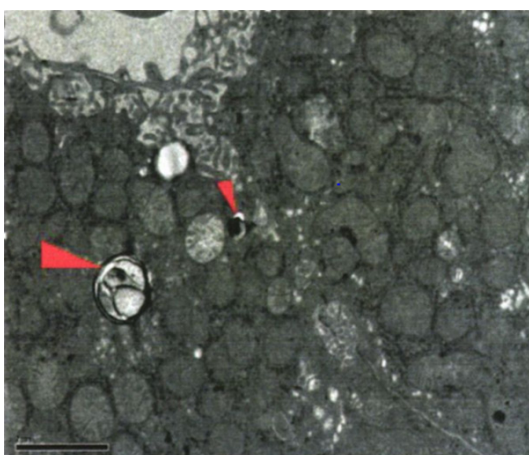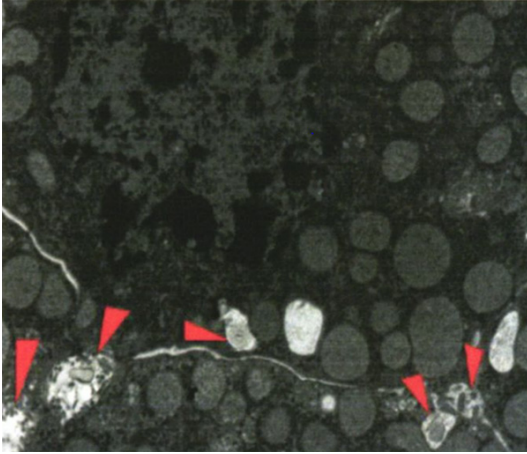Figure 5. Increase in both autophagosomes and Als under TH treatments.

Figure 6. TH treatment significantly increased the number of ALs.

have the best effect at 7 days, 14 days, 21 days and 28 days, which was administered for 7 days similar to glycyrrhizinatis group 7 days; Low dose group have a better recovery contrasted with glycyrrhizinate group at 14 days; Low dose group started pseudolobule distribution at 21 day, there are still many inflammatory reaction, but relieved; chitosan liver tissue status in low dose group improved significantly after 28 days administration. Other groups have certain effect, but worse than glycyrrhizinatis group. Results indicate that low dose chitosan can reduced hepatocellular damage and improve the liver in HF rats.

To confirm if the increase in autophagy related genes actually resulted in an increased number of autophagosomes and whether there was indeed more lipophagy, we examined samples by transmission electron microscopy (TEM). Total number of autophagosomes that had lipid droplets in them and the total number of autophagolysosomes (AL) with lipid droplets were measured. Together these bodies are taken as autophagic vacuoles (AV) (**Figure 4**). TH treatment increased the number of AVs, although the number of autophagosomes and ALs varied with treatment. In oleic acid treated hepatocytes there was an insignificant change in AVs after exendin treatment, although the autophagosome count was significantly increased by exendin-4. There was a clear increase in both autophagosomes and Als under TH treatments (**Figure 5**). TE treatment with or without exendin resulted in a similar number of autophagosomes, however, TH treatment significantly increased the number of ALs (**Figure 6**). While visualizing cells for AVs we observed that some large sized lipid droplets had 'shriveled' margins with distinct absence of autophagic vacuoles around them (**Figure 7**). We hypothesized that this may be a result of change in contents of the lipid droplet, perhaps due to transport of fatty acids for beta oxidation. To confirm enhanced-oxidation we determined the concentration of ketone bodies, the final breakdown product of beta oxidation. ß hydroxybutyrate served as a marker for oxidation. Exendin-4 treatment increased the production of ketone bodies in all the treatments in comparison to control. Fatty acids themselves also led to an increase in ketone bodies probably as a normal cellular response, which was further enhanced by exendin-4. The differ-

ed, but there is still a cell balloon-like degeneration and fatty degeneration. Low dose group

Figure 7. TH margins with distinct absence of autophagic vacuoles.

ence between exendin treated and untreated fat loaded cell was insignificant in the case of oleic acid. In contrast exendin treatment increased significantly ketone body formation in cells loaded with either palmitic or elaidic acid exposure.

Discussion

Non-alcoholic fatty liver disease is characterized by macrovesicular fat accumulation in more than 5% of the hepatocytes in the absence of known causes of secondary steatosis [21-25]. This accumulation ranges from scarce to panacinar steatosis and usually starts in Rapaport's zone. Despite the high prevalence of NAFLD and its potential for serious sequelae, the underlying etiologic factors that determine disease progression remain poorly understood; therefore, effective therapeutic strategies need to be further explored.

NAFLD is considered to cover a spectrum of disease activity. This spectrum begins as fatty accumulation in the liver (hepatic steatosis). A liver can remain fatty without disturbing liver function, but by varying mechanisms and possible insults to the liver may also progress to become non-alcoholic steatohepatitis (NASH), a state in which steatosis is combined with inflammation and fibrosis (steatohepatitis) [26-29]. NASH is a progressive disease: over a 10-year period, up to 20% of patients with NASH will develop cirrhosis of the liver, and 10% will suffer death related to liver disease.

Cigarette smoking is not associated with an increased risk of developing NASH [30].

The exact cause of NAFLD is still unknown. However, both obesity and insulin resistance probably play a strong role in the disease process [31]. The exact reasons and mechanisms by which the disease progresses from one stage to the next are not known. One debated mechanism proposes a "second hit", or further injury, enough to cause change that leads from hepatic steatosis to hepatic inflammation. Oxidative stress, hormonal imbalances, and mitochondrial abnormalities are potential causes for this "second hit" phenomenon [32, 33].

Thyroid hormones are potent mediators of several physiological processes, including embryonic development, cellular differentiation, metabolism, and cell growth. The liver is a typical target organ of THs. Autophagy is a mechanism involved in cellular homeostasis under basal and stressed conditions delivering cytoplasmic content to the lysosomes for degradation to macronutrients. Nowadays it is commonly accepted that autophagy plays a role in the hepatic lipid metabolism. Autophagy has many potentially beneficial functions that could prevent NASH development and progression.

The thyronines act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate long bone growth (synergy with growth hormone) and neural maturation, and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness [34, 35]. The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. They also stimulate vitamin metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis. Thyroid hormone leads to heat generation in humans. However, the thyronamines function via some unknown mechanism to inhibit neuronal activity; this plays an important role in the hibernation cycles of mammals and the moulting behavior of birds. One effect of administering the thyronamines is a severe drop in body temperature [34-37].

To the best of our knowledge, our study is the first to evaluate the associations between Thyroid hormones and NAFLD via inducing autophagy. The findings of this study demonstrated that MCD diet fed NAFLD model mice liver autophagy were significantly reduced, supplement the thyroid hormone levels significantly improve the level of NAFLD model mice liver autophagy, and reduce the degree of liver steatosis, suggesting Thyroid hormone autophagy has a regulatory role of liver cell, and may participate in the development of NAFLD. Furthermore, we emphasized that presence of NAFLD has a harmful effect on thyroid function and is also correlated with enhanced insulin resistance.

In conclusion, increasing knowledge on its exact role in the complex pathophysiology of Thyroid hormones and NAFLD might make autophagy a target for treatment of the metabolic syndrome or NAFLD. We should, however, always keep in mind that altering a key cellular process such as autophagy might lead to a better metabolic state; the use of such drugs to increase hepatic autophagy may offer a new therapeutic approach to NASH.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondences to: Dr. Youming Li, Department of Gastroenterology, The First Affiliated Hospital of Medical College, Zhejiang University, 79 Qingchun Road, Hangzhou 310000, China. E-mail: liyouminghz@sina.com

References

[1] Bieghs V and Trautwein C. Innate immune signaling and gut-liver interactions in non-alcoholic fatty liver disease. Hepatobiliary Surg Nutr 2014; 3: 377-385.

[2] Yu H, Jia W and Guo Z. Reducing liver fat by low carbohydrate caloric restriction targets hepatic glucose production in non-diabetic obese adults with non-alcoholic fatty liver disease. J Clin Med 2014; 3: 1050-1063.

[3] Drapkina J and Drapkina O. Epicardial fat as the predictor for essential hypertension and early vascular damage in patients with metabolic syndrome and non-alcoholic fatty liver disease. Endosc Ultrasound 2014; 3: S2.

[4] Wang XY, Zhang XH, Yao CH, Zhu HH and Zhang L. The impacts of obesity and metabolic abnormality on carotid intima-media thickness and non-alcoholic fatty liver disease in children from an inland Chinese city. J Clin Med 2014; 3: 323-333.

[5] Zeng W, Shan W, Gao L, Gao D, Hu Y, Wang G, Zhang N, Li Z, Tian X, Xu W, Peng J, Ma X and Yao J. Inhibition of HMGB1 release via salvianolic acid B-mediated SIRT1 up-regulation protects rats against non-alcoholic fatty liver disease. Sci Rep 2015; 5: 16013.

[6] Zhang L, Zhang Z, Li Y, Liao S, Wu X, Chang Q and Liang B. Cholesterol induces lipoprotein lipase expression in a tree shrew (Tupaia belangeri chinensis) model of non-alcoholic fatty liver disease. Sci Rep 2015; 5: 15970.

[7] Zhang Y, Zhang T, Zhang C, Tang F, Zhong N, Li H, Song X, Lin H, Liu Y and Xue F. Identification of reciprocal causality between non-alcoholic fatty liver disease and metabolic syndrome by a simplified Bayesian network in a Chinese population. BMJ Open 2015; 5: e008204.

[8] Liu Z, Que S, Zhou L and Zheng S. Dose-response relationship of serum uric acid with metabolic syndrome and non-alcoholic fatty liver disease incidence: a meta-analysis of prospective studies. Sci Rep 2015; 5: 14325.

[9] Zain SM, Mohamed Z, Pirmohamed M, Tan HL, Alshawsh MA, Mahadeva S, Chan WK, Mustapha NR and Mohamed R. Copy number variation in exportin-4 (XPO4) gene and its association with histological severity of non-alcoholic fatty liver disease. Sci Rep 2015; 5: 13306.

[10] Duseja A, Singh SP, Saraswat VA, Acharya SK, Chawla YK, Chowdhury S, Dhiman RK, Jayakumar RV, Madan K, Misra SP, Mishra H, Modi SK, Muruganathan A, Saboo B, Sahay R and Upadhyay R. Non-alcoholic fatty liver disease and metabolic syndrome-position paper of the Indian National Association for the study of the liver, endocrine society of India, Indian College of Cardiology and Indian Society of Gastroenterology. J Clin Exp Hepatol 2015; 5: 51-68.

[11] Zimmermann E, Gamborg M, Holst C, Baker JL, Sorensen TI and Berentzen TL. Body mass index in school-aged children and the risk of routinely diagnosed non-alcoholic fatty liver disease in adulthood: a prospective study based on the Copenhagen School Health Records Register. BMJ Open 2015; 5: e006998.

[12] Wolfs MG, Gruben N, Rensen SS, Verdam FJ, Greve JW, Driessen A, Wijmenga C, Buurman WA, Franke L, Scheja L, Koonen DP, Shiri-Sverdlov R, van Haeften TW, Hofker MH and Fu J. Determining the association between adipokine expression in multiple tissues and phenotypic features of non-alcoholic fatty liver disease in obesity. Nutr Diabetes 2015; 5: e146.

[13] Jiang W, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, Hu Y, Li J and Liu Y. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci Rep 2015; 5: 8096.

[14] Mirrakhimov AE and Polotsky VY. Obstructive sleep apnea and non-alcoholic Fatty liver disease: is the liver another target? Front Neurol 2012; 3: 149.

[15] Gelino S and Hansen M. Autophagy-An Emerging anti-aging mechanism. J Clin Exp Pathol 2012; Suppl 4.

[16] Wu J, Hu D and Zhang R. Depletion of Bmi-1 enhances 5-fluorouracil-induced apoptosis and autophagy in hepatocellular carcinoma cells. Oncol Lett 2012; 4: 723-726.

[17] Maier HJ and Britton P. Involvement of autophagy in coronavirus replication. Viruses 2012; 4: 3440-3451.

[18] Ma K, Guo L, Xu A, Cui S and Wang JH. Molecular Mechanism for Stress-Induced Depression Assessed by Sequencing miRNA and mRNA in Medial Prefrontal Cortex. PLoS One 2016; 11: e0159093.

[19] Das G, Shravage BV and Baehrecke EH. Regulation and function of autophagy during cell survival and cell death. Cold Spring Harb Perspect Biol 2012; 4.

[20] Kleiner BM. Macroergonomics: analysis and design of work systems. Appl Ergon 2006; 37: 81-89.

[21] Vargas V, Allende H, Lecube A, Salcedo MT, Baena-Fustegueras JA, Fort JM, Rivero J, Ferrer R, Catalán R, Pardina E, Ramón Y Cajal S, Guardia J, Peinado-Onsurbe J. Surgically induced weight loss by gastric bypass improves non alcoholic fatty liver disease in morbid obese patients. World J Hepatol 2012; 4: 382-388.

[22] Zhang BC, Li WM, Li XK, Zhu MY, Che WL and Xu YW. Tesaglitazar ameliorates non-alcoholic fatty liver disease and atherosclerosis development in diabetic low-density lipoprotein receptor-deficient mice. Exp Ther Med 2012; 4: 987-992.

[23] Sun WH, Song MQ, Jiang CQ, Xin YN, Ma JL, Liu YX, Ma L, Lin ZH, Li CY, Liu L, Zhang M, Chu LL, Jiang XJ, Wan Q, Zhou L, Ren R and Meng LF. Lifestyle intervention in non-alcoholic fatty liver disease in Chengyang District, Qingdao, China. World J Hepatol 2012; 4: 224-230.

[24] Bhat G, Baba CS, Pandey A, Kumari N and Choudhuri G. Life style modification improves insulin resistance and liver histology in patients with non-alcoholic fatty liver disease. World J Hepatol 2012; 4: 209-217.

[25] Tuyama AC and Chang CY. Non-alcoholic fatty liver disease. J Diabetes 2012; 4: 266-280.

[26] Anbalagan VP, Venkataraman V, Vamsi M, Deepa M and Mohan V. A simple Indian diabetes risk score could help identify nondiabetic individuals at high risk of non-alcoholic fatty liver disease (CURES-117). J Diabetes Sci Technol 2012; 6: 1429-1435.

[27] Tzanetakou IP, Doulamis IP, Korou LM, Agrogiannis G, Vlachos IS, Pantopoulou A, Mikhailidis DP, Patsouris E, Vlachos I and Perrea DN. Water soluble vitamin E administration in wistar rats with non-alcoholic fatty liver disease. Open Cardiovasc Med J 2012; 6: 88-97.

[28] Zhang J, Tan Y, Yao F and Zhang Q. Polydatin alleviates non-alcoholic fatty liver disease in rats by inhibiting the expression of TNF-alpha and SREBP-1c. Mol Med Rep 2012; 6: 815-820.

[29] O'Brien J and Powell LW. Non-alcoholic fatty liver disease: is iron relevant? Hepatol Int 2012; 6: 332-341.

[30] Miyazaki M, Kato M, Tanaka K, Tanaka M, Kohjima M, Nakamura K, Enjoji M, Nakamuta M, Kotoh K and Takayanagi R. Increased hepatic expression of dipeptidyl peptidase-4 in non-alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. Mol Med Rep 2012; 5: 729-733.

[31] Gariani K, Philippe J and Jornayvaz FR. [Diabetes and non-alcoholic fatty liver disease]. Rev Med Suisse 2012; 8: 1211-1214.

[32] Mao ZM, Song HY, Yang LL, Liu T, Li DF, Zheng PY, Liu P and Ji G. [Effects of the mixture of Swertia pseudochinensis Hara and Silybum marianum Gaertn extracts on CCl(4)-induced liver injury in rats with non-alcoholic fatty liver disease]. Zhong Xi Yi Jie He Xue Bao 2012; 10: 193-199.

[33] Tziomalos K, Athyros VG and Karagiannis A. Non-alcoholic fatty liver disease in type 2 diabetes: pathogenesis and treatment options. Curr Vasc Pharmacol 2012; 10: 162-172.

[34] Jourdan C, Linseisen J, Meisinger C, Petersen AK, Gieger C, Rawal R, Illig T, Heier M, Peters A, Wallaschofski H, Nauck M, Kastenmuller G, Suhre K, Prehn C, Adamski J, Koenig W, Roden M, Wichmann HE and Volzke H. Associations between thyroid hormones and serum metabolite profiles in an euthyroid population. Metabolomics 2014; 10: 152-164.

[35] Salvatore D, Simonides WS, Dentice M, Zavacki AM and Larsen PR. Thyroid hormones and skeletal muscle--new insights and potential implications. Nat Rev Endocrinol 2014; 10: 206-214.

[36] Afsar B and Ay M. The relationships between cortisol levels, insulin levels, and thyroid hormones with 24-h urinary sodium excretion in never treated essential hypertensive patients. ARYA Atheroscler 2014; 10: 159-163.

[37] DuRant SE, Carter AW, Denver RJ, Hepp GR and Hopkins WA. Are thyroid hormones mediators of incubation temperature-induced phenotypes in birds? Biol Lett 2014; 10: 20130950.