ISSN 0974-3618 (Print) www.rjptonline.org
0974-360X (Online)

RESEARCH ARTICLE

Effect of Biotin Treatment on the improvement of Lipid Profile and Foam Cells in Dyslipidemia rats

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ABSTRACT:

Introduction: This study aimed to assess the effects of increasing biotin concentrations on lipid profiles, CRP, and foam cells in Wistar rats with dyslipidemia risks. Materials and Methods: Thirty male Wistar rats (weighing 150-200grams) were divided into five groups and adapted for seven days. The negative control group received standard feed, while the positive control group received a high-fat diet. The treatment groups 1, 2, and 3 received a high-fat diet and biotin at different doses: 1.232mg/kg, 68.39mg/kg, and 97.72mg/kg, respectively, for six weeks. This study employed the colorimetric enzymatic method to examine the lipid profiles, a qualitative approach to examine the CRP, and painting Oil Red O and HE on histology slides to count the foam cells. Results: The negative control group indicated normal levels of lipid profiles and foam cells. The positive control group showed increased lipid profile levels and foam cells. Meanwhile, the treatment groups receiving an increase in biotin concentration showed a decreasing pattern of the foam cells, and their lipid profile levels (total cholesterol, triglycerides, and LDL) decreased. However, the HDL did not reduce. The results of all groups' CRP were negative. The one-way ANOVA test showed significance for the levels of total cholesterol, triglycerides, and LDL. The Kruskal-Wallis test was significant for the number of foam cells (a confidence level of 95%). Conclusion: The biotin treatment significantly improves Wistar rats' lipid profiles and the number of foam cells. However, the doses did not statistically affect the levels of HDL and CRP.

KEYWORDS: Biotin, Dyslipidemia, Lipid Profiles, CRP, Foam Cell.

INTRODUCTION:

Dyslipidemia is a lipid metabolism disorder caused by genetic and environmental interactions, as evidenced by an abnormal lipid profile test result. The examined lipid profiles include total cholesterol, triglyceride (TG), low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol levels.[1-3] Dyslipidemia can lead to atherosclerosis, increasing the risks of coronary heart disease, cardiovascular disease (CVD), and stroke.[4-6]

Received on 24.08.2022 Modified on 30.11.2022
Accepted on 10.01.2023
Research J. Pharm. and Tech 2023; 16(6):2779-2785.
DOI: 10.52711/0974-360X.2023.00457

Atherosclerosis is nodular arteriosclerosis spots initiated by adhesion of platelets and lipoprotein influx.[7] Excessively modified-coming LDL and cholesterol esters accumulation in intima macrophages result in the formation of foam cells, which significantly mark the progression stages from initial lesions to advanced atherosclerosis plaques.[8-9] Atherosclerotic lesions of humans and experimental animals reveal C-reactive protein (CRP) that becomes acute inflammatory protein increasing up to 1,000 times at the site of infection or inflammation.[10-13]

Preventive efforts are necessary to reduce dyslipidemia risks, for example, by giving supplements, such as biotin. Biotin is also called vitamin B7 or vitamin H. It is a water-soluble vitamin that acts as a prosthetic group in carboxylase of several metabolic pathways.[14] Ardabilygazir[15] asserts that biotin is a cofactor for carboxylase enzymes involved in synthesising fatty acids and energy production. Several pharmacological biotin concentrations influence gene expression in transcription and translation, as well as a wide range of systemic processes such as development, reproduction, and metabolism. Fernandez-Mejia et al.[16] propose a daily vitamin intake of 30g for adults and 35g for lactating mothers. Biotin is considered a safe vitamin, and an intake up to 300-fold greater than normal has been proven non-toxic.[16-18]

Biotin can lower TG levels and low-density lipoprotein (LDL) in the blood plasma of patients with type 2 diabetes and non-diabetic patients with hypertriglyceridemia.[19] The combination of atorvastatin drugs and biotin for patients with dyslipidemia results in decreased levels of total cholesterol, LDL cholesterol, and triglycerides.[20] Patients with secondary dyslipidemia who have taken Atorvastatin 20mg/day with biotin regularly show promising results in total cholesterol ratios: On the fourth and sixth weeks, HDL cholesterol was 3.5.[20]

Biotin carboxylase (AccC) is an excellent target for antibacterial agents.[21-29] Biotin is an agent that significantly lowers phospholipid levels in rats. Biotin was tested in healthy mice at doses of 97.7 mg/kg, and it could reduce serum TG levels by up to 35%. However, the reduction was not efficient and there was still lipogenic gene expression.[30] The analysis of signalling pathways and post-transcriptional mechanisms in the hypotriglyceridemic effects of biotin revealed that serum triglyceride and liver concentrations decreased. [31] Another study found that administering similar doses to rats reduced free fatty acid origin levels while having no effect on lipolysis. Furthermore, the study revealed that oxidation and absorption increased while fatty acid synthesis decreased. [32,33] The treatment of mice with a high-fat diet combined with biotin supplements of 300 μg/kg indicated a decrease in levels of total cholesterol, LDL cholesterol, and triglycerides.[34]

Administration of biotin as a supplement to Wistar rats is considered a preventive measure for dyslipidemia. A high-fat diet containing lard given to Wistar rats can cause an increase in LDL and the formation of foam cells. This study was conducted to investigate the effects of variations in biotin concentrations on lipid profiles, CRP levels, and foam cells in Wistar rats. The variation of biotin concentrations referred to a previous study indicating that the administration of biotin at 97.7 mg/kg could only lower the levels of TG up to 35% (21–24). As a result, the biotin concentration in mice was increased by 50%, from 97.7 mg/kg to 139.6 mg/kg in this study. The dose conversion for experimental animals was conducted from mice to Wistar rats. The increase in concentration is expected to bring better results.

MATERIALS AND METHODS:

Ethical Approval:

All procedures had been reviewed and approved by the ethics committee of the Faculty of Medicine, Universitas Muhammadiyah Semarang, number No. 382/KEPK. FKM/UNIMUS/2020, in July 2020. This procedure agrees with the 1964 Declaration of Helsinki, subsequent amendments, and the Principles of Laboratory Animal Care (NIH publication, vol. 25, no. 28, 1996 revision).

Animals and Study Design:

This study is an experimental study with post-randomized controlled group design. It involved 30 male Wistar rats, aged eight weeks, in the range of 150-200 grams body weight. All the rats were adapted for seven days before divided into experimental groups. The rats were kept in a room with 22ºC of temperature, sufficient lighting (lights were lit every evening from 5 pm to 7 am), and Ad libitum drink. We reared the rats in groups, namely control and treatment groups. Each group consisted of six members. The description of each group is as follows:

A. Rats in the Control Groups

The control groups consisted of a negative control group and a positive control group. The negative control group received standard feed, the chicken feed with high-fat diet AD II, and lard with a ratio of 1:10 for six weeks.

B. Rats in the Treatment Group

The treatment groups consisted of groups 1, 2, and 3. Groups 1 received high-fat diet and biotin doses of 1.232 mg/kg of BW (bodyweight); group 2 received high-fat diet and biotin doses of 68.39 mg/kg; group 3 received with high-fat diet and biotin doses of 97.72 mg/kg of BW. The rearing of the treatment groups was conducted for six weeks. The variation of biotin concentrations referred to previous research positing that the administration of biotin of 97.7 mg/kg BW in mice could only lower TG levels up to 35%. This study raised 50% of the biotin concentration for mice from 97.7 mg/kg to 139.6 mg/kg. The dose conversion for experimental animals was conducted from mice to Wistar rats. The increase in concentration is expected to bring better results. The followings are the calculations of conversion values from mice to rats:

Table 1: Dose Calculation Conversions[35]

	Mice (20 g)	Rats (200 g)
Mice (20 g)	1.0	7.0
Rats (200 g)	1.14	1.0

Dose Conversion from Mice to Rats:

The dose calculation conversions for experimental rats if the dose for mice is discovered

1. Doses for mice with 1.76mg/kg BW

Factors of dose conversion for mice to rats = 7

a. Absolute dose for mice weighing 20g

= 1.76mg/kg BW x 0.02 kg (from 20g/1000g)

= 0.0352mg.

b. Doses for mice

= 0.0352mg x 7 (conversion for the mice-rats)

= 0.2464mg (for rats 200g)

= 0.2464mg/0.2kg

= 1.232mg/kg BW

2. Doses for mice with 97.7mg/kg BW

Factors of dose conversion for mice to rats = 7

a. Absolute dose for mice weighing 20 g

= 97.7mg/kg BW x 0.02kg (from 20g/1000g)

= 1.954 mg.

b. Doses for mice

= 1.954mg x 7 (conversion for mice-rats)

= 13.678mg (for rats weighing 200 g)

= 13.678mg/0.2kg

= 68.39mg/kg BW

3. Doses for mice with 139.6mg/kg BW

Factors of dose conversion for mice to rats = 7

a. Absolute dose for mice weighing 20g

= 139.6mg/kg BW x 0.02kg (from 20g/1000g)

= 2.792mg.

b. Doses for mice

= 2.792mg x 7 (conversion for the mice-rats)

= 19.544mg (for rats weighing 200g)

= 19.544mg/0.2kg

= 97.72mg/kg BW

All rats in the control and treatment groups fasted for 8-10 hours at the end of the sixth week. Then, anesthesized intraperitoneally with the mixture of Ketamine 75-100 mg/kg and xylazine 5-10 mg/kg. Blood was drawn through the retro-orbital plexus to get serums. Rats were terminated to take their aorta for materials of histology preparations. The serum's lipid profiles and C-reactive protein were examined. Foam cells were checked in the histology preparations. The lipid profile test consisted of total cholesterol, triglycerides, LDL, and HDL using the CHOD-PAP method, while the CRP test used the agglutination method. The foam cell test used painting Oil Red O and HE considering the procedures of Koss in the anatomic pathology laboratory of Sentra Pathology Akurat (the Center of Accurate Pathology). All laboratories have implemented IEC 17025 standards (Testing Laboratory).

Foam Cell Count:

Following Koss's procedures, the cut aortic arch was painted with Oil Red O and HE[36]. The 100X magnification was conducted to discover obvious layers of the aorta. The result of this examination was then measured in 20 wide fields of view at 1000X magnification to connect and measure foam cells. These steps were conducted three times by the same researchers at different times.

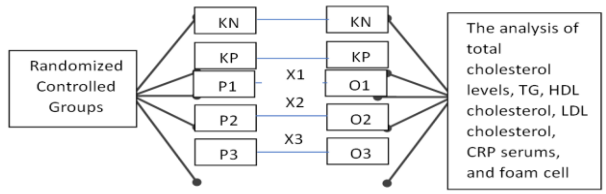

Figure 1: Experimental Design

Statistical Analysis:

The data were analysed using the standard error of mean (SEM). We employed the Shapiro-Wilk test to examine the data distribution. All the lipid profile data indicated normal distribution, and thus, the ANOVA statistical test was employed. Since the foam cell data showed abnormal distribution, the Kruskal-Wallis statistical test was employed. All analyses were performed using IBM Statistics SPSS 22 (SPSS Inc., Chicago, IL, USA). The differences were considered statistically significant at p> 0.05.

RESULTS:

Wistar Rats' Body Weight:

Wistar rats were weighed every week to investigate their conditions and determine the administration of biotin doses for each rat by considering their weight. Weight gain in rats is presented in Table 2.

Table 2: Weight Gain in Rats

Groups	Initial BW (g)	Final BW (g)	The Percentage of Weight Gain BW
Negative Control	162.33	295.00	81.72 %
Positive Control	163.33	277.00	69.59 %
Treatment 1	158.50	265.33	67.40 %
Treatment 2	160.00	248.33	55.21 %
Treatment 3	157.67	254.00	61.10%

BW=Body weight, g=gram

Data Analysis of Lipid Profiles of Wistar Rats

The data analysis of lipid profiles of Wistar rats is presented in Figure 2.

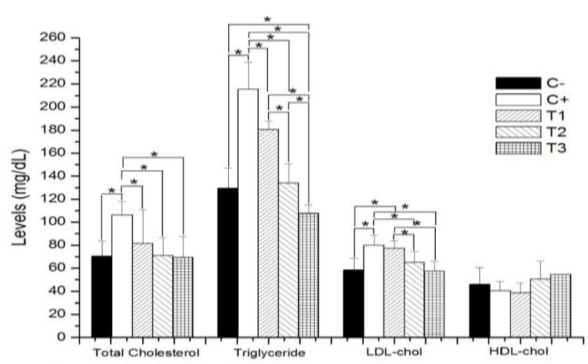

Figure 2. The Average Results of the Lipid Profile Test on Each

Group
* Represents a significant effect

Figure 2 shows the average results of the lipid profile test on each group. The average of total cholesterol levels of the treatment groups 1, 2, and 3 sequentially are 78.40mg/dl, 71.03mg/dl, and 69.50mg/dl. The average TG levels of the treatment groups 1, 2, and 3 sequentially are 180.60 mg/dl, 134.00 mg/dl, and 107.67 mg/dl. The average LDL cholesterol of the treatment groups 1, 2, and 3 sequentially is 38.83mg/dl, 50.50 mg/dl, and 54.67mg/dl. The average HDL cholesterol levels of the treatment groups 1, 2, and 3 sequentially are 77.43 mg/dl, 65.24 mg/dl, and 57.51mg/dl. The one-Way ANOVA test indicates the effects of an increase in biotin concentration on the results of total cholesterol, TG, and LDL cholesterol level tests.

Data Analysis Results of CRP of the Wistar Rats:

Data analysis results of the CRP was conducted descriptively. The analysis results are presented in Table 3.

Table 3: The CRP Serum Levels of the Wistar Rats

S. No	CRP Results	Number of Samples	Percentage
1	Negative	29	100%
2	Positive	0	0 %
	Total	29	100%

The CRP level test results in Table 3 indicated that 29 samples have negative results (100%), and no sample showed positive. Furthermore, the results show that the increase in biotin concentrations did not affect CRP levels of Wistar rats.

Data Analysis of Wistar Rats' Foam Cells:

The data of the Wistar rats' aortic foam cells were descriptively analyzed to discover the results of reading the number of foam cells. The number of foam cells was calculated using scoring systems by a specialist in anatomical pathology. The percentage of the foam cells from each group is presented in Table 4.

Table 4. The Percentage of Total Scores of Wistar Rats' Aortic Foam Cells

Treatment		∑ Foam Cell Score			Description
	0	1	2	3	
Negative Control	18	0	0	0	Score 0 = 100%
Positive Control	0	0	0	18	Score 3 = 100%
Treatment 1	0	0	5	13	Score 2 = 27.78% Score 3 = 72.78%
Treatment 2	0	5	13	0	Score 2 = 27.78% Score 2 = 72.78%
Treatment 3	0	15	3	0	Score 1= 83.33% Score 2 = 16.67%

Score 0: Not found in foam cells
Score 1: Found in foam cells < 10% from the wide field of view
Score 2: Found in foam cells 10-30% from the wide field of view
Score 3: Found in foam cells > 30 % of the wide field of view

Table 4 denotes that foam cells were not found in the negative control (score 0). The foam cells were 100% found in the positive control. In the treatment 1 group foam cells were found as 27.78% (score 2) and 72.22% (score 3). Foam cells were found in treatment 2 group as many as 27.78% with score 1 and 72.22% with score 2. Foam cells are found in samples of treatment 3 as many as 83.33% with score 1 and 16.67% with score 2.

The Shapiro-Wilk test obtained $p \leq 0.05$. This result means that the data distribution is abnormal. The subsequent data analysis is the non-parametric test or the Kruskal-Wallis test with $p = 0.000$, which indicates a significant effect.

The appearance of foam cells in the Wistar rats' aorta with 400x magnification is presented in Figure 3.

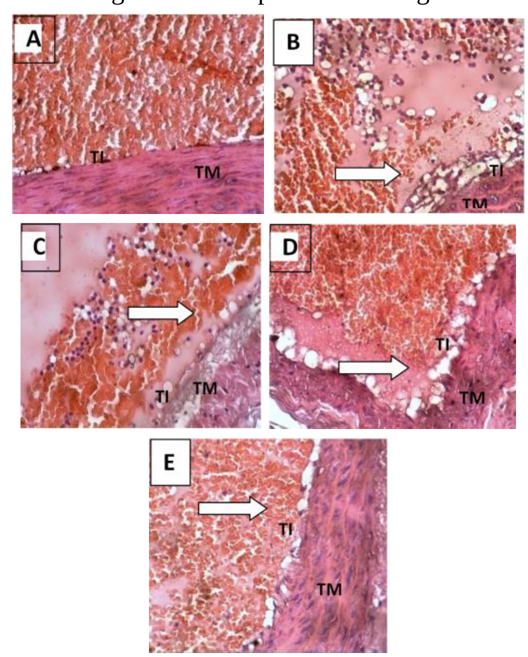

Figure 3. Microscopic Views of the Wistar Rats' Aorta with 400x Magnification:
A = Negative Control, B = Positive Control, C = Treatment 1, D = Treatment 2,
E = Treatment 3, TI = Tunica Intima, TM = Tunica Media

Artery vessel layers are composed of the tunica adventitia, located outermost, the tunica media layers, and the tunica intima layers. The foam cells were not found in the aortic cross-sectional areas of Wistar rats in the negative control groups (Figure A). Foam cells were found in the positive control groups (Figure B), as shown by the arrow, and in treatment group 1 with the administration of low-dose biotin (Figure C). Foam cells cover the tunica intima layers and are visible in the tunica media layers. Treatment group 2 indicates that the foam cells are still visible in the tunica intima layers, but their numbers have reduced (Figure D). Treatment group 3 indicates that foam cells are only visible in the tunica intima layers (Figure E).

DISCUSSION:

Lipid Profiles:

The research results indicated that administration of biotin could reduce total cholesterol levels, triglycerides, and LDL cholesterol significantly. The administration of biotin at 1.232mg/kg, 68.39mg/kg, and 97.72mg/kg in the treatment groups could decrease the total cholesterol by 26.22%, 33.16%, and 34.60%, respectively. These results are higher than those in the positive control group. The TG levels of the positive control groups decreased by 16.2%, 37.8%, and 50%, respectively, for groups 1, 2, and 3. Meanwhile, the positive control groups' LDL levels decreased by 2.99%, 18.3%, and 27.95%. HDL cholesterol levels increased, but they were not statistically significant. The HDL cholesterol levels of treatment group 1 decreased by 4.12% compared to the positive control groups. Meanwhile, the HDL cholesterol levels of treatment groups 2 and 3 increased by 24.69% and 34.98%, respectively.

These findings are consistent with those of Orhan et al.[34], who found that administering biotin to mice fed a high-fat diet significantly altered their lipid profiles. A study by Larrieta et al.[30] signified that the TG levels of the control groups were reduced by 35%. Meanwhile, this study found that increasing biotin levels in mice by 97.72mg/kg could reduce TG levels by 50%. This decrease in TG levels is associated with the reduction of excessive mRNA expression of lipogenic enzymes and transcription factors.[30] The concentration of free fatty acids also decreased in mice administered with biotin. The supplementation of biotin in tissue adipose increases acetyl-CoA carboxylase 1 and acetyl-CoA carboxylase 2 (enzymes that decrease fatty acid synthesis and increase the rate of fatty acid oxidation); this condition possibly decreases serum free fatty acid levels.[32,33]

C-Reactive Protein:

CRP is an acute inflammatory protein. However, large-scale prospective studies showed that CRP was also associated with chronic inflammation, such as cardiovascular diseases.[12,37] CRP was found in atherosclerotic lesions in humans and experimental animals, along with LDL and macrophages. In this case, CRP was considered to be involved in modulating the pathogenesis of atherosclerosis.[13] The increase in CPR serum levels becomes a strong predictor for cardiovascular disease in asymptomatic individuals.[11]

Experiment animals fed high-fat diets demonstrated a link between plasma levels and CRP in lesions and the formation and progression of atherosclerotic lesions. CRP levels in plasma were strongly correlated with the size of the intimal lesion of the aortic arch. CRP levels were found to reflect the progression of lesions[38], but CRP did not play a role, even in early atherosclerosis.[39]
This study revealed that the administration of biotin supplements did not affect CRP levels in Wistar rats. The limitation of the qualitative CRP test in this study was its ability to detect CRP only at 10mg/L. Therefore, a value <10mg/L is considered negative. Another more sensitive method is the high-sensitivity CRP assay (hs-CRP), which can detect CRP concentrations as low as 0.3mg/L.

Foam Cells:

Wistar rats received a high-fat diet and supplementation of biotin for six weeks. The number of foam cells formed in the arcus aorta reduced. This is inversely proportional to the concentration of biotin. The higher the concentration of biotin is administered, the increasingly lower number of foam cells in rats with dyslipidemia risk is. Foam cells are found in samples of treatment 1 with score 2(27.78%) and score 3(72.22%). Foam cells are found in samples of treatment 2 with score 1(27.78%) and score 2(72.22%). Foam cells are found in samples of treatment 3 with score 1 (83.33%) and score 2(16.67%). A large number of foam cells indicated the increase in the amount of oxidized LDL cholesterol accumulated by macrophages through a scavenger receptor (in contrast to the LDL receptor). Consequently, the number of LDL particles in intima layers increased.[40] Cholesterol and free fatty acid buildups in macrophages and other cells triggered the inflammation in the initiation and development of atherosclerotic lesions.[40] LDL cholesterol levels can reduce the risk of atherosclerotic cardiovascular disease (ASCVD).[41] This study showed that the LDL cholesterol levels decreased in the groups with supplementation of biotin. Treatment group 3 showed a decrease in LDL cholesterol up to 27.95%.

CONCLUSION:

The research results showed that the increase in the concentration of biotin affected Wistar rats' lipid profiles and a number of foam cells significantly. However, these results did not affect HDL cholesterol levels or CRP statistically. So it can be concluded that the administration of biotin could reduce total cholesterol levels, triglycerides, and LDL cholesterol significantly. We believe that our findings add to the important, albeit limited, knowledge of this biotin's functions. Furthermore, human requirements for biotin as a therapeutic agent in lipid metabolism should be studied in the future using novel approaches and cutting-edge techniques in order to broaden its applications.

ACKNOWLEDGEMENTS:

The authors would like to express their sincere gratitude to the Master of Clinical/Medical Laboratory Science Program, the Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang, and the Health

Laboratory Center of Jawa Tengah.

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