Title

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Title: Whole egg consumption compared to yolk free egg increases the cholesterol efflux capacity of high-density lipoproteins in overweight, postmenopausal women.

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Short Running Head: Eggs increase cholesterol efflux capacity

Abbreviations:

ABCA1- ATP binding cassette A1

ApoA-I, apolipoprotein A-I

ApoB, apolipoprotein B

CE, cholesteryl esters

CETP, cholesteryl ester transfer protein

CRP, C- reactive protein

CVD, cardiovascular disease

DG, diacylglycerol

DBP, diastolic blood pressure

HDL-C, high-density lipoprotein cholesterol

LC, liquid chromatography

LCAT, lecithin-cholesterol acyltransferase

LDL-C, low-density lipoprotein cholesterol

MetS, metabolic syndrome

MS, mass spectrometry

MS DIAL, mass spectrometry data-independent acquisition in liquid chromatography

m/z, mass to charge ratio

oxLDL, oxidized LDL

p, plasmalogen

PC, phosphatidylcholine

PE, phosphatidylethanolamine

PON1, paraoxonase 1

QTOF, quadrupole orthogonal acceleration time-of-flight

RCT, reverse cholesterol transport

SAA, serum amyloid A

SBP, systolic blood pressure;

TC, total cholesterol

TG, triacylglycerol

UPLC CSH, ultra performance liquid chromatography charged surface hybrid

Clinical Registry Number: Clinical Trials.gov NCT02445638

ABSTRACT

Background: Postmenopausal women are at higher risk for cardiovascular disease (CVD) than their younger counterparts. High-density lipoprotein cholesterol (HDL-C) is a biomarker for CVD risk, but the function of HDL may be more important than HDL-C in deciphering disease risk. While diet continues to be a cornerstone of treatment and prevention of CVD, little is known about how diet affects the functionality of HDL.

Objective: The objective of this study was to characterize the effects of whole eggs compared with yolk-free eggs on HDL function and composition in overweight, postmenopausal women and determine how changes in HDL composition are related to HDL functional parameters.

Design: The study was a 14-wk, single-blind, randomized crossover dietary trial with two 4-wk intervention periods in 20 overweight, postmenopausal women. The crossover treatments were frozen breakfast meals containing 100 g of liquid (~2) whole eggs versus 100 g of (~2) yolk-free eggs per day, separated by a 4-wk washout. Fasting blood samples were taken at the beginning and end of each treatment period to determine the effects on HDL composition and function.

Results: Cholesterol efflux capacity increased in the whole egg treatment (mean (SD) % change: + 5.69 (9.9) %) compared to the yolk-free egg treatment (-3.69 (5.3) %) ($p < 0.01$), but there were no other significant changes in HDL functions or antioxidant or inflammatory markers. Apolipoprotein A-I (ApoA-I), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and HDL-C also did not change in response to the egg treatment.

Conclusions: The consumption of 2 whole eggs per day in overweight, postmenopausal women showed a significant increase in cholesterol efflux capacity. This increase in cholesterol efflux capacity was seen without significant changes in ApoA-I, TC, LDL-C, or HDL-C, supporting the idea that HDL function rather than HDL-C should be addressed in this population.

Keywords: antioxidant, apolipoproteins, dietary cholesterol, HDL composition, HDL function, hypercholesterolemic, inflammation, lipids, menopause, phospholipids

INTRODUCTION

Cardiovascular disease (CVD) continues to be the leading cause of death in the US (1). CVD is often thought to be a disease that impacts men more than women, however, death rates from CVD for men and women are similar. Furthermore, after a woman reaches menopause her risk for CVD increases (2).

Although epidemiological, animal and clinical studies confirm the inverse correlation between high-density lipoprotein cholesterol (HDL-C) and CVD risk, pharmaceutical approaches to reduce CVD risk through increasing HDL-C have failed (3, 4), which has called into question the wisdom of strategies to reduce CVD risk by increasing HDL-C. Additionally, during menopause HDL-C may be positively correlated with CVD, but it is unknown whether higher HDL-C is correlated with changes in HDL function (5, 6).

Other components of the HDL particle as well as the functionality of the HDL particle may be more important than HDL-C in order to decrease CVD risk. The main cardio-protective function of HDL is thought to be the promotion of cholesterol efflux from macrophages as the first step in reverse cholesterol transport (RCT) (7). Other functions of HDL also confer protection against CVD, including antioxidant and anti-inflammatory functions (8, 9). Studies have shown that both prevalent and incident heart diseases are negatively correlated with cholesterol efflux capacity (10-12). Therefore, in order to develop broader strategies to decrease CVD risk, methods to improve the composition and function of HDL must be developed (13).

Diet interventions continue to be a cornerstone of CVD risk reduction therapy, yet there is little research on the effects of these dietary interventions on the function and composition of HDL. Although the recent Dietary Guidelines eliminated the restriction on dietary cholesterol (14), these guidelines as well as other dietary recommendations for the prevention and treatment

of CVD continue to recommend dietary patterns that limit cholesterol intake (14-16). In addition, previous studies have shown that incorporation of whole eggs in the diet increases specific phospholipid species and functional capacity of HDL (17). Thus, the incorporation of eggs, with around 200 mg of cholesterol each, into a CVD prevention diet, continues to be controversial. The question remains as to what impact eggs have on CVD risk and in particular, what impact do whole eggs have on the composition and function of HDL in postmenopausal women.

The objective of this study was to characterize the effects of whole eggs compared with yolk-free egg on HDL function and composition in overweight, postmenopausal women and determine how changes in HDL composition are related to HDL functional parameters. Our main hypothesis was that whole egg composition would increase the apolipoprotein A-I (ApoA-I) content of HDL and our secondary hypothesis was that whole egg consumption would increase HDL functionality, specifically cholesterol efflux capacity and antioxidant capacity.

METHODS

Subjects

Twenty overweight/obese postmenopausal women, aged 48-70, with HDL-C concentrations above 50 mg/dL and a BMI between 25 and 35 kg/m^2 were recruited for this study. Subjects were recruited through advertisements in Davis, California and the surrounding communities. Recruitment began in July of 2015 and the study ended in November 2016. Exclusionary criteria for the subject selection included smoking, hormone replacement therapy or any medications or supplements known to affect lipid metabolism. Women were further excluded if they had exhibited 3 or more traits of metabolic syndrome (MetS) which include high blood pressure (greater that 130/85 mm Hg), high fasting blood glucose (greater than 100 mg/dL), large waist circumference (greater than 89 cm), high triacylglycerols (greater than 150 mg/dL) and low concentrations of HDL-C (less than 50 mg/dL) (18). Women were also excluded for any documented chronic disease, including diabetes, cancer, hypertension or CVD. Additionally, participants were excluded if they were allergic to eggs, consumed more than 5 eggs per wk, followed any extreme diet or exercise patterns or had recent weight fluctuations (>10% in the last 6 months). If women were within one year of their last menses at screening, postmenopausal status was confirmed by follicle-stimulating hormone concentrations. Twenty-three women met the inclusion criteria and were enrolled in the study. The study was carried out at the Nutrition Department of the University of California (UC), Davis with the approval of the Institutional Review Board of UC Davis. Informed consent was confirmed prior to entry of subjects into the protocol. One participant withdrew due to difficulties with blood draws and two participants withdrew due to medical reasons as shown in the study flow diagram (**Figure 1**). The study was registered at Clinical Trials.gov NCT02445638.

Experimental Design

The study was a 14-wk, single-blind, randomized crossover dietary trial with two 4-wk intervention periods separated by a 4-wk washout. After a 2-wk lead-in period where all the women were instructed to not consume any eggs or egg containing products, women were randomized to one of two groups. Women were randomly assigned to either Group A or Group B with a computer random number generator in blocks of 2, 6, 6, and 6. Group A started with the consumption of frozen breakfast meals containing 100 g of liquid whole egg (equivalent to 2 whole eggs) followed by a 4-wk washout period before crossing over to consume frozen breakfast meals with the 100 g of yolk-free eggs (equivalent to 2 egg whites) for 4 wks. Group B started with the consumption of frozen breakfast meals containing 100 g of liquid yolk-fee eggs for 4-wk and crossed over to consume frozen breakfast meals with 100 g of liquid whole eggs for 4-wk with a 4-wk washout period between the two arms. The frozen breakfast meals were prepared from a single batch of frozen liquid whole egg or yolk-free egg substitute in the Ragle Human Nutrition Center on the UC Davis campus. The meals consisted of 4 rotating breakfasts: an egg sandwich, an egg burrito and 2 different quiches. The egg sandwiches, burritos and quiches were made with either 100 g of whole egg or 100 g of yolk-free egg. The main dish was frozen and later packaged with an 8-ounce fruit juice. An additional granola bar was added to the yolk-free breakfasts in order to match the calories in the whole egg breakfasts. An average of 410 kcal was provided by the breakfast; means and the macronutrient content of the meals are listed in **Table 1.** During the washout periods, the participants were instructed to not consume any eggs or egg containing products for the last 2 wks of the period. Fasting blood samples, anthropometrics, blood pressures, and 3-d diet records, which included one weekend day and 2 nonconsecutive weekdays were collected at the beginning and end of each 4-wk intervention

period. Women came to the Ragle Human Nutrition Center at the UC Davis campus every week to be weighed, pick up their frozen breakfast meals and turn in their empty food containers and daily intake forms from the previous week.

The participants were instructed by the study dietitian to eat their breakfast meals and not to consume any other eggs or egg containing foods during the intervention arms. Women were provided with lists of food items and ingredients that contain egg products, including non-obvious sources that might be missed by participants. In addition to avoiding any additional eggs and egg products, participants were instructed not to change their normal dietary intake or exercise patterns. Dietary compliance was measured with 3-d food records and with recorded breakfast meal consumption as well as the collection of empty breakfast containers. Diet records were analyzed by nutrition software (Food Processor SQL Version 10.2 ESHA, Salem, OR, USA).

Analytical Methods

Height was measured to the nearest 0.1cm using a wall-mounted stadiometer (Ayrton Corp, Prior Lake, MN) at screening for the study. Body weight was measured weekly to the nearest 0.1kg using a calibrated electronic scale (Scale-Tronix, Welch Allyn, Skaneateles Falls, NY). Blood pressure was measured on the right arm of the participants in a seated position after a 5-min rest with an automated sphygmomanometer (OxiMax, Welch Allyn, Skaneateles Falls, NY) at entry to the study and at the beginning and end of each 4-wk dietary intervention period. All measurements were performed in triplicate and the average 3 measurements was used.

Whole blood samples were collected after a 12-h overnight fast via venipuncture at the beginning and end of each 4-wk intervention period. EDTA-containing tubes (Becton Dickinson and Company, Franklin Lakes, NJ) were immediately centrifuged (Sorvall-Legend RT) at 1000

× g at 4° C for 15 min. Serum tubes (Becton Dickinson and Company, Franklin Lakes, NJ) were allowed to clot for 30 min at room temperature and then centrifuged (Sorvall-Legend RT) at 1000 × g at 4° C for 15 min. After centrifugation, aliquots of serum or plasma were frozen at -80° C for subsequent analyses. One additional plasma separation tube (Becton Dickinson and Company, Franklin Lakes, NJ) was collected and centrifuged at 1000 × g at 4° C for 10 min and shipped to the UC Davis Medical Center Pathology Laboratory for analysis.

A lipid panel (total cholesterol (TC), triacylglycerols (TG), HDL-C and calculated LDL-C) and basic metabolic profile panel were performed by the UC Davis Medical Center, Clinical Pathology laboratory using a clinical analyzer (Beckman Coulter DXC 800). TC, HDL-C and TG were directly measured and LDL-cholesterol was calculated using the Friedewald equation (19).

The concentration of oxidized LDL (oxLDL) was measured in plasma using a commercially available ELISA kit (Mercodia, Uppsala, Sweden), which detects modified apolipoprotein B-100 (ApoB-100) on LDL particles using murine monoclonal antibody 4E6 (20). OxLDL was expressed as arbitrary units/L (U/L). Intra-assay CV was 5.0% and inter-assay CV was 10.1% based on a low and a high control sample supplied with the kit.

Plasma C-Reactive Protein (CRP**)** concentration was measured according to instructions provided by a commercially available ELISA kit (Quantikine, R&D Systems, Minneapolis, MN) and read at 450 nm absorbance (Synergy H1 plate reader, BioTek, Winooski, VT). All samples, standards, and control were run in duplicate. Intra-assay CV and inter-assay CV were 4.8% and 14.6%, respectively.

Plasma serum amyloid A (SAA) concentrations were measured using a commercially available ELISA kit (Invitrogen, Carlsbad, CA) and the plates were read at 450 nm absorbance

(Synergy H1 plate reader, BioTek, Winooski, VT). All samples, controls, and standards were run in duplicate. Intra-assay CV and inter-assay CV were 4.8% and 9.0%, respectively.

HDL Isolation, Compositional and Functional Characterization

Sequential flotation ultracentrifugation for HDL isolation was done according to a modified technique as previously described (21, 22). Briefly, 2 mL plasma samples were first adjusted to a density of 1.063 g/mL with potassium bromide (KBr) solution (d = 1.340 g/mL). Adjusted plasma solutions were overlaid with KBr solution (d = 1.063) and ultracentrifugation was performed in an ultracentrifuge with a fixed-angle rotor (Beckman Optima MAX-TL ultracentrifuge rotor: TLA-110) 110,000 rpm for 3 h 10 min to isolate the combined VLDL and LDL fractions, which were removed by aspiration of the supernatant. The remaining HDL containing fraction was adjusted to 1.21 g/ml with KBr solution (d = 1.34 g/mL) and overlaid with KBr solution (d = 1.21 g/mL) and the second ultracentrifugation performed at 110,000 rpm for 3 h 20 min. The HDL fraction (1.21-1.063 g/mL) was collected from the top of the tube, followed twice by dialysis using Amicon Ultra-4, MWCO 10kDa Centrifugal filters (Millipore UFC800324) to remove excess salt (KBr). Isolated HDL was reconstituted in LCMS grade water and stored at -80° C for further analysis.

ApoA-I in isolated HDL fractions was measured using SimpleStep ELISA (Abcam, ab189575) according to manufacturer's instructions and the intensity read at 450 nm on a Powerwave X Microplate reader (BioTek Corporation, Winooski VT). All samples were run in duplicate and the intra-assay CV and inter-assay CV were 2.1% and 2.9%, respectively

Lipidomics: Isolated HDL was sent to the West Coast Metabolomics Center at UC Davis for analysis of complex lipids as previously described (23). Briefly, 25 µL of isolated HDL was added to 225 µL of methanol with the following internal lipid standards:

phosphatidylethanolamine (PE) (17:0/17:0), phosphatidylglycerol (PG) (17:0/17:0), lyso-phosphatidylcholine (LPC) (17:0), C17 sphingosine, C17 ceramide, sphingomyelin (SM) (d18:1/17:0), palmitic acid (deuterated (d3)), phosphatidylcholine (PC) (12:0/13:0), cholesterol (deuterated (d7)), triacylglycerol (TG) (17:0/17:1/17:0) (deuterated (d5)), diacylglycerol (DG) (12:0/12:0), DG (18:1/2:0), monoacylglycerol (MG) (17:0), and lyso-PE (LPE) (17:1). This was followed by the addition of 750 µL MTBE with an additional lipid standard, cholesteryl ester (CE) (22:1), and shaken at 4° C for 6 min. After the addition of 188 µL of distilled water, the sample was centrifuged at 14,000 g for 2 min. 350 µL supernatant was extracted, dried down, and reconstituted with 65 µL methanol/toluene (9:1, v/v) solution. 3 µL samples were injected in parallel into an Agilent 6530 QTOF in positive mode electrospray ionization (ESI+), and an Agilent 6550 QTOF in negative mode (ESI-), in order to capture as many lipid species as possible. When lipid species were identified in both positive and negative modes, the mode with the lowest CV according to the quality control samples was used. With a Waters UPLC CSH C18 column (1.7 um, 2.1 mm × 100 mm), LC separation was executed using a gradient method. Using the raw MS intensities, individual lipid species were calibrated with the following equation:

$$\mathrm{Conc}_{i,j} = \frac{\mathrm{Int}_{i,j}}{\mathrm{Int\_IS}_{k,j}} \times \mathrm{Conc\_IS}_{k,j}$$

$\mathrm{Conc}_{i,j}$ is the concentration of the lipid species of the sample where i is the lipid species and j is the sample. $\mathrm{Int}_{i,j}$ is the MS intensity where i is the lipid species and j is the sample. $\mathrm{Int\_IS}_{k,j}$ is the MS intensity for the internal standard k that belongs to the same lipid class as lipid species i. $\mathrm{Conc\_IS}_{k,j}$ is the concentration of the internal standard k in sample j. The final unit was µg/mL.

Relative proportions of each lipid class were determined by adding all lipid species of a lipid class together and dividing by the total amount of lipid.

Cholesterol efflux capacity of ApoB-depleted plasma was measured using a commercially available kit (Abcam, ab196985) with J774 macrophages as described previously (24) with the following modifications: The cells were cultured in Roswell Park Memorial Institute (RMPI) 1640 media containing 10% FBS until 90% confluence and loaded with half of the fluorescently-labeled cholesterol labeling reagent for 4 h to avoid declining cell adhesion levels as was seen with long incubation times. Plasma was depleted of ApoB containing lipoproteins with polyethylene glycol (PEG) precipitation. The cells were washed and incubated for 2 h with each subject's ApoB-depleted plasma (1%), ACAT inhibitors and cAMP, which was used to induce cholesterol efflux by ATP binding cassette A1 (ABCA1). Cellular supernatant was removed and cells lysed using M-PER® cell lysis buffer, a mammalian protein extraction reagent (#78501 Thermo Scientific). Fluorescently-labeled cholesterol in the supernatant and cells was measured separately on a Synergy H1 plate reader (BioTek, Winooski, VT) and used to calculate the percent cholesterol efflux as follows: [Fluorescence intensity of the media/(fluorescence intensity of the cell lysate + media)] X 100. All samples were run in duplicate and the intra-assay CV was 6.2%.

Lecithin cholesterol acyltransferase (LCAT) activity was assessed with a commercial kit (Roar Biomedical, Millipore Sigma, St. Louis, MO) according to the manufacturer's instructions. Briefly, the plasma samples were incubated with fluorescently labeled substrate for 2.5 h at 37° C and plates read at 340 nm excitation, 390 nm and 470 nm emission (Synergy H1 plate reader, BioTek, Winooski, VT). The activity was calculated using the ratio of the 470 nm emission peak to the 390 nm emission peak, representing intact substrate reagent and hydrolyzed substrate

reagent, respectively. Samples were run in duplicate and the intra-assay CV and inter-assay CV were 1.6% and 6.4%, respectively.

Cholesteryl ester transfer protein (CETP) activity was measured in duplicate using a fluorometric assay following manufacturer's instructions (Roar Biomedical, MAK106, Millipore Sigma, St. Louis, MO). The plasma samples were incubated with the fluorescently labeled substrate for 3 h at 37° C and the fluorescence intensities of the samples were measured on a Synergy H1 plate reader (BioTek, Winooski, VT), adjusting for the sample blank and compared to the standard curve to determine the amount of substrate transferred by the CETP enzyme. All samples were run in duplicate and the intra-assay CV between duplicates was 2.4%.

Inhibition of LDL particle oxidation by HDL was measured by incubating LDL samples (150 μg total protein) from a pool of healthy volunteers, with copper II sulfate (5 μM final concentration) in the presence and absence of isolated HDL (50 μg total protein) (25). The lag time or the length of time before the appearance of conjugated dienes was measured in a Shimadzu UV-1601 UV-Visible spectrophotometer (Shimadzu, Columbia, MD) at 234 nm absorbance every 5 min up to 180 min. Intra-assay CV for the lag time of the LDL control duplicates was 3.9%. Intra-assay CV for the lag time of LDL plus HDL duplicates was 5.0%. HDL inhibition of LDL oxidation is reported as percent increase. The effect of HDL on LDL oxidation was measured in $n = 18$ participants as the assay failed for 2 subjects and there was insufficient sample to rerun the assay.

PON1 arylesterase activity was determined as previously described (26). Briefly, a 190 μL of phenylacetate substrate was added to 10 μL of diluted (1:10) serum. The rate of phenylacetate hydrolysis was monitored at 25° C by measuring the change in absorbance of phenol at 270 nm over a 2 min period on a Synergy H1 plate reader (BioTek, Winooski, VT)

with path-length correction applied (Gen5 Software). The samples were run in triplicate and intra-assay CV and inter-assay CV were 3.5% and 14.9%, respectively.

Lipidomics Data Processing

Mass spectrometry (MS) data were processed using MS-DIAL (27). Lipid metabolites were identified by liquid chromatogram retention time, MS1 m/z, and MS2 fragmentations. The MS1 m/z and retention time data were used to search against the in house rt-mz library (23) and the MS2 data were searched against LipidBlast (28).

Statistical Analysis

Statistical analyses were performed with the limma package in R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria). The primary outcome of the study was the change in ApoA-I associated with HDL. A sample size of 20 was determined based on earlier studies (unpublished data), which detected significant changes in ApoA-I, the primary outcome, with an alpha set to 0.05 and power set to 0.90. Secondary outcomes included measures of HDL function including cholesterol efflux capacity, antioxidant capacity, (PON1, conjugated diene production, oxLDL) as well as HDL lipidomics. Exploratory measures included HDL associated functions (CETP and LCAT activity) as well as markers of inflammation (CRP and SAA).

Linear mixed models were generated for each outcome variable with treatment, order of treatment and their interaction as fixed effects and the subjects as the random effect (29). Continuous variables were assessed for normality and log transformed where appropriate before statistical analyses were performed. Spearman correlations were used to evaluate correlations between HDL functional variables and clinical measurements. Benjamini Hochberg correction was used to adjust for multiple comparisons with the false discovery rate set at 0.05 (30).

RESULTS

In our mixed linear model, none of the measurements were found to be affected by treatment order and there were no carryover effects. Subjects enrolled in the study were overweight, postmenopausal women with a mean (SD) age of 57.7 (5.3) with a mean (SD) BMI of 28.3 (2.9) kg/m^2. Baseline characteristics of the subjects (n = 20) are summarized in **Table 2**. There were no significant changes in body weight or BMI throughout the study (**Table 3).** Also, there were no significant differences in plasma TC, LDL-C, HDL-C, TC: HDL-C and TG with whole egg consumption compared to yolk-free egg consumption (**Table 3**).

There were no significant changes in dietary components for any of the time points with the exception of dietary cholesterol, which was significantly higher with whole egg consumption with a mean intake of 498.9 (59.7) mg compared to the mean intake 126.6 (53.8) mg with yolk-free egg consumption ($p < 0.001$) (**Table 4**). It is important to note that even though the carbohydrate content of the breakfast meals were different in the 2 treatment arms, the overall carbohydrate content of the diets was not different between treatments. Compliance as recorded on daily intake forms for consumption of the breakfast meals, and as assessed from empty food containers was 98%.

The mean change in ApoA-I on isolated HDL showed a tendency to increase in response to whole egg consumption vs yolk-free egg but did not reach statistical significance ($p = 0.075$) due to a high degree of inter-individual variability (**Figure 2**). ApoA-I on isolated HDL was positively correlated with HDL-C (rho = 0.414, $p = 0.002$) and TC (rho = 0.306, $p = 0.037$).

There was a significant increase in the mean (+/-SD) percent cholesterol efflux capacity in ApoB- depleted plasma with whole egg consumption (+5.69 (9.9) %) vs yolk-free egg (-3.69 (5.3) %) ($p < 0.01$) (**Table 5**) (Supplemental Figure 1). There were also significant increases in

the proportion of phosphatidylethanolamine (PE) in the HDL particle with whole egg consumption compared to yolk-free egg ($p = 0.007$, adjusted $p = 0.07$) (**Table 6**) (Supplemental **Figure 2**), but no other significant changes were seen in other lipid classes. In the lipid species of the HDL particle, phosphatidylcholine (PC) 40:5 2 significantly increased with egg consumption compared to yolk-free egg ($p < 0.001$, adjusted $p = 0.001$) **(Figure 3A).** PE 40:6 p (plasmalogen) also increased significantly with egg consumption vs yolk-free egg ($p < 0.001$, adjusted $p = 0.009$) (**Figure 3B)** as did PE 38:6 p ($p < 0.001$, adjusted $p = 0.012$) (**Figure 3C)**. Cholesterol efflux was negatively correlated with DG (rho = -0.384, $p < 0.001$, adjusted $p = 0.005$), and positively correlated with CE (rho = 0.28, $p = 0.006$, adjusted $p = 0.032$). Cholesterol efflux was also strongly and negatively associated with overall odd chain fatty acids (rho = -0.379, $p = 0.001$, adjusted p = 0.004) (**Supplemental Figure 3**) as well as odd chain fatty acids in SM (rho = -0.344, $p = 0.002$, adjusted $p = 0.007$), PC (rho = -0.33, $p = 0.003$, adjusted $p = 0.007$), ceramide (rho = -0.31, $p = 0.005$, adjusted $p = 0.009$), and TG (rho = -0.254, $p = 0.023$, adjusted $p = 0.032$). Additionally, cholesterol efflux was associated with 50 different HDL lipid species (**Supplemental Table 1**).

There was no significant change in mean LCAT activity between whole eggs vs yolk-free egg consumption ($p = 0.49$) (Table 5). LCAT activity was negatively correlated with HDL-C (rho = -0.412, $p < 0.001$) and positively correlated with CRP (rho = 0.556, $p < 0.001$). There was no significant change in CETP activity between whole egg and yolk-free egg consumption ($p = 0.78$) (Table 5).

The antioxidant capacity of HDL, measured as HDL's capacity to inhibit conjugated diene production in LDL particles incubated with copper sulfate, showed no significant change between whole egg intake and yolk-free egg intake ($p = 0.61$) (Table 5). PON1 activity was

measured as an additional marker for antioxidant protection and no difference was observed between whole egg consumption vs yolk-free egg ($p = 0.56$). PON1 was positively correlated with ApoA-I (rho = 0.317, $p = 0.012$) and negatively correlated with diastolic blood pressure (DBP) (rho = -0.342, $p = 0.008$).

CRP, a marker of inflammation, did not change significantly with egg consumption ($p = 0.12$) (Table 5). SAA, which is also a marker of inflammation and is carried on HDL particles, was similar in response to CRP and was also not significantly different with egg consumption vs yolk-free egg ($p = 0.11$) (Table 5). SAA was positively correlated with CRP (rho = 0.617, $p < 0.001$), body weight (rho = 0.361, $p = 0.001$), and both systolic (rho = 0.487, $p < 0.001$) and diastolic blood pressures (rho = 0.415, $p = 0.001$). There was also no significant difference in the amount of circulating oxLDL in plasma between the whole egg and the yolk-free egg treatments, (Table 5) ($p = 0.53$). However, oxLDL was positively correlated with TC:HDL (rho = 0.536, $p < 0.001$), TG (rho = 0.425, $p = 0.001$) and BMI (rho = 0.36, $p = 0.009$).

DISCUSSION

The results of this study show that despite a significant increase in dietary cholesterol intake of approximately 370 mg cholesterol/day from egg, no significant changes were observed in TC, LDL-C, HDL-C or TC: HDL-C in this cohort of mildly hypercholesterolemic women. Other studies have shown an increase in HDL-C with egg consumption, however most of these studies also included carbohydrate restriction and/or weight loss, thus previously observed changes in HDL-C may have been due to an interaction of egg yolk consumption with these other factors (17, 31, 32). It is also possible that the baseline lipid profiles of this cohort of women are indicative of a phenotype that may not respond to changes in dietary cholesterol or egg consumption. Women whose HDL-C values were > 50 mg/dL were specifically chosen for the current study because previous studies showed the greatest impact of dietary cholesterol on HDL-C in individuals with higher baseline HDL-C compared to individuals with clinically low HDL-C (i.e. < 50 mg/dL for women) (17).

ApoA-I, the main protein on HDL, provides structure and is involved in many of the functions of HDL, including RCT (33). ApoA-I in the isolated HDL fraction showed a tendency to increase with egg consumption compared to yolk-free egg. This result is in agreement with two previous studies which also reported no significant change in ApoA-I in response to eggs (31, 34), though a variable response pattern most likely existed and was not apparent from reported mean values. Indeed, a high degree of inter-individual variability in response was seen in our study (Figure 2). Previous studies incorporated both male and female subjects and it is known that gender-based differences in ApoA-I production and removal rates exist (35). The current study included only mildly hypercholesterolemic, overweight, postmenopausal women

with normal baseline HDL-C concentrations, thus the variation seen in this study was not due to gender or age differences, differences in obesity, or baseline lipid profiles.

Cholesterol efflux, the first step in RCT, which is considered the main cardio-protective function of HDL, increased with whole egg consumption compared to yolk-free egg consumption (Figure 3). Cholesterol efflux capacity has been negatively correlated with both incident and prevalent CVD (10-12). Although ApoA-I plays a significant role in cholesterol efflux, ApoA-I concentrations did not change in this study and other studies have shown that HDL particles with the same cholesterol and ApoA-I concentrations have different abilities to perform cholesterol efflux (36). Many factors affect cholesterol efflux including particle size and particle composition, including lipids (37). Egg yolks are high in phospholipids which may be preferentially incorporated into HDL particles (38). It has been suggested that the phospholipids in eggs are incorporated into the HDL molecule and this leads to an increase in cholesterol carrying capacity (38). PE increased in the whole egg treatment compared to the yolk-free egg, which was also seen with a longer egg intervention (17). PE has been shown to enhance the binding of the amphipathic helices of ApoA-I (39) which may increase cholesterol efflux. However, an association between PE content and cholesterol efflux capacity was not seen in this study and could be due to the fact that ApoB-depleted plasma was used for the cholesterol efflux assay whereas isolated HDL was used for lipidomic analysis. Specific lipid species PC 40:5, PE 40:6 p, and PE 38:6 p all increased with egg consumption and thus may be markers for egg intake. Cholesterol efflux capacity was positively correlated with the abundance of cholesteryl ester (CE) in HDL. Previous studies have also shown that enrichment of HDL with CE and phospholipids increased the ability of HDL to perform cholesterol efflux (40).

We observed no change in LCAT activity, which is in agreement with some egg feeding studies (41, 42), but not in egg feeding studies that included weight loss and carbohydrate restriction (31, 34, 43). Two of these studies showing an increase in LCAT activity were performed in the context of a study design that included weight loss and carbohydrate restriction (31, 34), both of which have been shown to affect LCAT activity. In another study an increase in LCAT activity was seen with 2 eggs per day compared with 0 and 1 egg per day (43), but this may have been due to longer duration of egg feeding (12 wks instead of 4 wks).

CETP also did not change in the current study and this is in agreement with other egg feeding studies (31, 34, 41-43). CETP transfers TG from very low density lipoproteins (VLDL) to HDL in exchange for cholesteryl esters (44). CETP activity is increased with hypertriglyceridemia, which was not seen in our population and changes in the TG content of HDL were also not observed. Thus, changes in CETP may be more likely to occur in the context of diets that increase TG, such as high carbohydrate diets.

Our results indicate that consumption of 2 whole eggs per day for 4 wks did not alter markers of oxidation or antioxidant capacity in this cohort of postmenopausal women. We observed no change in the amount of oxLDL, ability of HDL to inhibit LDL oxidation, or PON1 activity. PON1 is carried mainly on HDL where it serves to protect HDL as well as LDL from oxidation (45). In a previous study where participants consumed 1, 2, and 3 eggs per day for 3 consecutive 4-wk arms, consumption of 3 eggs, but not 1 or 2 eggs, significantly increased PON1 activity (43). Therefore, the increase in PON1 activity may have been due to the continuous consumption of eggs over the course of 12 weeks.

CRP is an acute phase protein that increases dramatically with inflammation, but is also elevated with chronic conditions such as obesity and is associated with increased risk for CVD in

postmenopausal women (46). CRP did not change significantly, which is in agreement with other studies (32) and the mean value for CRP on egg (2.26 mg/dL) is within the average risk concentration for CRP (47). SAA is an acute phase protein that associates with HDL during inflammation (48). A previous study found that egg consumption in MetS patients following a low carbohydrate diet decreased SAA (31). Although the participants in the present study were overweight they did not exhibit MetS, nor did they lose weight or follow a carbohydrate restricted diet. Thus, in the previous study, it may have been the higher baseline concentrations of SAA seen in MetS patients combined with weight loss that decreased SAA rather than egg intake.

Limitations to this study include the fact that these were free-living women and thus only their egg consumption was controlled and although their diets did not significantly change, it is possible that some other factor may have affected the HDL outcomes. Another possible limitation is the fact that ApoB depleted plasma was used for the cholesterol efflux assay whereas isolated HDL were used for the compositional analysis.

In conclusion, 2 whole eggs per day as compared to yolk-free eggs for 4 wks were shown to increase cholesterol efflux capacity of ApoB-depleted plasma in free-living, overweight, postmenopausal women. These results support the idea that HDL function rather than HDL-C should be assessed in this population, and suggest that the consumption of cholesterol in the form of eggs improves the cholesterol efflux capacity of HDL particles in mildly hypercholesterolemic, overweight, postmenopausal women.

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Author contributions: FMS and AMZ were responsible for study design, funding, oversight and had primary responsibility for final content of the manuscript. CZ generated the randomization scheme, ASB enrolled the participants and LSK assigned the participants to the interventions. LSK, ASB and CZ conducted the intervention, experimental activities, data collection and statistical analyses. RS and JMR provided support with laboratory analysis and other study activities. CHR conducted the cell-based cholesterol efflux assays. LSK wrote the first draft of the manuscript. All authors read, contributed to and approved the final manuscript.

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Table 1. Average macronutrient composition of whole egg and yolk-free egg breakfasts[1]

	Whole egg breakfasts	Yolk-free egg breakfasts
Total energy (kcal)	412.3 (10.5)	408.3 (9.9)
Protein (g)	19.9 (0.3)	18.9 (0.3)
% energy from protein	19.3	18.5
Carbohydrate (g)	50.0 (1.9)	65.4 (2.0)
% energy from carbohydrate	47.4	64.1
Fiber (g)	2.3 (1.4)	3.3 (1.4)
Total Fat (g)	15.7 (1.6)	8.7 (1.6)
% energy from total fat	34.3	19.2
Saturated Fat (g)	6.1 (0.8)	3.5 (0.8)
MUFA (g)	5.7 (0.9)	1.7 (0.9)
PUFA (g)	2.5 (0.3)	0.5 (0.3)
Trans Fat (g)	0.1 (0.2)	0.1 (0.2)
Cholesterol (mg)	384.7 (1.7)	12.7 (1.7)

[1]Values presented as mean (SD) of 4 breakfast types for whole egg and 4 breakfast types for yolk-free egg treatment.

Table 2. Baseline characteristics of postmenopausal women[1]

Characteristic	Mean (SD)
Age (y)	57.7 (5.3)
Weight (kg)	74.4 (8.6)
BMI (kg/m^2)	28.3 (2.9)
Waist circumference (cm)	90.4 (9.2)
Hip circumference (cm)	108.9 (6.0)
SBP (mmHg)	121.7 (14.8)
DBP (mmHg)	78.7 (5.6)
TC (mg/dL)	217.5 (28.5)
HDL-C (mg/dL)	63.8 (9.1)
ApoA-I[2](mg/dL)	185.2 (34.2)
LDL-C (mg/dL)	137.0 (23.3)
TC:HDL	3.4 (0.4)
TG (mg/dL)	83.8 (28.1)
Non-HDL-C (mg/dL)	153.7 (24.6)
Fasting Glucose (mg/dL)	86.9 (5.35)

[1] Values presented as mean (SD) (n = 20). [2]ApoA-I, apolipoprotein A-I on isolated high-density lipoproteins; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerol.

Table 3. Clinical measurements Pre-and Post-egg 4 week treatments[1]

Variable	Yolk-Free Egg		Whole Egg		
	Pre	Post	Pre	Post	*p*-value
Weight (kg)	74.4 (9.0)	74.7 (9.0)	74.5 (8.7)	74.5 (8.6)	0.34
BMI (kg/m^2)	28.3 (3.0)	28.5 (3.1)	28.4 (2.9)	28.4 (2.9)	0.35
SBP (mmHg)	120.8 (12.3)	119.8 (9.6)	120.6 (14.5)	121.0 (10.5)	0.69
DBP (mmHg)	77.9 (5.2)	77.5 (4.9)	78.3 (5.9)	78.5 (5.3)	0.67
TC (mg/dL)	220.0 (27.6)	216.4 (30.4)	220.2 (29.6)	223.0 (29.3)	0.35
HDL-C (mg/dL)	64.0 (7.8)	62.5 (6.3)	62.9 (8.8)	64.0 (8.0)	0.17
LDL-C, (mg/dL)	138.4 (22.6)	134.7 (28.2)	139.1 (26.1)	141.0 (27.4)	0.36
TC:HDL	3.5 (0.4)	3.5 (0.7)	3.6 (0.6)	3.5 (0.7)	0.48
TG (mg/dL)	87.4 (32.0)	97.0 (36.5)	91.2 (32.6)	89.8 (30.5)	0.29
Non-HDL-C (mg/dL)	155.9 (24.1)	153.9 (31.3)	157.3 (28.9)	158.9 (30.7)	0.59
F Glucose (mg/dL)	85.9 (6.3)	88.0 (9.2)	87.2 (5.0)	89.3 (8.20)	0.98

[1] Values are presented as mean (SD). Changes on whole egg vs yolk-free egg were compared using linear mixed model (n = 20). DBP, diastolic blood pressure; F Glucose, fasting glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; SBP, Systolic blood pressure; TC, total cholesterol; TG, triacylglycerol.

Table 4. Difference in dietary factors on whole egg vs yolk free [1]

Variable	Treatment	Pre-Treatment	Post-Treatment	*p*-value	Adj *p*- value
Total kcalories					
	Whole egg	1714.2 (421.0)	1702.0 (315.7)	0.64	0.70
	Yolk-free egg	1767.6 (366.4)	1847.0 (423.6)		
Total fat (g)					
	Whole egg	60.8 (19.0)	65.0 (15.9)	0.06	0.18
	Yolk-free egg	71.6 (22.2)	65.1 (22.3)		
Sat fat (g)					
	Whole egg	19.4 (8.2)	21.0 (6.8)	0.24	0.36
	Yolk-free egg	23.6 (9.0)	23.1 (10.6)		
MUFA (g)					
	Whole egg	14.2 (7.2)	16.0 (5.7)	0.01	0.06
	Yolk-free egg	16.0 (7.3)	13.9 (8.9)		
PUFA (g)	Whole egg	7.4 (4.5)	7.5 (3.6)	0.17	0.30
	Yolk-free egg	9.4 (8.2)	7.2 (4.4)		
Trans fat (g)	Whole egg	0.53 (0.61)	0.59 (0.43)	0.64	0.70
	Yolk-free egg	0.64 (0.66)	0.66 (0.68)		
Cholesterol (mg)					
	Whole egg	135.4 (62.2)	498.9 (59.7)	<0.001	<0.001
	Yolk-free egg	145.9 (53.3)	126.6 (53.8)		
Protein (g)	Whole egg	72.9 (16.6)	76.2 (18.8)	0.73	0.73
	Yolk-free egg	70.6 (19.5)	75.7 (21.6)		
CHO (g)	Whole egg	220.3 (71.7)	206.3 (46.7)	0.17	0.30
	Yolk-free egg	212.0 (39.5)	231.6 (56.8)		
Total Fiber (g)					
	Whole egg	24.4 (9.5)	22.7 (8.2)	0.44	0.58
	Yolk-free egg	25.8 (8.4)	22.1 (7.7)		
Alcohol (g)	Whole egg	5.6 (7.9)	4.2 (1.9)	0.08	0.18
	Yolk-free egg	6.1 (11.0)	9.0 (18.4)		

[1]Raw data are presented as mean (SD). Changes on whole egg vs yolk-free egg were compared using linear mixed model (n = 20). Benjamini Hochberg correction was used for multiple comparisons (n = 11).

Table 5. HDL function, enzyme activities, inflammatory and oxidative stress markers after 4 weeks of whole egg and yolk-free egg treatments[1]

Variable	Treatment	Pre-Treatment	Post-Treatment	*p*-value	*Adj. p*-value
Cholesterol efflux, %					
	Whole egg	26.0 (12.0)	31.6 (8.9)	0.001	0.01
	Yolk-free egg	29.5 (10.6)	25.8 (12.6)		
LCAT activity, 470/390 nm					
	Whole egg	0.94 (0.07)	0.93 (0.07)	0.49	0.69
	Yolk-free egg	0.95 (0.06)	0.95 (0.06)		
CETP activity, nmol/mL/hour					
	Whole egg	27.1 (12.1)	33.2 (11.7)	0.80	0.80
	Yolk-free egg	28.9 (14.8)	34.2 (14.9)		
HDL inhibition of LDL oxidation[2], %					
	Whole egg	19.2 (15.2)	22.7 (17.6)	0.61	0.69
	Yolk-free egg	15.9 (11.5)	17.8 (12.0)		
PON1 activity, kU/L					
	Whole egg	118.4 (26.8)	121.0 (27.1)	0.56	0.69
	Yolk-free egg	118.0 (29.1)	119.4 (29.9)		
CRP, mg/L	Whole egg	1.6 (1.5)	2.3 (1.9)	0.12	0.62
	Yolk-free egg	2.1 (1.7)	2.3 (2.5)		
SAA[3], mg/L	Whole egg	18.1 (9.9)	24.6 (20.1)	0.11	0.25
	Yolk-free egg	21.4 (11.8)	19.9 (9.7)		
oxLDL[2], U/L	Whole egg	42.9 (7.5)	42.6 (7.1)	0.30	0.53
	Yolk-free egg	43.1 (8.8)	51.5 (40.3)		

[1]Raw data are presented as mean (SD). Changes on whole egg vs yolk-free egg were compared using linear mixed model (n = 20). Benjamini Hochberg correction was used for multiple comparisons (n = 8). CETP, cholesteryl ester transfer protein; CRP, C-reactive protein; LCAT,

lecithin-cholesterol acyltransferase; LDL, low density lipoprotein; oxLDL, oxidized low density lipoprotein, PON1, paraoxonase; SAA, serum amyloid A.

[2]HDL inhibition of LDL oxidation was determined for $n = 18$.

[3] Log_{10} transformed before analyses.

Table 6. HDL lipid composition after 4 weeks of whole egg and yolk-free egg diets[1]

Lipid Class	Treatment	Pre-Treatment	Post-Treatment	*p*-value	*Adj. p*-value
PC					
	Whole egg	36.3 (3.0)	35.8 (2.7)	0.32	0.54
	Yolk-free egg	36.5 (2.5)	36.7 (3.4)		
CE					
	Whole egg	25.9 (3.5)	25.5 (4.2)	0.11	0.42
	Yolk-free egg	25.7 (4.5)	24.9 (3.6)		
TG					
	Whole egg	13.9 (5.9)	13.9 (5.9)	0.39	0.54
	Yolk-free egg	13.0 (5.4)	13.8 (5.4)		
Chol					
	Whole egg	9.4 (1.4)	9.0 (1.0)	0.33	0.54
	Yolk-free egg	9.2 (1.0)	9.2 (1.2)		
SM					
	Whole egg	8.5 (1.0)	8.3 (0.6)	0.53	0.58
	Yolk-free egg	8.4 (0.9)	8.4 (0.8)		
FA					
	Whole egg	2.7 (1.2)	2.9 (1.4)	0.40	0.54
	Yolk-free egg	2.9 (1.3)	2.7 (1.4)		
LPC					
	Whole egg	2.2 (0.5)	2.1 (0.4)	0.31	0.54
	Yolk-free egg	2.2 (0.5)	2.2 (0.5)		
PE					
	Whole egg	1.3 (0.4)	1.8 (0.9)	0.007	0.07
	Yolk-free egg	1.5 (0.6)	1.5 (0.5)		
DG					
	Whole egg	0.43 (0.1)	0.41 (0.1)	0.07	0.40
	Yolk-free egg	0.39 (0.1)	0.44 (0.1)		
Cer					
	Whole egg	0.24(0.04)	0.24 (0.03)	0.66	0.66
	Yolk-free egg	0.24 (0.03)	0.24 (0.03)		
LPE					
	Whole egg	0.03 (0.01)	0.03 (0.01)	0.45	0.55
	Yolk-free egg	0.03 (0.01)	0.03 (0.01)		

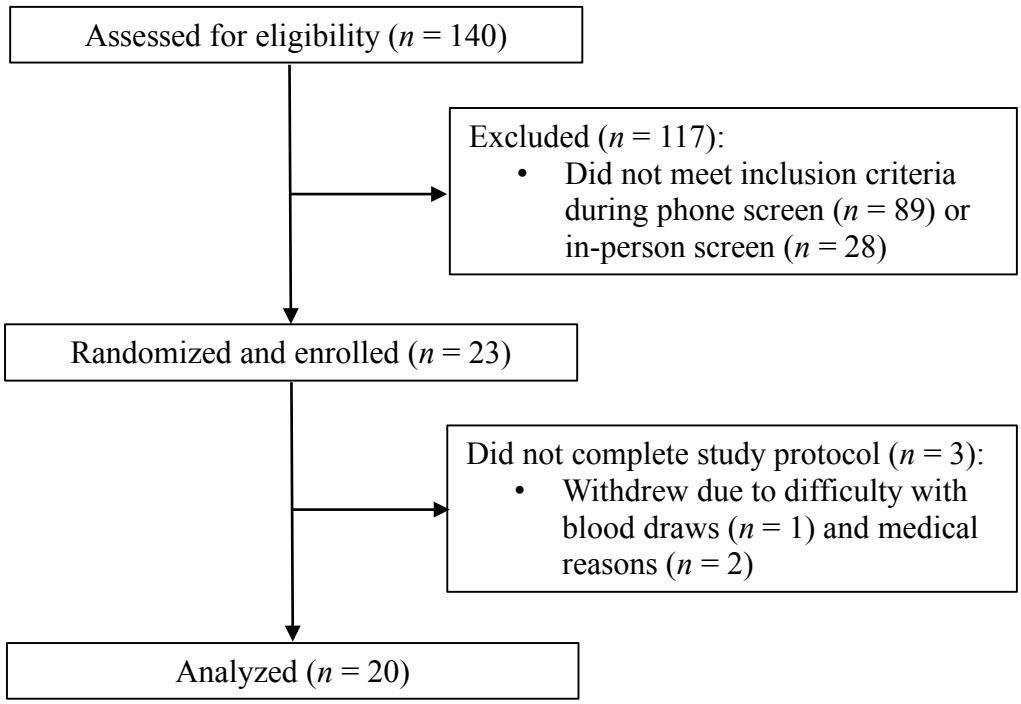
[1]Data are presented as mean % of total lipid mass (SD). Changes on whole egg vs yolk-free egg were compared using linear mixed model (n = 20). Benjamini Hochberg correction was used for multiple comparisons (n = 11). CE, cholesteryl ester; cer, ceramides; Chol, cholesterol; DG,

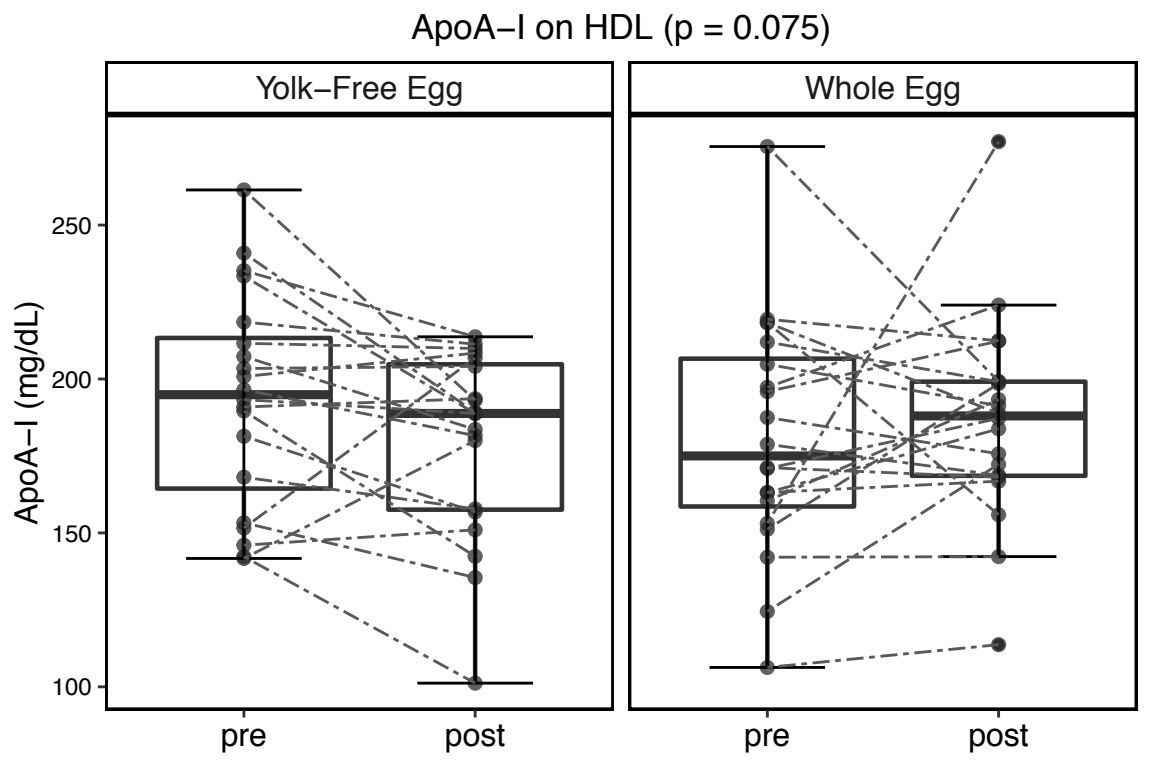
diacylglycerol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; TG, triacylglycerol.

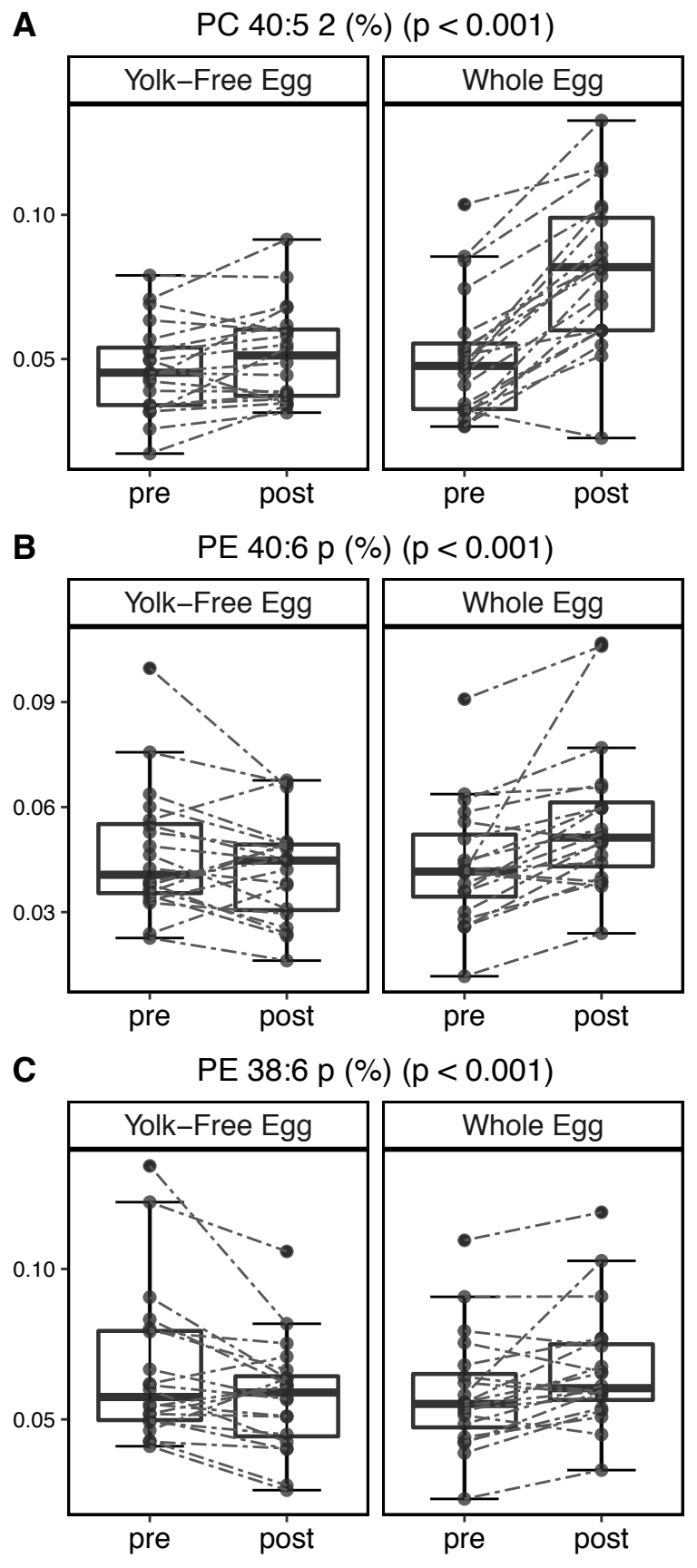
Figure 1: Study Flow Diagram

Figure 2. Changes in ApoA-I content of HDL in overweight, postmenopausal women in response to whole egg and yolk-free egg using linear mixed model (n = 20).

Figure 3. Changes in lipid content of isolated HDL in overweight, postmenopausal women in response to whole egg vs yolk-free egg using linear mixed model (n = 20). **A**. Change in PC 40:5 2 content of HDL in response to whole egg vs yolk-free egg. **B**. Change in PE 40:6 p content of HDL in response to whole egg and yolk-free egg. **C**. Change in PE 38:6 p content of HDL in response to whole egg vs yolk-free egg. PC, phosphotidylcholine; PE, phosphotidlyethanolamine; p, plasmalogen.


Assessed for eligibility (n = 140)
Excluded (n = 117):
• Did not meet inclusion criteria during phone screen (n = 89) or in-person screen (n = 28)
Randomized and enrolled (n = 23)
Did not complete study protocol (n = 3):
• Withdrew due to difficulty with blood draws (n = 1) and medical reasons (n = 2)
Analyzed (n = 20)


ApoA–I on HDL (p = 0.075)
Yolk–Free Egg
Whole Egg
ApoA–I (mg/dL)
250
200
150
100
pre
post
pre
post

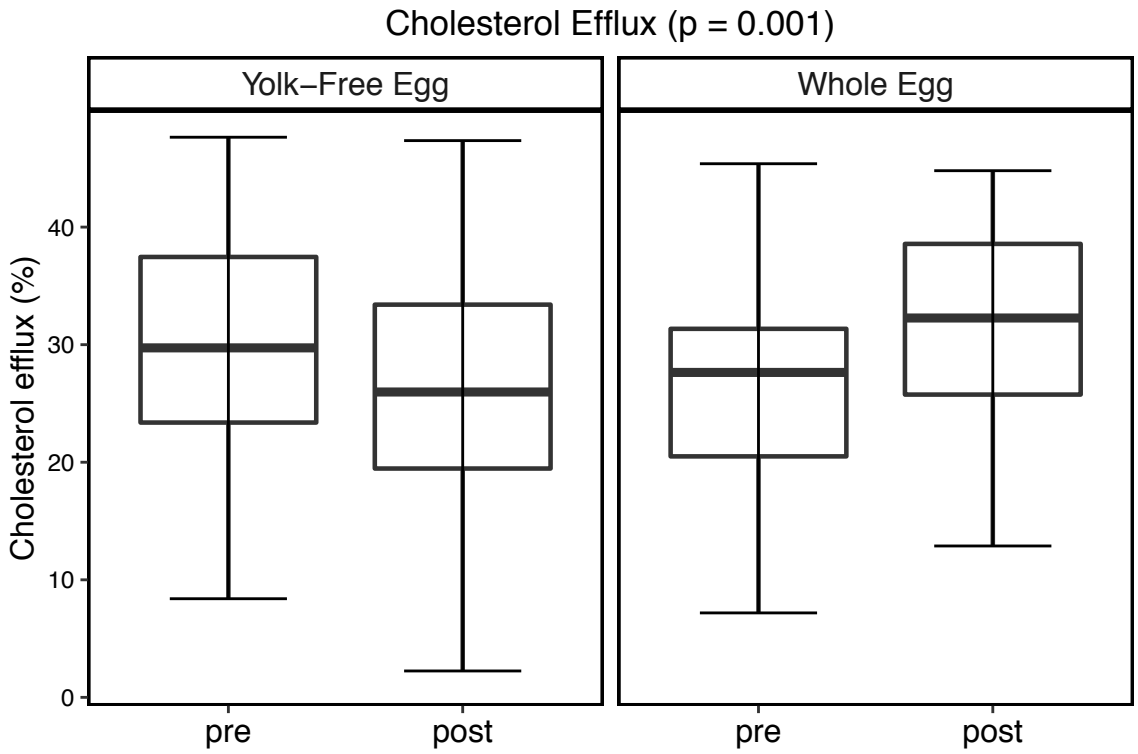

A
PC 40:5 2 (%) (p < 0.001)
Yolk−Free Egg
Whole Egg
0.10
0.05
pre
post
pre
post
B
PE 40:6 p (%) (p < 0.001)
Yolk−Free Egg
Whole Egg
0.09
0.06
0.03
pre
post
pre
post
C
PE 38:6 p (%) (p < 0.001)
Yolk−Free Egg
Whole Egg
0.10
0.05
pre
post
pre
post

Supplemental Table 1. Lipid species significantly associated with cholesterol efflux[1]

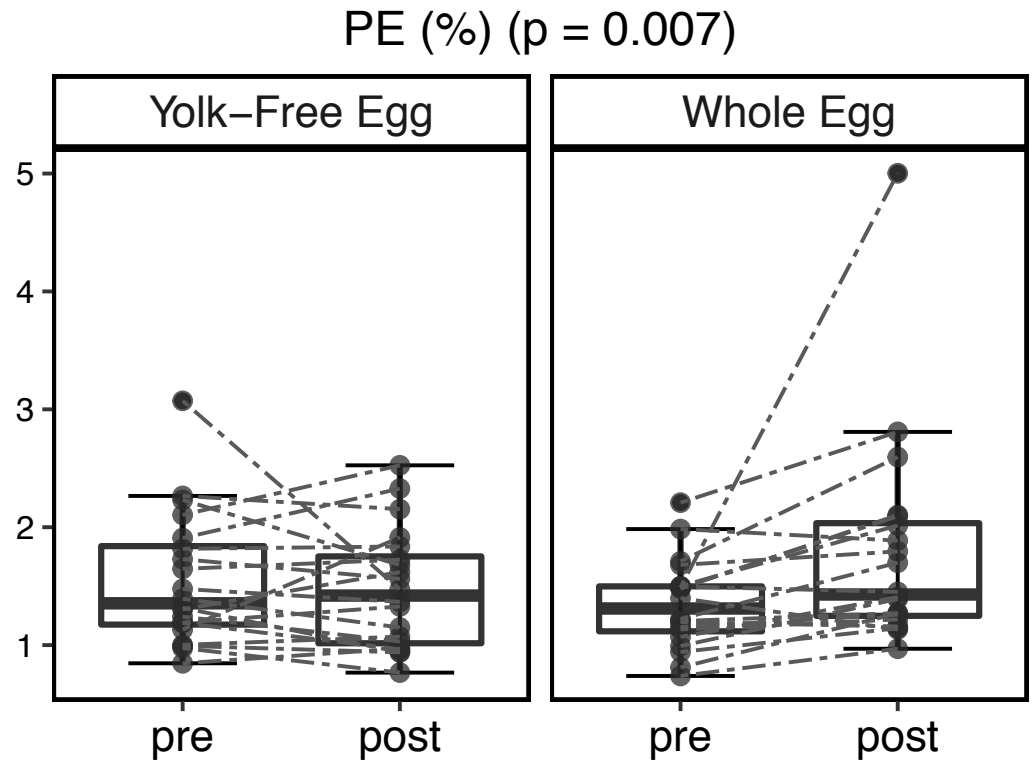
HDL Lipid Species	rho	*p*-value	adj *p*-value
CE 20:4	0.389	< 0.001	< 0.001
DG 36:3	-0.381	< 0.001	< 0.001
DG 38:5	-0.398	< 0.001	< 0.001
PC 35:3	-0.388	< 0.001	< 0.001
DG 36:2	-0.354	0.001	0.078
TG 53:3	-0.319	0.004	0.151
DG 38:6	-0.312	0.005	0.151
PC 35:2	-0.313	0.005	0.151
PC 37:2	-0.311	0.005	0.151
TG 53:4	-0.308	0.006	0.151
Cer 41:1 d	-0.301	0.007	0.151
PC 34:3 1	-0.298	0.007	0.152
SM 36:0 d	0.302	0.007	0.151
SM 41:2 d	-0.302	0.007	0.151
TG 54:4	-0.290	0.009	0.159
FA 20:4	0.286	0.01	0.159
TG 56:3	-0.288	0.01	0.159
Cer 39:1 d	-0.283	0.011	0.159
PC 36:4	-0.284	0.011	0.159
TG 53:5	-0.284	0.011	0.159
TG 53:2	-0.277	0.013	0.18
Gal-Gal-Cer 34:1 d	0.271	0.015	0.191
TG 54:2	-0.271	0.015	0.191
PC 36:3 1	-0.264	0.018	0.217
DG 34:2	-0.262	0.019	0.217
Cer 41:1 d 1	-0.259	0.021	0.227
GlcCer 40:1 d 1	0.256	0.022	0.227
GlcCer 42:1 d	0.255	0.023	0.227
GlcCer 42:1 d 1	0.252	0.024	0.227
TG 51:4 1	-0.253	0.024	0.227
DG 36:4	-0.250	0.026	0.227
TG 54:5 1	-0.249	0.026	0.227
GlcCer 40:1 d	0.248	0.027	0.227
PE 36:2	-0.247	0.028	0.227

TG 51:3	-0.246	0.028	0.227
Cer 42:2 d	-0.245	0.029	0.227
TG 54:3	-0.242	0.031	0.236
DG 34:3	-0.239	0.033	0.236
PC 37:3	-0.239	0.033	0.236
SM 39:1 d	-0.239	0.033	0.236
TG 50:5 1	-0.232	0.038	0.263
Gal-Gal-Cer 42:2 d	0.232	0.039	0.263
TG 52:5 1	-0.231	0.04	0.263
FA 20:3 1	0.227	0.043	0.279
FA 14:0	0.224	0.045	0.279
Ceramide 36:1 d	0.224	0.046	0.279
SM 40:0 d	0.224	0.046	0.279
DG 34:1	-0.223	0.047	0.279
TG 51:2	-0.221	0.049	0.285

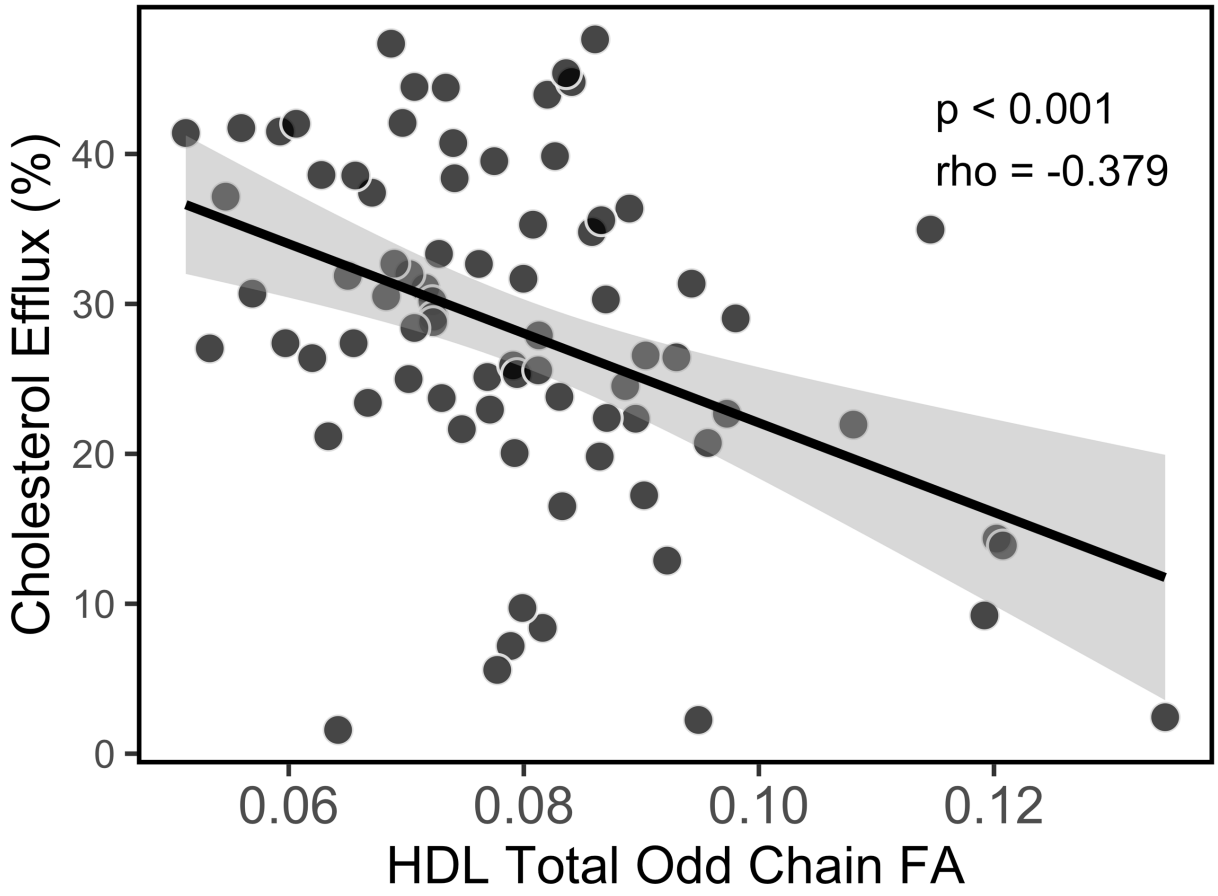
[1]Correlations with lipid species and cholesterol efflux using Spearman's test. Benjamini Hochberg correction was used for multiple comparisons. CE, cholesterol ester; Cer, ceramide, DG, diacylglycerol; FA, fatty acid; Gal-Gal-Cer, galacto-galactosylceramide; GlcCer, glucosylceramide; PC, phosphotidylcholine; PE, phosphotidlyethanolamine; SM, sphingomyelin; TG, triacylglycerol.



Supplemental Figure 1. Percent change in cholesterol efflux from J774 macrophages to participant's Apolipoprotein B (ApoB)-depleted plasma of overweight, postmenopausal women on whole egg vs yolk-free egg using linear mixed model (n = 20).



Supplemental Figure 2. Change in proportion of PE content of HDL in overweight, postmenopausal women in response to whole egg vs yolk-free egg. PE, phosphotidlyethanolamine.



Supplemental Figure 3. Scatterplot of total odd chain fatty acids in HDL with cholesterol efflux with Spearman's correlation coefficient. FA, fatty acids.