Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 20 (2009) 26 – 34
Journal of Nutritional Biochemistry

Habitual consumption of eggs does not alter the beneficial effects of endurance training on plasma lipids and lipoprotein metabolism in untrained men and women

Lisa M. Vislocky, Matthew A. Pikosky, Kristin Herron Rubin, Sonia Vega-López, P. Courtney Gaine, William F. Martin, Tosca L. Zern, Ingrid E. Lofgren, Maria Luz Fernandez*, Nancy R. Rodriguez

Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA

Received 24 August 2007; received in revised form 20 November 2007; accepted 26 November 2007

Abstract

Changes in plasma lipid and apolipoprotein profiles were evaluated in 12 healthy, unfit subjects (VO_{2peak} 39.1±2.8 ml·kg^{-1}·min^{-1}; 5 women, 7 men) at baseline and following endurance exercise training. The exercise protocol consisted of a 6-week endurance exercise training program (4–5 days week^{-1}; 60 min·session^{-1}; ≥65% HR_{max}). Subjects were randomly assigned to consume an egg- (n=6; 12 eggs·week^{-1}) or no-egg (n=6; 0 eggs·week^{-1})-based, eucaloric, standardized diet for 8 weeks. Both diets were macronutrient balanced [60% carbohydrate, 30% fat, 10% protein (0.8 g·kg^{-1}·day^{-1})] and individually designed for weight maintenance. Plasma lipids were measured twice within the same week at baseline and following exercise training. At baseline, subjects were normolipidemic with values of 163.9±41.8, 84.8±36.7, 60.6±15.4 and 93.1±52 mg dl^{-1} for total cholesterol, LDL cholesterol and HDL cholesterol and triglyceride concentrations, respectively. A two-way ANOVA was used to analyze diet and exercise effects and interactions. In both groups, endurance exercise training resulted in a significant 10% increase in HDL-C (P<.05), a 19% decrease in Apo B concentrations (P<.05) and reductions in plasma CETP activity (P<.05). Plasma LDL-C decreased by 21% (P=.06). No main effects of diet or interactions with plasma lipids or Apo B concentrations were observed. These data demonstrate that endurance training improved the plasma lipid profiles of previously unfit, normolipidemic subjects independent of dietary cholesterol intake from eggs.

1. Introduction

Cardiovascular disease (CVD), any of several diseases affecting the heart or blood vessels, is the number one cause of death in America [1]. Coronary heart disease (CHD), atherosclerosis of the coronary arteries specifically, accounted for more than half of CVD deaths in America in 2004 and is the primary killer of American men and women [1]. Major modifiable risk factors for developing CHD are abnormal blood cholesterol levels [i.e., elevated total cholesterol (TC) and LDL cholesterol (LDL-C) and low HDL cholesterol (HDL-C)]. To decrease the risk of developing CHD, the American Heart Association (AHA) and American College of Sports Medicine [2] along with the United States Department of Agriculture (USDA) and the Department of Health and Human Services (DHHS) [3] have established physical activity and dietary guidelines to favorably modify blood cholesterol levels. For healthy adults, these guidelines recommend at least 30 min of moderate-intensity, aerobic activity at least 5 days per week and a decrease in the consumption of foods high in saturated fats, trans-fatty acids and cholesterol.

It is well known that physical activity is associated with a decreased risk of coronary heart disease [1,4]. Data from

Abbreviations: AHA, American Heart Association; Apo B, apolipoprotein B; CETP, cholesterol ester transfer protein; CHD, coronary heart disease; CVD, cardiovascular disease; DHHS, Department of Health and Human Services; HDL-C, high-density lipoprotein cholesterol; HR_{max}, age-predicted maximal heart rate; IOM, Institute of Medicine; LCAT, lecithin: cholesterol acyltransferase; LPL, lipoprotein lipase; LDL-C, low-density lipoprotein cholesterol; USDA, United States Department of Agriculture; VO_{2max}, maximal oxygen consumption; VO_{2peak}, peak oxygen consumption

* Corresponding author. Tel.: +1 860 486 5547; fax: +1 860 486 3674.
E-mail address: maria-luz.fernandez@uconn.edu (M.L. Fernandez).

0955-2863/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.11.011

cross-sectional studies suggest aerobically trained individuals have blood lipid profiles which place them at lower risk of developing CHD when compared to sedentary controls [5–10]. However, conflicting data [11–19] make it unclear what specific changes in lipoprotein profiles occur following endurance exercise training to exert this protective effect [20]. However, in groups regularly expending 1200–2200 kcal of energy per week by briskly walking or jogging, HDL-C typically increases while TG levels decrease [20].

Associations between elevated levels of serum cholesterol and increased risk of CHD [21] and increased dietary cholesterol and elevations in serum lipids [22] were first identified in the 1960s. However, epidemiological, animal and human studies have failed to establish a definitive relationship between cholesterol consumption and CHD development [23–26]. Nonetheless, the message to avoid high-cholesterol eggs has been ubiquitously recommended by government agencies and health professionals for many years.

One study [24] investigating the dietary intake of 117,000 nurses and health professionals over 14 years reported no differences in the relative risk of CHD development between individuals consuming <1 egg·week^{-1} vs. >1 egg·day^{-1}. Ballesteros et al. [26] reported that consuming 2 eggs·day^{-1} by children 10–12 years old did not increase CHD risk as determined by the nonsignificant change in the LDL-C/HDL-C, an important marker of CHD risk [27,28]. Similarly, in men and premenopausal women consuming 3 eggs·day^{-1} for 30 days, Herron et al. [27,28] observed no change in CHD risk as determined by insignificant alterations in LDL-C/HDL-C. Still, in 2000, eggs comprised only 1.3% of total energy consumed by Americans [29].

The purpose of this investigation was to determine how endurance training would influence blood lipids, apoproteins and lipoprotein metabolism in healthy, previously sedentary adults. In addition, we evaluated whether a diet supplying cholesterol primarily from eggs and in excess of the upper limits proposed by the AHA [30], USDA and DHHS [3] and IOM [31] would impact the blood lipid responses observed with training. To achieve this objective, we studied 12 normolipidemic adult men and women over 6 weeks who were assigned to one of two groups: (1) Egg (12 eggs·week^{-1}; 468.5 mg cholesterol·day^{-1}) or (2) No Egg (0 eggs·week^{-1}; 159.2 mg cholesterol·day^{-1}). We hypothesized that 6 weeks of aerobic exercise training would favorably impact blood lipid profiles independent of egg (and hence, cholesterol) intake.

2. Methods and materials

2.1. Subjects

Twelve untrained, but otherwise healthy adults (n=12; 7 men, 5 women) ages 18–30 years were recruited from the university community. Eleven subjects (6 men, 5 women) completed the intervention. Prior to selection for the study, participants were asked to provide a complete medical history, activity log and record of dietary intake. Individuals reporting metabolic or cardiovascular abnormalities, gastrointestinal disorders (i.e., lactose intolerance, egg protein allergies), use of nutritional/sports supplements or anabolic steroids, or weekly exercise totaling >90 min were excluded from the study. In addition, potential qualifying subjects underwent a test of peak oxygen consumption (VO_{2peak}) to confirm they were untrained (<45 mL·kg^{-1}·min^{-1} and <40 mL·kg^{-1}·min^{-1} for men and women, respectively). Women were eumenorrheic, not taking oral contraceptives and nonpregnant as determined by self-report. Informed, written consent was obtained from all participants. The study protocol was approved by the University of Connecticut Institutional Review Board.

2.2. Experimental design

Study participants were randomly assigned to one of two weight maintaining dietary treatments for 8 weeks. Following 2 weeks of diet intervention only, the subjects began a 6-week endurance exercise training program while remaining in the same diet intervention group. Various plasma indices of lipid metabolism were assessed prior to the start of the diet intervention (to ensure differences did not exist between groups following randomization) and following 6 weeks of endurance training. Additional criterion measures [i.e., VO_{2peak}, body composition, resting energy expenditure (REE) and a 3-mile time trial performance] were assessed pre- and post-training.

2.3. Anthropometric, metabolic and performance assessments

Peak oxygen uptake (VO_{2peak}) testing was determined via breath-by-breath analysis of expired gases during testing using an open circuit respiratory apparatus (MedGraphics CPX/D, Medical Graphics Corporation, St. Paul, MN, USA) on a treadmill (Quinton MedTrack ST55, Bothell, WA, USA) [32]. Body composition was measured using dual-energy X-ray absorptiometry (DEXA, DPX-MD Lunar Corp., Madison, WI, USA) prior to the initiation of the diet intervention (untrained) and following the combined dietary intervention and exercise training (trained). Whole body scans were obtained while the subject was supine, wearing only a hospital gown and undergarment with all jewelry and metal removed. Scan times were approximately 10 min per assessment. REE was measured using open-circuit indirect calorimetry (MedGraphics CPX/D, Medical Graphics Corporation) before study participants began the diet intervention. Subjects drove or were driven to the metabolic laboratory immediately after waking, were fasted overnight (10 h) and rested quietly 15–20 min before REE was determined over a 20-min period with the subject lying in a quiet, temperature-regulated room. REE was assessed following 2 weeks of diet alone (untrained) and following 4 weeks of dietary intervention and exercise training

(trained). Time to complete a 3-mile (4.8 km) run was recorded and monitored by research personnel prior to and following 6 weeks of exercise training. Body weight and height were measured using a balance beam scale equipped with a measuring rod (Health-o-meter, Bridgeview, IL, USA). Body weight was assessed at baseline and weekly throughout the study to ensure weight maintenance. Calorie intake was adjusted accordingly if gains or losses of greater than 1% body weight occurred.

2.4. Dietary intervention

Energy needs were estimated for each individual (based on REE measurements along with individual dietary intervention and adjusted using reported physical activity data) to maintain energy balance throughout the entire study. Subjects were randomly assigned to a non-egg-based (No-Egg group; 0 eggs $week^{-1}$) or an egg-based (Egg group; 12 eggs $week^{-1}$) diet for 8 weeks. For all subjects, and therefore both diet interventions, protein intake was set at 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ (10% of total calories) and fat at 30% of total calories. Carbohydrates supplied the remainder of the available calories. All foods (meals and snacks) were prepared and provided by University of Connecticut catering services. Research personnel were present at all meals to weigh and serve the appropriate portions of food specific for each subject and to record intake for nutrient analysis. Meals between diet groups differed only in that eggs were substituted for other sources of high-quality protein at some meals for the Egg and No-Egg groups, respectively. Subjects were instructed not to consume any other foods other than what was supplied to them. Dietary compliance was verified by the maintenance of body weight during weekly weigh-ins.

2.5. Exercise training protocol

Exercise training was initiated following 2 weeks of dietary intervention. Subjects participated in supervised and monitored exercise sessions of 30–45 min which consisted of stretching and group runs/walks 3–5 days·$week^{-1}$. To minimize injury risks or compliance issues, training volumes followed a gradual weekly progression beginning with 3 sessions·$week^{-1}$, 30 min·$session^{-1}$ the first week until the goal of 5 sessions·$week^{-1}$, 45 min·$session^{-1}$ was achieved by Week 5 of the exercise intervention. For all training sessions, subjects were provided Polar HR monitors (Polar Electro Inc., Port Washington, NY, USA) to assist with keeping their exercise intensity within the desired HR range of 65–85% age predicted maximal heart rate (HR_{max}; HR_{max}=220−age). All data from each training session, i.e., total time, average heart rate, time spent in target heart rate zone, and estimated energy expenditure, were recorded.

2.6. Sample collection and storage

The effect of diet and exercise on TC, LDL-C, HDL-C, TG, Apo B, Apo C-I, Apo C-III, Apo E concentrations and the LDL/HDL ratio was examined at baseline and after 6 weeks of exercise. Two fasting (12 h) blood samples were initially collected, on different days, from each subject into tubes containing 0.15 g/100 g EDTA to determine baseline plasma lipids. Similarly, two fasting blood samples were collected (one at 5 and another at 6 weeks of training), from each subject into tubes containing 0.15 g/100 g EDTA to determine "training" plasma lipids. Plasma was separated by centrifugation at 1500×*g* for 20 min at 4°C, placed into vials containing phenyl methyl sulphonyl fluoride (0.05 g/100 g), sodium azide (0.01 g/100 g) and aprotinin (0.01 g/100 g) and stored at −80°C until analysis. Plasma samples were used to determine plasma lipid, apolipoprotein and cholesterol-specific enzyme concentrations.

2.7. Materials

Enzymatic total cholesterol and triglyceride (TG) kits were obtained from Roche-Diagnostics (Indianapolis, IN, USA). Free cholesterol, Apo C-III and apo E kits were ordered from Wako Pure Chemical (Osaka, Japan). Apo B kits, EDTA, aprotinin, sodium azide and phenyl methyl sulfonyl fluoride (PMSF) were obtained from Sigma Chemical (St. Louis, MO, USA). All consumables were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.8. Plasma lipids and apoproteins

Our laboratory has been participating in the Centers for Disease Control–National Heart, Lung and Blood Institute (CDC-NHLBI) Lipid Standardization Program since 1989 for quality control and standardization for plasma total cholesterol (TC), HDL-C and triglyceride (TG) assays. Coefficients of variance assessed by the Standardization Program during the study period were 0.76–1.42 for TC, 1.71–2.72 for HDL-C and 1.64–2.47 for TG.

TC was determined by enzymatic methods using Roche-Diagnostics standards and kits [33]. HDL-C was measured in the supernatant after precipitation of Apo B-containing lipoproteins [34] and LDL-C was determined using the Friedewald equation [35]. TG was determined using Roche-Diagnostics kits, which adjust for free glycerol. Means of the two blood draws were used to assess differences between treatment periods. Kits that utilize an immunoturbidimetric method were obtained from Sigma for the determination of Apo B concentrations. Turbidity was measured in a microplate spectrophotomer at 340 nm [36]. Apo C-III [37] and Apo E [38] were measured with a Hitachi Autoanalyzer 740 utilizing kits from Wako.

2.9. Plasma CETP and LCAT determinations

CETP activity was determined according to Ogawa and Fielding [39], which measures the mass transfer of cholesterol ester between HDL and Apo B containing lipoproteins. Thus, physiological CETP activity was found through an analysis of the decrease in HDL cholesterol ester mass between 0 and 6 h, without LCAT inhibition. Samples were incubated at 37°C for 6 h in a shaking water bath.

Following this period, total, HDL and free plasma cholesterol were measured, and previously described calculations were performed [40]. LCAT activity was determined by an endogenous self-substrate method, which involves mass analysis of the decrease in plasma free cholesterol between 0 and 6 h at 37°C. Assays were carried out concurrently with measurements of CETP. Both of these methods have been standardized in our laboratory.

2.10. Nutrient analysis

Daily diet records were analyzed for energy, macronutrient composition, cholesterol and fiber intake using the US Department of Agriculture National Nutrient Database for Standard Reference as part of Nutritionist Pro Software (First Data Bank, Inc., version 1.1).

2.11. Statistical analysis

Sample size analysis revealed that 11 subjects were needed to detect a difference from untrained to trained in all plasma lipid measures. Values are reported as means±S.E.M. Two-way ANOVA was used to analyze diet and exercise effects and interactions. Paired *t* tests were used to detect differences in physical characteristics, TC, LDL-C, HDL-C, TG, LDL/HDL, apolipoproteins, LCAT and CETP at baseline and following 6 weeks of exercise training. The level for statistical significance was set at $P<.05$.

3. Results

3.1. Baseline and post-training subject characteristics

Baseline (Untrained) and post-endurance training (Trained) characteristics for the Egg (n=3 males, 2 females) and No-Egg (n=3 males, 3 females) groups are presented in Table 1. No differences were noted between groups for any of the criterion measures at baseline or post-training. From pre- to post-training, the Egg group experienced an increase in REE and VO_{2peak} ($P<.01$) and a significant increase in the 3-mile time trial performance ($P<.05$). The No-Egg group also experienced an increase in VO_{2peak} ($P<.01$) and in 3-mile time trial performance ($P<.01$).

Table 1
Baseline (Untrained) and post endurance training (Trained) subject characteristics for Egg and Non-Egg diet groups[a]

Characteristic	Untrained		Trained	
	Egg	No Egg	Egg	No Egg
Age (years)	20.3±0.5	21.2±0.5	20.3±0.5	21.5±0.6
Height (cm)	170.5±4.3	167.2±7.2	170.5±4.3	167.2±7.2
Weight (kg)	79.9±8.4	76.6±8.5	79.6±8.0	75.7±8.5
REE (kcal day^{-1})	1591±183	1644±191	1709±179*	1739±189
VO_{2peak} (ml kg^{-1} min^{-1})	41.9±3.4	38.6±2.4	46.1±4.4*	41.8±2.7*
Body fat %	26.3±4.3	33.2±5.7	26.3±4.9	32.9±5.7
Fat free mass (kg)	55.8±5.6	47.2±6.8	55.8±5.7	47.8±6.9
Run time trial (min)	30:06±3:07	33:47±2:24	27:22±3:42*	29:06±2:06*

[a] Values expressed as mean±S.E.M. Egg group: n=3 males, 2 females; No-Egg group: n=3 males, 3 females.
* Indicates within-group differences from Untrained to Trained ($P<.05$).

Table 2
Energy and dietary carbohydrate, fat, protein, fiber, cholesterol, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) intake for Egg and No Egg groups[a]

Nutrient	Egg	No Egg
Energy [kcal $(kg\ BW)^{-1}\ day^{-1}$]	33±3	31±2
Carbohydrate (% energy)	60±1	61±1
Fat (% energy)	28±1	28±1
SFA (g day^{-1})	24.3±1.5	22.0±1.9
MUFA (g day^{-1})	22.3±1.6	21.4±1.1
PUFA (g day^{-1})	18.7±2.3	19.7±1.2
Protein (% energy)	12±1	11±1
Protein [kcal $(kg\ BW)^{-1}\ day^{-1}$]	0.91±0.06	0.80±0.07
Fiber (g day^{-1})	19.3±0.8	20.1±0.4
Cholesterol (mg day^{-1})	468.5±21.4*	159.2±4.5

BW, body weight; %, percent of total calories.
[a] Values are expressed as mean±S.E.M. Egg group: n=3 males, 2 females; No-Egg group: n=3 males, 3 females.
* Indicates significant difference as determined by independent *t* test ($P<.01$).

3.2. Dietary intervention

Mean daily energy, macronutrient intake, grams of protein per kilogram body weight, and grams of fiber and cholesterol intake are shown in Table 2. Average daily fiber, cholesterol, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are also shown in Table 2. No differences were observed between diet groups in energy intake expressed per kilogram body weight; percentage of total calories that carbohydrates, fat and protein represented in the diet; or fiber intake. The only dietary variable different between groups was the higher cholesterol intake in the Egg vs. No-Egg group (468.5±21.4 vs. 159.2±4.5, $P<.01$).

3.3. Plasma lipids and apoproteins

Plasma lipid and apoprotein responses to training were independent of diet group (i.e., Egg vs. No Egg). Therefore, all plasma lipid, apolipoprotein, LCAT and CETP data were pooled and differences in the concentrations of these variables between Untrained and Trained are presented

Table 3
Plasma lipid values in healthy adults at baseline (Untrained) and after 6 weeks of endurance training (Trained)[a]

	Untrained	Trained	Paired *t* test
TC (mg dl^{-1})	163.9±41.8	151.2±26.0	NS
LDL-C (mg dl^{-1})	84.8±36.7	65.6±19.1	NS
HDL-C (mg dl^{-1})	60.6±15.4	66.0±15.9	$P<.05$
TG (mg dl^{-1})	93.1±52.0	105.3±48.7	NS
LDL/HDL	1.5±1.0	1.1±0.4	$P<.05$

[a] Values are expressed as mean±S.E.M. for 11 subjects.

Table 4
Plasma apoproteins, LCAT and CETP activities in healthy adults at baseline (Untrained) and after 6 weeks endurance training (Trained)[a]

	Untrained	Trained	*P* value
Apo B (mg dl^{-1})	71.5±9.0	57.8±13.3	<.05
Apo C-I (mg dl^{-1})*	3.8±1.4	3.7±1.0	NS
Apo C-III (mg dl^{-1})	15.4±3.7	15.0±3.5	NS
Apo E (mg dl^{-1})	3.5±0.7	3.2±0.8	NS
LCAT (μmol h^{-1} L)*	17.3±5.0	18.2±4.1	NS
CETP (μmol h^{-1} L)*	22.2±6.9	15.3±5.7	<.05

[a] Values are expressed as mean±S.E.M. for 11 subjects. *n=8.

(Tables 3 and 4). There were no differences in TC, LDL-C or TG in response to the exercise treatment (Table 3). However, a borderline significant decrease (21%) in plasma LDL-C (P=.06) was observed. In addition, subjects experienced a 10% increase in HDL-C and a 27% decrease in LDL/HDL following the 6-week progressive, aerobic training intervention. A significant negative correlation was observed between changes in LDL-C vs. HDL-C (Fig. 1; R^2=0.3413, P<.05). Although subjects experienced no significant decrease in LDL-C in response to training, concentrations of plasma Apo B were reduced by 19% (P<.05; Table 4). No differences were observed in the plasma concentrations of Apo C-I, Apo C-III or Apo-E following exercise training.

3.4. Plasma LCAT and CETP

Plasma LCAT and CETP activity changes in response to exercise training were also independent of diet group (i.e., Egg vs. No Egg). Therefore, all plasma LCAT and CETP measures were pooled and differences in the concentrations of these variables from Untrained to Trained are reported (Table 4). Plasma LCAT activity was unresponsive to aerobic exercise training, but plasma CETP decreased 32% from Untrained to Trained (P<.05).

4. Discussion

The present study compared the plasma lipid and apolipoprotein profiles of normolipidemic subjects following 6 weeks of progressive, aerobic exercise training while consuming an egg or no-egg diet for 8 weeks. The major findings were that short-term, aerobic exercise training without weight loss or changes in body composition improved plasma lipid and lipoprotein profiles of subjects regardless of daily egg consumption. More specifically, both diet groups experienced increases in HDL-C, borderline significant decreases in LDL-C, an improved LDL-C/HDL-C and decreased plasma Apo B concentrations and CETP activity. These findings are significant in that observations (1) support the effectiveness of aerobic exercise training for positively impacting plasma lipids, lipoproteins and lipoprotein metabolism, and (2) suggest that eggs can be routinely incorporated into the diets of healthy, normolipidemic individuals beginning an endurance training program without negatively impacting the improvements in cardiovascular risk factors usually reported with aerobic training. Furthermore, eggs have been reported to increase plasma HDL-C in men and pre-menopausal women [28,29].

4.1. Effects of aerobic exercise training on plasma lipid profiles

Endurance exercise training is often associated with improvements in plasma lipid profiles. Most cross-sectional

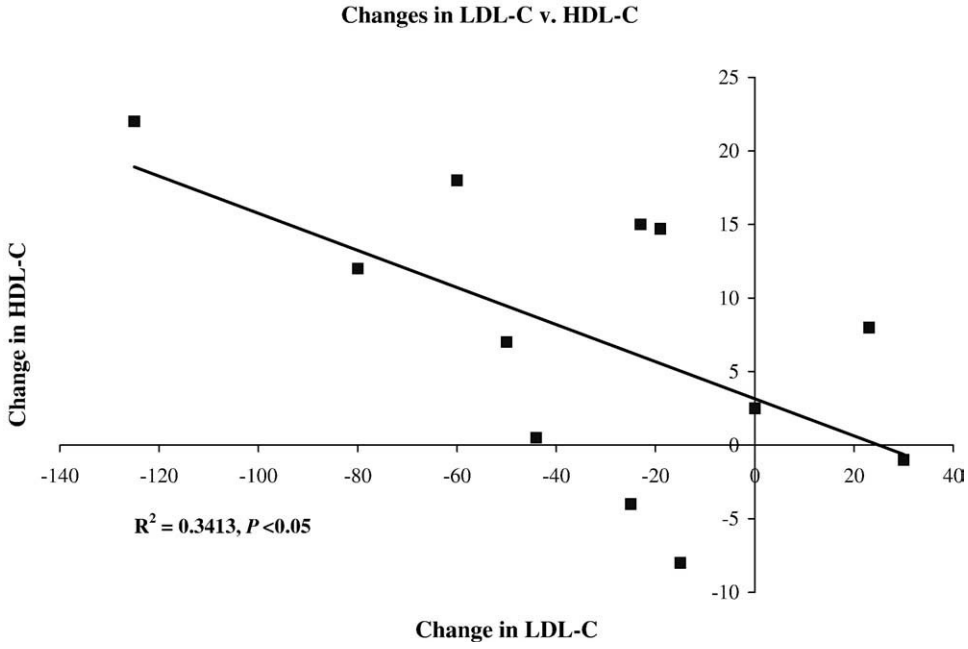

Fig. 1. Pearson correlation between changes in LDL-C vs. HDL-C in 11 healthy adults before and after a 6-week aerobic training program.

studies comparing trained athletes with sedentary counterparts report that trained individuals have more favorable lipid profiles [6–10] characterized by any one or more of the following: higher circulating levels of HDL-C; lower plasma levels of TC, LDL-C, VLDL and TG; or lower LDL/HDL. However, results from longitudinal studies are less consistent. In general, many studies report favorable changes in lipid profiles [13,14,17,19] that usually reflect increases in HDL-C and decreases in TG. However, results are inconsistent [41] and discrepancy in the data has been attributed to differences in training volume [20], baseline plasma lipid concentrations [12], lack of dietary control during training and prior to measurements [41], and changes in body weight/fat which occur during the training period among other potential variables [41]. The present study controlled for training and diet variables, baseline lipid values and maintenance of body weight.

We observed favorable alterations in plasma lipids in both diet groups following 6 weeks of progressive aerobic exercise training without changes in body weight, lean body mass or body fat. Concentrations of HDL-C increased, the LDL/HDL ratio improved, decreases in LDL-C were borderline significant and changes in LDL-C were negatively correlated with changes in HDL-C. No changes in TC or TG were observed. Overall, blood lipid profiles became less atherogenic. These results are not unique [11,14,19] among studies employing a comparable training protocol and controlling for diet and body weight/fat changes in a similar population. Altena et al. [19] observed increases in HDL-C and decreases in LDL-C and TC following just 4 weeks of endurance training (5 days·week^{-1}; 30 min·session^{-1}; 75% HR_{max}) without weight loss in a similar population. Thompson et al. [14] also reported increases in HDL-C, but also noted decreases in TG, following 14 weeks of exercise training (5 days·week^{-1}; 60 min·session^{-1}; 80% HR_{max}) without weight loss in adult men with high intakes of cholesterol (~400 mg·day^{-1}). Different from our study, Thompson investigated only adult men, who generally experience greater decreases in TG concentrations than females [41], which may partially account for this difference in TG responses between studies. Also, the small sample size in the present study may have limited detection of these changes in study males. In another investigation, Thompson et al. [11] reported a 10% increase in HDL-C in overweight men engaging in 12 months of aerobic exercise training (4 days·week^{-1}; 60 min·session^{-1}; 60–80% HR_{max}) without weight loss. Grandjean et al. [16] used a similar study design and reported decreases in HDL-C (5.5%) in women following 12 weeks of endurance training (4d·week^{-1}; 45 min·session^{-1}; 50–70% VO_{2max}) with weight maintenance. Differences in study populations and diet design may partially account for contradictory findings. In total, data from the present study are consistent with the majority of similar works documenting a benefit of aerobic exercise training on plasma lipid profiles.

Observations from three recent studies suggest aerobic exercise training might actually exert protective cardiovascular effects by shifting the size, and hence atherogenicity, of the HDL and LDL subparticles [10,17,19], even without impacting pre-training total plasma lipid concentrations [18,19]. For example, Halverstadt et al. [17] report that following 24 weeks of aerobic training (3–4 days·week^{-1}; 40 min·session^{-1}; 50–70% VO_{2max}), older men and women experienced decreases in TC, LDL-C and TG plasma levels; increases in HDL-C concentrations; as well as increases in mean HDL-C particle size (larger subfractions are more protective against CHD). They noted a tendency for LDL-C particle size to increase (larger subfractions are less atherogenic). Similarly, Altena et al. [19] endurance trained healthy men and women for 4 weeks (5 days·week^{-1}; 30 min·session^{-1}; 75% HR_{max}) and observed a less atherogenic blood lipid profile characterized by decreases in plasma TC and LDL-C concentrations, no change in HDL-C levels, but increases in HDL_2 (a larger size HDL-C subfraction) and in mean LDL particle size. Finally, following 8 months of endurance training (32.2 km jogging·week^{-1}; 65–80% VO_{2max}), Kraus et al. [18] document no changes in LDL-C plasma concentrations, but increased and decreased levels of larger and smaller LDL subparticles, respectively, creating a less atherogenic profile overall.

4.2. Effects of endurance exercise training on plasma apolipoproteins

Less often, studies characterize changes in plasma apolipoproteins in response to endurance exercise training. However, some apolipoproteins are important markers of lipoprotein remodeling making them potentially important to evaluate. In the present study, 6 weeks of endurance training decreased apolipoprotein B (Apo B), but failed to alter apolipoproteins C-I, C-III or E regardless of diet group.

Apo B is the major apolipoprotein of LDL and is required for VLDL secretion as well as LDL uptake. Both LDL and VLDL have been associated with increased risk of CHD; therefore circulating Apo B concentrations are considered an independent risk factor for CHD [42]. The decreased concentrations of Apo B following aerobic exercise training we observed support the reports of some [11], but contradict the observations of others [15,16] from analogous studies. However, regardless of the direction of the Apo B changes, Apo B alterations typically mirror those of LDL-C [20]. We observed such a change in this study, with decreased Apo B levels paralleling the borderline significant decreases in LDL-C concentrations.

Apo C-I inhibits the binding of lipoproteins to receptors, is the principal plasma inhibitor of CETP activity and prevents fatty acid uptake by tissues [43], while Apo C-III inhibits the activity of lipoprotein lipase (LPL), thereby decreasing lipolysis and elevating plasma TG concentrations [43]. We are not aware of any other exercise training studies which evaluated these apolipoprotein response. In the present study, endurance training failed to alter plasma

Apo C-III concentrations which may, in part, explain the lack of change in TG levels.

Apo E is the major ligand for the removal of chylomicron remnants returning to the liver and can contribute to increases in plasma TG by displacing Apo C-II (LPL activator) from TG-rich lipoproteins, thereby limiting their interaction with LPL [44]. There are essentially no exercise training studies in healthy individuals with comparable controls that have measured Apo E concentrations. In the present investigation, no changes were identified in plasma Apo-E concentrations after 6 weeks of aerobic exercise training. The importance of this finding requires further research.

4.3. Effects of exercise on plasma LCAT and CETP activity

Lecithin cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP) are enzymes involved in the modification of plasma lipoprotein particles, especially HDL. LCAT is responsible for the conversion of free cholesterol into cholesterol esters (CE) which are taken up by discoidal HDL to form spherical HDL. CETP is a glycoprotein that circulates in plasma bound primarily to HDL [45]. It is known to transfer CE from HDL to LDL and other TG-rich lipoproteins and from TG-rich lipoproteins to HDL and LDL [46].

With respect to LCAT, cross-sectional investigations report increased LCAT activities [47,48] in endurance-trained individuals. Longitudinal studies analogous to the current study in terms of design, control and populations report increases [49] or no changes [16] in plasma LCAT activity. In the present study, no changes were observed in LCAT activity from pre- to post-endurance training.

Plasma CETP activity decreased following 6 weeks of endurance training in the current study. These findings parallel those of Seip et al. [50] who reported decreases in CETP activity in 57 healthy, normolipidemic men and women following 9–12 months of aerobic exercise training (3–5 days·week^{-1}; 45–60 min·session^{-1}; 65–85% HR_{max}) without weight loss. However, these results conflict with the observations of at least one cross-sectional investigation [47] reporting elevated concentrations of CETP in aerobically trained persons which presumably reflect an enhanced reverse cholesterol transport system. CETP is known to be both pro- and anti-atherogenic depending on the metabolic context in which the concentrations are interpreted [46]. When decreases in CETP are accompanied by significant increases in HDL-C concentrations, as they were in the current study, the metabolic consequences appear to be antiatherogenic and reflect an enhanced reverse cholesterol transport [46].

4.4. Effects of high dietary cholesterol/egg consumption on plasma lipids, apolipoproteins and lipid metabolism

Despite years of research failing to implicate habitual egg consumption in the development of atherosclerosis, eggs have long been promoted as a heart unhealthy food because of the high cholesterol content of its yolk (213 mg). High dietary cholesterol was first associated with elevated plasma cholesterol levels [22] which were first associated with increased CHD risk [21] during the 1960s. Even though egg intake has not been associated with CHD risk, researchers have observed increases in plasma cholesterol levels in humans following habitual intake of eggs providing cholesterol in excess of the upper limit recommended by the AHA, USDA and DHHS for promoting heart health [26–28,51,52]. However, this response, which typically occurs in 1/3 of the population, has been characterized by increases in both LDL-C and HDL-C, which maintain the LDL-C/HDL-C, an important marker for CHD risk. We have documented this response in children [26], younger adults [27,28] and the elderly [51,52].

In the present study, despite consuming 12 eggs·week^{-1} (468.5 mg cholesterol·day^{-1}; 365 mg·day^{-1} additional dietary cholesterol from eggs), the Egg group experienced no increases in LDL-C. In fact, following 6 weeks of aerobic exercise, the blood lipid profiles of the Egg group were the same as those of the Non-Egg group characterized by increases in HDL-C, borderline significant decreases in LDL-C and decreases in the LDL-C/HDL-C. Furthermore, it suggests that routine aerobic exercise expending ~2500 kcal·week^{-1} is an important component of interventions designed to optimize or improve heart health in healthy, normolipidemic populations.

4.5. Conclusion

We conclude that an aerobic exercise program is an effective intervention for modestly improving plasma lipid and lipoprotein profiles in healthy, normolipidemic previously unfit subjects. Furthermore, although the small sample size might limit the generalization of these results, the findings suggest that persons habitually consuming eggs supplying cholesterol in excess of that recommended by the AHA, USDA, DHHS and IOM still experience favorable changes in plasma lipids, apolipoproteins and lipid metabolism that commonly occur in response to aerobic exercise training. Combined, the results of these interventions will be valuable in the regular reevaluation of the AHA, ACSM, USDA and DHHS physical activity and dietary guidelines with regard to what exercise and dietary interventions are effective for optimizing blood lipid levels to minimize CHD risk in the young, healthy adult population.

Acknowledgment

This study was supported in part by the American Egg Board. The authors are grateful to those who willingly participated in these investigations and for the efforts of all

the graduate students and staff who helped make this project possible.

References

[1] Heart disease and stroke statistics — 2007 update. Dallas: American Heart Association; 2007.

[2] Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Circulation 2007;116:1081–93.

[3] Dietary guidelines for Americans, 2005. US Department of Health and Human Services & US Department of Agriculture; 2005. Available at: www.healthierus.gov/dietaryguidelines.

[4] McKechnie R, Mosca L. Physical activity and coronary heart disease: prevention and effect on risk factors. Cardiol Rev 2003;11(1):21–5.

[5] Durstine JL, Grandjean PW, Davis PG, Ferguson MA, Alderson NL, DuBose KD. Blood lipid and lipoprotein adaptations to exercise: a quantitative analysis. Sports Med 2001;31(15):1033–62.

[6] Buyukyazi G. Differences in blood lipids and apolipoproteins between master athletes, recreational athletes and sedentary men. J Sports Med Phys Fitness 2005;45(1):112–20.

[7] Lippi G, Schena F, Salvagno GL, Montagnana M, Ballestrieri F, Guidi GC. Comparison of the lipid profile and lipoprotein(a) between sedentary and highly trained subjects. Clin Chem Lab Med 2006;44 (3):322–6.

[8] Yataco AR, Busby-Whitehead J, Drinkwater DT, Katzel LI. Relationship of body composition and cardiovascular fitness to lipoprotein lipid profiles in master athletes and sedentary men. Aging (Milano) 1997;9 (1–2):88–94.

[9] Tsopanakis C, Kotsarellis D, Tsopanakis AD. Lipoprotein and lipid profiles of elite athletes in Olympic sports. Int J Sports Med 1986;7(6): 316–21.

[10] Herbert PN, Bernier DN, Cullinane EM, Edelstein L, Kantor MA, Thompson PD. High-density lipoprotein metabolism in runners and sedentary men. JAMA 1984;252(8):1034–7.

[11] Thompson PD, Yurgalevitch SM, Flynn MM, Zmuda JM, Spannaus-Martin D, Saritelli A, et al. Effect of prolonged exercise training without weight loss on high-density lipoprotein metabolism in overweight men. Metabolism 1997;46(2):217–23.

[12] Couillard C, Despres JP, Lamarche B, Bergeron J, Gagnon J, Leon AS, et al. Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the health, risk factors, exercise training and genetics (heritage) family study. Arterioscler Thromb Vasc Biol 2001;21(7): 1226–32.

[13] LeMura LM, von Duvillard SP, Andreacci J, Klebez JM, Chelland SA, Russo J. Lipid and lipoprotein profiles, cardiovascular fitness, body composition, and diet during and after resistance, aerobic and combination training in young women. Eur J Appl Physiol 2000;82 (5–6):451–8.

[14] Thompson PD, Cullinane EM, Sady SP, Flynn MM, Bernier DN, Kantor MA, et al. Modest changes in high-density lipoprotein concentration and metabolism with prolonged exercise training. Circulation 1988;78(1):25–34.

[15] Blumenthal JA, Matthews K, Fredrikson M, Rifai N, Schniebolk S, German D, et al. Effects of exercise training on cardiovascular function and plasma lipid, lipoprotein, and apolipoprotein concentrations in premenopausal and postmenopausal women. Arterioscler Thromb 1991;11(4):912–7.

[16] Grandjean PW, Crouse SF, O'Brien BC, Rohack JJ, Brown JA. The effects of menopausal status and exercise training on serum lipids and the activities of intravascular enzymes related to lipid transport. Metabolism 1998;47(4):377–83.

[17] Halverstadt A, Phares DA, Wilund KR, Goldberg AP, Hagberg JM. Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. Metabolism 2007;56(4):444–50.

[18] Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, et al. Effects of the amount and intensity of exercise on plasma lipoproteins. N Engl J Med 2002;347(19):1483–92.

[19] Altena TS, Michaelson JL, Ball SD, Guilford BL, Thomas TR. Lipoprotein subfraction changes after continuous or intermittent exercise training. Med Sci Sports Exerc 2006;38(2):367–72.

[20] Durstine JL, Grandjean PW, Cox CA, Thompson PD. Lipids, lipoproteins, and exercise. J Cardiopulm Rehabil 2002;22(6):385–98.

[21] Kannel WB, Dawber TR, Friedman GD, Glennon WE, McNamara PM. Risk factors in coronary heart disease. An evaluation of several serum lipids as predictors of coronary heart disease; The Framingham Study. Ann Intern Med 1964;61:888–99.

[22] Grande F, Anderson JT, Chlouverakis C, Proja M, Keys A. Effect of dietary cholesterol on man's serum lipids. J Nutr 1965;87(1):52–62.

[23] Song WO, Kerver JM. Nutritional contribution of eggs to American diets. J Am Coll Nutr 2000;19(5 Suppl):556S–62S.

[24] Hu FB, Stampfer MJ, Rimm EB, Manson JE, Ascherio A, Colditz GA, et al. A prospective study of egg consumption and risk of cardiovascular disease in men and women. JAMA 1999;281(15): 1387–94.

[25] McNamara DJ. The impact of egg limitations on coronary heart disease risk: do the numbers add up? J Am Coll Nutr 2000;19(5 Suppl): 540S–8S.

[26] Ballesteros MN, Cabrera RM, Saucedo Mdel S, Fernandez ML. Dietary cholesterol does not increase biomarkers for chronic disease in a pediatric population from northern Mexico. Am J Clin Nutr 2004;80 (4):855–61.

[27] Herron KL, Vega-Lopez S, Conde K, Ramjiganesh T, Roy S, Shachter NS, et al. Pre-menopausal women, classified as hypo- or hyperresponders, do not alter their LDL/HDL ratio following a high dietary cholesterol challenge. J Am Coll Nutr 2002;21(3):250–8.

[28] Herron KL, Vega-Lopez S, Conde K, Ramjiganesh T, Shachter NS, Fernandez ML. Men classified as hypo- or hyperresponders to dietary cholesterol feeding exhibit differences in lipoprotein metabolism. J Nutr 2003;133(4):1036–42.

[29] Federation of American Societies of Experimental Biology. Report on nutrition monitoring in the United States. Washington (DC): U.S. Government Printing Office; 1999.

[30] Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, et al. AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000;102(18):2284–99.

[31] Food and Nutrition Board (FNB) and Institute of Medicine (IOM). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington (DC): National Academies Press; 2005. p. 21–37.

[32] Kasch FW, Wallace JP, Huhn RR, Krogh LA, Hurl PM. Vo2max during horizontal and inclined treadmill running. J Appl Physiol 1976;40(6): 982–3.

[33] Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20(4): 470–5.

[34] Warnick GR, Benderson J, Albers JJ. Dextran sulfate-mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28(6):1379–88.

[35] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499–502.

[36] Rifai N, King ME. Immunoturbidimetric assays of apolipoproteins a, ai, aii, and b in serum. Clin Chem 1986;32(6):957–61.

[37] Fredenrich A, Giroux LM, Tremblay M, Krimbou L, Davignon J, Cohn JS. Plasma lipoprotein distribution of apoc-iii in normolipidemic and hypertriglyceridemic subjects: comparison of the apoc-iii to apoe ratio in different lipoprotein fractions. J Lipid Res 1997;38(7):1421–32.

[38] Cohn JS, Tremblay M, Amiot M, Bouthillier D, Roy M, Genest Jr J, et al. Plasma concentration of apolipoprotein e in intermediate-sized remnant-like lipoproteins in normolipidemic and hyperlipidemic subjects. Arterioscler Thromb Vasc Biol 1996;16(1):149–59.
[39] Ogawa Y, Fielding CJ. Assay of cholesteryl ester transfer activity and purification of a cholesteryl ester transfer protein. Methods Enzymol 1985;111:274–85.
[40] Vega-Lopez S, Vidal-Quintanar RL, Fernandez ML. Sex and hormonal status influence plasma lipid responses to psyllium. Am J Clin Nutr 2001;74(4):435–41.
[41] Leon AS, Sanchez OA. Response of blood lipids to exercise training alone or combined with dietary intervention. Med Sci Sports Exerc 2001;33(6 Suppl):S502–15 [discussion S528–509].
[42] Vega GL, Denke MA, Grundy SM. Metabolic basis of primary hypercholesterolemia. Circulation 1991;84(1):118–28.
[43] Shachter NS. Apolipoproteins c-i and c-iii as important modulators of lipoprotein metabolism. Curr Opin Lipidol 2001;12(3):297–304.
[44] Huang Y, Liu XQ, Rall Jr SC, Taylor JM, von Eckardstein A, Assmann G, et al. Overexpression and accumulation of apolipoprotein e as a cause of hypertriglyceridemia. J Biol Chem 1998;273(41): 26388–93.
[45] Tall AR. Plasma cholesteryl ester transfer protein. J Lipid Res 1993;34 (8):1255–74.
[46] Barter P. CETP and atherosclerosis. Arterioscler Thromb Vasc Biol 2000;20(9):2029–31.
[47] Gupta AK, Ross EA, Myers JN, Kashyap ML. Increased reverse cholesterol transport in athletes. Metabolism 1993;42(6):684–90.
[48] Tsopanakis C, Kotsarellis D, Tsopanakis A. Plasma lecithin: cholesterol acyltransferase activity in elite athletes from selected sports. Eur J Appl Physiol Occup Physiol 1988;58(3):262–5.
[49] Marniemi J, Dahlstrom S, Kvist M, Seppanen A, Hietanen E. Dependence of serum lipid and lecithin: cholesterol acyltransferase levels on physical training in young men. Eur J Appl Physiol Occup Physiol 1982;49(1):25–35.
[50] Seip RL, Moulin P, Cocke T, Tall A, Kohrt WM, Mankowitz K, et al. Exercise training decreases plasma cholesteryl ester transfer protein. Arterioscler Thromb 1993;13(9):1359–67.
[51] Greene CM, Zern TL, Wood RJ, Shrestha S, Aggarwal D, Sharman MJ, et al. Maintenance of the LDL cholesterol:HDL cholesterol ratio in an elderly population given a dietary cholesterol challenge. J Nutr 2005;135(12):2793–8.
[52] Homma Y, Kobayashi T, Yamaguchi H, Ozawa H, Homma K, Ishiwata K. Apolipoprotein-e phenotype and basal activity of low-density lipoprotein receptor are independent of changes in plasma lipoprotein subfractions after cholesterol ingestion in Japanese subjects. Nutrition 2001;17(4):310–4.