Original Article

J Babol Univ Med Sci
Vol 19, Issu 7; Jul 2017. P:35-41

Introduction and Optimization of a Dietary Model for Inducing Hyperlipidemia in Rats

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J Babol Univ Med Sci; 19(7); Jul 2017; PP: 35-41

Received: Jan 3rd 2017, Revised: Feb 22th 2017, Accepted: May 10th 2017.

ABSTRACT

BACKGROUND AND OBJECTIVE: Inducing hyperlipidemia in laboratory animals through diet is a good way to study metabolic disorders. This study was conducted to provide an effective and accessible diet for generating hyperlipidemia and non-alcoholic fatty liver models in rats.

METHODS: In this experimental study, 40 male Wistar rats (180-200 g) were divided into 5 equal groups including 2 control groups receiving regular diet for 4 (C1) or 8 weeks (C2), and 3 experimental groups receiving high fat diet along with 0.2% (E1) or 0.1% thiouracil (E2) for 8 and 4 weeks (E3). Finally, the concentration of total cholesterol (TC), LDL, HDL, and triglyceride (TG) was measured and the fat accumulation in the liver tissue was measured quantitatively.

FINDINGS: All experimental groups had significantly higher TC, TG, LDL and lower HDL compared to control ($p<0.0001$). The cholesterol level was significantly higher in E1 (642.66±133.01), E2 (848.16±146.17) and E3 (406.83±116.28) groups, compared with the C1 (64.87±16.10) and C2 (76.83±11.37) groups ($p<0.0001$). The degree of fat accumulation in the groups E1 (3.70±0.34), E2 (3.45±0.32) and E3 (2.83±0.25) was significantly higher than the groups C1 (0.25±0.01) and C2 (0.33±0.03) ($p <0.0001$).

CONCLUSION: The high-fat diet introduced in this study can cause hyperlipidemia and non-alcoholic fatty liver rats within 4 weeks.

KEY WORDS: *High-fat diet, Hyperlipidemia, Non-alcoholic fatty liver, Rat*

Please cite this article as follows:

Amirabadizadeh A, Foaddodini M, Khatamsaz S, Moktari M, Shariati M, Hosseini M, Hajipoor F. Introduction and Optimization of a Dietary Model for Inducing Hyperlipidemia in Rats. J Babol Univ Med Sci. 2017;19(7):35-41.

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[DOR: 20.1001.1.15614107.1396.19.7.5.8]
[DOI: 10.22088/jbums.19.7.5]

Introduction

So far, many efforts have been made to replace animal models in various diseases for humans, including animal models of diabetes, atherosclerosis, Parkinson's disease, hyperlipidemia, etc. (1–4). Ideal animal models for the study of human diseases should be the best in terms of the many characteristics, including availability, price and similarity to human conditions. Therefore, quick and cost-effective access to these models is the first step in empirical studies. Several animal models are used today to examine the pathogenesis of metabolic syndrome (5). The term “High Fat Diet” was proposed by Masek et al. for the first time in 1959 to create hyperlipidemia (6). Further studies have argued that a high fat diet would increase blood glucose and has multiple effects on the muscle and physiology of the liver (7). Hyperlipidemia models have often been performed on laboratory mice, rats, guinea pigs, rabbits, pigeons and quails over the past several years. So far, a variety of high fat diets have been developed to achieve models with high levels of cholesterol, triglycerides and high LDL. All of these models produce hyperlipidemia after a specific period of 6, 7 or 8 weeks, while the results of the reports presented in these models are highly diverse and dispersed (4, 7–10).

Several diets are now commercially available to cause obesity and hyperlipidemia in animals. Due to the high cost of these diets and many constraints in their preparation, researchers are always interested in using an effective and accessible native hyperlipidemia (11). Regarding the fact that the use of high fat diets for domestic researchers due to high costs and limited access is not always possible, the purpose of this study is to develop a diet for the development of hyperlipidemia and non-alcoholic fatty liver syndrome, which is accessible, low cost and easy to construct, and most importantly, it has acceptable performance.

Methods

M Lab animals: This experimental study was approved by the ethics committee of Islamic Azad University, Kazeroon Branch, (registration code: 214.1395IR.BUMS.REC) and was conducted on 40 5-week-old male Wistar rats (180 – 200 g), purchased from the Experimental Medicine Research Center of Birjand University of Medical Sciences. The animals were grouped and kept without intervention for one week so that they would adapt to the new environment. During the experiment, animals had free access to healthy urban water and the diet specified for each group, and at the time of intervention, they were kept in 20 – 24 °C temperature conditions, 35 – 45% moisture and 12 – hour light – dark cycle in cages made from propylene (Razi Rad Co., Iran).

Grouping and designing of the experiment: Animals were randomly divided into 5 equal groups (each group included 8 rats). The first and second groups were treated are control group 1 and control group 2 for 4 (C1) and 8 (C2) weeks, respectively, with normal laboratory animal food (Javanehkhorasan Co., Iran). Experimental group 1 (E1) and experimental group 2 (E2) were treated with high fat diet number 1 (H1) and number 2 (H2) for 8 weeks, respectively. Experimental group 3 (E3) also received H2 diet for 4 weeks. In this study, two types of high fat diet were prepared and the details of their ingredients are as follows:

High fat diet 1 (H1) containing 66% wheat flour, 35% corn starch, 7% egg yolk, 6.6% wheat bran, 4% animal fat, 1% cholesterol, 1% salt, 0.2% colic acid±0.2% thiouracil.

High fat diet 2 (H2) containing H1 ingredients±0.1% thiouracil.

The diet used in this study is based on the study of Pengzhan et al. (12), which was evaluated by altering the type and concentration of its ingredients and the duration of use in this study. For this diet, colic acid, cholesterol and salt were purchased from Merck Co., Germany. Propylthiouracil tablets (Iran Hormones Co., Iran) were used to provide thiouracil (Fig 1).



Figure 1. The sample of food used to induce hyperlipidemia

Corn starch, wheat flour and wheat bran were supplied from the confectionery supply stores. The animal fat used in this diet was prepared for a source of visceral fat in sheep. At the end of the intervention (4 or 8 weeks), animals were sacrificed after anesthesia with ether, and their liver tissue was isolated immediately and fixed in a fixative buffer solution containing 10% formalin. Plasma was obtained from the blood samples and the concentration of TSH, T3 hormones and blood fats (triglycerides, total

[DOR: 20.1001.1.15614107.1396.19.7.5.8]
[DOI: 10.22088/jbums.19.7.5]

cholesterol, low density cholesterol and high density cholesterol) were measure using the diagnostic kits of Bionic Co. and Pars Azmoon Co. (Iran) and an auto – analyzer (Roche Hitachi 912, Japan). Tissue sections were also prepared form the upper outer lobe of each liver through tissue passage and stained with hematoxylin and eosin. 3 slides were prepared from each rat and from each section, 4–6 fields were observed using an optical microscope (BB.1153-PLI EUROMEX, Netherland) and 300 dpi images were prepared using a camera (Image Focus v2, Netherland) connected to a microscope. Then, fat accumulation syndrome in hepatocytes (such as ballooning, the presence of foam cells, vacuolation and fat deposition) was evaluated based on the method described by Brunt et al. (13).

In this method, the percentage of hepatocyte cells in each image is counted with the presence of fatty acids and is classified in four grades according to the intensity of involvement. Grade Number 1: There is no indication of fat accumulation in the cell, Grade Number 2: A maximum of 33% of the liver cells have fatty lipid syndrome, Grade Number 3: 33 – 66% of the liver cells show fat accumulation syndrome, Grade Number 4: More than 66% of the liver cells have a fatty tissue syndrome. Finally, the grading results of the groups were compared with each other.

Statistical analysis: Biochemical data associated with the groups were entered into the SPSS 22 statistical software and after determining the distribution of data using ANOVA test, the groups were compared and since the distribution of variances was equal among the groups, Tukey's post hoc test was used to compare the groups two by two. The groups were compared with their corresponding control group according to the treatment duration, and $p < 0.05$ was considered significant.

Results

Comparison of mean blood lipids in different groups showed that there was a significant difference between the groups in all parameters ($p<0.001$). In addition, there was no significant difference in blood lipids levels between control groups of 4 weeks (C1) and 8 weeks (C2). After 8 weeks of treatment with high fat diets, the mean plasma triglyceride in E1 group ($p=0.003$, 98.83±16.85) and E2 group ($p=0.011$, 68.00±4.92) increased significantly compared to the C2 group (50.87±6.10). In addition, the mean plasma triglyceride ($p=0.021$, 63.50±3.78) in the E3 group was significantly higher than group C1 (48.33±7.94) (table 1). Comparison of cholesterol levels between groups showed that there was a significant difference between the groups. While the comparison of control groups did not show any significant difference, cholesterol levels in rats receiving high fat diet increased significantly compared to control groups.

The highest level of cholesterol (848.16±146.17) was in the E2 group that received H2 diet for 8 weeks, which increased more than 12 times compared with the C2 group (76.83±11.37) ($p <0.0001$). Cholesterol levels in E1 group were significantly higher than that of C2 group ($p<0.0001$). The cholesterol level in the E3 group (406.83±116.28) increased by about 6 times compared with the C1 group (44.33±4.63), which was statistically significant ($p<0.0001$).

In addition to triglyceride and cholesterol, treatment of rats with both high fat diets (H1, H2) within both 4 weeks and 8 weeks significantly increased LDL-c levels and significantly decreased HDL-c levels ($p <0.05$). There was no significant difference in T3 and TSH levels between control groups. The comparison of T3 levels between the groups showed that its level in high fat diet induced hyperlipidemia rat decreased compared to control groups. The highest decline was observed in E1 group (10.35±1.80), which was significantly lower than C2 group ($p=0.002$, 19.88±3.80), but compared to E2 ($P=0.83$) and E3 ($p=0.36$) groups, did not show any significant difference (Fig 2).

Overall, diets increased TSH hormone in rats compared with control groups, whereas the mean plasma TSH concentration in the E1 group (0.25±0.01) was significantly higher than C1 ($p<0.0001$, 0.106±0.039), E2 ($p=0.025$, 0.22±0.02), and E3 ($p<0.0001$, 0.17±0.03) groups, respectively (Fig 3). Histologic examination of the liver indicates a significant difference in the accumulation of fatty particles between different groups. A sample of liver tissue micrographs belonging to different groups is presented in Fig 4. A comparison between the grades of hepatic steatosis is presented in Fig 5. Groups receiving hyperlipidemic diets had significantly higher degrees of liver steatosis compared to control groups. The E1 group, which received H1 diet for 8 weeks, had the highest grade of steatosis (3.70±0.34) among the experimental groups, which was significantly higher than C2 ($P<0.0001$, 0.25±0.014) and E3 ($p=0.002$, 2.83±0.25) groups.

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Table 1. Mean values of blood lipids in the studied groups

Group	HDL cholesterol Mean±SD	LDL cholesterol Mean±SD	Total cholesterol Mean±SD	Triglyceride Mean±SD
C1	26.33±3.32a	44.33±4.63a	64.87±16.10 a	48.33±7.94 a
C2	27.25±4.34a	49.16±3.42a	76.83±11.37 a	50.87±6.10 a
E1	16.16±1.47b	602.83±128.34b	642.66±133.01b	98.83±16.85c
E2	16.16±2.22b	679.66±181.85b	848.16±146.17c	68.00±4.92 b
E3	17.66±5.60b	369.50±119.36c	406.83±116.28b	63.50±3.78 b

C1: The control group receiving the usual diet for 8 weeks; C2: The control group receiving the usual diet for 4 weeks; E1: The group receiving hyperlipidemia diet (containing 0.2% thiouracil) for 8 weeks; E2: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 8 weeks; E3: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 4 weeks. (a, b, c) The same letters in each column indicate lack of difference and non-identical letters indicate a significant difference ($p < 0.05$).

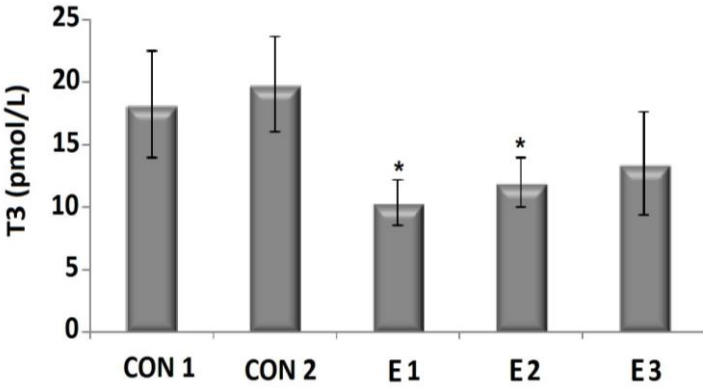


Figure 2. Comparison of mean triiodothyronine (T3) hormone in the studied groups

Values are presented as mean±SD for 8 rats for each group. C1: The control group receiving the usual diet for 8 weeks; C2: The control group receiving the usual diet for 4 weeks; E1: The group receiving hyperlipidemia diet (containing 0.2% thiouracil) for 8 weeks; E2: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 8 weeks; E3: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 4 weeks. * Significant differences ($p \leq 0.05$) compared to the control group.

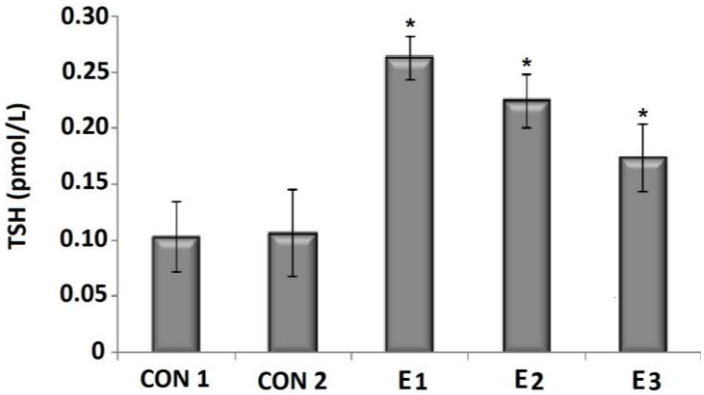


Figure 3. Comparison of mean thyroid stimulating hormone (TSH) in the studied groups

Values are presented as mean±SD for 8 rats for each group. C1: The control group receiving the usual diet for 8 weeks; C2: The control group receiving the usual diet for 4 weeks; E1: The group receiving hyperlipidemia diet (containing 0.2% thiouracil) for 8 weeks; E2: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 8 weeks; E3: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 4 weeks. * Significant differences ($p \leq 0.05$) compared to the control group.

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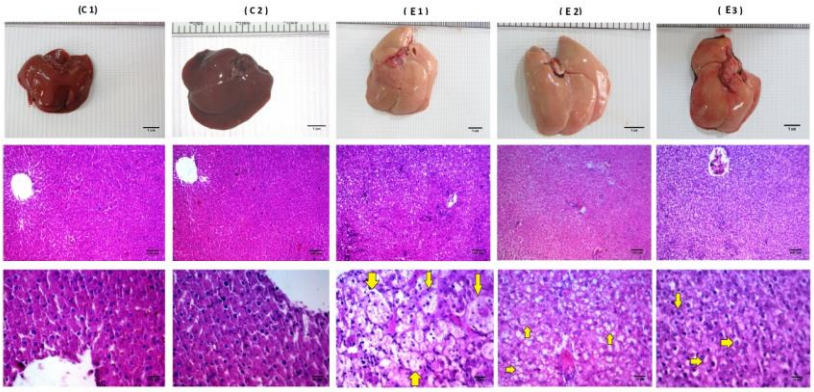


Figure 4. Macroscopic view (first row) and microscopic view (second and third row) of liver (100x zoom [second row] and 400x zoom [third row]). Stained with hematoxylin and eosin technique. C1: The control group receiving the usual diet for 8 weeks; C2: The control group receiving the usual diet for 4 weeks; E1: The group receiving hyperlipidemia diet (containing 0.2% thiouracil) for 8 weeks; E2: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 8 weeks; E3: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 4 weeks. Yellow arrows indicate the accumulation of fat in the liver cells

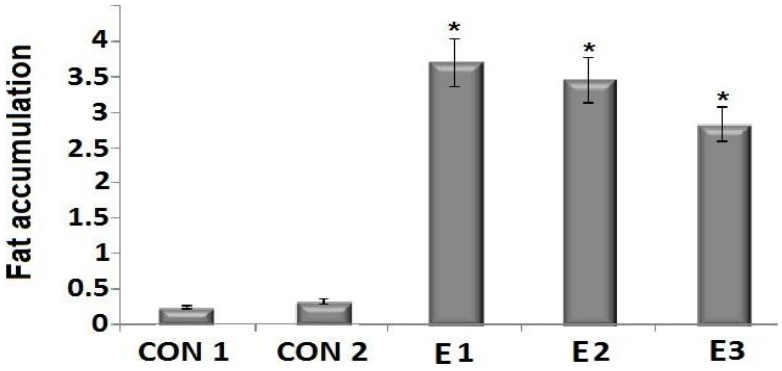


Figure 5. Comparison of mean accumulation index of lipid droplets in the liver tissue in the studied groups

Values are presented as mean±SD for 8 rats for each group. C1: The control group receiving the usual diet for 8 weeks; C2: The control group receiving the usual diet for 4 weeks; E1: The group receiving hyperlipidemia diet (containing 0.2% thiouracil) for 8 weeks; E2: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 8 weeks; E3: The group receiving hyperlipidemia diet (containing 0.1% thiouracil) for 4 weeks.
* Significant differences ($p \leq 0.05$) compared to the control group

Discussion

The results of this study showed that in all experimental groups, blood lipids increased significantly in rats. These results showed that even in a shorter period and a lower dose of thiouracil (E3 group), this diet can induce favorable hyperlipidemia. The histological examination of the liver also showed that changes in liver steatosis could be observed in the experimental groups compared with control groups, and highest accumulation of fatty vesicles was observed in the E1 group, which was treated for 8 weeks with H1 diet. In order to create a hyperlipidemic model for 7 weeks, Elhemely et al. treated the rats with a diet containing 1% cholesterol, 0.2% colic acid, 0.2% methylthiouracil, 7% egg yolk, 4% bovine tallow, 1% sodium chloride, 45% wheat flour, 6.6% wheat bran, and 3% corn starch (14). The results of their research showed that the diet significantly increased total cholesterol, triglyceride and LDL-c (increased total cholesterol by 5 times, triglyceride by 2 times, LDL by 8 times) and decreased HDL-c (more than 50%). Our findings also showed similar results, as total cholesterol increased by 8 times, triglyceride increased by 2 times, and LDL-c increased by 12 times, while HDL-c decreased by about 60% in the E1 group that received the same diet as the study of Elhemely. Therefore, it can be deduced that the results of this diet can be repeated and can even be adjusted to the desired level of hyperlipidemia in animals, based on the need, during the intervention period. Based on a study by Fatemi et al., an 8-week high fat diet containing

[DOR: 20.1001.1.15614107.1396.19.7.5.8]
[DOI: 10.22088/jbums.19.7.5]

cholesterol, tail, casein, yeast, and salt resulted in a significant increase in total cholesterol and LDL-c (15). But in comparison with the present study, the increase in the mentioned parameters was much lower. Homayounfar et al. used a high-calorie diet with ruminant fat for 12 weeks in order to develop a syndrome model.

Finally, there was a significant difference in weight and lipid profiles of the rats in the hyper and control group, but the increased blood lipids was much less than the present study; In the mentioned study, the mean total cholesterol in the group receiving a high fat diet was 60.70±6.88 mg / dl after 12 weeks (16), whereas in the present study, the experimental group 3, which received the weakest and shortest recommended diet, showed the mean total cholesterol level of 406.83±116.28 mg / dl.

The most important pathologic characteristic of fatty liver is lipid metabolism (the triglyceride form) within the liver cells, which is called steatosis. Liver steatosis can ultimately lead to cirrhosis of the liver or complete liver failure (17). To detect liver steatosis, at least 5% of the liver cells must have signs of fat accumulation (18). In this study, 33 – 66% of liver cells in rats receiving hyperlipidemia had lipid accumulation symptoms, with the largest extent of involvement in the E1 group and the lowest in the E3 group. In the study of Meli et al., a high fat diet was purchased from Gessate Co. (Italy) to induce fatty liver in rats, in which 58% of energy was supplied by the source of fat. The researchers treated the animals with a high fat diet for 5 and 8 weeks. Finally, the histological findings of the liver showed that the diet was able to damage the liver through fat accumulation by about 50% and 66% within 5 weeks and within 8 weeks, respectively (19).

The results of this study showed that TSH was significantly increased in the hyperlipidemia receiving groups, depending on the dose of thiouracil, the time of intervention, and similarly, T3 was decreased in the recipient groups. This means that the rats receiving the diet also had hypothyroidism, although the syndrome was much milder in the E3 group. Recent studies have shown that liver and thyroid play a complex role in the metabolism of fats and homeostasis so that animals with hypothyroidism have higher cholesterol levels than rats with normal thyroid function (20).

The results of this study showed that the high fat diet introduced in this study can be used as a cheap, accessible and high – efficiency method for researchers interested in the scope of metabolism and animal models.

Acknowledgments

Hereby, we express our deepest sense of gratitude and indebtedness to Research Deputy of Birjand University of Medical Sciences for their financial support.

[DOR: 20.1001.1.15614107.1396.19.7.5.8]
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