RESPONSE OF EXERCISE TRAINING ON HIGH DENSITY LIPOPROTEIN-CHOLESTEROL AND ITS SUBFRACTIONS
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Abstract:
INTRODUCTION: Low level of HDL-C is an important independent risk factor for atherosclerotic cardiovascular disease. HDL-C levels can be modified by exercise training. This is because of various enzymes involved in HDL metabolism and the interaction of that with physical activity. Endothelial lipase is one such enzyme and the beneficial effect of exercise training on increasing the levels of HDL-C and its subfractions are linked to endothelial lipase enzyme involved in HDL metabolism. OBJECTIVES: The objective is to find out the response of exercise training on HDL cholesterol and its subfraction levels.

METHODOLOGY: INTERVENTIONAL STUDY: 60 apparently healthy subjects of 30-65 years of age newly enrolled in fitness centre were selected. Baseline assessment of fasting lipid profile and HDL subfractionation was done. Subjects underwent 24 weeks of supervised aerobic exercise training. After 24 weeks, fasting lipid profile and HDL subfractionation was repeated. RESULTS: By paired t test, statistically significant difference of total cholesterol, total HDL cholesterol, its subfraction and total cholesterol HDL ratio before and after exercise training was noted (p<0.05).

CONCLUSION: There was a beneficial effect of exercise training on High density lipoprotein cholesterol and its subfraction levels and was explained by endothelial lipase enzyme involved in HDL-C metabolism.

Keyword: High Density Lipoprotein Cholesterol, Endothelial Lipase, Exercise Training.

AIMS AND OBJECTIVES: The aim is to find out the response of exercise training on HDL cholesterol and its subfraction levels.

REVIEW OF LITERATURE: HDL METABOLISM: The liver and small intestine synthesize and secrete nascent HDL as relatively small molecules. Nascent HDL consists of discoid phospholipid bilayers and free cholesterol. On the other hand, discoidal HDL and Pre HDL is also generated by apolipoproteins and phospholipids from chylomicrons and VLDL particles. LCAT and the LCAT activator, apo A1 bind to the newly formed discoidal particles. The surface phospholipid and free cholesterol in the discoidal HDL are converted into cholesteryl esters and lysocleicin by LCAT. Thus a non polar cholesteryl ester core is generated forming a spherical HDL covered by a surface film of polar lipids and apolipoproteins. The spherical HDL3 thus generated from discoidal HDL accepts cholesterol from peripheral tissues via the following: scavenger receptor B1 (SR-B1) the ATP-binding cassette transporters A1(ABCA1) and G1(ABCG1). Then the cholesterol in the surface of spherical HDL3 is esterified by LCAT, increasing the size of particles to form the less dense HDL2. HDL2 concentrations are inversely related to incidence of atherosclerosis because they reflect the efficiency of reverse cholesterol transport. HDL2 has two fates, one in which HDL3 is reformed after selective delivery of cholesteryl esters to liver via SR-B1 receptors and the other in which HDL3 is reformed after hydrolysis of HDL2 phospholipid and triacylglycerol by both hepatic and endothelial lipase. This interchange of HDL2 and HDL3 is called the HDL cycle.

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HDL METABOLISM
HDL consists of two major subfractions – HDL2 and HDL3. HDL2 is larger in size with diameter of 10 – 20 nm. It contains more lipid and has a lower density of 1.063 – 1.2512. HDL3 is smaller in size with diameter of 5 -10 (nm), contains less lipid and has a density of 1.125 – 1.210. The major apolipoprotein present in HDL2 is apo A-I with little apo A-II but HDL3 contains both apo A-I and apo A-II in appreciable quantities

ENDothelial Lipase:
Endothelial lipase, a new member of triglyceride lipase family (i.e. lipoprotein lipase, hepatic lipase and pancreatic lipase) was reported by two independent research groups in 1999. The amino acid sequence of endothelial lipase enzyme shares varying degrees of homology to lipoprotein lipase - 45%, hepatic lipase - 40%, pancreatic lipase - 27% 16. Unlike LPL and HL, this enzyme is synthesized by endothelial cells and to lesser extent in macrophages and smooth muscle cells. As a lipase, endothelial lipase has primarily phospholipase A1 activity17. Hence it has been suggested that LPL, HL and EL define a spectrum of lipase activities, with LPL acting mainly as a triglyceride lipase, EL acting almost exclusively as a phospholipase and HL exerting both triglyceridase and phospholipase activities. EL enzyme is coded by LIPG gene which was mapped to chromosome 18q21.1 by ishada et all 18. It contains 11 exons and spans 71.4 kb. LIPG gene variation was associated with variation in HDL- C levels 19-20. Also antibody inhibition of Endothelial lipase activity in mice showed a significant increase of HDL cholesterol levels 21-22. Viral vector mediated overexpression of LIPG gene in mice showed a reduction in HDL cholesterol levels 23. Hence it has been suggested that LIPG gene variation which was mapped to chromosome 18q21.1 by ishada et all 18. It contains 11 exons and spans 71.4 kb. LIPG gene variation was associated with variation in HDL- C levels 19-20. Also antibody inhibition of Endothelial lipase activity in mice showed a significant increase of HDL cholesterol levels 23. Viral vector mediated overexpression of LIPG gene in mice showed a reduction in HDL cholesterol levels 24. Thus endothelial lipase activity influences HDL metabolism.

MECHANISM OF ACTION OF ENDOTHelial LIPASE:
Endothelial lipase has high phospholipase A1 activity compared to triglyceridase activity. McCoy et al showed that the ratio of triglyceridase to phospholipase activity of endothelial lipase is 0.65 compared to the ratios for lipoprotein lipase (139.9) and hepatic lipase (24.1) 24. Thus endothelial lipase has primarily phospholipase A1 activity with minimal triglyceridase activity. Phospholipase A1 enzyme hydrolyze the sn-1 fatty acids from phospholipid substrates (phosphatidylcholine, phosphatidyserine and phosphatidyl ethanolamine). Endothelial lipase bound to heparin sulfate proteoglycan hydrolyzes HDL2 phospholipids to free fatty acids and lysosphosphatidyl choline, which enters the cells or underlying tissues. The resulting HDL3 shows reduced phospholipid content and decreased particle size. Thus endothelial lipase decreases HDL2 levels.

MECHANISM OF ACTION OF ENDothelial LIPASE EXERCISE TRAINING:
Physical inactivity increases the risk of atherosclerotic cardiovascular disease. A number of studies in adults have documented the association of reduced cardiovascular disease risk with increased physical activity. Over 50 observational studies, primarily of men, have established that physical fitness, on the job of physical activity and leisure time physical activity, have a protective effect against cardiovascular disease 26. Higher levels of physical fitness and leisure time physical activity are associated with lower risk of mortality, independent of other risk factors 26. In addition to decreasing myocardial oxygen demand and increasing myocardial efficiency and electrical stability, other potential mechanisms by which physical activity may reduce atherosclerotic cardiovascular disease risk include increasing HDL, reducing blood pressure, reducing obesity, improving insulin sensitivity, decreasing platelet aggregation and increasing fibrinolysis 26. Regular aerobic exercise have shown an increase of serum HDL cholesterol levels and reducing the cardiovascular disease risk 27. Aerobic exercise training have shown a reduction of 9% of triglyceride, 2% of total cholesterol, 2% of LDL cholesterol and increase of 3% of HDL cholesterol in men older than 18 years of age28. Exercise training showed an increase of lipoprotein lipase activity and therefore it improves HDL cholesterol and reduces triglyceride levels. Studies conducted to detect the role of endothelial lipase affecting HDL cholesterol level at baseline and in response to exercise training have suggested that exercise training attenuates the proinflammatory cytokines 29 , thereby downregulating the LIPG mRNA. LIPG gene expression is upregulated by inflammatory mediators 30-32. Further, interlukein IL-1 , tumour necrosis factor and biomechanical forces have induced EL mRNA expression in human endothelial cells 33. Mechanical forces such as shear fluid stress also increase endothelial lipase mRNA 34. Exercise training also alter endothelial shear stress thereby altering endothelial lipase mRNA 35-37. Downregulation of LIPG by exercise not only increase HDL-cholesterol levels but also modulates the local process of vascular inflammation or atherosclerosis in humans.

MATERIALS AND METHODS:
The study protocol was approved by the Institutional ethics committee of Madras Medical College, Chennai.

- INTERVENTIONAL STUDY
INCLUSION CRITERIA:
60 apparently healthy subjects of 30-65 years of age who are newly enrolled in a fitness centre was selected for the study.

EXCLUSION CRITERIA:
Subjects with history of smoking, hypertension, diabetes mellitus, BMI > 35 kg/m2 and any other acute or chronic illness are excluded.

SAMPLE COLLECTION:
5ml of venous blood sample was collected from all subjects after 10-12 hours of over night fasting in Clot activator tubes.

BASELINE ASSESSMENT:
Baseline assessment of, Fasting cholesterol, Fasting HDL2 and HDL3 subfraction.

EXERCISE TRAINING:
Subjects underwent 24 weeks of supervised aerobic exercise training consisting of atleast 3 sessions per week with each session lasting for atleast 20 – 40 minutes. The average duration of exercise training of subjects per week was 2 hours 25 minutes.

ASSESSMENT AFTER 24 WEEKS OF EXERCISE TRAINING:
After 24 weeks of exercise training, Fasting cholesterol and Fasting HDL2 and HDL3 subfractions were done for 51 subjects. Nine subjects were irregular in attending exercise training and also discontinued from fitness centre, so were excluded from the study.

FASTING LIPID PROFILE:

ESTIMATION OF PLASMA TOTAL CHOLESTEROL:
It is estimated by colorimetric enzymatic method using ERBA diagnostics Mannheim kit in merck microlab 300 semi-automated analyzer.

ESTIMATION OF PLASMA TRIGLYCERIDE:
It is estimated by colorimetric enzymatic method using ERBA diagnostics Mannheim kit in merck microlab 300 semi-automated analyzer.

ESTIMATION OF HDL-CHOLESTEROL AND ITS SUBFRACTIONS:

METHODOLOGY: It is estimated by Dual precipitation method.
adapted from the original procedure proposed by Gidetz et al 38.

ESTIMATION OF LDL CHOLESTEROL:
It is calculated by Friedewald Formula given below: LDL cholesterol = TC – (HDL + TG/5.0) mg/dl. (Provided triglyceride level was less than 400 mg/dl)

ESTIMATION OF VLDL CHOLESTEROL:
It is calculated by using the formula given below: VLDL cholesterol = Triglycerides/5 mg/dl. (Provided triglyceride level was less than 400 mg/dl)

STATISTICAL ANALYSIS:

<table>
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<tr>
<th>TABLE 1: EFFECT OF EXERCISE ON LIPID PROFILE, HDL SUBTRACTIONS AND HDL RELATED ATHEROSCLEROTIC RISK FACTOR (TC/CHOL RATIO)</th>
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<tr>
<td><strong>BASELINE MEAN</strong></td>
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<td>TOTAL CHOLESTEROL/ HDL RATIO</td>
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RESULTS:
- In table 1, data are presented as Mean ± 2 * SD. Statistical analysis was performed using Microsoft Excel worksheet. P value less than 0.05 was considered to be statistically significant.
- Table 1 shows the effect of exercise training on HDL cholesterol and its subfraction, and HDL related atherosclerotic risk factor (total cholesterol/HDL-C).
- Paired t-test was used to compare the baseline data with data after exercise.

OBSERVATION: Total cholesterol, total HDL cholesterol, HDL 3 subfraction, HDL 2 subfraction and total cholesterol/HDL ratio showed statistically significant difference between baseline data and data after exercise training.

DISCUSSION:
Lipid and lipoprotein levels can be modified by exercise training. However HDL Cholesterol levels can be influenced by genetic and environmental factors as well and also by the interaction of genetic variation with physical activity2-8. Heizmann et al and Cohen et al have previously shown that lipoprotein lipase and hepatic lipase gene variation have an influence on plasma HDL-C levels39-40. Genome wide association studies shows that LIPG gene which codes for endothelial lipase enzyme may affect HDL-C and its subfraction level8. In this study, initially 60 subjects were recruited. After 24 weeks of supervised aerobic exercise training, nine subjects were excluded from the study because they were irregular in exercise training and also discontinued from the fitness centre. Baseline as well as post exercise training blood samples were analysed for fasting HDL cholesterol and its subfractions. The results of this study shows that there is a statistically significant difference of total cholesterol, total HDL cholesterol and its subfractions before and after 24 weeks of aerobic exercise training. From observing the mean of various parameters studied, it is inferred that all parameters are decreased (except HDL-C and its subfractions which are increased) after exercise training.

After exercise training, there is a statistically significant difference of HDL cholesterol, its subfraction and total cholesterol/HDL ratio. The beneficial effects of exercise training in increasing HDL2 levels is explained by the fact that exercise decreases inflammatory cytokines like TNF- and IL-1, which inturn decreases the endothelial lipase gene expression and hence its activity, leading to increase of HDL 2 Cholesterol and total HDL-C levels.

CONCLUSION: There was a beneficial effect of exercise training on High density lipoprotein cholesterol and its subfraction levels and was explained by endothelial lipase enzyme involved in HDLC metabolism.

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