Spring 2022

The Effects of Exercise Training on Cholesterol Efflux Capacity in the HERITAGE Family Study

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Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Exercise Science

Arnold School of Public Health

University of South Carolina

2022

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ACKNOWLEDGEMENTS

This study was supported by multiple grants from the National Institutes of Health: R01HL146462 (Sarzynski); R01NR019628 (Gerszten and Sarzynski); HL45670, HL47317, HL47321, HL47323, 47327 (all in support of HERITAGE); U54 GM104940 (pilot grant, Sarzynski); 8P20 GM-1033528 (COBRE project PI, Sarzynski); R01HL136724 (Rohatgi).

ABSTRACT

Cholesterol efflux capacity (CEC) has been associated with cardiovascular disease risk independent of HDL-C levels. However, the effect of regular exercise on CEC is not yet fully understood. Therefore, we examined the effects of exercise training on CEC in the HERITAGE Family Study. In HERITAGE, subjects participated in an endurance training program that consisted of exercise on a cycle ergometer three times per week for 20 weeks. Global and non-ABCA1 CEC were measured at baseline and post-training in 542 subjects via a cell-based radiolabeled CEC assay. Neither global nor non-ABCA1 CEC significantly changed with training in the whole cohort. Changes in HDL-C and apoA-I were weakly, positively correlated with changes in global efflux at a nominal level ($p<0.05$), while change in LPL activity was significantly, positively associated with change in non-ABCA1 CEC ($r=0.17$, $p=0.0001$). HDL-P concentration and mean HDL-P size were significantly, but weakly, positively correlated with training-induced changes in both global ($r=0.12$, $p=0.003$) and non-ABCA1 CEC ($r=0.14$, $p=0.001$). In conclusion, moderate to vigorous endurance exercise training had no effect on CEC in a large cohort of mostly healthy adults. Future studies should investigate the potential impact of exercise training on CEC in clinical populations, as well as in combination with dietary interventions resulting in weight loss.

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CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD), which affects nearly half of the US adult population, is the leading cause of death in the United States.[1,2] Total costs directly and indirectly related to treating patients with CVD amount to $363.3 billion annually.[2] High-density lipoprotein cholesterol (HDL-C) level is one of the strongest risk factors for CVD, with an inverse association with adverse cardiac events and the development of CVD.[3–5] Despite strong epidemiological associations, treatments successful in increasing HDL-C have been unable to produce a reduction in mortality or adverse cardiac events.[6–9] Furthermore, Mendelian randomization studies have been unsuccessful in linking HDL-related genes to alterations in CVD risk, further calling into question the causal relationship between HDL-C and CVD.[10,11] As such, recently a collective shift in the HDL-C hypothesis has led researchers io investigate whether HDL particle function may be of more clinical significance than quantity of cholesterol cargo (i.e., HDL function hypothesis).[12]

One such functionality that HDL exhibits is its primary role in reverse cholesterol transport, including the process of cholesterol efflux. Cholesterol efflux is the first step in reverse cholesterol transport wherein cholesterol which has entered the tunica of the artery is evacuated back through the endothelium and packaged into a nascent HDL particle for transport back to the liver.[13]

Epidemiologically, cholesterol efflux capacity (CEC) displays a strong inverse relationship with prevalent and incident risk of coronary artery disease, independent of HDL-C levels and may serve as an important clinical indicator of CVD risk.[14–17]

Given this relationship, it is necessary to identify determinants of CEC and methods to increase CEC. Lifestyle, body mass index (BMI), age, genetics, and inflammatory status along with several other variables have been identified as potential determinates or covariates of cholesterol efflux capacity.[18] Therefore, more recent investigations in the field have shifted focus to the specific effects of modifications of diet and exercise in altering cholesterol efflux capacity.[19] Koba et al. observed improvements in cholesterol efflux capacity in patients with acute coronary syndrome following an outpatient cardiac rehabilitation program.[20] Notably however, these improvements in CEC were observed in combination with an improvement in HDL-C concentrations. Additionally, further studies were unable to reproduce these results in patients with peripheral artery disease.[21] However, Sarzynski et al. recently reported improvements in cholesterol efflux capacity following high intensity exercise training only, suggesting intensity of exercise may be influential in altering HDL particle function.[22]

Limitations in existing studies on CEC and exercise include small sample sizes, homogenous populations, short duration, and variability in exercise prescription.[23] Therefore, direct effects of regular exercise on CEC remain to be elucidated, including whether exercise-induced changes in CEC differ by individual characteristics (age, sex, ethnicity, metabolic profile). Moreover, it is

unknown how exercise-induced changes in CEC relate to changes in other lipid metabolism phenotypes. Therefore, the purpose of the current study was to examine the effects of exercise training on cholesterol efflux capacity in a large, diverse cohort of Black and White adults. We hypothesized that exercise would increase CEC and changes in CEC would be related to changes in HDL particle size. We tested these hypotheses with the following aims:

Aim 1: Investigate the effects of 20-weeks of endurance exercise training on CEC in individuals from the HERITAGE Family Study. 1.1 Examine whether exercise-induced changes in CEC differ by subject characteristics, including sex, race, and metabolic profile.

We hypothesized that exercise training would result in improvements in CEC and these improvements would be more prominent in men, Black subjects, and individuals with metabolic disease.

Aim 2: Examine the association of changes in CEC with concomitant changes in lipid and lipoprotein phenotypes.

We hypothesized that CEC would increase in association with other key determinants of lipid metabolism such as triglycerides, lipoprotein lipase, and HDL size and HDL particle subclass traits.

CHAPTER 2

BACKGROUND

The role of HDL in the cholesterol metabolism process known as reverse cholesterol transport is well established.[24] Reverse cholesterol transport involves the removal of cholesterol from macrophage foam cells in the artery wall for transport to the liver for further metabolism or excretion. In the efflux process, the first step in reverse cholesterol transport, cholesterol is removed from macrophages in the arterial wall via one of four previously identified mechanisms; aqueous diffusion-mediated efflux, scavenger type B class 1 (SR-B1)-mediated efflux, ATP binding cassette sub-family A member 1 (ABCA1)-mediated efflux or ATP binding cassette subfamily G member 1 (ABCG1)-mediated efflux. Following efflux, the HDL particle serves as the extracellular receptor for cholesterol.[25]

In a cross-sectional analysis, Khera et al. identified a strong, inverse relationship between cholesterol efflux capacity (CEC) and carotid intima-media thickness as well as prevalence of coronary artery disease.[26] The results from this study were mirrored in three subsequent longitudinal studies that found inverse relationships between CEC and incident cardiovascular events.[15,27–29] These studies found graded associations between CEC and incident atherosclerotic CVD, with individuals with the highest CEC having 36%-67% lower risk of CVD (**Figure 2.1**).[28] In a recent longitudinal study involving cohorts

from the MESA cohort, Shea et al. identified CEC as a potential protective mechanism against coronary heart disease.[17] CEC has also been found to be inversely associated with left atrial structural remodeling in patients with atrial fibrillation.[30] A systematic review and meta-analysis of 20 trials that examined the association between CEC and cardiovascular outcomes, conducted by Lee et al., supports these conclusions.[31]

Several clinical trials have examined the effect of drugs on cholesterol transport with varying success. Cholesterol ester transport protein (CETP)-inhibitors have been shown to increase non ABCA1-mediated efflux.[23] Statin therapies, however, have produced mixed results with respect to CEC. Both in-vitro and animal models involving statins have been inconclusive on mechanistic effects of this drug class on CEC with varying success dependent on drug administered. Niacin has shown mild increases in SRB1-mediated efflux, although this was also associated with increases in HDL-C concentrations and otherwise studies have not identified consistent results from niacin therapies.[23] While drug therapies have yet to provide conclusive evidence of effectiveness, exercise has been shown to alter some aspects of HDL particle function and subclass profile.[32,33]

A clear relationship between exercise and CEC is yet to be definitively established. Initially, Olchawa et al. found that both CEC and HDL-C concentrations were higher in individuals with greater fitness levels.[34] Previous studies involving interventions to improve CEC combined diet and exercise. While Aicher et al. saw no improvement in CEC following a lifestyle intervention

program, the authors did observe a decrease in HDL-C and no change or slight reductions in some efflux mechanisms.[35] This observation may be partially explained by the emerging U-shaped association between HDL-C and cardiovascular events outlined by Riggs and Rohatgi.[36] Other lifestyle interventions have reported more success in patients with obesity, metabolic syndrome, and dyslipidemia.[17,37–40] Multiple studies have concluded that exercise interventions alone alter HDL particle subclass and function in patients with metabolic syndrome and obesity.[32,41] Koba et al. reported that following cardiac rehabilitation, patients displayed increased HDL-C concentrations and CEC.[20]

Despite these positive findings, few intervention studies with clear conclusions exist which have isolated exercise from diet. A recent review cited inconsistency in methods as well as insufficient study length as current sources of uncertainty in the literature focusing on exercise and CEC.[19] Sang et al. found no change in CEC following a walk/run training program.[42] Sarzynski et al. found improvements in CEC in subjects from the STRRIDE-PD and E-MECHANIC randomized trials.[22] However, the improvements were only observed in subjects randomized to the high intensity or high dose training groups. Findings published by Hernaez et al. found a positive association between leisure time physical activity and improvements in HDL functionality in a one-year study of high cardiovascular individuals using self-reporting methods.[43] These findings as well as the vast heterogenous responses identified may be suggestive of a dose-response relationship between CEC and exercise.

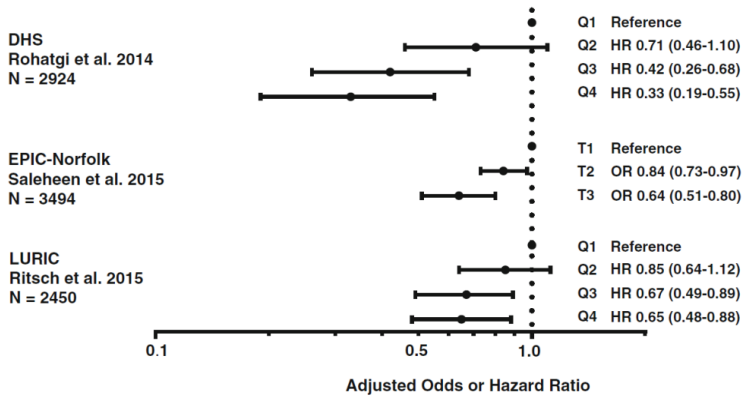

Figure 2.1. Cholesterol efflux capacity (CEC) and risk of incident cardiovascular events across three cohorts. The depicted hazards ratio (HR) and odds ratio (OR) with 95 % CI are adjusted for traditional risk factors. Q quartile, T tertile. From Bhatt and Rohatgi, 2016.

CHAPTER 3

METHODS

The HERITAGE Family Study (HERITAGE) was a multisite exercise intervention study with the stated principle aim to determine genetic factors associated with cardiovascular, metabolic, and hormonal response to aerobic training. Detailed methods of HERITAGE have been previously outlined in detail.[44] Briefly, researchers recruited Black and White families, mostly consisting of two parents and at least three biological children. Subjects were between the ages of 17 and 65 years and were considered inactive (i.e., no regular physical activity in the 3 months prior to study) but otherwise healthy. Normotensive and mildly hypertensive individuals (BP < 160/100 mmHg) without medication were included in the study and individual BMI was required to be less than 40 kg/m^2. A detailed medical history for each patient was obtained and physical examination was completed prior to onset of the exercise program.

At baseline, subjects submitted to a comprehensive health screening which included an electrocardiogram at rest and during exercise testing in conjunction with a battery of questionnaires related to food intake, behavior and health habits. The training program consisted of submaximal aerobic exercise on a cycle ergometer, 3 times per week for twenty weeks. Intensity of training was determined relative to individual VO_{2max} measured at baseline. Intensity began at the heart rate associated with 55% of VO_{2max} and increased every four weeks.

Volume was also modified as training progressed, beginning at 30 minutes of exercise, and increasing every 4 weeks. These modifications were offset so that an alteration in volume/intensity occurred every 2 weeks throughout the program with a final intensity of 75% of VO_{2max} for 50 minutes for the final 6 weeks (**Table 3.1**).

Lipid and lipoprotein profile measurements. Blood samples for analysis of plasma lipid and lipoprotein levels were obtained twice at baseline and 24h and 72h after the last exercise session following a 12 hour overnight fast. Cholesterol and triglyceride levels were determined using the Technicon RA-1000 analyzer. Plasma very low-density lipoprotein (VLDL) were first isolated via ultra-centrifugation. High-density lipoprotein (HDL) was measured following precipitation of low-density lipoprotein (LDL) with heparin and $MnCl_2$. Infranatant concentrations of Apoprotein (Apo) B and Apo-A1 were measured via electrophoresis. LDL-C, LDL-TG, and VLDL-Apo B were then calculated from the difference. Cholesterol concentrations of HDL subfractions were also determined following precipitation. Lipoprotein traits were adjusted for changes in exercise induced changes in hemodilution. Extensive quality-control procedures were implemented to ensure high quality lipid assays[45] and other study data.[46]

Comprehensive lipoprotein analysis was performed on fasting plasma samples collected before and after completion of exercise training by NMR spectroscopy at LabCorp, Inc (Morrisville, N.C.) using the LipoProfile-4 algorithm.[47] A total of 24 NMR-derived lipoprotein subclass traits will be included in the proposed analysis, including concentration of large, medium, small HDL,

LDL, and triglyceride-rich lipoprotein (TRLP) particles and their average particle sizes.

Preparation of ApoB-Depleted Plasma (Polyethylene Glycol-Precipitation). EDTA plasma collected from participants was depleted of apoB (apolipoprotein B)-containing lipoproteins utilizing the polyethylene glycol precipitation method. A solution of 20% polyethylene glycol (molecular weight 6000, Sigma-Aldrich) was prepared in 200 mmol/L, pH 7.4 glycine buffer. For every 10 µL of whole plasma, 4 µL of polyethylene glycol was added (plasma:polyethylene glycol, 10:4 ratio). The mixture was then incubated for 20 minutes at room temperature and subsequently centrifuged at 16 000 rpm for 30 minutes at 4 °C. Following centrifugation, the supernatant was transferred to a fresh tube and centrifuged a second time to ensure complete removal of ApoB-containing lipoproteins.

^{3}H cholesterol efflux capacity assay. Measurement of the efflux of radiolabeled (^{3}H) cholesterol from J774 macrophages to apoB depleted plasma was performed with the use of a previously described method.[26] Briefly, cholesterol efflux capacity was measured using J774 mouse macrophage cells in the presence and absence of cAMP, thus providing values for global efflux, as well as non-ABCA1 dependent efflux. Cells were incubated with ^{3}H-labeled cholesterol and 2 µg/mL acyl–coenzyme A:cholesterol acyltransferase inhibitor (Sandoz, Sigma-Aldrich). Cells were then incubated overnight in 0.2% BSA with or without 8-(4-chlorophenylthio)-cyclic AMP. After washing, the cholesterol labeled cells were incubated with 2.8% apolipoprotein B (apoB)-depleted plasma for 4 hours. The amount of ^{3}H-labeled cholesterol released was measured

through liquid scintillation counting. Cholesterol efflux capacity is calculated as the amount of effluxed cholesterol expressed as a fraction (%) of the initial cell content of cholesterol. Results were normalized to the measured efflux by a pooled reference apoB-depleted sample evaluated on every plate. All samples were run in duplicate and the average value is reported.

This assay quantifies total efflux mediated by known pathways of cholesterol efflux from macrophages, including ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1), scavenger receptor B1, and aqueous diffusion.[48] The assays were performed with and without stimulation by cAMP, thus providing values for global efflux, as well as non-ABCA1 dependent efflux. ABCA1-dependent efflux was calculated as the difference between global (+cAMP) and non-ABCA1 (-cAMP) efflux.

Study sample size. Measures of CEC at baseline and post-training were available in 542 HERITAGE subjects, which represents the maximum sample size for the proposed analyses. Of the 542 subjects, 56% are female, 37% Black, and 34% parents and 66% offspring.

*Statistical analysis***.** To examine the effects of exercise training on CEC in Aim 1, paired t-test were performed to evaluate the difference in mean CEC values between baseline and post-training. Paired t-tests were performed in the total sample (primary analysis) and separately in men, women, White subjects, Black subjects, parents, offspring, those with or without clinically significant weight loss (5% or more), and those with or without metabolic syndrome (secondary analysis). Additionally, to examine whether the mean change in CEC

after training differed between subgroups, two sample t-tests were performed across generation (parents/offspring), sex, ethnicity, clinically significant weight loss (yes/no) and metabolic syndrome (yes/no) groups. For subgroup analyses, given there are five subgroups and two CEC traits, we used a Bonferroni-corrected p-value threshold of 0.005 to determine statistical significance.

In Aim 2, Pearson's correlations were used to examine the associations between change in CEC and concomitant change in lipid and lipoprotein traits as well as measures of body composition in the total sample. Correlation models were tested as univariate models. To account for multiple testing (13 lipid traits x 2 CEC traits = 26 tests, 10 measures of body composition x 2 CEC traits = 20 tests) Bonferroni-corrected p-value thresholds of 0.002 and 0.0025 were used to determine statistical significance for correlation models of lipid traits and measures of body composition respectively.

Table 3.1. Overview of the 20-week training program in HERITAGE.

Weeks	Frequency (sessions/wk)	Intensity (% VO_2max)	Duration (min/session)*
2	3	55	30
2	3	55	35
2	3	65	35
2	3	65	40
2	3	70	40
2	3	70	45
2	3	75	45
6	3	75	50

*Does not include 5 min warm-up or 3 min cool-down

CHAPTER 4

RESULTS

Cholesterol efflux data was obtained on a total of 542 subjects. Baseline characteristics of the subjects, as well as changes observed in response to exercise training are displayed in **Table 4.1**. There were wide inter-individual differences in percent change of CEC in response to exercise training (**Figure 4.1**). Neither global nor non-ABCA1 CEC significantly changed with training in the whole cohort. In subgroup analyses, the magnitude of training response was nominally ($p<0.05$) different between those with and without metabolic syndrome ($p=0.04$), with global efflux significantly decreasing among individuals with metabolic syndrome (mean change in CEC = -0.05, SD=0.22, $p=0.03$). However, this difference was not statistically significant after accounting for multiple testing and no other differences in efflux training response were observed among other subgroups (**Table 4.2**).

Subject and clinical characteristics were not found to be associated with change in cholesterol efflux (data not shown). Baseline fat mass ($r=-0.09$, $p=0.04$), measured by underwater weighing, and baseline waist circumference ($r=-0.09$, $p=0.047$) both displayed nominal ($p<0.05$) yet weak, inverse associations with change in non-ABCA1 CEC. However, these values did not remain significant upon accounting for multiple testing and no other significant associations between baseline measures nor changes in body composition and

changes in CEC were observed (**Table 4.3**). Additionally, there were no differences in mean change in CEC between subjects who experienced clinically significant weight loss (N=33) and non-obese subjects (N=509) (data not shown).

Changes in HDL-C and apoA-I were weakly, positively correlated with changes in global efflux at a nominal level ($p<0.05$), while change in LPL activity was significantly, positively associated with change in non-ABCA1 CEC (**Table 4.4**). Additionally, changes in several HDL subclass traits were nominally correlated with global efflux and non-ABCA1 efflux (**Table 4.5**). Notably, total HDL-P concentration and mean HDL-P size were significantly, but weakly, positively correlated with training-induced changes in both global and non-ABCA1 CEC, while the concentration of large HDL-P were significantly, but weakly, positively associated with global CEC.

Table 4.1. HERITAGE subject characteristics at baseline and in response to exercise training (n=542).

Characteristic	Baseline	Delta	p-value
Age (yrs)	35.11 (13.5)		
BMI (kg/m^2)	26.3 (5.4)	-0.13 (0.85)	0.0006
VO_{2max} (L/min)	2.35 (0.74)	0.39 (0.2)	<0.0001
HDL-C (mmol/L)	1.07 (0.28)	0.04 (0.12)	<0.0001

Values shown as mean (SD).

Table 4.2. Changes in cholesterol efflux capacity in subgroups of HERITAGE (n=542).

Trait	Male (n=238)	Female (n=304)	Black (n=198)	White (n=344)	Parent (n=186)	Offspring (n=356)	Metabolic Syndrome (n=98)	No Metabolic Syndrome (n=397)	Weight Loss (n=33)	No Weight Loss (n=509)
Global Efflux	-0.02 (0.20)	-0.006 (0.19)	-0.002 (0.20)	-0.02 (0.19)	-0.01 (0.21)	-0.01 (0.18)	-0.05* (0.22)	-0.003 (0.18)	-0.02 (0.19)	0.01 (0.19)
Non-ABCA1 Efflux	0.002 (0.19)	0.002 (0.16)	-0.001 (0.18)	0.004 (0.17)	0.007 (0.18)	-0.001 (0.17)	-0.03 (0.17)	0.008 (0.17)	-0.03 (0.20)	0.0003 (0.17)

Values displayed as mean (SD). *p=0.04 for mean difference between groups with and without metabolic syndrome.
No other subgroups showed p<0.05 for differences of mean training response.

Table 4.3. Correlations of baseline values and changes in measures of body composition with change in CEC in HERITAGE (n=542)

		Global Efflux		Non-ABCA1 Efflux	
Variable		r	p-value	r	p-value
BMI (kg/m^2)	Baseline	0.001	0.99	-0.06	0.18
	Delta	0.02	0.69	-0.01	0.87
Total Body Mass (kg)	Baseline	0.003	0.9365	-0.04	0.37
	Delta	0.005	0.91	0.004	0.93
Fat Weight (kg)	Baseline	0.02	0.65	-0.09	0.04
	Delta	0.02	0.60	-0.003	0.95
Fat Free Weight (kg)	Baseline	-0.001	0.98	0.008	0.85
	Delta	-0.02	0.74	0.02	0.61
Percent Body Fat (%)	Baseline	0.008	0.86	-0.08	0.07
	Delta	0.05	0.31	-0.04	0.41
Waist Circumference (cm)	Baseline	-0.03	0.43	-0.09	0.046
	Delta	0.07	0.13	-0.02	0.60
Waist/Hip Ratio	Baseline	-0.05	0.27	-0.10	0.03
	Delta	0.07	0.11	-0.04	0.41
Total Abdominal Fat Area (cm^2)	Baseline	-0.01	0.75	-0.07	0.10
	Delta	-0.03	0.48	-0.03	0.54
Visceral Abdominal Fat Area (cm^2)	Baseline	0.0003	0.99	-0.06	0.12
	Delta	-0.03	0.54	-0.02	0.66
Subcutaneous Abdominal Fat Area (cm^2)	Baseline	-0.02	0.69	-0.06	0.14
	Delta	-0.03	0.56	-0.02	0.60

Table 4.4. Correlations of change in CEC with changes in lipid and lipoprotein phenotypes in HERITAGE (n=542).

	Global Efflux		Non-ABCA1 Efflux	
Variable	r	p-value	r	p-value
HDL-C (mmol/L)	0.10	0.02	0.12	0.006
LDL-C (mmol/L)	0.002	0.96	-0.05	0.22
VLDL-C (mmol/L)	-0.02	0.69	-0.03	0.43
TG (mmol/L)	0.02	0.62	-0.001	0.97
ApoA-I (mmol/L)	0.09	0.04	0.04	0.38
ApoB (mmol/L)	0.04	0.37	-0.01	0.76
TC (mmol/L)	0.02	0.67	-0.03	0.54
LPL activity (nmol/mL/min)	0.07	0.12	**0.17**	**0.0001**

Values in bold meet Bonferroni corrected threshold of significance ($p<0.002$).

Table 4.5. Correlations of change in CEC with concomitant changes in lipoprotein subclass phenotypes in HERITAGE (n=537).

	Global Efflux		Non-ABCA1 Efflux	
Variable	r	p-value	r	p-value
Total HDL-P µmol/L)	**0.17**	**<0.0001**	0.12	0.007
Large HDL-P (µmol/L)	0.12	0.004	0.09	0.03
Medium HDL-P (µmol/L)	0.12	0.005	0.11	0.01
Small HDL-P (µmol/L)	0.03	0.55	-0.01	0.90
HDL-P Size (nm)	0.12	0.003	**0.14**	**0.001**

HDL-P, HDL particle. Values in bold meet Bonferroni corrected threshold of significance ($p<0.002$).

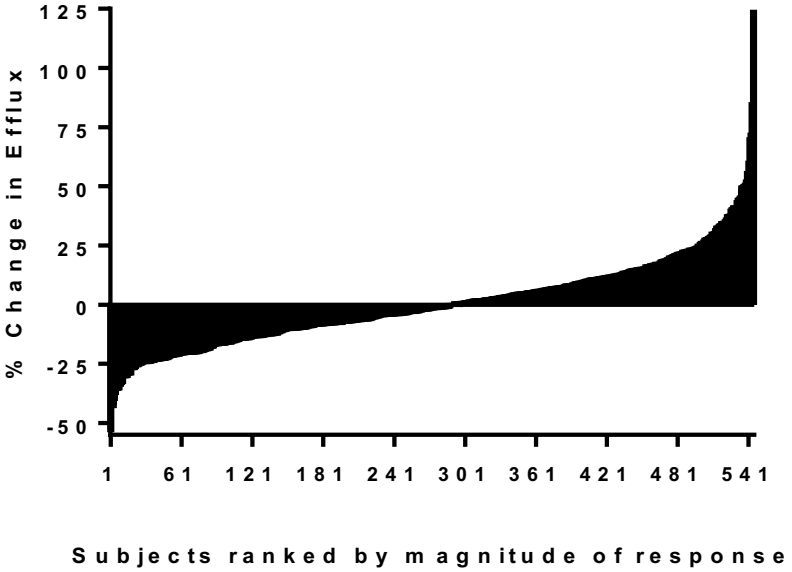

Figure 4.1. Distribution of Individual responses of percent change in cholesterol efflux capacity in HERITAGE (n=542).

CHAPTER 5

DISCUSSION

We found that 20 weeks of endurance exercise training did not change measures of CEC in the total cohort or in subgroups. Several existing studies support the current findings of no effects of regular exercise on CEC. A case-control study conducted by Sang et al. found no change in CEC in 39 patients with metabolic syndrome following a 10 week walk/run training program.[42] In a randomized controlled trial, Woudberg et al. found no effect of 12 weeks of combined aerobic and resistance exercise training on CEC in obese women.[41] Dokras et al. also reported no change in CEC in women with polycystic ovarian syndrome who received an intensive lifestyle intervention consisting of caloric restriction, moderate intensity physical activity and weight loss medication.[49] In the PREDIMED-Plus randomized controlled trial, 391 older adults with metabolic syndrome were assigned to a 6 month lifestyle intervention of diet plus moderate physical activity or a control group. HDL triglyceride metabolism was improved but no change in CEC was observed.[50]

Conversely, other interventions have shown increases in CEC. Boyer et al. found CEC increased by 14.5% in 113 middle aged men (48 $\pm$ 8.5 yrs) with abdominal obesity and dyslipidemia that participated in a 1-year lifestyle intervention, which included a calorically restricted diet as well as 160 minutes of moderate to vigorous physical activity per week.[39] Khan et al. observed

improvements in CEC in 53 patients with metabolic syndrome following a 12 week diet and exercise intervention resulting in weight loss.[37] Lesna et al. also reported improvements in CEC in obese females following a 9 week diet and exercise program, with the increases in CEC correlated with weight loss.[51]

The above studies which found improvements in CEC following diet and exercise interventions all reported clinically significant weight loss. In HERITAGE, however, subjects were instructed to remain weight stable throughout the course of the study. While there was a slight reduction in weight over the course of the intervention, this reduction was not associated with changes in CEC nor would this decline in weight be considered clinically significant. Thus, the lack of dietary or lifestyle interventions in the present study as well as the lack of clinically significant weight loss may serve as partial explanations for the lack of improvement in CEC. Another explanation may be that almost all of the aforementioned studies were performed in individuals with existing health issues and/or disease states (e.g., pre-diabetes, dyslipidemia, obesity, metabolic syndrome, etc.), whereas the HERITAGE cohort consisted of mostly healthy adults.

As stated, the previously described studies largely were lifestyle and not solely exercise interventions, and as such, did not include supervised and standardized exercise programs. It has been proposed that a dose-response relationship may exist between exercise intensity and improvements in CEC.[22] While exercise intensity in the present study was moderate-to-vigorous, it remained below doses reported by Sarzynski et al. to be effective at increasing

CEC until the final six weeks of exercise training. Specifically, the authors found global CEC significantly increased 6.2% in the high-amount/vigorous intensity group (16 kcal/kg/week at 75% intensity) in comparison to other STRRIDE-PD groups. In E-MECHANIC, non-ABCA1 CEC significantly increased 5.7% in the highest amount group (20 kcal/kg/week at 65-85% intensity) compared to control with no change observed in other groups. It is possible that continued exercise training (past the assigned 20 weeks) and/or higher intensities in the present study may have improved CEC. Although, to the authors' knowledge, no proposed mechanisms for such a relationship between exercise intensity and CEC exist.

Positive associations between LPL activity and non-ABCA1 efflux were observed in the current study. LPL is an important factor in the maturation of HDL precursors.[52] Thus, an increase in LPL activity would expectedly result in an increased CEC capacity due to increased formation of HDL-P that can then accept cholesterol. HDL-P concentration and mean size also significantly correlated with changes in CEC in the present study. An increase in HDL-P concentration increases the number of HDL particles available for efflux and mean particle size increases through continued efflux and cholesterol esterification.[53]

The current study had several limitations. Most notable is a lack of a control group as individual baseline values served as the control in this instance. Additionally, only a single exercise dose was prescribed across all subjects. Thus, generalizability to modes and/or doses/higher intensities is limited and a

dose-response relationship between exercise training and CEC could not be assessed in the present study. However, strengths of the current study include the large sample size, diverse population, high adherence (≥95%), and stringent supervision and standardization of the exercise program that meets the national physical activity guidelines.

In conclusion, moderate to vigorous endurance exercise training had no effect on CEC in a large cohort of mostly healthy young-to-middle aged Black and White adults. This is the largest study to date to assess the effects of exercise training on CEC and our null findings are supported by several previous studies. While it appears evident that exercise training has no effect on CEC in healthy populations, future research should be directed at investigating the potential impact of exercise training on CEC in clinical populations, as well as in combination with dietary interventions resulting in weight loss.

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