**Exercise Training Protects the LDL I Subfraction from Oxidation Susceptibility in an Aged Human Population**

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**ABSTRACT**

**Background:** Exercise training is considered an effective strategy to improve metabolic disease. Despite this, less is known regarding exercise training in the prevention and susceptibility of LDL subfraction oxidation, particularly in an aged population. **Methods:** Eleven aged (55 + 4 yrs) and twelve young (21 + 2 yrs) participants were randomly separated into an experimental or control group as follows: young exercise(*n*=6); young control (*n*=6); aged exercise (*n*=6) and aged control (*n*=5). The participants assigned to the exercise groups performed 12 weeks of moderate intensity (55-65% *V*O2max) exercise training. Venous blood was extracted at baseline, and 48 hrs following 12 weeks of exercise and assayed for a range of metabolites associated with lipid composition and lipoprotein susceptibility to oxidation. **Results:** Although there was no difference in the oxidation potential (time ½ max) of LDL I, II or III between groups at baseline (p>0.05), there was an increase in time ½ max for LDL I following exercise within the aged exercise group (*p*<0.05). Moreover, α-tocopherol concentration was selectively lower in the aged exercise group, compared to the young exercise at baseline. The lipid composition of LDL I, LDL II, LDL III, VLDL, HDL2, HDL3 and serum lipid hydroperoxides remained unchanged as a function of exercise training and aging (*p*>0.05). **Conclusion:** The primary finding of this study demonstrates that adaptations in LDL resistance to oxidation occur following 12 weeks of exercise training in the aged, and this may be of clinical significance, as oxidation of LDL has been implicated in atherosclerosis.

Keywords: Atherosclerosis, Lipid oxidation, Exercise, LDL cholesterol

**INTRODUCTION**

Oxidative stress can be defined as an imbalance between the production of pro-oxidants, such as reactive oxygen species (ROS), and their destruction, leading to macromolecular damage and disruption of redox signalling [1]. The oxidation and modification of LDL cholesterol by ROS is considered a key event in the initiation and development of fatty streaks during atherogenesis [2]. The uptake of cholesterol from oxidized LDL by macrophages occurs as an unregulated process, and is a necessary step in the generation of lipid laden foam cells, which is characteristic of fatty streaks [3].

The ageing process is associated with an increase in ROS production leading to oxidative stress [4]. Furthermore, the “oxidative stress theory” of aging proposes that a progressive accumulation of irreversible oxidative damage by ROS is a key feature of the aging process. Evidence linking aging and oxidative damage to tissues has been documented in a number of species including humans [5], while the effect of aging on the susceptibility of LDL to oxidation has been controversial.

Exercise training is regarded as an effective and non-invasive method of improving health in older subjects [6]. The link between exercise and oxidative stress has been well documented, and chronic moderate intensity exercise training has been shown to increase antioxidant defences in both healthy and diseased populations [7,8]. Furthermore, improvements in plasma and lipoprotein lipid content often seen following chronic aerobic exercise may prevent atherosclerotic development [9]. In relation, Durstine et al [10] suggests that improvements in blood lipids and lipoproteins occur with weekly energy expenditures between 1200-1500kcal, and as such an exercise intervention targeted to improve the lipid profile should be designed to meet this criteria.

There is currently a lack of research examining the effects of exercise on LDL susceptibility to oxidation, particularly within an aged population, and the reported effects of exercise training are inconclusive. Given the limited evidence, and the enhanced oxidative stress within the aged, it is feasible to hypothesise that chronic exercise training may render the LDL particle less susceptible to oxidation in an aged population. Thus, the primary aim of this study was to investigate the effects of chronic exercise training on LDL fraction oxidation and plasma lipid and lipoprotein composition in an aged population.

**METHODS and MATERIALS**

*Human subjects and experimental design*

Following ethical approval from a local Research Ethics Committee, twenty-three (n=23) apparently healthy male participants were recruited for the study (Anthropometric data is provided in table 1). Participants were recruited via a university population by email circulation and a poster campaign. Informed consent was obtained from each participant prior to the completion of a medical and exercise history questionnaire. Participants were excluded if they used multi-vitamins or antioxidants in the past 2 months, had a diagnosis of heart disease, dyslipidaemia, type 1 and type II diabetes mellitus, orthopaedic limitations and had any other health problem that may interfere with exercise. All participants were non-smokers and only participated in sporadic recreational type activity as assessed by a physical activity questionnaire.

*Experimental design*

Participants were divided into two groups classified as young (18-25yrs, *n*=12) and aged (50-65yrs, *n*=11), and further separated into an experimental or control group as follows: group 1, young exercise(*n*=6); group 2, young control (*n*=6); group 3, aged exercise (*n*=6) and group 4, aged control (*n*=5). Prior to and following exercise training, participants performed a graded maximal exercise test to assess aerobic fitness (*V*O2max). Participants were asked to refrain from exercise and alcohol consumption for 48 hours prior to all experimental testing. Blood pressure, stature, body mass and % total body fat were assessed using standard laboratory procedures before and following exercise training.

*Progressive exercise test*

The progressive maximal test enables the determination of heart rate (HR) and oxygen uptake (VO2) at maximal effort (highest workload achieved). Following a self-determined warm up and familiarisation phase, all participants performed a continuous and progressive incremental treadmill test to exhaustion. A standard protocol was employed to determine maximal oxygen uptake in young healthy subjects. The test began at a speed determined suitable for each subject, with a 1% increase in treadmill gradient at each minute until volitional exhaustion. The Modified Bruce protocol was employed to determine maximal oxygen uptake at peak effort in aged subjects. This protocol employs a slow starting speed (2.73kph) with small increments in speed (0.2kph) and/or gradient (1.2%) every 20 seconds. Oxygen uptake and heart rate was recorded during the last 5 seconds of every stage, while a heart rate corresponding to 55-65% VO2max was calculated from the VO2-HR relationship and used as an attainable HR zone during the exercise training phase.

*Exercise training programme*

The participants assigned to the exercise group performed 12 weeks of moderate intensity (55-65% VO2max) exercise training while those in the non-exercise control group maintained their normal daily lifestyle for 12 weeks. Throughout the 12 week exercise training programme, the exercising participants were required to complete 4 sessions of unsupervised aerobic exercise per week, set at an intensity of 55-65% VO2max and with a duration designed to expend 400kcal per session. This equates to a total weekly caloric expenditure of 1600kcal per participant. Caloric expenditure was calculated based on 5kcal expended per litre of oxygen consumed. The exercise generally consisted of continuous outdoor activity involving running or cycling. Heart rate (HR) training zones were calculated based on the VO2-HR relationship. All participants in the exercise groups were fully instructed on the use of the HR device (Polar Electro, Finland) with regards to monitoring their personalised target HR.

*Exercise compliance*

Participants recorded HR during each exercise session using a polar HR monitor (Polar, Kempele, Finland), and were kindly asked to record weekly exercise sessions detailing average HR, exercise duration, and mode of exercise whether treadmill walking or walking outdoors. From the data gathered, exercise frequency, duration and intensity was collated for each participant and group to determine the number of exercise bouts successfully completed throughout the intervention.

*Dietary intake assessment*

Dietary intake was assessed at weeks one, six and twelve of the intervention using a seven day unweighed diet dairy completed using a food photography atlas to determine portion size (Ministry of Agriculture Fisheries and Food, Food Portion Atlas, 1993). All dietary information was analysed using the Netwisp nutritional analysis programme (Netwisp, Version 3.0).

*Haematology*

Fasting supine venous blood was collected using the vacutainer system (Becton-Dickinson, Oxford, UK) at baseline and 48hrs following 12 weeks intervention. All laboratory tests involving blood sampling were carried out between 7am and 10am to minimise biological variation.

*Lipoprotein isolation*

Lipoproteins were isolated by density gradient ultracentrifugation according to the method of McEneny et al [11]. In brief, 1.8ml of heparinised-plasma was added to a 3ml bell-top ultracentrifuge tube and the density was adjusted to d=1.019kg/L by the addition of 0.03226g of solid potassium bromide (KBr). The plasma was then overlaid with KBr solution (d= 1.019kg/L) and a Beckman Tube Topper Sealer (Beckman Coulter, UK) was used to seal the tube. The solution was ultracentrifuged for 60 min at 100,000rpm and 4oC in a Beckman Table top Ultracentrifuge (Beckman Instruments, UK) using a Beckman fixed angle rotor (TL100.3). Following ultracentrifugation, VLDL/IDL (1.2ml) was removed by slicing the tube at 1.4cm using a Beckman tube slicer (Beckman Coulter, UK). The supernatant (VLDL/IDL) and infrantant (1.8ml LDL/HDL) were aliquoted into 2ml eppendorf tubes. To isolate LDL, 1.2ml of LDL/HDL in a 3ml ultracentrifuge tube was adjusted to d=1.090kg/L by application of 0.12276g of solid KBr. The density-adjusted solution was then overlaid with 0.6ml KBr solution (d=1.070kg/L) and overlaid to fill the tube with KBr solution (d=1.063kg/L). The tube was then sealed and ultracentrifuged for 120 mins at 100,000rpm and 4oC. Following ultracentrifugation, the ultracentrifuge tube was sliced at 1.4cm and both the supernatant (1.2ml LDL) and infranatant (1.8ml HDL/plasma proteins) were aliquoted into 2ml Eppendorf tubes. All samples were frozen at -80oC until required for further analysis.

*LDL subfractionation*

All cholesterol subfractionation was performed as outlined by McEneny et al [12]. The subfractionation of LDL into LDL I, LDL II and LDL III was achieved by three sequential ultracentrifugation steps using a 3ml polycarbonate open-top ultracentrifuge tube. 1.0ml of LDL (from the above supernatant) was adjusted to a density of 1.063 kg/L by adding 0.00283 g solid KBr. This was then overlaid with 1.0ml KBr solution (d=1.063kg/L) and 1.0ml KBr solution (d=1.035kg/L). Samples underwent ultracentrifugation for 40 min at 100,000rpm and 4oC. Following ultracentrifugation, the top 1.0ml was removed and aliquoted into 2ml Eppendorf tubes as LDL I. The remaining solution was then overlaid with 1.0ml KBr solution (d=1.045kg/L) and ultracentrifuged for 40 min at 100,000rpm and 4oC. Following ultracentrifugation, the top 1.0ml was removed and aliquoted into 2ml Eppendorf tubes as LDL II. 200µl KBr solution (d=1.30kg/L) was then pipette mixed into the remaining infranatant to adjust the density to 1.085kg/L. The solution was then overlaid with 1.0ml KBr solution (d=1.063kg/L) and ultracentrifuged for 180 min at 100,000rpm and 4oC. Following ultracentrifugation, the top 1.0ml was removed and aliquoted into 2ml Eppendorf tubes as LDL III.

*HDL subfractionation*

In a 3ml bell-top ultracentrifuge tube, 0.0972g of solid KBr was added to 1.2ml of cHDL (from the infranatant above) giving a final density of 1.125 kg/L. HDL was then overlaid with KBr solution (d=1.125kg/L) and ultracentrifuged for 120 min at 100,000rpm and 4oC. Following ultracentrifugation, 0.8mL HDL2 was recovered by tube slicing at 1.8cm and aspirated as supernatant into 2ml Eppendorf tubes. The remaining supernatant (2.2ml HDL3/plasma proteins) was then density adjusted to 1.210 kg/L by adding 0.2902g of solid KBr. The solution was then overlaid with KBr solution (d=1.210kg/L) and ultracentrifuged for 120 min at 100,000rpm and 4oC. Following ultracentrifugation, 0.8ml HDL3 was removed by tube slicing at 1.8cm and aspirated into 2ml Eppendorf tubes.

For all subfraction methodologies, the final infranatant was assessed by gel electrophoresis to ensure complete harvesting of the lipoprotein classes (results not shown).

*Lipoprotein oxidation*

This assay was performed according to the method of McDowell et al [13]. Isolated lipoprotein subfractions (50μg protein/ml) were incubated with 50 μl of 40uM CuSO4 and phosphate buffered saline (PBS) in a total volume of 1 ml and vortex mixed (final CuSO4 concentration 2uM). 300μl of sample was then pipetted in triplicate into a 96 well UV microtitre plate. The kinetics of lipoprotein oxidation was determined by observing the change in absorbance at 37 ºC in a SpectraMax-190 spectrophotometer (Molecular Devices Corp, USA). Absorbance at 234nm was recorded every 2 min over a period of 8 hours. The susceptibility of lipoprotein to oxidation was recorded as the T½max. The T½max is the point of time halfway between the lagtime and the beginning of the decomposition phase, which has a close correlation to lag time (r=0.987, p<0.001). Intra assay and inter assay CV for this method is 2% and 7.9% respectively.

*Lipid analysis*

To calculate the concentration of lipids in serum, VLDL, LDL I, LDL II, LDL III, HDL2 and HDL3 were assayed for total cholesterol (TC; Total Cholesterol Reagent: Randox, Crumlin, UK), free cholesterol (FC; Wako Free Cholesterol) and triglyceride (TG; ILab Triglycerides Reagent: ILab). The assays were performed as per manufacturer’s instructions, using an ILab Autoanalyser. The intra assay co-efficient of variation for FC, TG, and TC was 1.70%, 1.36% and 1.26%, respectively.

*Total superoxide dismutase analysis*

Total superoxide dismutase (SOD) activity was measured using the OxiSelect Assay (Cell Biolabs Inc.). The master mix was prepared using 5μl Xanthine solution, 5μl Chromagen solution, 10μl 10XSOD assay buffer and 60μl deionised water. 10μl of sample was added to 80μl of master mix in a 96 well micro-titer plate. A blank was created using 10μl of deionised water and 80μl of master mix. 10μl of 1X Xanthine Oxidase solution was added to each well and pipette mixed. Samples were incubated at room temperature in the dark for 1hr. The absorbance was recorded at 490nm on a BioTek EL808 (USA) spectrophotometer. Absorbance against a standard curve in the range of 0-5U/μl was recorded and the concentration of SOD was subsequently determined. Intra-assay CV was 5.9%.

*Lipid hydroperoxides analysis*

Total serum hydroperoxide concentration was measured using the FOX1 assay as described by Wolff [14]. FOX1 reagent was prepared by dissolving 0.007606g xylenol orange, 0.0098g Ammonia ferrous sulphate, 1.822g Sorbitol, 0.137ml sulphuric acid in a 100ml solution using HPLC-grade H2O. The final FOX1 reagent comprised of 100µM Xylenol orange, 250µM ammonium ferrous sulphate, 100mM Sorbitol and 25mM Sulphuric Acid. 50µl of serum sample was added to 900µl of FOX1 reagent. A blank was created using 50µl of deionised water and 900µl of FOX1 reagent. Samples were vortexed and incubated at room temperature in the dark for 30minutes. The spectrophotometer was autozeroed using a blank, and the absorbance recorded at 560nm using a UVmini-1240 spectrophotometer (Shimadzu, China). Absorbance against a standard curve of H202 in the range of 0-5uM was recorded. Intra-assay CV was 15.2% and inter-assay CV was 16%.

*Serum lipid soluble antioxidant analysis*

Serum α-tocopherol, γ-tocopherol, retinol, β-carotene, α-carotene and lycopene were determined using High Performance Liquid Chromatography (HPLC) according to Thurnham et al [15]. Tocopherol acetate as an internal standard was produced by the addition of 1g of tocopherol acetate to 100ml heptane. 1ml of tocopherol actetate/heptane solution was added to 249ml ethanol to create a 250ml internal standard solution. Serum samples (200µl) were mixed with 100µl 10mM sodium dodecyl sulphate (SDS) in a glass tube followed by the addition of 200µl of internal standard solution and 500µl heptane containing 0.5g/l of BHT. Each sample was mixed vigorously and centrifuged at 2000rpm for 5min to allow separation of organic and aqueous phases. 350µl of the heptane supernatant was transferred to a glass tube. A further 500µl of heptane was added to the infranatant, vortexed and centrifuged at 2000rpm for 5min. 350µl of supernatant was removed and combined with 350µl of the supernatant previously extracted. The 750µl of combined supernatant underwent evaporation for 2hrs. The residue was reconstituted with 150µl of mobile phase (750ml acetonitrile, 200ml methanol, 50mls dichloromethane and 500mg butylated hydroxytoluene (BHT)), vortexed and stored in the dark until ready for analysis. During analysis, samples were combined with the mobile phase at a flow rate of 1ml/min at 21oC. The UV detector was set at 325nm and 450nm to detect retinol and carotenoids respectively. Data was analysed by Empower software. Intra- and inter-assay CV was <5%.

*Statistical analysis*

The method of Altman [16] was used to determine the number of subjects (n=28, excluding a 20% drop out rate) required for this study (using previously published lipid data, [17]). Data were analysed using parametric statistics following confirmation of a normal distribution by Shapiro Wilks tests. Between (group; young control, young exercise, aged control, aged exercise) and within subject factor changes (exercise; pre vs. post 12 week intervention) were determined using a 2-way repeated measures ANOVA. Following a significant interaction effect (exercise x group), within subjects factors were analysed using a Bonferroni corrected paired samples t-tests, while the between subject differences were analysed using a one-way ANOVA with a posterior Tukey honestly significance difference test. The alpha was established at P<0.05 and all values are expressed as mean ± SD unless stated otherwise.

**EXPERIMENTAL RESULTS**

*Participant characteristics*

There was a difference in age, body mass index (BMI), % body fat and aerobic fitness between groups (*p*<0.05) as indicated in Table 1. Maximal aerobic capacity increased in both young and aged groups following exercise training (*p*<0.05), while no change was observed in the control groups (Table 2. *p*<0.05).

*Serum lipid concentration*

There was no difference in baseline serum lipid concentration between young and aged groups (*p*>0.05), and also no change in total cholesterol, free cholesterol and serum triglycerides following exercise training in either group (Table 3. *p*>0.05 for all comparisons).

*Lipoprotein lipid composition*

The concentration of lipid components in VLDL, HDL2, HDL3 (data not shown) and in LDL I, LDL II and LDL III (Table 4) were not different between young and aged groups, nor were there any changes observed following exercise training (*p*>0.05 for all comparisons).

*Oxidation of LDL subfractions*

Although there was no difference in the oxidation potential (time ½ max) of LDL I, II or III between groups at baseline (p>0.05), there was an increase in time ½ max for LDL 1 following exercise training in the aged exercise group only (Figure 1, *p*<0.05). There was no difference between or within groups for LDL II and III following exercise training (*p*>0.05, data not shown).

*Lipid soluble antioxidants, Superoxide dismutase and lipid hydroperoxides*

α-Tocopherol was selectively lower in the aged exercise group, compared to the young exercise group at baseline (Table 5, *p*<0.05), with no changes observed following exercise training for any lipid soluble antioxidant within both groups (*p*>0.05). Superoxide dismutase or lipid hydroperoxides did not differ between groups or as a function of exercise training (Table 5, *p*>0.05).

*Exercise compliance and dietary intake*

Adherence to exercise training related to 97% of participants completing an average of 46 of the prescribed 48 exercise sessions, and this was identical for both the young and aged exercising groups. There was no change in mean weekly dietary intake for total energy (kcal) and nutrients through exercise training (data not shown, *p*>0.05)

**DISCUSSION**

The principle aim of this study was to investigate the effect of chronic exercise training on LDL fraction oxidation and plasma lipid and lipoprotein composition in an aged population. The major finding of our work shows that 12 weeks of moderate intensity exercise training improves the resistance of LDL I to oxidation in an aging population. This is the first study to examine the effects of 12 weeks of exercise training on the susceptibility of LDL subfractions to oxidation in both the young and aged.

Previous work by Ziegler et al [18] has examined the effects of 8 weeks (twice weekly) moderate intensity exercise on *in vitro* LDL oxidisability in 13 middle aged men and women with coronary artery disease, and showed an improvement in LDL oxidation rate following exercise training. Similarly, Elosua et al [19] found an increase in the resistance of LDL to oxidation in young subjects following moderate intensity exercise training. Although our study found no change in LDL II or LDL III resistance to oxidation, we did however observe an improvement in LDL I susceptibility to oxidation in the aged subjects following exercise training. It has previously been demonstrated that individuals partaking in regular exercise have greater amounts of LDL I particles in comparison to sedentary individuals. This is thought to occur as a result of an increase in lipoprotein lipase activity (LPL), which acts to hydrolyse triglycerides within LDL, generating a less dense, more buoyant LDL molecule [20]. Trained athletes have higher LPL activity at rest, while LPL activity can often increase after a single bout of exercise [21,22]. This suggests the increase in resistance to oxidation in LDL I, as opposed to no change in either of the LDL II or LDL III subfractions might reflect an exercise-induced increase in LDL I particle number due to an up-regulation in LPL. However, this is speculative as measurements of LDL oxidisability only reflect LDL composition and its environment, and do not take into account particle number given that LDL concentration is standardized prior to oxidation during the analysis process.

LDL susceptibility to oxidation is influenced by several factors including LDL composition and density [23]. As such, a weekly caloric expenditure of 1600 kcal was chosen based on improvements in lipoprotein composition observed with weekly expenditures greater than 1000 kcal [10]. Most studies report an increase in HDL-C following chronic aerobic exercise [24]. The chronic exercise effect on LDL-C is less convincing with studies showing both a decrease [25] and no change in concentration [26]. Elosua et al [19] found no change in LDL-C or HDL-C after 16 weeks of moderate intensity exercise, despite an increase in the resistance of LDL to oxidation. In the present study, no adjustments were made to HR training intensity during the course of the 12 weeks to reflect changes in aerobic capacity. Both the young and aged participating in exercise significantly increased maximal aerobic capacity following chronic exercise, therefore, it cannot be determined accurately whether subjects were expending 400kcal per session at an intensity of between 55-65% VO2max. Exercise training interventions lasting 3 to 6 months have been reported to improve VO2max by as much as 36%, particularly in coronary artery disease (CAD) patients [27]. In this study, VO2max increased by 10% and 14% in young and aged exercise groups respectively. Despite no significant change in body mass in either group, it can be postulated that the observed increase in VO2max induced by exercise training is related to changes in cardiovascular and skeletal muscle function. These adaptations may include changes in stroke volume and skeletal muscle capillary and mitochondrial density leading to enhanced oxygen delivery and utilization [28].

The absence of change in LDL lipid composition despite a decrease in LDL I susceptibility to oxidation in the aged subjects following chronic exercise training, suggest that alternative mechanisms may exist to explain the improvement in the resistance of LDL I to oxidation. Despite no decrease in lipid hydroperoxides, a change that would reflect a decrease in free radical attack on LDL lipids, there are additional factors that were not measured within the present study that include LDL endogenous antioxidants, which may contribute to the observed change in LDL I oxidisability. In fact, Parks et al [29] demonstrated that a reduction in LDL susceptibility following 12 weeks of moderate intensity (60-70% HRmax) exercise (20min/d, 5d/week) and diet control (10% fat content) was attributed to an increase in LDL α-tocopherol content. α-tocopherol acts as a chain breaking antioxidant by trapping peroxyl radicals [30] and supplementation with α-tocopherol has been shown to decrease the susceptibility of LDL to oxidation [31]. Enhanced circulating lipophilic and lipophobic antioxidants may also offer the LDL particle additional protection. For example, antioxidant compounds in foods consumed by the participants as part of their normal dietary intake may have contributed towards a protective effect. Physiological concentrations of ascorbic acid, ubiquinol-10 and β-carotene inhibit LDL oxidation *in vitro* [32], and α-tocopherol and ascorbic acid can function together in a cyclic process. Ascorbic acid acts to replenish α-tocopherol by reducing α-tocopheryl radicals that were formed following α-tocopherol’s role in donating a labile hydrogen to a lipid or lipid peroxyl radical [33]. In this study,there was no increase in plasma antioxidants, including the tocopherols, carotenoids, retinol and lycopene as a result of exercise in any group. Plasma α-tocopherol content was significantly lower at baseline in the aged exercise, in comparison to their young exercised counterparts. This may be a consequence of insufficient dietary intake given the correlation of α-tocopherol with diet in aged subjects [34]. Dietary analysis found no difference in dietary intake of the main macromolecules throughout the intervention, however, the dietary analysis software used in this study was not descriptive enough to provide quantities of antioxidant intake, therefore, it remains a possibility that intake of other food derived antioxidants, which were not measured, may have had a protective effect.

Some chronic exercise based studies within the literature, like those previously cited [18, 19] have focused solely on LDL as a homogenous molecule. However, it has been shown that LDL is a heterogeneous particle existing as a set of subspecies [35] LDL subfractions (LDL I, LDL II and LDL III) differ in their antioxidant content and Tribble et al [36] showed that the larger LDL I subfraction contains more α-tocopherol and ubiquinol-10 than the smaller more dense LDL III particle, therefore, offering greater resistance to oxidation in the larger LDL particles. This study was limited by the absence of LDL subfraction antioxidant content as previously indicated, so any measurement of lipid antioxidant function may offer a more conclusive explanation for the increase in the resistance of LDL I to oxidation in the aged exercise following exercise training as opposed to no change in LDL II and LDL III oxidative resistance.

The oxidative resistance of LDL is also influenced indirectly by antioxidant enzymes that catalyze the conversion of ROS to a less reactive or inert species. An increase in antioxidant enzyme activity has been observed in animal models following exercise, however, to date, the effect of chronic exercise in humans remains controversial. Trained subjects have often demonstrated a higher concentration of antioxidant enzymes in comparison to the untrained [37]. We found no change in SOD in any group following exercise training. Similarly, Elosua et al [19] found no change in SOD following 16 weeks of moderate intensity aerobic training although an increase in both glutathione peroxidase and reduced glutathione were noted. This may be due to the repeated production of free radicals, as a result of chronic exercise, inducing gene expression of glutathione peroxidase [38]. Nuclear erythroid 2 p45-related factor 2 (Nrf2) has been implicated in the increased transcription of a number of antioxidant genes including SOD3 [39]. Furthermore, acute exercise induced oxidative stress has been shown to increase the activation of Nrf2 [40]. As such, the chronic stimulus in this study may not have been sufficient to stimulate the activation of Nrf2 and the subsequent up-regulation of SOD gene expression. Future research may benefit from the quantification of other antioxidant enzymes including glutathione peroxidase and catalase.

Although the aged exercise group in the current study showed an increase in the resistance of LDL I to oxidation, this was not the case in the young. This may be due to the duration and frequency of the training phase adopted. The duration of Elosua et al’s [19] study was 16 weeks in length with a frequency and duration of exercise increasing periodically from four 30 minute bouts per week to five 50 minute bouts per week after 8 weeks. Another possible reason for the lack of change may be as a consequence of limited statistical power. Following statistical analysis the observed retrospective power of LDL I susceptibility to oxidation was 0.61, suggesting that a greater number of young participants are required for a change in LDL I susceptibility to oxidation to occur following exercise training. That said, there was sufficient power to detect a significant difference in LDL I oxidative susceptibility in the aged following exercise training.

**CONCLUSION**

This study demonstrates that adaptations in LDL resistance to oxidation occur following 12 weeks of exercise training in an aged human population. It is well known that the uptake of cholesterol from oxidized LDL by macrophages is a necessary step in the generation of lipid laden foam cells, which is characteristic of fatty streaks, and as such, the findings of our work is of significant clinical interest as exercise training can decrease the susceptibility of LDL oxidation and thus inhibit the development of atherosclerosis.Moreover,it is highly recommended that future exercise and clinically related studies focus on LDL subfractions rather than LDL as a homogeneous particle. Areas of potential influence of exercise on LDL oxidation that warrant investigation include LDL antioxidant content and capacity.

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Table 1. Participant characteristics

|  |  |  |
| --- | --- | --- |
|  | Young  (*n*=12) | Aged  (*n*=11) |
|  |  |  |
| Age (years) | 21 ±2 | 55±3† |
| Stature (m) | 1.80±0.8 | 1.76±0.6 |
| Body Mass (kg) | 78.4±10.7 | 86.3±10.5 |
| BMI (kg.m-2) | 24.0±2.0 | 27.7±2.4 |
| Body fat (%) | 15.5±2.5 | 25.0±2.9† |
| VO2max (ml.kg-1.min-1) | 48±7 | 35±5† |
|  |  |  |

BMI, body mass index. †Significant difference between groups (*p*<0.05).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *Young control* | | *Young exercise* | | *Aged control* | | *Aged exercise* | |
|  | Pre | *Post* | *Pre* | *Post* | *Pre* | *Post* | *Pre* | *Post* |
| Body mass (kg) | 76.6±13.2 | 76.1±14.3 | 80.2±8.6 | 81.3±9.4 | 82.7±10.9 | 82.4±10.9 | 89.4±10.2 | 87.1±9.1 |
| BMI (kg.m-2) | 24.1±2.5 | 23.9±2.8 | 24.0±1.8 | 24.3±2.1 | 27.9±2.6 | 27.8±2.8 | 27.6±2.5 | 26.9±2.2 |
| Body fat (%) | 16.2±2.2 | 18.4±2.9 | 15.0±3.0 | 15.6±3.0 | 25.9±1.8 | 25.2±2.4 | 24.4±3.6 | 23.8±3.0 |
| Systolic BP  (mmHg) | 139±7 | 136±15 | 142±11 | 135±11 | 131±11 | 132±11 | 134±17 | 128±11 |
| DiastolicBP (mmHg) | 81±9 | 77±7 | 74±12 | 72±7 | 81±8 | 80±7 | 81±12 | 82±6 |
| VO2max  (ml.kg-1.min-1) | 47±9 | 47±9 | 50±6 | 57±7\* | 34±7 | 37±7 | 36±3 | 40±4\* |

Table 2 Anthropometric and physiological indices at baseline and following exercise training for young and aged groups

All data are mean (±SD). BMI; body mass index, BP; blood pressure. \*difference between pre and post training (*p*<0.05).

Table 3. Serum lipid and oxidative stress indices at baseline and following exercise training for young and aged groups

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *Young control* | | *Young exercise* | | *Aged control* | | *Aged exercise* | |
|  | *Pre* | *Post* | *Pre* | *Post* | *Pre* | *Post* | *Pre* | *Post* |
| TC | 4.63±0.93 | 4.43±1.20 | 4.15±1.02 | 4.30±1.14 | 5.90±1.47 | 5.84±0.92 | 5.09±0.74 | 5.27±1.17 |
| FC | 1.22±0.25 | 1.27±0.18 | 1.18±0.23 | 1.09±0.30 | 1.56±0.40 | 1.54±0.23 | 1.37±0.16 | 1.43±0.29 |
| TG | 1.52±1.10 | 1.92±0.74 | 1.26±0.57 | 0.89±0.37 | 1.46±0.84 | 1.67±0.51 | 1.33±0.47 | 1.20±0.30 |
| LOOH | 1.03±0.25 | 1.06±0.13 | 1.08±0.28 | 0.97±0.30 | 1.17±0.26 | 0.87±0.13 | 0.91±0.17 | 0.80±0.17 |
| SOD | 0.25±0.05 | 0.25±0.04 | 0.25±0.06 | 0.23±0.06 | 0.27±0.14 | 0.30±0.12 | 0.33±0.11 | 0.31±0.11 |

FC; free cholesterol, TC; total cholesterol, TG; triglycerides, LOOH; lipid hydroperoxide, SOD; superoxide dismutase.

All indices expressed as mmol/L except for SOD in U/μl.

Table 4. LDL subfraction lipid composition at baseline and following exercise training for young and aged groups

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *Young control* | | *Young exercise* | | *Aged control* | | *Aged exercise* | |
|  | *Pre* | *Post* | *Pre* | *Post* | *Pre* | *Post* | *Pre* | *Post* |
| LDL I TC | 1.23±0.53 | 1.96±0.91 | 1.01±0.35 | 1.82±0.91 | 2.31±1.19 | 3.12±0.98 | 1.23±0.28 | 1.68±0.91 |
| FC | 0.46±0.14 | 0.49±0.18 | 0.42±0.18 | 0.70±0.39 | 0.77±0.25 | 0.89±0.35 | 0.56±0.21 | 0.49±0.32 |
| TG | 0.11±0.04 | 0.14±0.04 | 0.07±0.04 | 0.11±0.04 | 0.18±0.11 | 0.21±0.07 | 1.05±0.04 | 0.18±1.05 |
| LDL II TC | 0.63±0.11 | 0.74±0.28 | 0.60±0.32 | 0.63±0.21 | 0.98±0.63 | 1.12±0.56 | 0.74±0.28 | 0.67±0.21 |
| FC | 0.28±0.07 | 0.28±0.11 | 0.25±0.11 | 0.32±0.11 | 0.25±0.21 | 0.42±0.18 | 0.25±0.14 | 0.14±0.04 |
| TG | 0.07±0.02 | 0.07±0.04 | 0.07±0.04 | 0.07±0.04 | 0.07±0.07 | 0.04±0.07 | 0.07±0.04 | 0.04±0.02 |
| LDL III TC | 0.67±0.25 | 0.56±0.14 | 0.95±0.81 | 0.56±0.32 | 1.37±1.05 | 1.02±0.70 | 0.67±0.35 | 0.74±0.35 |
| FC | 0.25±0.11 | 0.21±0.07 | 0.35±0.28 | 0.28±0.14 | 0.28±0.25 | 0.25±0.21 | 0.18±0.11 | 0.14±0.04 |
| TG | 0.07±0.04 | 0.07±0.04 | 0.07±0.04 | 0.07±0.04 | 0.11±0.07 | 0.07±0.04 | 0.07±0.04 | 0.07±0.04 |

All indices expressed as mmol/L

Table 5. Serum antioxidants at baseline and following exercise training for young and aged groups

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *Young control* | | *Young exercise* | | *Aged control* | | *Aged exercise* | |
|  | *Pre* | *Post* | *Pre* | *Post* | *Pre* | *Post* | *Pre* | *Post* |
| α-Tocopherol | 15.42±3.35 | 13.49±2.93 | 16.38±4.27 | 14.33±2.11 | 11.66±3.59 | 10.64±1.17 | 9.43±4.82**‡** | 12.99±2.10 |
| γ-Tocopherol | 2.24±0.78 | 1.74±0.32 | 2.03±0.32 | 2.14±1.10 | 1.51±0.43 | 1.52±0.21 | 1.31±0.76 | 1.68±0.50 |
| α-Carotene | 0.02±0.04 | 0.01±0.01 | 0.02±0.04 | 0.01±0.01 | 0.02±0.04 | 0.04±0.04 | 0.00±0.00 | 0.00±0.00 |
| β-Carotene | 0.03±0.06 | 0.01±0.01 | 0.02±0.02 | 0.02±0.05 | 0.02±0.04 | 0.01±0.01 | 0.00±0.00 | 0.01±0.00 |
| Retinol | 0.06±0.04 | 0.07±0.01 | 0.08±0.03 | 0.07±0.03 | 0.05±0.04 | 0.07±0.04 | 0.05±0.05 | 0.05±0.04 |
| Lycopene | 0.03±0.02 | 0.03±0.01 | 0.03±0.02 | 0.05±0.08 | 0.02±0.02 | 0.04±0.03 | 0.03±0.03 | 0.04±0.02 |

**‡** difference between groups. All indices expressed as μmol/L.

**Titles and Legends**

*Supplementary Figure 1:*

Title: Oxidation potential of LDL I at baseline and following 12 weeks of exercise training in young and aged participants.

Values are means ±SD. YC; Young Control, YE; Young Exercise, AC; Aged Control, AE; Aged Exercise. \*difference between baseline and post training (P<0.05).