

## ORIGINAL ARTICLE

# THE EFFECT OF $\alpha$ -LIPOIC ACID IN BLOOD LIPID LEVELS AND MALONDIALDEHYDE IN ATHEROSCLEROTIC-INDUCED NEW ZEALAND WHITE RABBIT

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$\alpha$ -Lipoic acid (ALA) is a naturally occurring cofactor that serves as an acyl carrier in oxidative decarboxylation of  $\alpha$ -keto acids in carbohydrate metabolism. Current findings suggest that  $\alpha$ -lipoic acid and its reduced form, dihydrolipoic acid (DHLA) may act as antioxidants and are able to quench free radicals in vitro and in vivo. However, the mechanism underlying the process is still unknown. In this study, atherosclerotic lesions were induced in six groups of adult male NZW rabbits labelled as group K, A, B, C, D, E (n=6) by giving 100g/head/day of 2% cholesterol-rich diet for ten weeks. While group K acted as a control, the rest were supplemented with ALA orally (1.4, 2.8, 4.2, 8.0 and 10mg/kg, respectively). In week ten, venous blood samples drawn from ear lobes were analysed for complete lipid profile and peroxidation index. The results showed a significant reduction of total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) levels in most of the treated groups as compared to the control whereas apo-A levels showed a significant increase in group C and D. However, microsomal lipid peroxidation index, malondialdehyde (MDA) was found to be not significantly different. These findings suggest that  $\alpha$ -lipoic acid may act as a lipid lowering agent in dose dependent manner in premature stage of atherosclerosis but was unable to inhibit lipid peroxidation processes in matured stage of atherosclerosis in rabbits fed a high cholesterol diet.

**Key words :** *Lipoic acid, atherosclerosis, lipid profiles, lipid peroxidation, antioxidant*

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### Introduction

$\alpha$ -Lipoic acid, also known as 1,2-dithiolane-3-pentanoic acid, 1,2-dithiolane-3-valeric acid or 6,8-thioctic acid has generated considerable clinical interest as a cellular thiol-replenishing and redox-modulating agent (1). Biologically,  $\alpha$ -lipoic acid (ALA) functions as a cofactor of oxidative decarboxylation reactions in glucose metabolism to yield energy. It has been used for a long time in the western world to treat complications associated with diabetes (2). To carry out this function, the disulfide group of the lipoic acid dithiolane ring is reduced to

its dithiol form, dihydrolipoic acid, DHLA (fig 1). Current findings suggest that ALA not only acts as a cofactor in glucose metabolism, but may also act as an antioxidant in vitro and in vivo. In vitro experiments have shown that both ALA and DHLA are potent scavengers of reactive oxygen species.  $\alpha$ -lipoic acid quenches singlet oxygen, hydroxyl radical and hypochlorous acid (3) while DHLA scavenges hydroxyl radicals, hypochlorous acid, superoxide anions radicals and peroxy radicals (4).

The observations of ALA as an antioxidant in vitro and in vivo were previously based on hyperglycaemic ambience (2-5). However, until

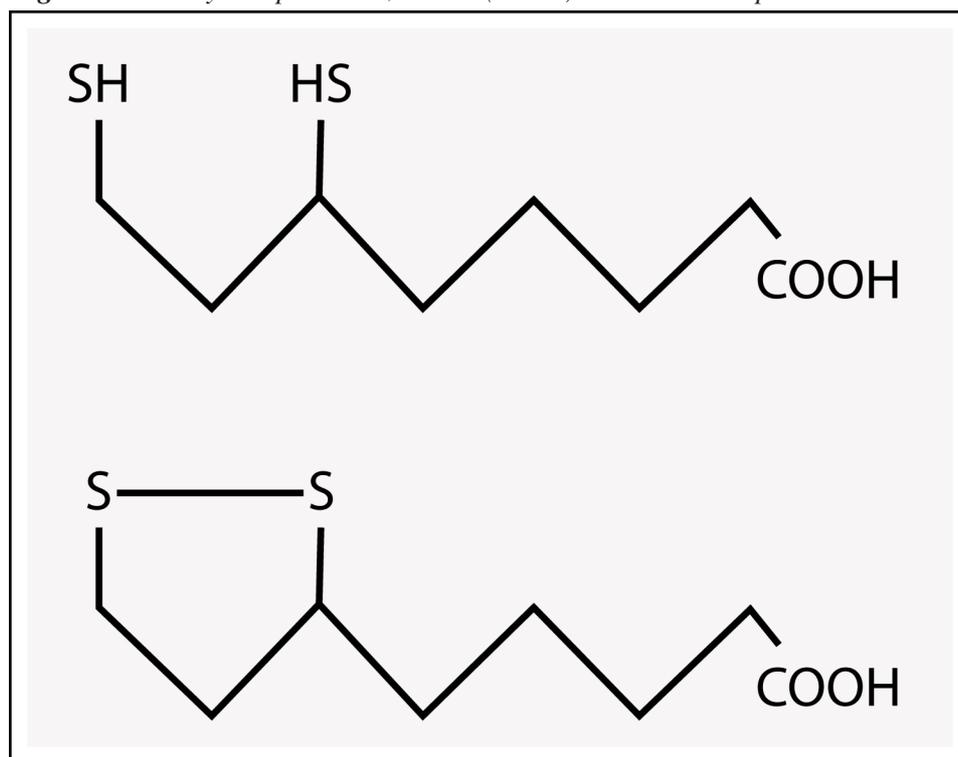
now, no experimental design has addressed the question as to whether  $\alpha$ -lipoic acid could work in atherosclerotic atmosphere in vivo in preventing proliferation and propagation of this degenerative disease. Lipid peroxidation, the oxidative deterioration of the polyunsaturated fatty acids (PUFA), leads to the formation of hydroperoxides, short-chain aldehydes, ketones and other oxygenated compounds. This process is considered responsible for the development of various diseases like atherosclerosis (6), diabetes (7), cancer (8) and may be one of the main contributing factor towards aging (9). Atherosclerosis, characterised by deposition of cholesterol in and around cells at the intimal layer of the arterial wall is the most important cause of mortality world wide. Although intensive research has been carried out throughout the globe, the precise mechanism of atherogenesis is still uncovered. Hypercholesterolemia most often associated with an elevation of plasma low density lipoprotein-cholesterol (LDL-C) and other related lipids, is believed to be the prime risk factor of atherosclerosis and coronary heart disease (10).

In the present study, we sought to determine whether  $\alpha$ -lipoic acid was able to exert its antioxidant effect and alter blood lipid levels in atherosclerosis-induced animals.

## Materials and Methods

**Experimental animals.** 36 three month-old male New Zealand White (NZW) rabbits with an average body weight of 2-3 kg were purchased from the Institute for Medical Research Kuala Lumpur (IMRKL). The animals were placed in individual cages and segregated into six groups (n=6) labelled as K (control), A, B, C, D and E. The animals were induced atherosclerotic lesion by giving 100g/head/day of 2% cholesterol-rich diet (ICN Biomedical, USA) for ten weeks. Group A to E were supplemented orally with  $\alpha$ -lipoic acid (Sigma) at a dose of 1.4, 2.8, 4.2, 8.0 and 10.0mg/kg respectively whereas group K acted as a control. Drinking water was given *ad libitum*. Prior to treatment (day=0) 25 ml of ear lobe venous blood samples were drawn into EDTA venoject tubes placed in a transport ice bucket and were centrifuged at 3000 rpm in a refrigerated bench top centrifuge for 10 minutes at 4 °C. The plasma samples were analysed for prediet baseline value of TC, LDL-C, HDL-C, TG, apo-A, apo-B and peroxidation index, MDA. In week ten, the animals were sacrificed by exsanguination through common carotid artery after withdrawing the same volume of blood samples from ear vein as previous and the plasma samples

**Figure 1:** Dihydrolipoic acid, DHLA (above) and oxidised lipoic acid



obtained were aliquoted and kept in  $-70^{\circ}\text{C}$  for a maximum of 7 days before analyses. All experimental procedures in these animals were performed in accordance with protocols approved by the Animal Care & Use Committee, Faculty of Medicine, UKM.

**Lipid profiles estimation.**

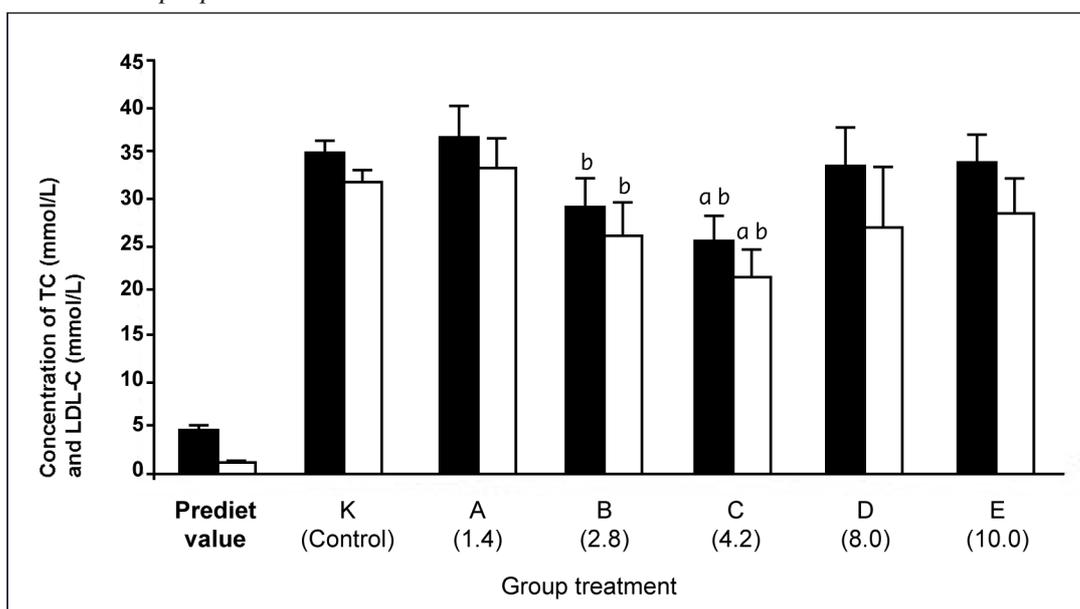
All plasma lipids were estimated using a commercial rapid test kit (Human). Plasma cholesterol was estimated enzymatically according to principle involving extraction of cholesterol ester by cholesterol esterase(11). The free cholesterol will react with cholesterol oxidase to form cholestenone. The indicator, quinoneimine was formed from hydrogen peroxide, 4-aminoantipyrine and 4-chorophenol under catalytic influence of peroxidase. For this, 0.02 ml plasma was mixed with 2 ml cholesterol reagent, vortexed and incubated at room temperature for 10 minutes. The absorption of pink-coloured quinoneimine was measured at 500nm spectrophotometrically and was multiplied with 5.17 to obtain total cholesterol levels. HDL-C level was estimated by precipitation technique with phosphotungstic acid and magnesium chloride. 0.2 ml of plasma sample was mixed with 0.5 ml HDL

precipitant reagent following incubation at room temperature for 10 minutes. 0.1 ml supernatant obtained after centrifugation for 10 minutes at 4000 g, mixed with 1 ml cholesterol reagent was incubated at room temperature for 10 minutes. The absorbance value measured at 500nm was multiplied with 4.52 to estimate HDL-C level. TG level was estimated by the same method, where 0.01 ml plasma samples was mixed with 1 ml of reagent solution and incubated at  $37^{\circ}\text{C}$  for 5 minutes. The absorbance was measured 500nm spectrometrically and was multiplied with 2.28 to obtain TG level. LDL-C level were calculated using Friedwald formula :  $\text{LDL-C} = \text{TC} - \text{HDL} - [\text{TG}/2.2]$  mmol/L (Friedwald et al. 1972). apo-A and apo-B levels were analysed at 340nm by turbidimetric technique using a commercial kit (Human).

**Lipid peroxidation estimation.**

Plasma samples obtained were used to study lipid peroxidation in vivo. Malondialdehyde (MDA) as thiobarbituric acid reactive substance was measured at 532nm spectrophotometrically (12) whereas the microsomal protein concentration was determined by Lowry’s method (13).

**Figure 2:** Level of total cholesterol, TC and low density lipoprotein-cholesterol, LDL-C in high cholesterol diet, pretreatment (far left bars) and post-treatment of various doses of alpha-lipoic acid (ALA). Number in the bracket below the group label indicated the dose (mg/kg).  $p < 0.05$  considered statistically significant. <sup>a</sup> $p < 0.05$  from control, <sup>b</sup> $p < 0.05$  from group A. Prediet bars provided is for comparison purposes.



## Statistical analysis.

All data were expressed as mean  $\pm$  standard deviation. Statistical analysis was done by one-way ANOVA and  $p < 0.05$  were considered significant. Tukey post-tests were performed for multiple group comparison.

## Results

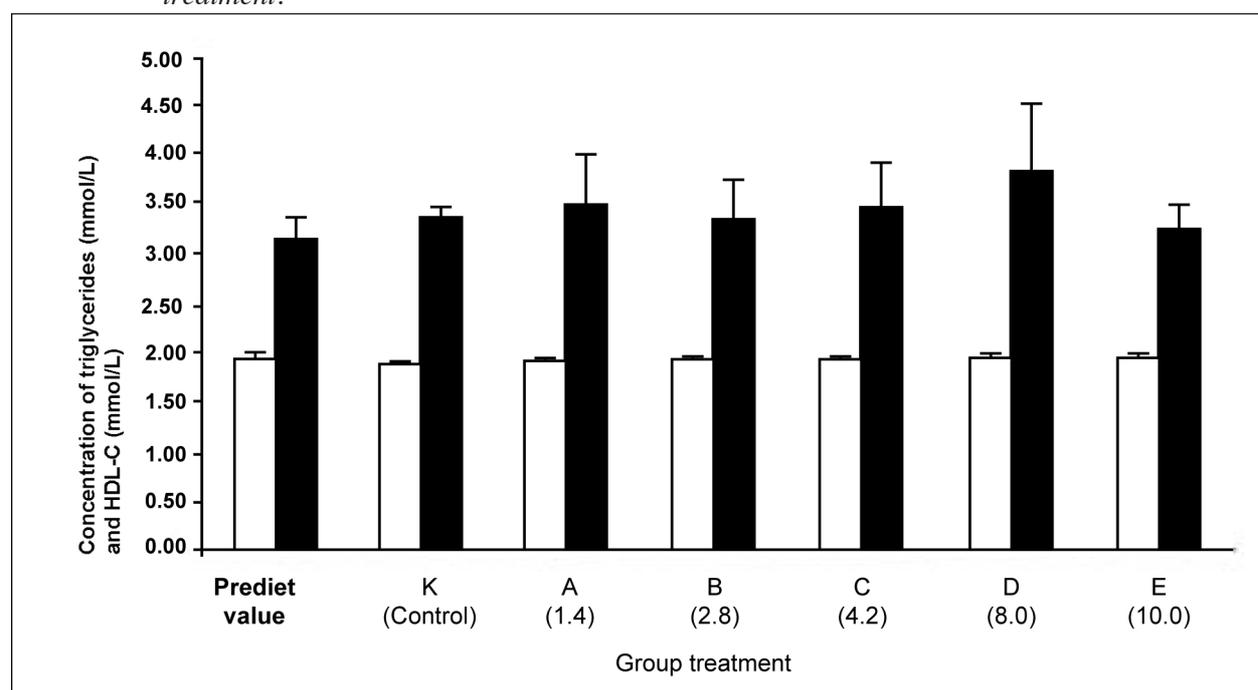
Prediet baseline value for lipids and lipoproteins in NZW rabbits at the start of the study were  $4.63 \pm 0.15$  mmol/L for TC,  $3.15 \pm 0.22$  mmol/L for TG,  $1.92 \pm 0.08$  mmol/L for HDL-C,  $1.28 \pm 0.18$  mmol/L for LDL-C,  $19.46 \pm 5.09$  mg/dL for apo-A,  $4.72 \pm 0.91$  mg/dL for apo-B whereas MDA level was  $0.04 \pm 0.01$  nmol/mg protein. At the end of week ten, venous blood were once again withdrawn and analysed as appropriate. The analysis revealed that the level of TC was significantly reduced in most of the treatment groups compared to control, with group C (4.2 mg/kg of ALA treatment) exhibiting the lowest TC level ( $24.45 \pm 3.34$  mmol/L) followed by group B and A ( $29.02 \pm 3.22$  mmol/L and  $36.32 \pm 3.37$  mmol/L, respectively) (fig.2). In group D and E, post-treatment TC levels however increased. The levels of low density lipoprotein cholesterol (LDL-C) were observed to

reduce significantly in a similar pattern to that of TC levels with group C showing the lowest concentration ( $20.94 \pm 3.48$  mg/dL). Similar to TC level, LDL-C level increased in both group D and E. Neither TG nor HDL-C gave a significant difference between control and treatment group (fig. 3). For lipoprotein profiles, apo-A levels were increased significantly in group D ( $54.99 \pm 9.61$  mg/dL) followed with group C ( $40.08 \pm 9.30$  mg/dL) (fig 4), whereas apo-B did not show any significant difference. Plasma lipid peroxidation index, MDA also did not show a significant difference in any of the treated groups (fig.5).

## Discussions

As the world rapidly progresses, the society becomes more affluent with the lifestyle changes. High consuming power has led to the people consuming more food rich in fat, sugar, salt and cholesterol. Sedentary lifestyle, increased stress coupled with environmental pollution and the changing lifestyle have significantly contributed towards increased incidence of atherosclerotic-related diseases. These days however, more and more people are now beginning to look upon natural products or naturopath (14) as an alternative supplement in preventing diseases.  $\alpha$ -lipoic acid

**Figure 3:** Level of triglycerides, TG and high density lipoprotein-cholesterol, HDL-C in high cholesterol diet, pretreatment (far left bars) and post-treatment of various doses of alpha-lipoic acid (ALA). Non of these parameter reveals a significant different between prediet (day=0) values or post-treatment.

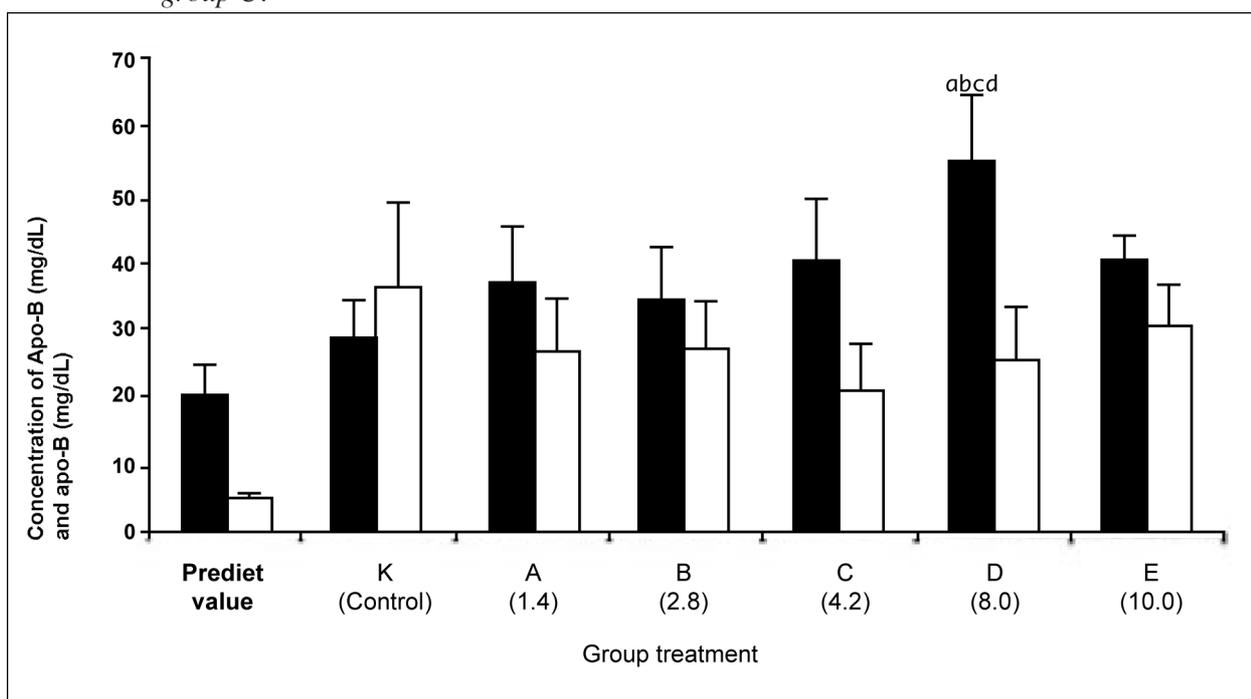


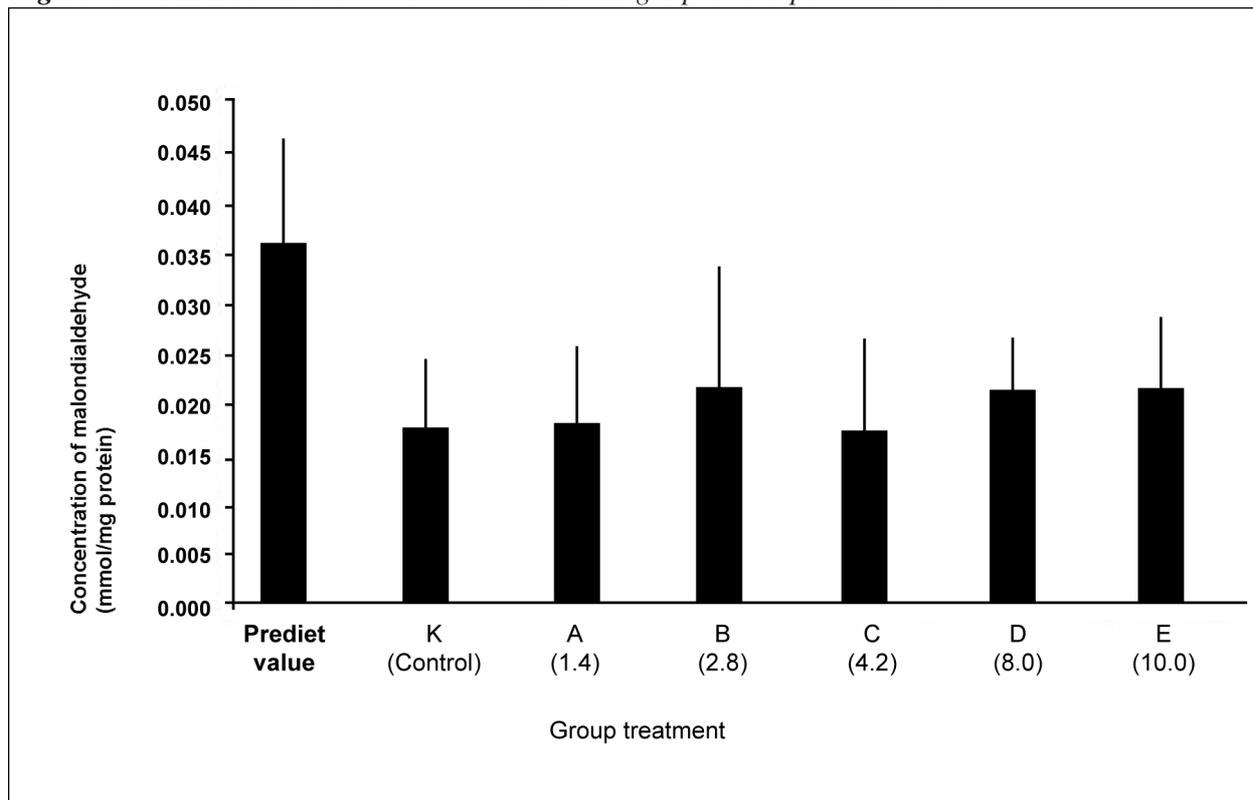
extracted in 1950s and being realised by scientist to have an antioxidant effect in the last decade, may play a significant role in disease prevention measures.  $\alpha$ -lipoic acid is a fat and water-soluble, sulphur containing coenzyme which is involved in energy production. A related metabolic functions of  $\alpha$ -lipoic acid is its role in blood glucose disposal (2) through the glucose-metabolizing enzymes, pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase eventhough some researchers suspects a more direct role in cellular glucose uptake at the cellular membrane (15). However, the involvement of this substance in cardiovascular related diseases have not been studied. Current findings suggest that pathogenesis of atherosclerosis involves oxidation of LDL-C particles via free radical activity (5-9). Elevated concentration of LDL-C in the circulation may further contribute the so-called bad cholesterol to penetrate into the vascular wall followed by macrophage activation to form foam cell. Cellular interaction was believed to be involved in propagating advanced-stage atherosclerosis lesion particularly MCP-1 and M-CSF molecule which play a role in macrophage

recruitment and migration whereas cytokins such as IL-1 and PDGF contribute towards SMC proliferation at the site of lesion (17).

Previous studies noted that ALA through its reduced form DHLA exhibits a protective effect against free radical activity eventhough its precise mechanism is still not understood. In our present study, MDA level was not significantly different in the treated group compared to the control. This seems to be in contrast to previous findings that suggest ALA would be a potent metabolic antioxidant to quench free radicals in vitro and in vivo (9,17-18). For the plasma lipid analysis, a significant reduction of TC and LDL-C were observed. This data may suggest a new lipoate activity in vivo. Both TC and LDL-C levels were maximally reduced by 4.2mg/kg of ALA supplementation. The reduced levels however were still high compared to the prediet baseline value. Following 8.0 and 10.0mg/kg, these lipid levels increased. These results suggest that ALA supplementation may increase lipid and lipoprotein regulation. The mechanism of how ALA is able to reduce LDL-C and TC concentration is unknown.

**Figure 4:** Level of apo-A and apo-B in high cholesterol diet, pretreatment (far left bars) and post-treatment of various doses of alpha-lipoic acid (ALA). Supplementation 4.2 mg/kg and 8.0 mg/kg of ALA gives a significant increase in apo-A concentration indicating ALA capable to enhance apo-A protein synthesis probably through the hepatic system although matured form HDL-C do not simultaneously increase. apo-B reduces accordingly but do not achieved statistically significant.  $p < 0.05$  was considered statistically significant; <sup>a</sup> $p < 0.05$  significant from control, ; <sup>b</sup> $p < 0.05$  significant from group A, ; <sup>c</sup> $p < 0.05$  significant from group B, ; <sup>d</sup> $p < 0.05$  significant from group C.



**Figure. 5** MDA concentration do not reveals changes pre- and post- treatment.

Probably it may be via lipoprotein lipase (LPL) activity or through cholesterol metabolism by the liver. Chiba et al. reported increased LPL activity and HDL-C level in cholesterol fed NZW rabbit after administration of NO-1886 (19). ALA probably initiates LDL receptor synthesis in the liver which in turn increases the uptake of cholesterol back to the hepatic system and increased synthesis of apoprotein A for reverse cholesterol transport (20). However, at higher doses, a reverse effect may occur (18). These data may provide information that beyond the optimum dose, ALA could be toxic to living tissue. However, the threshold concentration for this molecule to exhibit its optimum effect needs further investigations. This study also reveals that prolonged intake of high cholesterol diet may cause retention of LDL-C and TC even with supplementation of ALA. Reduction of LDL-C and TC would probably depend on the cholesterol concentration in the diet. More and more workers have now started using diets with less than 2 % cholesterol for induction of atherosclerosis. With consideration to the dose of ALA, progression of atherosclerotic disease probably could be reduced to some extent. Although the present data do not allow the conclusion that ALA supplementation could prevent atherosclerosis related free radical activity, the data are in agreement with a model in which ALA supplementation at an optimum dose

may contribute to reduction of bad cholesterol in the circulatory system. (21-24)

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