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ORIGINAL RESEARCH

Moderate- and High-Intensity Exercise Improves Lipoprotein Profile and Cholesterol Efflux Capacity in Healthy Young Men

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BACKGROUND: Exercise is associated with a reduced risk of cardiovascular disease. Increased high-density lipoprotein cholesterol (HDL-C) levels are thought to contribute to these benefits, but much of the research in this area has been limited by lack of well-controlled subject selection and exercise interventions. We sought to study the effect of moderate and high-intensity exercise on HDL function, lipid/lipoprotein profile, and other cardiometabolic parameters in a homogeneous population where exercise, daily routine, sleep patterns, and living conditions were carefully controlled.

METHODS AND RESULTS: Male Army recruits (n=115, age 22±0.3 years) completed a 12-week moderate-intensity exercise program. A subset of 51 subsequently completed a 15-week high-intensity exercise program. Fitness increased and body fat decreased after moderate- and high-intensity exercise (P<0.001). Moderate-intensity exercise increased HDL-C and apolipoprotein A-I levels (6.6%, 11.6% respectively), and decreased low-density lipoprotein cholesterol and apolipoprotein B levels (7.2%, 4.9% respectively) (all P<0.01). HDL-C and apolipoprotein A-I levels further increased by 8.2% (P<0.001) and 6.3% (P<0.05) after high-intensity exercise. Moderate-intensity exercise increased ABCA-1 (ATP-binding cassette transporter A1) mediated cholesterol efflux by 13.5% (P<0.001), which was sustained after high-intensity exercise. In a selected subset the ability of HDLs to inhibit ICAM-1 (intercellular adhesion molecule-1) expression decreased after the high (P<0.001) but not the moderate-intensity exercise program.

CONCLUSIONS: When controlling for exercise patterns, diet, and sleep, moderate-intensity exercise improved HDL function, lipid/lipoprotein profile, fitness, and body composition. A sequential moderate followed by high-intensity exercise program showed sustained or incremental benefits in these parameters. Improved HDL function may be part of the mechanism by which exercise reduces cardiovascular disease risk.

Key Words: ATP-binding cassette transporters ■ cholesterol ■ cholesterol efflux capacity ■ exercise ■ HDL-C

Exercise has been shown to reduce overall mortality and cardiovascular disease (CVD) risk, in a dose-dependent manner.[1–3] However, the mechanisms by which CVD risk is reduced by exercise have not been fully elucidated. Also, the optimal type, volume, and intensity of exercise have not been clearly determined for CVD risk reduction.

Exercise has consistently been shown to increase high-density lipoprotein cholesterol (HDL-C) levels, and HDL-C levels have been shown in large-scale epidemiologic studies to be inversely related to CVD risk.[4–6] However, the effect of exercise on other lipids and lipoproteins is less consistent.[4,7,8] Despite the strength of the epidemiologic data, and the beneficial effects of

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CLINICAL PERSPECTIVE

What Is New?

- This is the first study to show that well-controlled moderate-intensity exercise, and sequential moderate- and high-intensity exercise regimes plus uniform external variables are associated with enhanced high-density lipoprotein function and an improved cardiometabolic profile in healthy young men.

What Are the Clinical Implications?

- A moderate-intensity exercise program may be important for primary prevention in healthy young men.
- Most of the beneficial cardiometabolic effects were attained after a 12-week moderate-intensity exercise program; there was only minimal additional benefit from higher intensity exercise.
- Improved high-density lipoprotein function may be part of the mechanism by which exercise reduces cardiovascular disease risk.

Nonstandard Abbreviations and Acronyms

HCAEC	human coronary artery endothelial cells
HOMA-IR	homeostatic model assessment of insulin resistance
ICAM	intercellular adhesion molecule
TC	total cholesterol

increasing HDL-C levels in animal studies, large-scale clinical outcome trials of effective HDL-raising therapies have not reduced CVD events.[9] In light of this, attention has shifted away from using HDL-C to reduce CVD risk toward strategies with the potential to improve HDL function. HDLs have several putative atheroprotective functions.[5] They accept the cholesterol that effluxes from macrophages in the artery wall in the first step of reverse cholesterol transport. Cholesterol efflux is inversely associated with cardiovascular events independent of HDL-C level, HDL size, and traditional cardiovascular risk factors and may underlie at least part of the cardioprotective effects of HDLs.[10] HDLs also have anti-inflammatory and antioxidant properties and have been shown to inhibit monocyte activation and proinflammatory cytokine and chemokine synthesis.[11–13] However, knowledge about the effects of exercise on cholesterol efflux and the other cardioprotective functions of HDLs is limited.[14]

In addition to its ability to increase HDL-C, several studies have indicated that exercise is associated with lower levels of circulating markers of inflammation and decreased CVD risk.[15–18] More recently, the CANTOS study (Canakinumab Antiinflammatory Thrombosis Outcome Study) established that inhibiting systemic inflammation was causally related to decreased cardiovascular events in people with prior myocardial infarction.[19] However, the effects of exercise on markers of systemic inflammation in healthy populations are inconsistent.[15,20–22] Exercise does, however, decrease inflammation in endothelial cells in patients with established cardiovascular risk factors, but the effect on healthy individuals is not known.[23] In contrast to the chronic effects of exercise, acute endurance exercise has been shown to increase CRP (C-reactive protein) and inflammatory cytokine levels. These inconsistent results may be a reflection of heterogeneity in the study populations and poor control of external factors such as diet, exercise, and sleep patterns.[20,24]

These limitations were overcome in the present study by investigating the effect of a moderate exercise and a sequential moderate- and high-intensity exercise program on serum lipids and lipoproteins, HDL function, and key inflammatory and metabolic parameters in a healthy, homogeneous, population of male army recruits where the exercise program, daily schedule, living conditions, and sleep pattern were tightly controlled and dietary choices were limited.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient Population

Male Australian Regular Army recruits from all specializations were invited to voluntarily participate in the study at the start of their 12-week Army Recruit Course; ≈400 eligible recruits were invited to participate in the study. Study enrollment capacity of 140 was met. Of those who were enrolled, 115 completed the recruit course. Twenty-five soldiers did not complete the course because of voluntary discharge from the army, injury, or illness. As volunteer numbers exceeded study capacity, priority was given to those intending to become infantry soldiers because they were eligible to participate in both the moderate- and high-intensity program. Remaining participants were selected randomly.

As part of this course, each participant undertook a moderate-intensity, mixed strength and endurance exercise program. Each participant was assessed at baseline and at the end of the program. A subset of 51

subjects who were enlisted as infantry soldiers subsequently completed a 15-week higher intensity mixed strength and endurance exercise program immediately after the initial exercise program as part of their Initial Employment Training (Figure S1). These subjects were reassessed at the end of the high-intensity program.

Inclusion criteria included male sex, age > 18 years and <35 years, meeting the Army's initial entry medical and fitness standards, and completing the recruit course. Standard medical requirements included no significant medical disease including cardiovascular disease, hypertension, and diabetes. Hence, the absolute cardiovascular risk in this healthy young cohort is low. Entry fitness tests included achieving 15 pushups within 2 minutes, 45 situps to a cadence (1 repetition every 3 seconds), and a level of 7.5 on the multistage fitness test. The multistage fitness test is a 20-m shuttle run test that has been validated previously as an accurate assessment of aerobic fitness.[25] The test consists of shuttle running between 2 markers placed 20 m apart at increasing speeds. The results of this test have been validated as a reliable means to convert to an estimated VO_2 max.[25] Achieving level 7.5 on the beep test requires average fitness. Participants with evidence of an active infection during testing were excluded.

All participants lived at the army base throughout the study, participated in the same daily schedule and sleep routine, and ate from a set number of food options during set meal timings, from the same kitchen. Because of the controlled nature of the group, there was close to 100% compliance with the program. All aspects of the physical activity training were supervised, and soldiers were required to participate in all parts of the program in order to complete the course. The only reason for missing an exercise session was acute illness or injury. At each phase of the study, participants underwent fasting blood tests, a fitness test, anthropometry, and blood pressure recording.

This study was approved by Departments of Defence and Veterans' Affairs Human Research Ethics Committee, protocol number 767-14. All participants gave written informed consent to participate in the study and the study conforms to the principles outlined in the Declaration of Helsinki.

Exercise Program

The exercise programs consisted of a structured military mixed strength and endurance physical fitness training program, as well as occupational-specific activities that were consistent for each participant. The fitness program incorporated strength-based circuit training, run sessions (set distance middle distance runs, interval and farklet sessions), and swim sessions. Most sessions were 1 hour long. Occupational-specific activities included graded endurance marches with graded load bearing (pack marching), obstacle courses, ropes courses, carrying heavy equipment/stretches, and combat drills. The full details of the exercise program are the property of the Army and cannot be documented in detail. The majority of the occupational-specific activities were done as a group and were performed at the same pace within groups. The programs were set and run by qualified Army physical training instructors.

The exercise dose was controlled as were the frequency and type of exercise performed. The dose was controlled for as either the duration of exercise or the distance of an activity was kept constant between participants. Given the nature of the program and inherent individual variability, exercise intensity could not be completely controlled. However, all participants completed the same activities in a group environment with a physical training instructor giving set instructions to guide the level of intensity required (ie, number of repetitions) for circuits and level of intensity for farklet training (ie, 70% maximal speed). The dose and intensity of exercise gradually increased throughout both programs.

The intensity of each physical activity session was estimated by conversion to metabolic equivalents of task (METs) based on the 2011 Compendium of Physical Activity as a means to determine which activities in the program fell into the moderate- or high-intensity category.[26] Moderate-intensity training was classified as physical training activities estimated to be between 4.3 and 6 METs, and high-intensity activities were >6 METs.

Moderate-Intensity Exercise Program

The 12-week moderate-intensity exercise program was part of the Army Recruit Course. It is a mixed strength and endurance program with ≈75% of the activities being combined strength and endurance activities (endurance activity with weighted load, ie, pack marching); ≈10% of the program could be classified as solely endurance focused (running sessions) and <15% could be classified as predominantly strength focused (ie, weight-based circuits). The average total physical activity time/wk was ≈9.3 hours, or 1.3 h/d. The average METs for each activity was 5.9, indicating moderate-intensity. This is a graded exercise program with increasing intensity (ie, increasing intensity of circuits, progressive increase in distance traveled, progressive increase in weights/load carried and speed) as well as increasing volume (progressive increase in distance for load bearing and nonload bearing endurance activities). The majority of the activities were done within a group at the same pace and with the same load bearing.

The program culminated in a final moderate- to high-intensity physical challenge that consisted of ≈6.7 hours of physical activity. The activity consisted of ≈160 minutes of moderate intensity and 240 minutes of high intensity with an average METS of 6.5.

High-Intensity Exercise Program

The high-intensity program consisted of a 15-week physical training program that was part of the Army's Infantry Initial Employment Training. It is a mixed strength and endurance program with ≈90% of the activities being combined strength and endurance activities (endurance activity with weighted load, ie, pack marching), ≈5% of the program could be classified as solely endurance focused (running sessions), and 5% could be classified as predominantly strength focused (ie, weight-based circuits). The average total physical activity time per week was ≈14.1 h/wk, or 2 h/d. The average METs for each activity was 7.5, indicating high intensity. As with the moderate-intensity program this is a graded exercise program with one of the core components being increasing intensity (eg, increasing intensity of circuits, progressive increase in weights/load carried and speed) as well as increasing volume (progressive increase in distance for load bearing and nonload bearing endurance activities). The intensity and volume of exercise were higher in this program compared with the moderate-intensity program. The majority of the activities were done within a group at the same pace and with the same load bearing.

The program culminated in a final high-intensity physical challenge that consisted of ≈7.1 hours of physical activity. The entire activity was high intensity (>6 METs) and the average METs was 8.7 METs.

Fitness Testing

A multistage fitness test (20-m shuttle run) at baseline and at the end of each phase of training was conducted.[25] The test consists of shuttle running between 2 markers placed 20 m apart at increasing speeds (using prerecorded "beeps" at decreasing intervals).[25] All participants were required to pass this test before enlistment and hence were familiar with the format. The level achieved was converted to distance in meters as well as an estimated VO_2 max as validated in previous studies.[25] All tests were conducted by Army physical training instructors in a standardized format.

Anthropometry and Clinical Parameters

At baseline and at the end of each exercise program height, weight, heart rate, and blood pressure were recorded. Manual blood pressure was recorded by a nurse or a qualified allied health practitioner. The average of 2 sitting readings with an interval of 2 minutes was recorded. Body fat percentage was calculated using a Harpenden skinfold caliper and the 4-site system validated by Durnin and Wormersley.[27]

Blood Collection and Biochemical Analyses

Fasting blood (35 mL) was collected at baseline and at 2 days after the moderate- and high-intensity exercise programs. An aliquot (20 mL) of each specimen was sent to an accredited commercial laboratory (Laverty Pathology, NSW, Australia) for quantification of HDL-C, low-density lipoprotein cholesterol (LDL-C), triglycerides, total cholesterol (TC), apoB (apolipoprotein B), apoA-I (apolipoprotein A-I), hsCRP (high-sensitivity CRP), insulin, and blood glucose levels. LDL-C was calculated using the Friedewald formula.[28] Plasma was isolated from the remaining blood, aliquoted, and stored at −80 °C until further analysis.

HDL Isolation and Preparation of apoB-Depleted Serum

HDLs were isolated from plasma by sequential ultracentrifugation (1.063<d<1.210 g/mL) and dialyzed against endotoxin-free PBS (10 mmol/L phosphate buffer, 2.7 mmol/L KCl, 137 mmol/L NaCl [pH 7.4]), sterile filtered (0.22 µm filter, Merck Millipore, Billerica, MA), and analyzed within 48 hours. For cholesterol efflux, EDTA plasma was coagulated with 1 M $CaCl_2$ (1:40 (v/v)) for 2 hours at 37 °C, then centrifuged (2000*g*, 30 minutes). Serum was collected and depleted of apoB-containing lipoproteins with PEG-6000.[29]

Determination of Lipid and Apolipoprotein Concentrations in Plasma, apoB-Depleted Serum, and Isolated HDLs

ApoA-I and apoA-II concentrations were determined immunoturbidometrically using sheep antihuman apoA-I and apoA-II polyclonal antibodies.[30] TC, unesterified cholesterol, triglycerides, and phospholipid concentrations were determined enzymatically.[31–33] Cholesteryl ester concentrations were calculated as the difference between the total and unesterified cholesterol concentrations. Total protein concentration was determined by the bicinchoninic assay.[34] All analyses were performed in triplicate using a Beckman Coulter AU480 Autoanalyzer (Beckman Coulter, Brea, CA).

Cholesterol Efflux

Cholesterol efflux was determined in the group that completed both the moderate- and high-intensity exercise program (n=51). Chinese hamster ovary cells expressing the ABCA1 (ATP-binding cassette

transporter A1) under control of a tetracycline-inducible promoter were seeded into 24-well plates (40 000 cells/well in 500 µL of Ham's F-12 medium/10% (v/v) heat-inactivated fetal calf serum).[29] The following day the medium was changed to Ham's F-12/10% (v/v) heat-inactivated fetal calf serum/1 µCi/mL ^{3}H-cholesterol±tetracycline (1 µg/mL). The cells were then incubated for 24 hours, washed with PBS, and incubated for 1 hour in serum-free Ham's F-12 medium containing 0.1% (w/v) BSA±tetracycline. ApoB-depleted serum was added to the medium (final concentration 1% [v/v]). After 4 hours of incubation, the medium was removed and centrifuged (2000*g*, 5 minutes, 25 °C) and 100 µL of each sample was added to scintillation fluid and counted (Tricarb scintillation counter, Perkin Elmer, Waltham, MA). Total counts were determined after cell lysis with 0.1% (v/v) Triton X-100. Efflux (%) was calculated as radioactivity in the medium relative to the total radioactivity (cells+medium) after subtracting background efflux. ABCA1-specific cholesterol efflux was calculated as the difference in efflux between cells incubated±tetracycline and normalized to a standard sample of apoB-depleted serum.

Determination of pre-β_1-HDL Concentration

The plasma concentration of pre-β_1-HDL was determined using a commercial ELISA kit (Sekisui Diagnostics Burlington, MA).

Anti-Inflammatory Properties of Isolated HDLs

The anti-inflammatory properties of isolated HDLs were determined in a subgroup of participants (n=19) that completed both exercise programs and had the greatest absolute change in fitness from baseline as defined by the multistage fitness test. Human coronary artery endothelial cells (HCAECs) (Cell Applications Inc., San Diego CA) were grown to 90% confluence in 5% CO_2 in Meso Endo Growth Medium (Cell Applications) and seeded onto 24-well tissue culture plates (5×10^4 cells/well) in Meso Endo Growth Medium (500 µL). The HCAECs were preincubated for 16 hours at 37 °C with isolated HDLs (final apoA-I concentration 1 mg/mL), then incubated (5 hours, 37 °C) with tumor necrosis factor-α (TNF-α) (final concentration 0.1 ng/mL).

The HCAECs were then labeled with a human PE-Cy5-vascular cell adhesion molecule (VCAM)-1 CD106 (1:5 in 10% (v/v) FBS) (Becton Dickinson Pty Ltd, Franklin Lakes, NJ) and FITC-intercellular adhesion molecule (ICAM)-1 CD54 (Beckman Coulter Immunotech, Pasadena, CA) (1:5 [v/v] conjugated antibodies in 10% [v/v] FBS) or respective isotype controls (murine VCAM-1 CD106 PE-Cy5 IgG1 1:5 [v/v] in 10% [v/v] FBS) (Becton Dickinson) and ICAM-1 IgG1-FITC (1:50 [v/v] in 10% [v/v] FBS) (Beckman Coulter Immunotech)). Expression of VCAM-1 and ICAM-1 was quantified by flow cytometry (FACSVerse™, BD Biosciences) and analyzed using FlowJo™ software v10 (BD Biosciences).

HDL Particle Size

HDL particle size was determined in the participants (n=51) that completed both exercise programs. Ultracentrifugally isolated HDLs were electrophoresed for 375 v.h on 4% to 20% nondenaturing gradient polyacrylamide gels (Mini-Protean TGX Precast Gels; Bio-RAD, Hercules, CA), then fixed, stained, scanned, and analyzed (ImageQuant TL™ 8.1 Software, GE Healthcare Life Sciences, Marlborough, MA).[35] Particle diameters were determined by reference to a High Molecular Weight marker kit (VWR International, Radnor, PA).[35]

Homeostatic Model Assessment of Insulin Resistance

Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (mU/L)×fasting glucose (mmol/L)/22.5.[36] Insulin and fasting blood glucose levels were measured by Laverty Pathology, NSW, Australia.

Statistical Analysis

Data are expressed as mean±SEM. Variables that were not normally distributed were log transformed before analysis and median and interquartile ranges are reported. Paired *t* tests were used to identify statistically significant differences between values at baseline and after moderate-intensity exercise. For the subjects who completed the moderate- as well as the high-intensity exercise interventions, a repeated measures ANOVA was conducted with Bonferroni post hoc pairwise comparison testing for multiple comparisons. An ANCOVA was also conducted on this cohort controlling for age, body mass index (BMI), body fat percentage, fitness, and HDL-C. Correlations were assessed using Spearman correlation. A 2-tailed *P* value <0.05 was considered significant.

RESULTS

Moderate-Intensity Exercise Program

Effects of Moderate-Intensity Exercise on Anthropometry and Fitness in all Participants

Of the enrolled 140 participants, 115 completed the recruit course and were included in the analysis.

Baseline fitness was high as all participants were required to pass the Army entry fitness test before inclusion in the study. Nevertheless, the moderate-intensity exercise program significantly improved fitness as well as decreased BMI and percentage of body fat (all $P<0.001$) (Table 1). Diastolic blood pressure and heart rate decreased after the moderate-intensity exercise program (both $P<0.01$); however, systolic blood pressure was unchanged.

Effects of Moderate-Intensity Exercise on Plasma Lipid and Apolipoprotein Levels, Glucose Metabolism, and Inflammation in All Participants

Moderate-intensity exercise increased HDL-C and apoA-I levels by 6.6% and 11.6% (respectively) and decreased LDL-C, apoB, and apoA-II levels by 7.2%, 4.9%, and 11.1% respectively (Table 1, $P<0.001$ for all except apoB $P<0.01$). Moderate-intensity exercise had no effect on TC levels but increased plasma triglyceride levels ($P<0.001$). Fasting blood glucose levels ($P<0.001$), insulin levels ($P<0.001$), HOMA-IR ($P<0.001$), and hsCRP ($P<0.01$) were all increased by moderate-intensity exercise. Results remained significant after adjustment for age, BMI, body fat percentage, fitness, and HDL-C.

Moderate- and High-Intensity Exercise Program

The following results apply to the subgroup of 51 participants who commenced the high-intensity exercise program immediately after completing the moderate-intensity exercise program.

The Effects of Sequential Moderate- and High-Intensity Exercise on Anthropometry and Fitness

Both the high- and moderate-intensity exercise programs were associated with a significant increase in fitness level and a decrease in percentage of body fat, BMI, and heart rate relative to baseline (Table 2). However, there was no incremental improvement in these parameters between the moderate- and the high-intensity programs. Diastolic blood pressure decreased after moderate-intensity exercise ($P<0.01$) but was not significantly different from baseline after moderate- followed by high-intensity exercise.

Effects of Sequential Moderate- and High-Intensity Exercise Program on Plasma Lipid and Apolipoprotein Levels, Glucose Metabolism, and Inflammation

In the subgroup that completed both exercise programs, HDL-C and apoA-I levels increased after moderate-intensity training by 8.2% ($P<0.05$) and 13.5% ($P<0.001$) respectively and increased further after the high-intensity exercise training program (8.2%, $P<0.001$ and 6.3%, $P<0.05$ respectively) (Table 2). ApoA-II levels decreased after moderate-intensity exercise ($P<0.01$) but were increased relative to baseline and moderate-intensity exercise after the high-intensity exercise program (both $P<0.001$). In this subgroup LDL-C and apoB levels did not change in response to either the moderate- or high-intensity exercise program. TC levels were comparable at baseline and after moderate-intensity exercise but increased significantly relative to baseline and moderate-intensity exercise after the high-intensity exercise program ($P<0.01$ and $P<0.05$, respectively). All of these changes remained significant after adjusting for age, BMI, percentage of

Table 1. Effects of Moderate-Intensity Exercise on Fitness, Anthropometry, Clinical Characteristics, Plasma Lipid and Apolipoprotein Levels, Markers of Glucose Metabolism, and Inflammation in All Participants

Characteristics (n=115)	Baseline	Moderate-intensity	*P* value
Clinical parameters			
Age, y	21.9±0.3	22.1±0.3	<0.001
Body mass index, kg/m^2	24.4±0.3	23.8±0.2	<0.001
Body fat, %	15.6±0.4	12.5±0.3	<0.001
Systolic BP, mm Hg	127.4±1.4	126.5±1.2	0.56
Diastolic BP, mm Hg	65.8±0.9	61.9±0.8	<0.01
Heart rate, beats/min	65.3±1.4	60.3±1.7	<0.01
Fitness, multistage fitness test meters	1546±29	1900±25	<0.001
Est. VO_2 max, mL/kg per min	45.1±0.12	50.1±0.26	<0.001
Pathology			
HDL-C, mmol/L	1.36±0.03	1.45±0.04	<0.001
Low-density lipoprotein cholesterol, mmol/L	2.65±0.07	2.46±0.06	<0.001
Total cholesterol, mmol/L	4.32±0.08	4.29±0.07	0.76
Triglycerides, mmol/L*	0.66 (0.46–0.86)	0.82 (0.52–1.12)	<0.001
nonHDL-C, mmol/L	2.95±0.08	2.84±0.07	0.06
apoA-I, mg/mL	1.38±0.01	1.54±0.02	<0.001
apoA-II, mg/mL†	0.45±0.01	0.40±0.01	<0.001
apoB, mg/mL	0.82±0.02	0.78±0.02	<0.01
Glucose, mmol/L	4.56±0.04	5.01±0.04	<0.001
Insulin, mU/L	5.41±0.24	6.87±0.35	<0.001
Homeostatic model assessment of insulin resistance	1.11±0.05	1.55±0.09	<0.001
High-sensitivity C-reactive protein, mg/L*	0.40 (0.30–1.0)	0.60 (0.40–1.30)	<0.01

Data represent the mean±SEM or median and interquartile range where data are log transformed. Paired *t* tests were used for statistical analysis. Apo indicates apolipoprotein; BP, blood pressure; and HDL-C, high-density lipoprotein cholesterol.

*Raw data median and interquartile range reported, significance is based on log transformed data.

†n=61.

Table 2. Effects of Sequential Moderate and High-Intensity Exercise Programs on Fitness, Anthropometry, Clinical Characteristics, Plasma Lipid and Apolipoprotein Levels, Markers of Glucose Metabolism, and Inflammation in Participants Who Completed Both Exercise Programs

Characteristics (n=51)	Baseline	Moderate intensity	High intensity
Clinical parameters			
Age, y	21.7±0.4	21.9±0.4*	22.3±0.4*,†
Body mass index, kg/m^2	24.5±0.3	23.9±0.3‡	24.0±0.3§
Body fat, %	15.4±0.5	12.3±0.3†	12.7±0.3†
Systolic BP, mm Hg	127.4±1.4	126.5±1.4	125.3±1.3
Diastolic BP, mm Hg	65.8±1.2	61.9±1.1‡	68.3±1.6‖
Heart rate, beats/min	65.4±1.4	59.6±1.7§	56.8±1.9‡
Fitness (multistage fitness test meters)	1585±41	1949±35*	1993±39†
Est. VO_2 max, mL/kg per min	46.1±0.7	50.8±0.5*	51.8±0.6*
Pathology			
HDL-C, mmol/L	1.46±0.05	1.58±0.06§	1.71±0.06*,†
Low-density lipoprotein cholesterol, mmol/L	2.49±0.10	2.39±0.09	2.49±0.10
Total cholesterol, mmol/L	4.24±0.11	4.35±0.12	4.58±0.10‡,¶
Triglycerides, mmol/L¶	0.60 (0.50–0.70)	0.70 (0.6–1.00)†	0.7 (0.6–1.0)†
Non-HDL-C, mmol/L	2.78±0.11	2.77±0.10	2.87±0.11
apoA-I, mg/mL	1.41±0.03	1.60±0.04†	1.70±0.04†,‡
apoA-II, mg/mL	0.44±0.01	0.40±0.01‡	0.49±0.01*,†
apoB, mg/mL	0.82±0.03	0.79±0.03	0.82±0.03
Pre β1-HDL, µg/mL	4.16±0.17	3.86±0.17	4.22±0.21
Glucose, mmol/L	4.58±0.07	5.03±0.07*	4.85±0.08‡
Insulin, mU/L	5.29±0.32	6.78±0.40‡	6.47±0.88
Homeostatic model assessment of insulin resistance	1.10±0.08	1.54±0.11‡	1.43±0.21
High-sensitivity C-reactive protein, mg/L¶	0.50 (0.20–1.10)	0.60 (0.40–1.50)	1.00 (0.40–2.00)‡

Data represent the mean±SEM or median and interquartile range where data are log transformed. Results are from repeated measures ANOVA with Bonferroni post hoc pairwise comparison testing for multiple comparisons. Apo indicates apolipoprotein; BP, blood pressure; and HDL-C, high-density lipoprotein cholesterol.

*P<0.001 compared with baseline.
†P<0.001 moderate vs high intensity.
‡P<0.01 compared with baseline.
§P<0.05 compared with baseline.
‖P<0.01 moderate vs high intensity.
¶P<0.05 moderate vs high intensity.
#Raw data median and interquartile range reported, significance is based on log transformed data.

body fat, fitness, and HDL-C. Exercise had no effect on plasma pre-β_1 HDL levels.

Triglyceride and blood glucose levels increased after moderate-intensity exercise (both P<0.001), and these increases persisted after the high-intensity exercise program (P<0.001 and P<0.05, respectively). Plasma insulin levels and HOMA-IR significantly increased relative to baseline after moderate- (both P<0.01) but not high-intensity exercise. hsCRP levels were increased after the high- (P<0.01) but not moderate-intensity exercise program compared with baseline but did not differ between the moderate- and high-intensity exercise program.

Effects of Sequential Moderate- and High-Intensity Exercise on the Cholesterol Efflux Capacity and Anti-Inflammatory Properties of HDLs

In the group that completed both exercise programs (n=51), moderate-intensity exercise improved ABCA1-mediated cholesterol efflux from Chinese hamster ovary cells to apoB-depleted serum by 13.5% (P<0.001) relative to baseline (Figure 1A). This improvement was sustained after the high-intensity program (17.3%, P<0.001) but did not differ between the moderate- and high-intensity exercise program. The changes in ABCA1-mediated cholesterol efflux remained significant after adjusting for age, BMI, percentage of body fat, fitness, and HDL-C.

The ability of isolated HDLs to inhibit ICAM-1 and VCAM-1 expression in TNF-α activated HCAECs was evaluated in the subset of 19 individuals who completed both exercise programs and who had the greatest absolute change in fitness from baseline (defined by the greatest improvement in the multistage fitness test). In these individuals the ability of isolated HDLs to inhibit ICAM-1 expression in TNF-α activated HCAECs was improved after the combined moderate- and high-intensity exercise program (Figure 1B, P<0.001) but not after the moderate-intensity program alone. Neither high- nor moderate-intensity exercise altered the ability of isolated HDLs to inhibit VCAM-1 expression in TNF-α activated HCAECs (Figure 1C). In line with the larger cohort, moderate-intensity exercise improved ABCA1-mediated cholesterol efflux relative to baseline (P<0.001) in this small subset and this was sustained after the high-intensity exercise program (P<0.001).

Cholesterol efflux was positively correlated with apoA-I and HDL-C at baseline (both P<0.01), and after moderate (both P<0.01) and high-intensity exercise (P<0.05 and P<0.01 respectively) (Figure S2A and S2B). It was not correlated with apoA-II at any point (not shown). Cholesterol efflux was positively correlated with pre-β HDL at baseline (P<0.05) and after moderate-intensity exercise (P<0.01) (Figure S2C). ApoB, LDL-C, fitness level, and percentage of body fat did not correlate with cholesterol efflux at baseline or after either exercise intervention (Figure S2D through S2G).

The change in cholesterol efflux capacity in response to exercise was assessed to see if it was associated with change in fitness, body fat

percentage, and key lipid and metabolic parameters (Figure 2). The change in cholesterol efflux capacity after moderate-intensity exercise was positively associated with the change in apoA-I, HDL-C, pre-β HDL, apoB, LDL-C, and TC (all $P<0.01$ except LDL-C $P<0.05$) levels (Figure 2A through 2F). This association remained significant only for apoA-1 and TC (both $P<0.05$) at the end of the high-intensity exercise intervention. Change in cholesterol efflux capacity was positively associated with change in triglycerides ($P<0.05$), insulin resistance ($P<0.01$), and fitness ($P<0.05$) after sequential moderate- and high-intensity exercise but not after the moderate-intensity exercise program. Cholesterol efflux capacity was not significantly correlated with percentage of body fat or glucose level.

Effects of Sequential Moderate- and High-Intensity Exercise on HDL Composition and Size

In the 51 participants who completed both exercise programs, moderate- and high-intensity exercise had no effect on HDL particle size (Figure S3).

HDL composition was assessed in a subgroup of participants who completed both training programs and who had the greatest absolute change in fitness from baseline (n=19). The apoA-I/apoA-II molar ratio increased relative to baseline after moderate- and high-intensity exercise ($P<0.01$) (Table 3). The wt% triglycerides in the isolated HDLs increased relative to baseline after moderate ($P<0.05$) but not high-intensity exercise. Moderate- and high-intensity exercise had no effect on the wt% phospholipid, unesterified cholesterol, cholesteryl ester, or total protein in the isolated HDLs.

Relationship Between Fitness, HDL Function, Plasma Lipids, Markers of Inflammation, and Glucose Homeostasis

Correlations were assessed across the whole cohort. Baseline fitness was inversely correlated with hsCRP ($P<0.01$), plasma insulin levels ($P<0.01$), and HOMA-IR ($P<0.05$). This inverse relationship persisted after moderate-intensity exercise (hsCRP $P<0.01$; insulin $P<0.05$; HOMA-IR $P<0.05$) but was no longer apparent

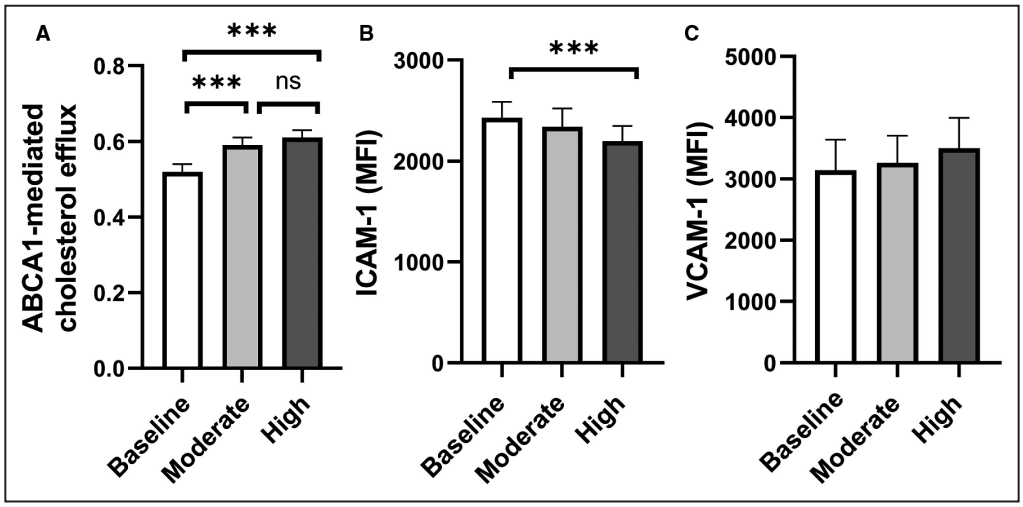

Figure 1. Exercise increases ABCA1-mediated cholesterol efflux and improves the anti-inflammatory properties of HDLs.
ABCA1-specific cholesterol efflux at baseline and after moderate- and high-intensity exercise. Chinese hamster ovary cells with tetracycline-inducible expression of human ABCA1 were loaded with ^{3}H-cholesterol and incubated for 4 hours in the absence or presence of apoB-depleted serum as described in the Methods. The percentage of efflux was calculated as radioactivity in the medium relative to the total radioactivity (cells+medium). ABCA1-specific cholesterol efflux was calculated as the difference in efflux between cells incubated with and without tetracycline and normalized to a standard sample of apoB-depleted serum (n=51) (**A**). ICAM-1 and VCAM-1 expression in TNF-α activated HCAECs at baseline and after moderate- and high-intensity (**B** and **C**). HCAECs were preincubated for 16 hours with ultracentrifugally isolated HDLs at baseline and after moderate- and high-intensity exercise, then activated for 5 hours with TNF-α. Expression of ICAM-1 (n=19) (**B**) and VCAM-1 (n=19) (**C**) was quantified by flow cytometry. Data represent the mean±SEM of triplicate measurements. Repeated measures ANOVA was conducted with Bonferroni post hoc pairwise comparison testing for multiple comparisons. ***$P<0.001$. ABCA1 indicates ATP-binding cassette transporter A1; apoB, apolipoprotein; HCAEC, human coronary artery endothelial cell; HDL, high-density lipoprotein; ICAM-1, intercellular adhesion molecule 1; MFI, mean fluorescence intensity; TNF-α, tumor necrosis factor alpha; and VCAM-1, vascular cell adhesion molecule 1.

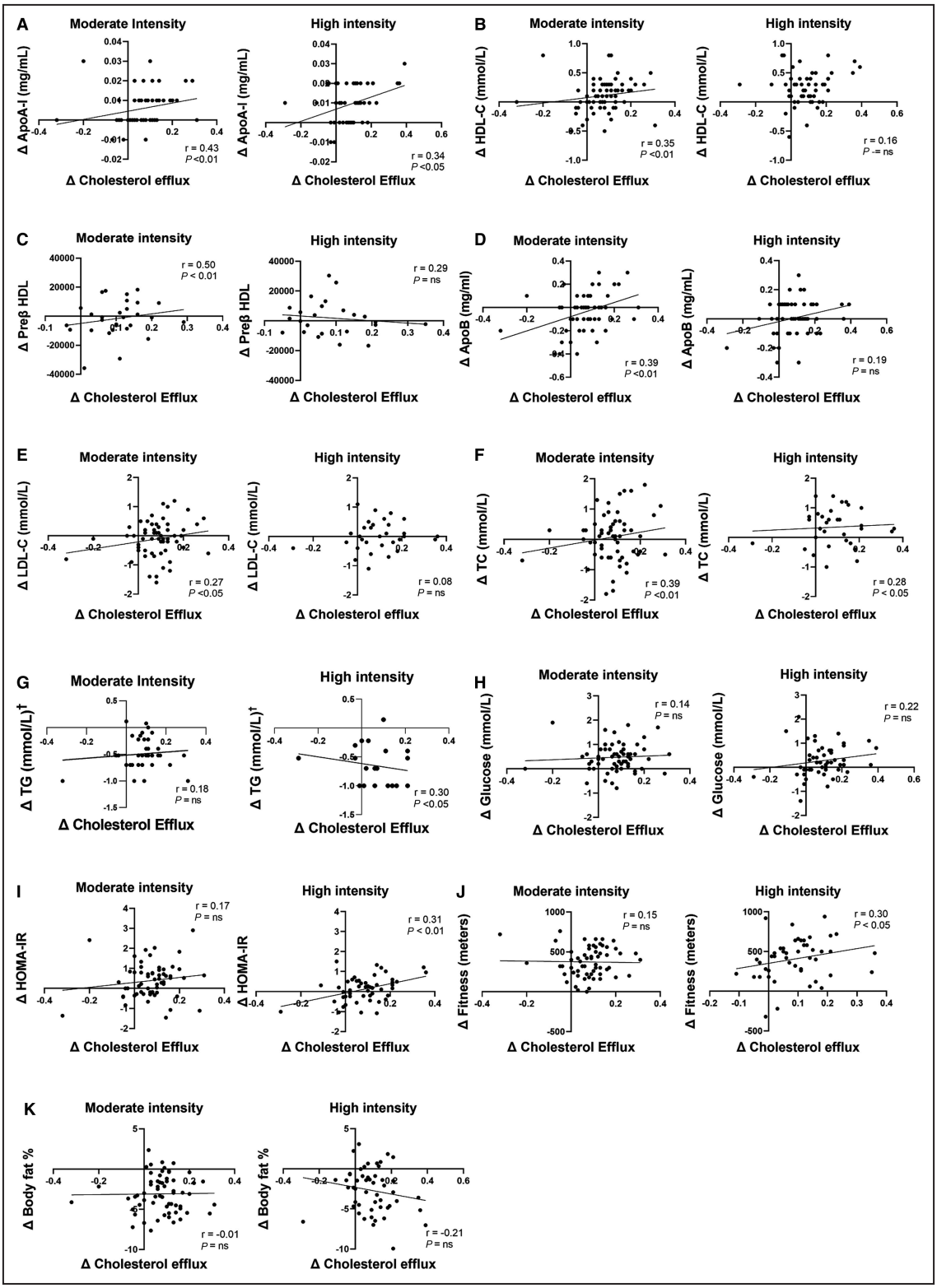

Figure 2. Correlation between change in cholesterol efflux capacity compared with change in key lipid, lipoproteins, and metabolic parameters from baseline to after moderate- and to after high-intensity exercise. Correlation between change in cholesterol efflux capacity and change in: (**A**) ApoA-I, (**B**) HDL-C, (**C**) pre-β HDL, (**D**) ApoB, (**E**) LDL-C, (**F**) TC, (**G**) TG, (**H**) glucose level, (**I**) HOMA-IR, (**J**) fitness, (**K**) body fat %. Graph shown is linear regression analysis. r value based on Spearman correlation. *P*<0.05 is considered significant. †ln. ApoA-I indicates apolipoprotein A-I; ApoB, apolipoprotein; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; and TG, triglycerides.

after high-intensity exercise (Figure S4A through S4C). Fitness level after high-intensity exercise was inversely correlated with blood glucose levels (P<0.05) but not at baseline or after moderate-intensity exercise (Figure S4D). Fitness was inversely correlated with triglycerides at baseline (P<0.01), and positively correlated with triglyceride levels after moderate-intensity exercise (P<0.05) (Figure S4E). Fitness and triglyceride levels were not correlated after high-intensity exercise. Baseline fitness and fitness after moderate- and high-intensity training were not correlated with levels of HDL-C, LDL-C, apoA-I, apoB, or cholesterol efflux (not shown).

The relationship between change in fitness level and the change in apoA-I, apoA-II, HDL-C, apoB, LDL-C, TC, cholesterol efflux, and percentage body fat was also assessed (Figure S5). There was no association between change in fitness and change in apoA-I or HDL-C after the moderate- or high-intensity exercise program; change in fitness level was, however, negatively associated with change in apoA-II levels after the moderate- (P<0.01) and sequential high-intensity exercise program (P<0.05) (Figure S5A through S5C). There was a negative association between change in fitness and change in apoB (P<0.01), LDL-C (P<0.01), TC (P<0.05), and percentage of body fat (P<0.001) after the moderate-intensity exercise program (Figure S5D through S5G). Only the association with change in percentage of body fat persisted after the high-intensity exercise program (P<0.01). Change in fitness level was, by contrast, significantly associated with change in cholesterol efflux capacity after the combined moderate- and high-intensity exercise program (P<0.05) but not after the moderate-intensity program (Figure S5H).

Table 3. The Effect of Sequential Moderate- and High-Intensity Exercise Programs on HDL Composition

Weight (%) (n=19)	Baseline	Moderate intensity	High intensity
Phospholipid	27.7±1.0	28.9±0.6	28.6±0.6
Cholesteryl esters	23.4±0.7	22.7±0.5	23.0±0.6
Unesterified cholesterol	5.0±0.4	4.8±0.3	4.7±0.3
Triglyceride*	1.3 (1.1–2.0)	1.7 (1.4–2.8)†	1.8 (1.5–2.2)
Protein	42.3±1.6	41.5±1.5	41.8±1.1
apoA-I/apoA-II (mol/mol)	1.9±0.1	2.2±0.1‡	2.1±0.1‡

Data represent the mean±SEM or median and interquartile range. Repeated measures ANOVA was conducted with Bonferroni post hoc pairwise comparison testing for multiple comparisons. Apo indicates apolipoprotein.

*Raw data median and interquartile range reported, significance is based on log transformed data.

†P<0.05 compared with baseline.

‡P<0.01 compared with baseline.

DISCUSSION

This study shows that moderate-intensity exercise has a beneficial effect on fitness, body composition, lipid and lipoprotein profile, and HDL function in a homogeneous, well-controlled group of male army recruits with a high level of baseline fitness. This study showed that such benefits can be achieved with moderate-intensity exercise, and that a sequential high-intensity exercise program showed either a sustained or incremental benefit. On the other hand, we found that the subjects showed unexpected but subtle exercise-associated increases in plasma hsCRP, triglyceride levels, and insulin resistance. The absolute changes in these parameters were small and the clinical significance is unknown. It does, however, raise the possibility that some of the benefits of exercise could be partly offset by mildly increased systemic inflammation and impaired glycemic control.

This study found that moderate- and high-intensity exercise are both associated with a less atherogenic lipid/lipoprotein profile, as evidenced by the increase in apoA-I and HDL-C levels. Most of the improvements seen after moderate-intensity exercise were sustained after high-intensity exercise. However, there was a clear benefit of a sequential high-intensity exercise program after the moderate-intensity program with a further incremental increase in HDL-C and apoA-I levels. This was independent of age, BMI, percentage of body fat, fitness, and HDL-C. As there was no high-intensity exercise-only group, it is not known whether the benefits that were observed after the high-intensity exercise program are due to exercise intensity or exercise volume given that those who participated in the high-intensity exercise program were subjected to both a more intense and longer duration program. Previous studies have demonstrated an improvement in lipid/lipoprotein profile with exercise but have not shown an added benefit of high- over moderate-intensity exercise.[4,7,37] Those studies also showed that exercise does not change or modestly decreases LDL-C levels. This is consistent with the present results, where moderate-intensity exercise reduced apoB and LDL-C levels and also showed that the change in fitness level was negatively correlated with apoB and LDL-C levels after moderate-intensity exercise.

We unexpectedly found a small increase in plasma triglyceride levels after moderate and high-intensity exercise. Triglyceride levels typically decrease or do not change in response to exercise, at least in subjects with high baseline triglyceride levels.[4,38] Increased triglyceride levels in response to exercise have been reported in a number of studies that assessed the metabolic effect of exercise.[39] The reason for this increase is not known. Its clinical importance is also unclear given that there is substantial interindividual variability in triglyceride levels.[40]

To our knowledge, this is the first study to show that that both moderate- and sequential moderate- and high-intensity exercise is associated with improved cholesterol efflux in healthy young men. This is a key finding as there is increasing evidence that HDL function, rather that HDL-C levels, may be responsible for the inverse relationship between HDL-C and cardiovascular risk.[5] The current results showing that exercise improves ABCA1-mediated cholesterol efflux is in line with studies where cholesterol efflux increased in soccer players[41] and endurance athletes[42] compared with controls. It is important to note that, even though SR-B1 (scavenger receptor class B type 1)-mediated cholesterol efflux from Fu5AH rat hepatoma cells and cAMP-inducible efflux (attributed to ABCA1) from RAW-264.7 mouse macrophages was measured in those studies, compared with ABCA1-specific efflux in the present study, the results are consistent. Interestingly, change in fitness level was significantly associated with change in cholesterol efflux capacity only after the sequential moderate- and high-intensity exercise programs. It is unclear if there is an intensity or exercise volume threshold associated with improvements in cholesterol efflux capacity, but the data does support the conclusion that exercise increases cholesterol efflux.

The limited and inconsistent effects of exercise on cholesterol efflux in the literature may be due to variability in the study populations, differing amounts and intensity of exercise, and the use of different methods to measure efflux.[41–44] In the STRRIDE-PD (Studies of Targeted Risk Reduction Interventions through Defined Exercise, in Individuals with Pre-Diabetes) randomized control trial, 3 different exercise programs with varied intensity or volume were evaluated. That study established that combined ABCA1-, ABCG1-, and SR-B1-mediated cholesterol efflux from J774 macrophages increased with a high-volume vigorous intensity exercise program, but not after high-volume moderate-intensity exercise or low-volume moderate-intensity exercise program.[43] In contrast to STRRIDE-PD, efflux improved with both moderate- and the sequential moderate- and high-intensity exercise programs in the present study. Baseline fitness and the volume and intensity of exercise were higher in the current study compared with STRRIDE-PD. The other difference in the current study was that all the participants were healthy young men compared to STRRIDE-PD, which included older participants with cardiovascular risk factors (prediabetic and overweight).

In the E-MECHANIC (Examination of Mechanisms of Exercise-induced Weight Compensation) randomized control trial, by contrast, high- but not low-volume moderate-intensity exercise increased non-ABCA1-mediated cholesterol efflux. This effect was no longer apparent after adjusting for change in HDL-C.[43] Importantly, and in contrast to the current results, exercise did not affect cAMP-induced (presumably ABCA1-mediated) cholesterol efflux in the E-MECHANIC trial.[43] Despite these differences, what does seem clear is that cholesterol efflux may well be a key factor that contributes to the observed inverse association between exercise and cardiovascular risk.

There is also controversy about the effect of exercise on HDL size. Some studies have shown that exercise increases the number of large HDL particles,[43,45] whereas others have shown no difference in particle size[7] or a decrease in either small or medium HDL particles with or without an increase in large HDL particles.[45,46] In general, mean HDL size and the number of large HDL particles are inversely associated with cardiovascular risk.[47] However some studies suggest that very high HDL-C and large HDL particles may increase cardiovascular disease risk and mortality.[48,49] There was, by contrast, no change in HDL size after exercise in the present study, even though the triglyceride content of HDL increased. This may be because the participants were already very fit and their baseline HDLs were large and therefore able to accommodate the modest increase in triglyceride content by altering the conformation of apoA-I, which would not require a quantum change in particle size.[50]

The present study found that in a small subgroup of 19 recruits with the greatest improvement in fitness after the combined high- and moderate-intensity exercise program that HDLs decreased ICAM-1, but not VCAM-1, expression in TNF-α activated HCAECs. Endothelial dysfunction and increased endothelial cell adhesion molecule expression are initiating events in atherogenesis. Increased soluble ICAM-1 plasma levels in apparently healthy individuals are consistently associated with an increased risk of future cardiovascular events, although this is not the case for VCAM-1 expression.[51] In line with the current results, previous studies have reported that exercise decreases ICAM-1 and, to a lesser extent, VCAM-1 expression. These differential effects of exercise on ICAM-1 and VCAM-1 may reflect differences in the mechanisms by which HDLs reduce their expression.[23] They are also consistent with ICAM-1 playing an important role as a biomarker in healthy individuals, whereas VCAM-1 may be more important in established disease.[51]

A small increase in hsCRP was seen after the sequential moderate- and high-intensity exercise program. The clinical significance of these results is unclear because of the large within subject variability in hsCRP. We do, however, note that acute bouts of intense exercise in marathon runners have been shown to increase proinflammatory cytokines and hsCRP levels.[52,53] This is consistent with the negative correlation between fitness and hsCRP levels in the current study and may reflect an acute stress response being attenuated in the “fittest” individuals.

One of the more unexpected findings to emerge from the present study was that blood glucose, plasma insulin levels, and insulin resistance increased after moderate-intensity exercise. The increase in blood glucose but not insulin levels persisted after high-intensity exercise. Exercise is generally associated with increased insulin sensitivity and decreased or unchanged fasting blood glucose.[37] The clinical significance is not clear because of the small absolute and wide biological variability in these parameters. However, the current results do suggest that moderate- to high-intensity exercise may increase metabolic stress and may lead to insulin resistance. This possibility is supported by the increase in hsCRP levels after moderate- and high-intensity exercise. Excessive high-intensity interval training has recently been shown to impair mitochondrial function and increase insulin resistance in a heathy cohort and some authors have postulated that that there may be an upper limit of intense physical activity that can be performed without having a negative effect on metabolic health.[54–56] Interestingly there was a negative correlation between fitness level and hsCRP, insulin and HOMA-IR levels, suggesting that the less fit individuals in the cohort had a differential inflammatory and metabolic response to the same level of exercise, perhaps owing to relatively increased metabolic stress.

Strengths of the Study

The main strength of this study was its highly controlled nature, where all participants lived in the same environment and participated in the same exercise regime and work schedule. Exercise compliance was strictly controlled, with all sessions being supervised. As all the participants were required to complete each session as part of the Army training program, compliance was near 100%. The only reason for missing an exercise session was acute illness. As all exercise was supervised there was no recall bias in terms of level of physical activity. All participants had similar sleep patterns and similar incidental activity and there was limited variation in meal times and meal choices. The ability to control for so many external variables is unique and allows the observed changes to be related to the effects of exercise without needing to account for multiple confounders.

Limitations

The limitations of the study are its focus on young men and thus a lack of generalizability to the broader population. The volume of exercise in this study was also very high, which may also preclude generalizability to the broader population.

As the number of volunteers was more than could be included in the study, preference was given to infantry soldiers as they could participate in both moderate- and high-intensity exercise programs. This may have introduced selection bias. Additional selection bias may have been introduced as participation in the high-intensity exercise program was confined to infantry soldiers who are self-selected by virtue of choosing this career path of an infantry soldier. There is no “non exercise” control group; however, the baseline parameters for each individual acted as an internal control. Participants were drawn from a larger cohort who were not assessed. Apart from the non assessed group being predominantly being non infantry soldiers, there was no known systematic difference between these groups.

Although the exercise program was controlled with each participant performing the same activity in a group environment with a military personal training instructor guiding the activity, there remains inherent interindividual variability in intensity. Hence although the exercise “dose” was controlled it varied across the program and between individuals. The average intensity of each activity was estimated using the physical activity compendium to obtain an estimate of exercise intensity in METS. However, there are inherent limitations in this method and the actual intensity of each activity will vary between individuals based on resting energy expenditure and individual variability in exercise performance.

Diet and weight loss were not specifically controlled and thus some of the findings may reflect the effects of diet and weight loss rather the exercise intervention per se. However, soldiers ate at set meal times, from a set selection of meal choices that were prepared in the same kitchen, limiting the variability in diet composition between subjects. The amount of food consumed at each meal was not measured and may have varied between subjects. Soldiers also had limited access to snacks between meals and this was also not standardized.

It is also likely that energy consumption increased as energy expenditure increased. With respect to weight loss, the subjects were lean to begin with, and HDL-C and apoA-I significantly increased between moderate- and high-intensity exercise without changing percentage of body fat or BMI. Hence, it is likely that much of the improvement in the lipid and lipoprotein profiles, at least in the high-intensity exercise group, can be attributed to the exercise intervention. Fitness was also not measured using the “gold standard” of VO_2 max due to logistic contraints. However, the validated multistage fitness test did provide a reliable estimate of VO_2 max.[25]

The Chinese hamster ovary cells transfected with ABCA1 for the efflux studies are not as widely used for efflux as other cell types. However, we have validated the efflux protocol in these cells and published the outcomes of these studies in multiple reports.[57,58] The ability of HDLs to inhibit expression of ICAM-1 and VCAM-1 in

endothelial cells was also measured only in a subgroup of participants. Subgroup selection was based on those with the greatest gains in fitness during the moderate-intensity exercise intervention as it was hypothesized that they may have a greater change in these variables. This may have introduced inherent bias that preclude the results from being generalized to the wider population. It is also hard to draw strong conclusions from a small, select subgroup. However, it was not technically feasible to evaluate the anti-inflammatory properties of HDLs in all of the study subjects.

A key limitation of the study is the absence of a high-intensity exercise-only group. This means that the changes seen after the high-intensity program cannot be clearly attributed high-intensity exercise per se and may reflect the duration of exercise rather than intensity.

CONCLUSIONS

This is the first study to show that moderate-intensity exercise is associated with enhanced HDL function and that this improvement is sustained after a sequential high-intensity exercise intervention in a cohort of healthy young men where the exercise regimen and external variables were well controlled. It also demonstrates that exercise improves fitness and body composition and is associated with a less atherogenic lipoprotein profile. Most of these benefits were attained after a 12-week moderate-intensity exercise program, with the longer and higher intensity exercise program showing a sustained or incremental benefit. This study is important for understanding the mechanism(s) by which exercise reduces CVD risk and provides insights into the intensity and volume of exercise that brings about significant improvements in the cardiometabolic profiles of healthy young men.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Figures S1–S5

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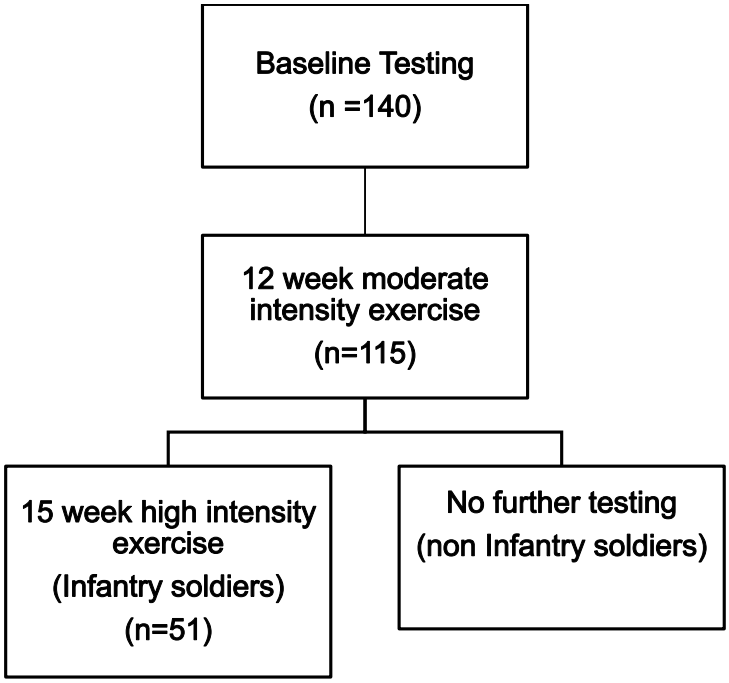
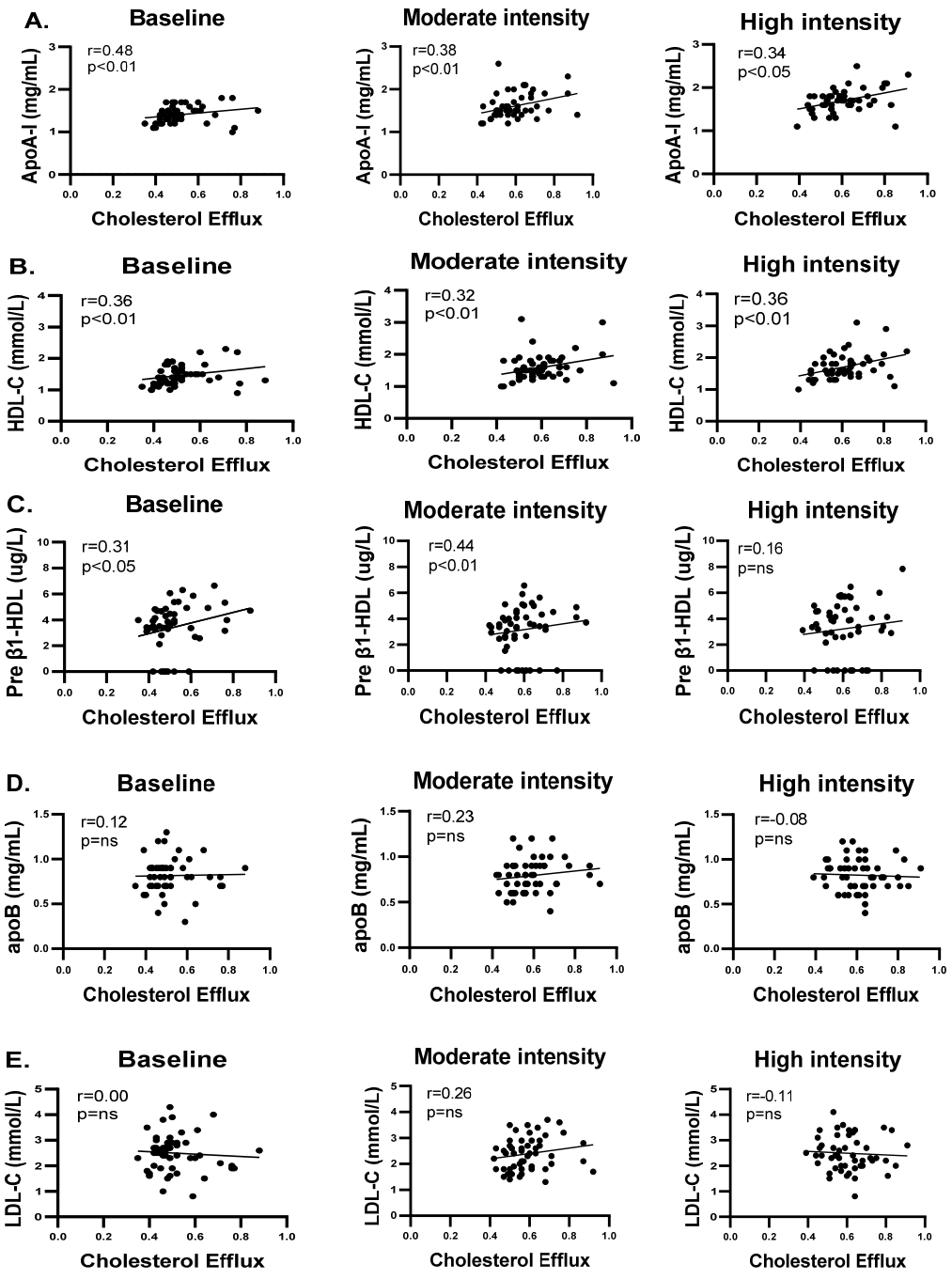
SUPPLEMENTAL MATERIAL

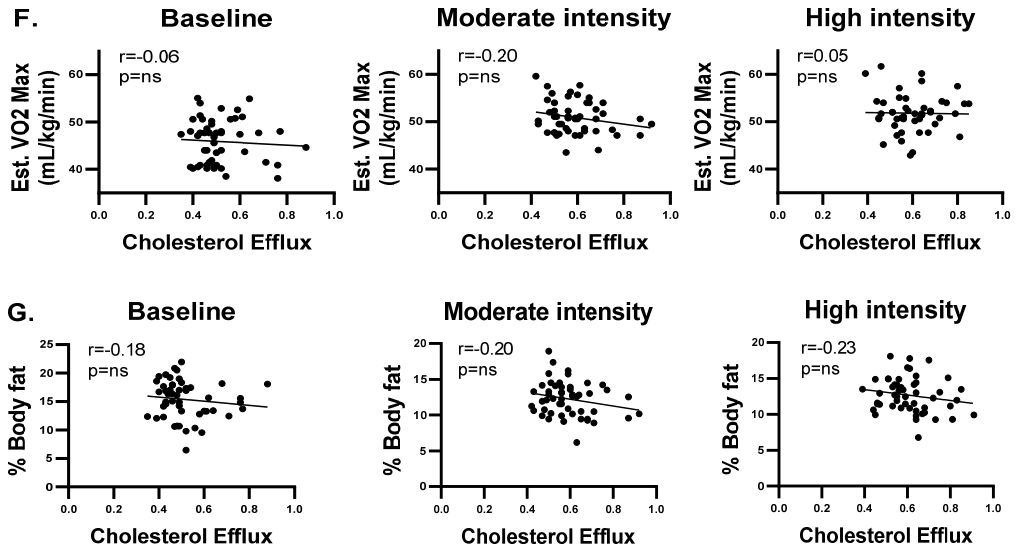
Figure S1.



Study design for assessing the effects of moderate and high intensity exercise in healthy young men

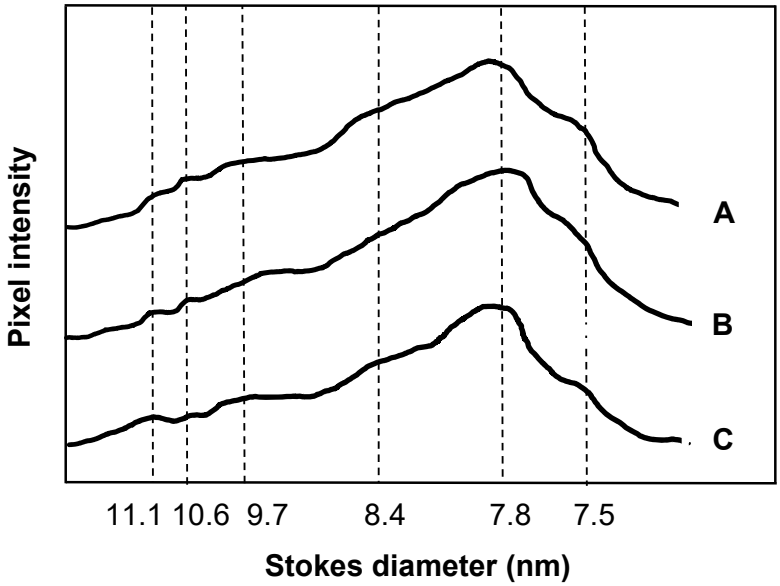
Figure S2. Correlation between, apoA-I, apoA-II, apoB, HDL-C, LDL-C, fitness, % body fat and cholesterol efflux capacity at baseline and after moderate and high intensity exercise





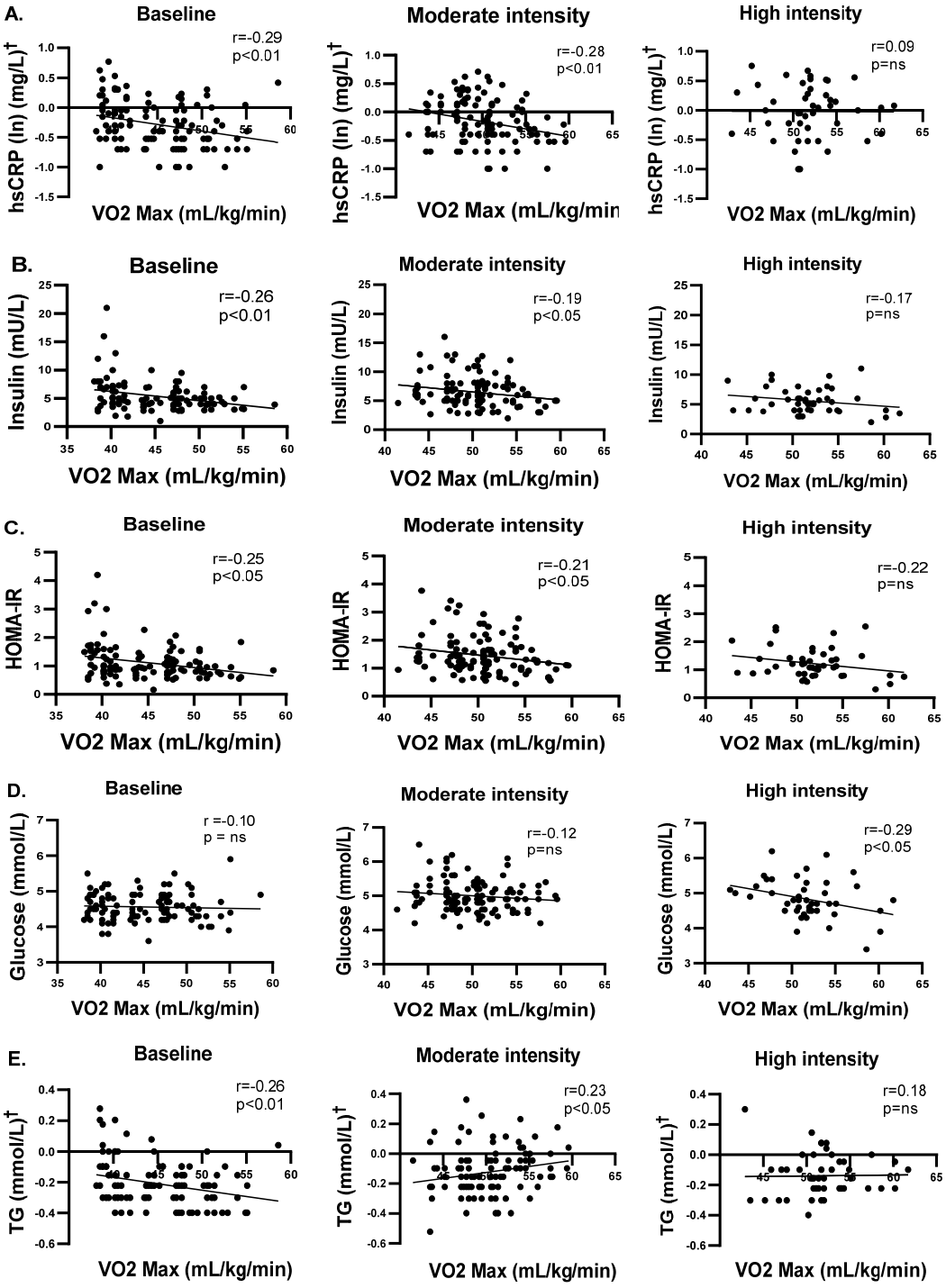
Linear regression analysis of cholesterol efflux, clinical variables and lipid/lipoprotein levels at baseline, and after moderate and high intensity exercise. r value based on Spearman Correlation. $p<0.05$ is considered significant.

Figure S3. Moderate and high intensity exercise does not affect HDL particle size.



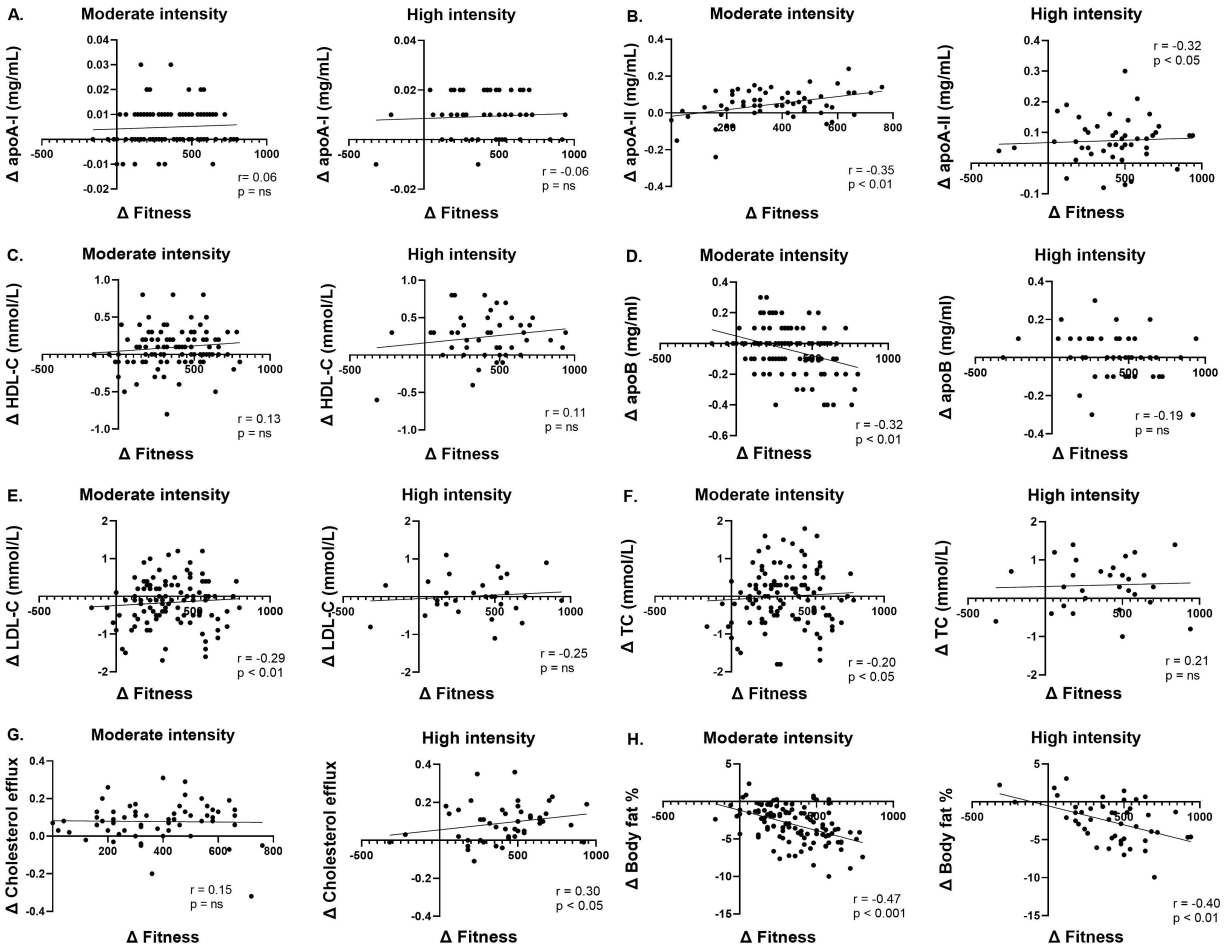
HDLs were isolated by ultracentrifugation, electrophoresed on 4–20% non-denaturing gradient polyacrylamide gels, stained and scanned. Particle diameters were determined by comparing the migration distances to those of high molecular weight standards. A representative gel from a single participant is shown. **Panel A**: Baseline, **Panel B**: Post-moderate intensity excise, **Panel C**. Post-high intensity exercise.

Figure S4. Correlation between fitness level and hsCRP, insulin, HOMA-IR, glucose and triglyceride levels at baseline and after moderate and high intensity exercise



Linear regression analysis of cholesterol efflux, clinical variables, metabolic parameter and triglyceride (TG) levels at baseline, and after moderate and high intensity exercise. r value based on spearman correlation. $p<0.05$ is considered significant. †ln. hsCRP, high sensitivity C-reactive protein, HOMA-IR, homeostatic model assessment of insulin resistance.

Figure S5. Correlation between change in fitness compared with change in apoA-I, apoA-II, HDL-C, apoB, LDL-C, TC % body fat and cholesterol efflux capacity from baseline to after moderate and high intensity exercise



Graph shown is linear regression analysis. r value based on Spearman Correlation. $p<0.05$ is considered significant.