International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-4, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

The Arterial Wall Lysosomal Stabilizing and Hypolipidemic Effect of Mono and Poly unsaturated Fatty Acids

Al-Khafaji AT[1], Majid WJ[2], Thwaini MM[3]

[1]Dept of Medicine, [2] Dept of Biochemistry and [3] Dept of Pathology, Thi qar college of Medicine, Nasiriyah, Iraq.

Manuscript No: IJPRS/V3/I4/00440, Received On: 27/11/2014, Accepted On: 05/12/2014

ABSTRACT

Both mono- and polyunsaturated fat lowered LDL-Cholesterol levels when they replaced saturated fat. On the other hand, many studies indicated that polyunsaturated fat lowered HDL-Cholesterol, whereas monounsaturates did not. This study was designed to address the controversy regarding a differential effect of monounsaturates versus polyunsaturates on serum lipids and on lysosomal stability of experimentally induced hyperlipidemia in rats, as parameters critically affected the incidence of ischemic heart diseases. The statistical analysis showed that oleic acid was more effective in reducing serum levels of triglycerides, total cholesterol, LDL- cholesterol and VLDL- cholesterol. In addition, oleic acid also significantly more effective in elevation of HDL-cholesterol and stabilizing of aortic wall lysosomes, in hyperlipidemic rats in comparison with normal saline treated group.

KEYWORDS

Monounsaturated, Polyunsaturated, Fatty Acid, Hyperlipidemia, Lipid Profile, Lysosomes

INTRODUCTION

Dietary fat composition is the primary determinant of serum total cholesterol, low-density lipoprotein (LDL) cholesterol, very low - density lipoprotein (VLDL) cholesterol and triglycerides, which are the main blood lipid risk factors for cardiovascular disease). In general, favorable serum lipid profile attributed to lower fat intake has been confounded by accompanying reduction in saturated fat intake[1].

For more than forty years, Keys *et al* and Hegsted *et al* independently developed several equations to predict changes in total cholesterol and LDL-C that would accompany changes in dietary fat and cholesterol intake[2-5]. Mattson and Grundy reported that both mono and polyunsaturated fat lowered LDL-Cholesterol levels when they replaced saturated fat[6].

***Address for Correspondence:**
Adnan T. Al-Khafaji
Dept of Medicine, Thi qar college of Medicine,
Nasiriyah, Iraq.
E-Mail Id: university_of_thi_qar@yahoo.com

On the other hand, many studies indicated that polyunsaturated fat lowered HDL-Cholesterol, whereas monounsaturates did not. The recommendations of this study were appeared in many studies as evidence that monounsaturates are preferred over polyunsaturates in decreasing the incidence of ischemic heart diseases[7-9].

Many mechanisms were postulated to explain the hypolipidemic effects of unsaturated fatty acids; these include increase fecal excretion of neutral steroids and/ or bile acids, reduction of cholesterol absorption, decrease endogenous cholesterol synthesis and re-distribution of circulating cholesterol between plasma and tissue pools[10-11].

However, the hypolipidemic difference between mono and polyunsaturated fatty acids is a subject of controversy. This study was designed to address the controversy regarding a differential effect of monounsaturates versus polyunsaturates on serum lipids and on lysosomal stability as

parameters critically affected the incidence of ischemic heart diseases.

MATERIAL AND METHODS

Effect on Lipid Profile

Forty male albino rats weighing 250-300g were used in this study. Hyperlipidemia was induced in rats by feeding high cholesterol diet containing 1% cholesterol, 0.5% cholic acid and 0.2% propylthiouracil for twenty weeks[12-13].

Rats were divided into four groups, three of them were treated by 1ml/ day of oleic acid, 1ml/day of linoleic acid and a combination of 0.5ml of each oil/ day respectively, from the first day and continue all over the period of induction of hyperlipidemia[14].

The fourth group was given 1 ml normal saline/ day for the same period to serve as untreated control. After twelve weeks, blood samples from animals were collected by cardiac puncture. Serum total cholesterol, triglycerides, VLDL-cholesterol and HDL-cholesterol were determined by an enzymatic methods (bio Merieux-France), while HDL- cholesterol was measured using the special formula stated by Nash[12,14].

Effect on Stability of Lysosomes in Arterial Wall

Lysosomal stability was determined according to the method of Sniki *et al.,* aorta (from the aortic arch to the iliac bifurcation) of the anesthetized rats in all groups were quickly removed and washed with cold physiological saline solution. The adventitia was carefully removed and the intima and media were minced with scissor and homogenized in 9 volumes (w/v) of 0.25 M sucrose solution containing 5mM Tris-HCl buffer (PH 7.0) using (IKa – Labortechnik Homogenizer).

The homogenate was centrifuged at 15000 RPM for 30 minutes. The precipitate was suspended in 1 ml of homogenizing buffer per gram of the original aorta and used as lysosomal fraction. The lysosomal fraction was incubated with 100 U/ml of venom phospholipase A2 at 37 ºC for 15 minutes, and then centrifuged at 15000 RPM for 30 minutes. The degree of the lysosomal injury was evaluated by measuring the acid phosphatase activity (by an enzymatic method, Randox-France) in the resulting supernatant. Acid phosphatase activity in the supernatant of aorta of the treated groups was expressed as a percentage of that in non treated group[14].

Student t- test was used to determine the significancy among groups.

RESULTS

Table 1 showed that both linoleic and oleic acid were significantly decrease serum levels of triglycerides, total cholesterol, LDL- cholesterol and VLDL- cholesterol in hyperlipidemic rats in comparison with normal saline treated group. However, the statistical analysis showed that oleic acid was more effective in reducing these parameters in comparison with linoleic acid.

In addition, only oleic acid significantly increased serum levels of HDL- cholesterol in hyperlipidemic rats in comparison with normal saline treated group. However, the statistical analysis showed that oleic acid was more effective in reducing these parameters in comparison with linoleic acid. Using a combination of oleic and linoleic acids didn't induced further significant effects in comparison with oleic acid alone.

Oleic acid was significantly stabilizing the lysosomes of aorta wall, linoleic acids exerted no significant effects on the stability of wall lysosomes, and in addition it inversely affected the lysosomal stabilizing effects of oleic acid when both acids were used in combination (Table 2).

DISCUSSION

Elevation of serum cholesterol is associated with high incidence of ischemic heart diseases. The important approach in prevention of atherosclerosis is to keep the serum lipids within the normal limit. Many mono and polyunsaturated fatty acids were used for these purposes[15-16]. The hypolipidemic difference between mono and polyunsaturated fatty acids is a subject of controversy.

Table 1: The release of acid phosphatase from rat aortic lysosomes induced by phospholipase A2 in hyperlipidemic rats treated orally with 1 ml/ day for 12 weeks of oleic, linoleic acids and their combination.

Groups	Treatment	Triglycerides mg/dl	Total cholesterol mg/dl	HDL-cholesterol mg/dl	LDL-cholesterol mg/dl	VLDL-cholesterol mg/dl
1st n=10	Linoleic acid 1ml/day for 12 weeks	85.28±12.16^{c}	106.16±10.18^{c}	49.82±6.25^{bc}	25.62±3.82^{c}	46.23±3.94^{c}
2nd n=10	Oleic acid 1ml/ day for 12 weeks	70.53±8.92^{b}	82.68±6.73^{b}	67.77±5.58^{b}	18.12±5.22^{b}	32.86±3.94^{b}
3rd n=10	Linoleic acid 0.5 ml+ Oleic acid 0.5 ml/day for 12 weeks	81.68±10.16^{b}	96.56±8.88^{b}	62.14±6.16^{b}	17.87±5.23^{b}	42.66±5.43^{b}
4th n=10	Normal saline 1ml/ day for 12 weeks	201±28.53^{a}	185.75±26.65^{a}	46.44±9.76^{a}	35.12±2.98^{a}	102.24±16.83^{a}

Similar letter vertically means not significant

Table 2: The release of acid phosphatase from rat aortic lysosomes induced by phospholipase A2 in hyperlipidemic rats treated orally with 1 ml/ day for 12 weeks of oleic, linoleic acids and their combination.

Groups	Treatment	Acid phosphatase activity in the treated group compared with normal saline treated hyperlipidemic group
1st n=10	Linoleic acid 1 ml/day for 12 weeks	94.64%
2nd n=10	Oleic acid 1 ml/day for 12 weeks	70.72%
3rd n=10	Linoleic acid 0.5 ml+ Oleic acid 0.5 ml/day for 12 weeks	80.42%
4th n=10	Normal saline 1 ml/day for 12 weeks	100%

Acid phosphatase activity expressed as a percentage of that of normal saline treated hyperlipidemic rats.

Some authors states that linoleic acid can decrease serum cholesterol and prevent atherosclerosis, a switch from (50-60% linoleic acid) to (little linoleic acid) in diet cause rise in serum cholesterol. On the other hand, the degree of hyperlipidemia was increased with the increase of linoleic acid[10,15,17]. Our results showed that linoleic acid was significantly decrease serum levels of triglycerides, total cholesterol, LDL- cholesterol and VLDL-cholesterol. These findings were in agreement with that mentioned by many authors[15-18.] Our study showed that oleic acid was more effective in reducing serum lipids in comparison with linoleic acid. In addition, only oleic acid significantly increased serum levels of HDL-cholesterol in hyperlipidemic rats in comparison with normal saline treated group. This fact was also recorded previously[15]. Siddique *et al.*, found that olive oil (83% oleic and 7% linoleic acid) was more efficient than sunflower (50-60% linoleic acid) in reducing serum cholesterol and triglycerides[17]. However, the most important effect of oleic acid is the elevation of HDL-cholesterol, so ischemic heart diseases were negatively correlated with HDL- cholesterol level[10,18-19]. Accordingly, diet with high oleic acid is required to decrease the atherosclerosis. On the other hand, vessel wall lysosomal function is essential to prevent accumulation of lipids in the vessels wall. The factor which affected lysosomal function is phospholipase A2 which present in lysosomes of vessel wall and produce lysophospholipids by hydrolyzing phospholipids, these lysophospholipids affect the stability of lysosomal membrane by its detergent action resulting in lysosomal dysfunction[20-21]. Our study also showed that the highest lysosomal stability was recorded with the using of oleic acid alone, while, linoleic acid showed the lowest lysosomal stability. Furthermore lysosomal stability was proportional with the hypolipidemic effects of unsaturated fatty acids[21]. These results supported the idea that hyperlipidemia increases the incorporation of LDL-cholesterol into cell and increase phospholipase A2 activity especially in vessels wall which induced lysosomal dysfunction. Accordingly, it appeared that oils with high oleic acids such as olive oil are more effective in decreasing serum lipids, stabilizing lysosomal enzymes and decreasing ischemic heart diseases**.**

REFERENCES

1. Barr, S. L., Ramakrishnan, R., Johnson, C., Holleran, S., Dell, R. B., & Ginsberg, H. N. (1992). Reducing total dietary fat without reducing saturated fatty acids does not significantly lower total plasma cholesterol concentrations in normal males. *The American Journal of Clinical Nutrition*, *55*(3), 675-681.
2. Keys, A., Anderson, J., & Grande, F. (1957). Prediction of serum-cholesterol responses of man to changes in fats in the diet. *The Lancet*, *270*(7003), 959-966.
3. Keys, A., Anderson, J. T., & Grande, F. (1965). Serum cholesterol response to changes in the diet: IV. Particular saturated fatty acids in the diet. *Metabolism*, *14*(7), 776-787.
4. Hegsted, D. M. (1986). Serum-cholesterol response to dietary cholesterol: a re-evaluation. *The American Journal of Clinical Nutrition*, *44*(2), 299-305.
5. Hegsted, D. M., Ausman, L. M., Johnson, J. A., & Dallal, G. E. (1993). Dietary fat and serum lipids: an evaluation of the experimental data. *The American Journal of Clinical Nutrition*, *57*(6), 875-883.
6. Mattson, F. H., & Grundy, S. M. (1985). Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research*, *26*(2), 194-202.
7. Artaud-Wild, S. M., Connor, S. L., Sexton, G., & Connor, W. E. (1993). Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox. *Circulation*, *88*(6), 2771-2779.
8. Reaven, P., Parthasarathy, S. A. M. P. A. T. H., Grasse, B. J., Miller, E., Steinberg, D., &

Witztum, J. L. (1993). Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. *Journal of Clinical Investigation*, *91*(2), 668.

9. Ginsberg, H. N., Karmally, W., Barr, S. L., Johnson, C., Holleran, S., & Ramakrishnan, R. (1994). Effects of increasing dietary polyunsaturated fatty acids within the guidelines of the AHA step 1 diet on plasma lipid and lipoprotein levels in normal males. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *14*(6), 892-901.

10. Cleeman, J. I., Grundy, S. M., Becker, D., & Clark, L. T. (2001). Expert panel on Detection, Evaluation and Treatment of High blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III). *Jama*, *285*(19), 2486-2497.

11. Sempos, C. T., Cleeman, J. I., Carroll, M. D., Johnson, C. L., Bachorik, P. S., Gordon, D. J., ... & Rifkind, B. M. (1993). Prevalence of high blood cholesterol among US adults: an update based on guidelines from the second report of the National Cholesterol Education Program Adult Treatment Panel. *Jama*, *269*(23), 3009-3014.

12. Somochowiec, L., Kadlubowska, D., and Rozewicka, L. (1976). Investigation in experimental atheroseclerosis. *Athero-seclerosis*, 233, 305-317.

13. Al-Jubouri, H. H., and Al-Bayati, Z. F. (1981). Hyper responsiveness of arteries from atheroseclerosed rats to noradrenaline and to syste*mic electrical stimulation. IRCS Journal of Medical Science*, 9, 317-324.

14. Al-Snafi, A. E. (2002). The best lysosomal stabilizing and hypolipoproteinemic mono/ polyunsaturated fatty acid combination. *Medical Journal of Tikrit University*, *8*, 148-153.

15. Pollak, O. T., and Del, D. (1972). Monograph on atheroseclerosis. NewYork, 52.

16. Durrington, P. N. (1996). Lipid and lipoprotein disorders. *In Oxford textbook of Medicine*, 3rd ed. Vol 2, Edited by DJ Weatherall Ledingham and DW Warrell. Oxford, 1399-1415.

17. Mensink, R. P., Zock, P. L., Kester, A. D., & Katan, M. B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *The American Journal of Clinical Nutrition*, *77*(5), 1146-1155.

18. Gardner, C. D., & Kraemer, H. C. (1995). Monounsaturated versus polyunsaturated dietary fat and serum lipids A meta-analysis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *15*(11), 1917-1927.

19. Edward CRWD, Bouchier AD, Haslett C and Chilvers ER. Davidsons principle and practice of medicine, 19th ed. ELBS with Churchill Livingstone, NewYork 2002: 308-312.

20. Goldstein, L. J., & Brown, S. M. (1976). The low density lipoprotein pathway in human fibroblasts. A receptor mediated mechanism for the regulation of cholesterol metabolism. *Current Top Cell Research*, 11, 147-148.

21. Sasaki, N., Matsuok, U. N., Shirai, K., Saito, Y., and Kumigi, A. (1981). Studies on phospholipid metabolism in arterial wall. The properties of phospholipase A2. *J Japan Atheroseclerosis Society*, 4, 69-73.

