METABOLISM CLINICAL AND EXPERIMENTAL XX (2012) XXX–XXX

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in individuals with metabolic syndrome

Christopher N. Blesso[a], *Catherine J. Andersen*[a], *Jacqueline Barona*[a,b], *Jeff S. Volek*[a,c], *Maria Luz Fernandez*[a,*]

[a] *Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA*
[b] *School of Microbiology, University of Antioquia, Medellin, Colombia*
[c] *Department of Kinesiology, University of Connecticut, Storrs, CT 06269, USA*

ARTICLE INFO

Article history:
Received 15 June 2012
Accepted 27 August 2012

Keywords:
Eggs
Lipoproteins
Metabolic syndrome
Carbohydrate restriction
NMR spectroscopy

ABSTRACT

Objective. We investigated if daily egg feeding, along with carbohydrate restriction, would alter lipoprotein metabolism and influence atherogenic lipoprotein profiles and insulin resistance in men and women with metabolic syndrome (MetS).

Methods. In a randomized, single-blind, parallel design, participants consumed either 3 whole eggs/day (EGG, $n = 20$) or the equivalent amount of yolk-free egg substitute (SUB, $n = 17$), as part of a moderately carbohydrate-restricted diet (25%–30% energy) for 12 weeks. Plasma lipids, apolipoproteins (apos), oxidized LDL (oxLDL), cholesteryl ester transfer protein (CETP) and lecithin-cholesterol acyltransferase (LCAT) activities were assessed at baseline and week 12. Lipoprotein particle concentrations and sizes were measured by nuclear magnetic resonance spectroscopy.

Results. Atherogenic dyslipidemia improved for all individuals as evidenced by reductions in plasma triglycerides, apoC-III, apoE, oxLDL, VLDL particle diameter, large VDL, total IDL, small LDL, and medium LDL particles ($P < 0.05$). Furthermore, there were increases in HDL-cholesterol, large LDL and large HDL particles ($P < 0.05$) for all individuals. However, there were greater increases in HDL-cholesterol and large HDL particles, and reductions in total VLDL and medium VLDL particles for those consuming EGG compared to SUB ($P < 0.05$). Plasma insulin and insulin resistance (HOMA-IR) were reduced, while LCAT activity, and both HDL and LDL diameters increased over time in the EGG group only ($P < 0.05$).

Conclusions. Incorporating daily whole egg intake into a moderately carbohydrate-restricted diet provides further improvements in the atherogenic lipoprotein profile and in insulin resistance in individuals with MetS.

Abbreviations: Apo, apolipoprotein; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; CRD, carbohydrate-restricted diet; CVD, cardiovascular disease; EGG, 3 whole eggs per day; HDL-C, HDL-cholesterol; LCAT, lecithin-cholesterol acyltransferase; LDL-C, LDL-cholesterol; LPL, lipoprotein lipase; MetS, metabolic syndrome; NCEP: ATP III, National Cholesterol Education Program Adult Treatment Panel III; NMR, nuclear magnetic resonance; oxLDL, oxidized LDL; SUB, egg substitute; TC, total cholesterol; TG, triglycerides.

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

0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.metabol.2012.08.014

1. Introduction

Metabolic syndrome (MetS) is characterized by a constellation of risk factors for both type 2 diabetes and cardiovascular disease (CVD). A major contributor to the elevated CVD risk observed in MetS is the atherogenic dyslipidemia associated with this condition, of which insulin resistance and visceral adiposity are the primary underlying causes [1]. Atherogenic dyslipidemia is characterized by a distinct lipoprotein profile including low plasma HDL-cholesterol (HDL-C), elevated fasting triglycerides (TG), and a predominance of small, dense LDL particles [2]. Small LDL is particularly susceptible to oxidation and subendothelial retention [2], and MetS is often associated with elevated plasma oxidized LDL (oxLDL) [3]. In addition, alterations in HDL particles are well-established in MetS, with reductions in HDL-C related to an increased proportion of small HDL compared to large HDL [4]. There is evidence that the small HDL particles that predominate in MetS are functionally defective and more susceptible to catabolism [5]. Furthermore, a greater proportion of small HDL particles are observed in those with CVD [6]. In contrast, large HDL is strongly inversely associated with CVD risk [6,7]. Moreover, MetS is often associated with increases in large, buoyant VLDL subclasses, which are believed to be a result of insulin resistance [8]. Hepatic overproduction of large VLDL is an important contributor to the appearance of other lipoprotein abnormalities in MetS [8].

Dietary strategies aimed at weight loss are recommended as a first-line treatment for MetS [9]. One dietary strategy, carbohydrate restriction, is particularly effective for weight loss and improving atherogenic dyslipidemia [10]. Carbohydrate intake in the context of insulin resistance has been shown to induce *de novo* lipogenesis and may preferentially route metabolic products towards TG-rich VLDL production [11]. Consequently, restricting carbohydrates in those with MetS is associated with global improvements in atherogenic dyslipidemia, including reductions in plasma TG, large VLDL, and small LDL subclasses with increases in large HDL [10]. Another dietary strategy that is compatible with carbohydrate-restricted diets and has been associated with improvements in lipoprotein particle characteristics is daily egg consumption. In healthy populations, daily egg intake consistently shifts the proportion of small LDL and small HDL subclasses towards their larger, more buoyant forms [12]. Furthermore, daily egg intake has been shown to increase lecithin-cholesterol acyltransferase (LCAT) activity, potentially improving reverse cholesterol transport [13,14]. Thus, incorporating daily egg intake into a carbohydrate-restricted diet may provide additional benefits for the atherogenic dyslipidemia observed in MetS. Somewhat counterintuitively, there is speculation that whole egg consumption may promote a shift towards more atherogenic lipoproteins in the context of insulin-resistance and type 2 diabetes, and that this may explain observational research linking eggs and CVD risk in these populations [15].

There is a lack of clinical interventions examining the effects of egg intake on lipoprotein subclasses in individuals with MetS. Therefore, we investigated if daily egg feeding, along with a moderately carbohydrate-restricted diet, would influence lipoprotein particle characteristics, lipoprotein metabolism, and oxLDL. We hypothesized that carbohydrate restriction would have favorable effects on lipoprotein particles, apolipoproteins, and oxLDL in all participants. Additionally, participants consuming 3 whole eggs per day would experience greater overall improvements in lipoprotein particle concentrations, particle size, and LCAT activity compared to participants consuming yolk-free egg substitute.

2. Methods

2.1. Study design

Forty men and women aged 30–70 years and classified with MetS were recruited and enrolled in a 12-week diet intervention. Participants were included in the study if they met the NCEP:ATP III revised criteria [1] for MetS at screening. Exclusion criteria included self-reported diabetes, coronary heart disease, history of stroke, renal problems, liver disease, cancer, current pregnancy or lactation, and allergy to eggs. All participants were asked to follow a moderately carbohydrate-restricted diet (CRD) for the entire 12 weeks (25%–30% of energy from carbohydrate, 25%–30% of energy from protein, and 45%–50% of energy from fat). The diet was *ad libitum*, as there were no specific recommendations or restrictions for energy intake. Participants were given comprehensive dietary guidelines and instruction on how to follow a CRD from trained graduate students. In addition to the CRD, participants were randomized into two groups at baseline and asked to consume either 3 whole eggs per day (EGG), or the equivalent amount of yolk-free egg substitute (SUB) for the entire 12-week study period (Sysco Corporation, Houston, TX). Each daily serving (1/2 cup) of EGG contained approximately 534 mg cholesterol, 0 g carbohydrate, 16 g protein, 12 g fat, and equivalent to 186 kcal. A daily serving of the cholesterol/fat-free SUB contained approximately 2 g carbohydrate, 14 g protein, and equivalent to 60 kcal. Egg products were given as a liquid and they were equal in both consistency and color. Participants were blinded to their group assignment and were given egg products biweekly. Participants were asked to avoid eating eggs outside of the study allocation. Compliance was monitored by use of weekly questionnaires and collection of empty product containers. Participants were asked to maintain their normal physical activity, medications, and dietary supplement usage upon starting the 12-week study.

A total of 37 participants ($n = 37$) completed the 12-week study (25 women and 12 men) and their data were used for the subsequent analyses. The study was approved by the University of Connecticut-Storrs Institutional Review Board. All participants signed the written, informed consent prior to screening.

2.2. Dietary intake analysis

Participants filled out 5-day dietary intake records at baseline, week 6 and week 12 of the study period. Each 5-day record consisted of 3 weekdays and 2 weekend days. Dietary records were analyzed using the Nutrition Data System for Research (NDSR) (Nutrition Coordinating Center, University of Minnesota).

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

2.3. Blood collection and processing

Fasted blood draws were performed on participants after a 12-h overnight fast at baseline and week 12. Antecubital venous blood samples were collected into EDTA-coated tubes and then centrifuged at 2200 × *g* for 20 min at 4 °C for plasma separation. A preservative cocktail (1 mL/L sodium azide, 1 mL/L phenylmethylsulfonyl fluoride, and 5 mL/L aprotinin) was added to plasma, and aliquots were subsequently frozen at −80 °C and stored until analysis.

2.4. Plasma lipids

Fasting plasma total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were determined at baseline and week 12 using an automated clinical chemistry analyzer (Cobas c 111, Roche Diagnostics, Indianapolis, IN). Plasma LDL-cholesterol (LDL-C) was estimated with the Friedewald equation [16].

2.5. Plasma insulin, HOMA-IR

Fasted plasma insulin was measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden) which utilizes two anti-insulin monoclonal antibodies to capture and detect insulin. The intra-assay variability was less than 5% for plasma insulin. The Homeostasis Model Assessment (HOMA-IR) equation was used to estimate basal insulin resistance based on fasting plasma insulin and plasma glucose measurements [17].

2.6. Lipoprotein particle concentrations and average particle diameter

VLDL, IDL, LDL, and HDL particle concentrations and average particle diameters were quantified using nuclear magnetic resonance (NMR) spectroscopy at baseline and week 12. NMR spectroscopy is a technique used to analyze lipoprotein particles in unfractionated plasma and is based on the natural magnetic distinctness of the individual lipoprotein subclasses. The signals emanated by the terminal lipid methyl group protons of lipoproteins are used to identify particular subclasses and their concentration. NMR spectroscopy was conducted with a 400-MHz NMR spectrometer (Bruker Biospin, Billerica, MA) by LipoScience (Raleigh, NC). NMR simultaneously quantifies >30 lipoprotein fractions that are grouped into 10 subclasses based on diameter: large VLDL (>60 nm), medium VLDL (35–60 nm), small VLDL (27–35 nm), IDL (23–27 nm), large LDL (21.2–23 nm), medium LDL (19.8–21.2 nm), small LDL (18–19.8 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm) and small HDL (7.3–8.2 nm). The lipoprotein subclass NMR signal amplitudes are directly proportional to their particle concentrations. The weighted average lipoprotein diameters were calculated based on the concentration and diameter of lipoprotein subclasses.

2.7. Plasma apolipoproteins and oxidized LDL (oxLDL)

Plasma apolipoproteins (A-I, A-II, B, C-II, C-III, E) were quantified using a human apolipoprotein multiplex assay kit (EMD Millipore Corp., Billerica, MA) and a Luminex IS 200 analyzer (Luminex Corp., Austin, TX). This system utilizes antibody-immobilized fluorescent dye-labeled microspheres to simultaneously quantify apolipoproteins in plasma. The intra-assay variability was less than 2% for plasma apolipoproteins.

Plasma oxLDL was measured using a sandwich ELISA kit (Mercodia AB, Uppsala, Sweden) according to the manufacturer's instructions. This kit utilizes the 4E6 murine monoclonal antibody which binds to oxidized apo B-100. Reactions were carried out in 96-well plates and quantified using a microplate reader (BioTek Instruments, Inc., Winooski, VT). The intra-assay variability was less than 4.5% for plasma oxLDL.

2.8. Lecithin-cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP)

Plasma LCAT activity was measured with a commercial fluorometric assay kit (Roar Biomedical Inc., New York, NY). The plasma samples were incubated with a fluorescently labeled substrate for 5 h at 37 °C. Substrate hydrolysis by LCAT resulted in an increase in emission at 390 nm and a decrease in emission at 470 nm. The ratio of the two emission intensities (390 nm / 470 nm) was used to express LCAT activity.

Plasma CETP activity was determined by a CETP activity assay kit (Roar Biomedical Inc., New York, NY). CETP activity was quantified by measuring the transfer of a fluorescent neutral lipid from a donor molecule to an acceptor molecule after incubation with plasma for 3 h at 37 °C. The CETP-mediated transfer of the fluorescent neutral lipid resulted in an increase in fluorescence (excitation = 465 nm, emission = 535 nm) which was measured by a microplate reader (BioTek Instruments, Inc., Winooski, VT). The intra-assay variability for LCAT and CETP assays was each below 4%.

2.9. Statistical analysis

We based our power calculation on predicted changes in HDL. In our previous report when we compared whole egg versus egg substitute, all individuals had a mean HDL of 45 mg/dL at baseline and those individuals in the egg had an increase of 13 mg/dL after 12 weeks. When we consider an alpha of < 0.05, we need 16 individuals per group to observe the predicted differences [13]. Repeated-measures ANOVA were used to determine the effects of diet and time for parameters measured throughout the study period. Egg or egg substitute assignment (EGG vs. SUB) was the between-subjects factor and time the within-subjects factor. Post-hoc multiple comparisons were tested using the Bonferroni correction. Independent *t* tests were used to determine differences in absolute changes in parameters between groups. Bivariate Pearson correlations were used to assess relationships between biochemical measures. Differences with a $P < 0.05$ were considered significant. SPSS version 20 for Windows was used for statistical analysis and data are reported as mean ± SD unless otherwise stated.

3. Results

3.1. Baseline characteristics of participants

Participants ($n = 37$) were middle-aged (51.9 ± 7.7 y) and with a mean BMI of 30.4 ± 5.5 kg/m^2 for the EGG and 30.6 ± 5.1 kg/m^2

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

for the SUB groups. Approximately 2/3 of the participants that completed the study were female in both the EGG (7 male, 13 female) and SUB groups (5 male, 12 female).

3.2. Dietary intake and weight changes

Although no specific guidelines or restrictions on energy intake were given, there was a 24% energy reduction for all participants from baseline to week 12 ($P < 0.0001$, data not shown), with no differences between groups. In accordance with following a moderately carbohydrate-restricted diet, there were reductions over time for both the percent of energy intake coming from carbohydrate (40.9 ± 7.4 vs. 28.3 ± 9.5%, $P < 0.0001$) and the total amount of carbohydrate consumed per day (211.9 ± 51.8 vs. 114.5 ± 55.0 g/d, $P < 0.0001$) for all participants. Total fat (90.7 ± 24.7 vs. 81.6 ± 31.4 g/d) intake and total protein intake (86.4 ± 22.6 vs. 88.2 ± 28.4 g/d) did not change from baseline for both groups. Total energy intake from fat increased from 38.6% ± 6.4% to 45.7% ± 7.4% ($P < 0.0001$), while energy intake from protein increased from 17.3% ± 3.0% to 23.9% ± 4.1% ($P < 0.0001$). At week 12, there were significant differences between groups for dietary cholesterol intake (EGG vs. SUB, $P < 0.0001$) and dietary choline intake (EGG vs. SUB, $P < 0.0001$). The EGG group increased dietary cholesterol intake by 106% from baseline (359.9 ± 177.7 vs. 740.8 ± 139.7 mg/d, $P < 0.0001$), while the SUB group decreased dietary cholesterol intake by 38% from baseline (344.9 ± 133.1 vs. 213.4 ± 83.3 mg/d, $P < 0.01$). The EGG group increased choline intake by 52% from baseline (332.1 ± 127.4 vs. 505.5 ± 113.3, $P < 0.0001$), while the SUB group decreased choline intake by 29% from baseline (350.1 ± 90.3 vs. 247.8 ± 99.9 mg/d, $P < 0.01$).

After 12 weeks, there was a 4% weight loss from baseline for both the EGG group (87.3 ± 20.2 vs. 83.7 ± 20.5 kg, $P < 0.0001$) and the SUB group (85.6 ± 16.2 vs. 82.2 ± 14.9 kg, $P < 0.0001$).

3.3. Plasma lipids

Plasma total cholesterol (TC) and LDL-C levels were not altered after 12 weeks, regardless of group ($P > 0.1$) (Table 1). Plasma TC and LDL-C for the EGG group did not change over time, even in the presence of consuming additional cholesterol provided by the eggs. In contrast, the dietary intervention significantly improved markers of atherogenic dyslipidemia associated with MetS (Table 1). Plasma HDL-C ($P < 0.0001$) increased by 13.6%, while plasma TG ($P < 0.0001$) was reduced by 24.0% in all participants over time. Consequently, the TG/HDL-C ratio decreased significantly from baseline to week 12 for both the EGG group (3.2 ± 2.1 vs. 1.9 ± 1.4, $P < 0.0001$) and the SUB group (3.4 ± 2.6 vs. 2.4 ± 1.5, $P < 0.05$). The EGG group had a larger HDL-C response than the SUB group, displaying greater percent increases from baseline for the EGG group (19.1%) compared to the SUB group (9.9%) ($P < 0.05$). The LDL-C/HDL-C ratio, a commonly used marker of atherosclerosis risk, improved in all participants mostly due to increases in HDL-C (Table 1). The LDL-C/HDL-C ratio decreased by 9.5% in the SUB group and 13.5% in the EGG group from baseline. Interestingly, only participants in the EGG group had a significant decrease in the LDL-C/HDL-C ratio over time ($P < 0.01$).

3.4. Fasting plasma glucose, insulin, and homeostasis model assessment (HOMA-IR)

There were no significant effects of the intervention on fasting plasma glucose (data not shown). In contrast, plasma insulin

Table 1 – Plasma lipids, insulin, and insulin resistance as determined by HOMA of participants at baseline, 6 weeks, and 12 weeks of the diet intervention.

Variable	Baseline	Week 6	Week 12	Change	P-value (time)	P-value (time × group)
Total Cholesterol *mg/dL*[1]						
EGG	192.4 ± 30.4	190.3 ± 33.0	192.0 ± 28.1	−0.4	>0.1	NS
SUB	200.5 ± 38.5	197.4 ± 42.0	197.8 ± 40.2	−2.7		
LDL-C, *mg/dL*						
EGG	114.2 ± 29.1	113.6 ± 30.4	113.2 ± 27.6	−1.0	>0.1	NS
SUB	122.0 ± 35.1	121.7 ± 37.1	119.8 ± 37.5	−2.2		
HDL-C, *mg/dL*						
EGG	49.9 ± 14.3[a]	55.0 ± 15.0[b]	58.5 ± 14.8[c]	+8.6*	<0.0001	NS
SUB	50.1 ± 13.8[a]	51.65 ± 12.8[ab]	54.8 ± 14.9[b]	+4.7		
Triglycerides, *mg/dL*						
EGG	140.9 ± 58.0[a]	108.6 ± 56.2[b]	100.3 ± 54.0[b]	−40.6	<0.0001	NS
SUB	142.4 ± 63.5	120.0 ± 43.8	116.2 ± 50.5	−26.2		
LDL-C/HDL-C						
EGG	2.5 ± 0.9[a]	2.2 ± 0.8[a]	2.1 ± 0.7[b]	−0.4	<0.05	NS
SUB	2.6 ± 0.9	2.5 ± 0.9	2.4 ± 1.1	−0.2		
Insulin, *pmol/L*						
EGG	55.8 ± 44.0[a]	ND	44.3 ± 35.9[b]	−11.5	<0.01	NS
SUB	42.4 ± 22.1	ND	36.4 ± 15.3	−6.0		
HOMA-IR						
EGG	2.3 ± 2.2[a]	ND	1.8 ± 1.7[b]	−0.5	<0.01	NS
SUB	1.7 ± 1.0	ND	1.4 ± 0.6	−0.3		

Values are mean ± SD. n = 20 (EGG), n = 17 (SUB). *P*-values are for time effects and time x group interactions for all subjects analyzed by repeated measures ANOVA. Values with different superscripts in a row are significantly different using post-hoc multiple comparisons with Bonferroni correction, $P < 0.017$. *Indicates significant differences in EGG vs. SUB using independent samples *t* test, $P < 0.05$. [1]ND = not determined.

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

and HOMA-IR decreased from baseline to week 12 in all participants (Table 1). When analyzed separately, participants in the EGG group had significant decreases in plasma insulin ($P < 0.05$) and HOMA-IR ($P < 0.05$) over time, while significant changes were not observed in the SUB group.

3.5. *Lipoprotein particle diameter*

As shown in Fig. 1A, the mean VLDL particle size (diameter) decreased significantly from baseline to week 12 for both the EGG and SUB groups. There were no significant differences in changes in VLDL diameter between the groups. For all participants, changes in plasma TG were positively associated with changes in VLDL size ($r = 0.625$, $P < 0.001$) (Fig. 1B).

In contrast to VLDL size, the mean LDL particle size significantly increased from baseline to week 12 only for the EGG group (Fig. 1C). For all participants, 12-week changes in the plasma TG/HDL-C ratio were inversely associated with changes in LDL size ($r = -0.371$, $P < 0.025$) (Fig. 1D).

The mean HDL particle size increased significantly from baseline to week 12 only for the EGG group (Fig. 2A). In addition, the EGG group experienced a significantly greater increase in HDL particle size after 12 weeks compared to the SUB group (EGG: $+0.22 \pm 0.30$ vs. SUB: $+0.05 \pm 0.22$ nm, $P < 0.05$). For all participants, 12-week changes in HDL size were positively associated with changes in HDL-C ($r = 0.623$, $P < 0.001$) (Fig. 2B).

3.6. *VLDL and IDL particle concentrations*

The EGG group experienced significantly greater decreases in total VLDL particles and medium VLDL particles after 12 weeks compared to the SUB group ($P < 0.05$) (Table 2). Total VLDL and medium VLDL particles decreased by 19.2% and 31.1%, respectively, in the EGG group ($P < 0.05$). In comparison, total VLDL particles decreased by only 0.4% and medium VLDL actually increased by 15.5% in the SUB group. For all participants, there was a 47.2% reduction in large VLDL particles ($P < 0.001$) and a 32.1% reduction in IDL particles ($P < 0.05$).

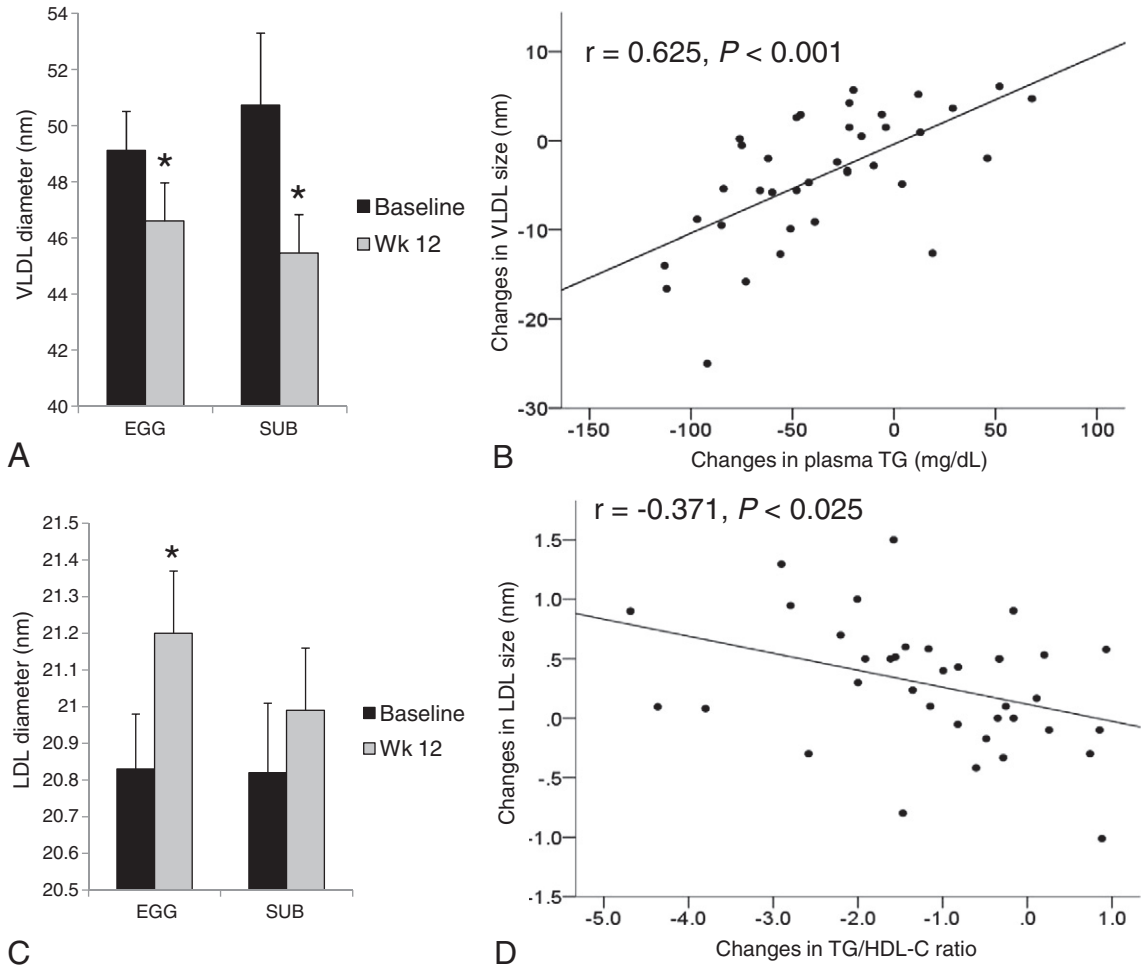

Fig. 1 – (A) Changes in VLDL particle size (diameter) from baseline to week 12 for both the EGG and SUB groups. Values are means ± SEM. *Indicates significantly different from baseline at $P < 0.05$. (B) Pearson correlation between the changes in plasma triglycerides (TG) and VLDL particle size from baseline to week 12. (C) Changes in LDL particle size (diameter) from baseline to week 12 for both the EGG and SUB groups. Values are means ± SEM. *Indicates significantly different from baseline at $P < 0.01$. (D) Pearson correlation between the changes in plasma TG/HDL-C ratio and changes in LDL particle size from baseline to week 12.

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

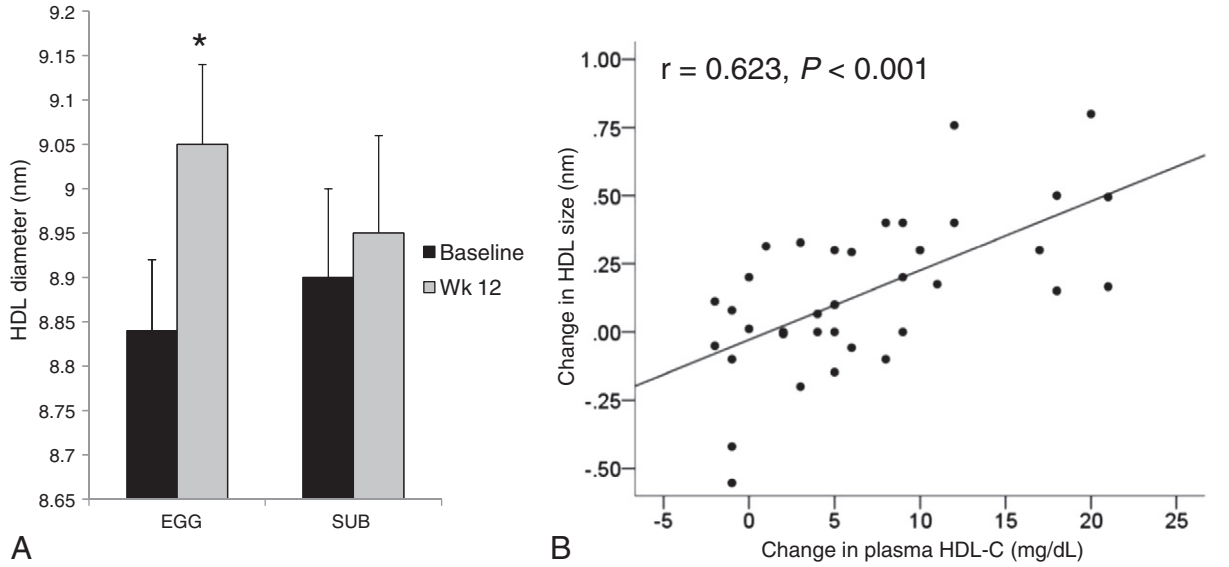

Fig. 2 – (A) Changes in HDL particle size (diameter) from baseline to week 12 for both the EGG and SUB groups. Values are means ± SEM. *Indicates significantly different from baseline at $P < 0.01$. (B) Pearson correlation between the changes in HDL-C and changes in HDL particle size from baseline to week 12.

3.7. *LDL particles and oxLDL concentrations*

After the 12-week study, there were no significant changes in the total concentration of LDL particles for either group (Table 3). However, there were significant changes seen in specific LDL subclasses, with increases in large LDL particles ($P < 0.01$), and decreases in both medium LDL ($P < 0.05$) and small LDL ($P < 0.05$) in all participants. Large LDL increased from baseline by 22.7% for the EGG group and 11.1% for the SUB group. Medium LDL and small LDL decreased by 23.6% and 22.0% in the EGG group, respectively, while 5.3% and 7.3% reductions were observed in the SUB group.

As shown in Table 3, plasma oxLDL decreased by 6.8% from baseline to week 12 for all participants ($P < 0.05$). Reductions in oxLDL were associated with reductions in LDL-C ($r = 0.558$, $P < 0.001$), and medium + small LDL particles ($r = 0.358$, $P < 0.05$) (Fig. 3A). In contrast, oxLDL was not associated with the number of large LDL particles (data not shown).

3.8. *HDL particle concentrations*

Compared to baseline, there were no significant changes in the total concentration of HDL particles for either group (Table 4). In contrast to total HDL, large HDL particles increased

Table 2 – Total number of IDL, VLDL, large, medium, and small VLDL particles for participants at baseline and after 12 weeks of the diet intervention.

Variable (*nmol/L*)	Baseline	Week 12	Change	P-value (time)	P-value (time × group)
Total VLDL					
EGG	82.7 ± 25.3[a]	66.8 ± 33.2[b]	−15.9*	<0.05	<0.05
SUB	77.6 ± 29.5	77.2 ± 34.3	−0.3		
Large VLDL					
EGG	3.6 ± 2.6[a]	1.9 ± 2.2[b]	−1.8	<0.001	NS
SUB	3.7 ± 3.7[a]	2.0 ± 2.1[b]	−1.7		
Medium VLDL					
EGG	29.9 ± 13.5[a]	19.9 ± 20.3[b]	−9.3*	NS	<0.05
SUB	25.8 ± 13.1	29.8 ± 18.9	+4.0		
Small VLDL					
EGG	49.9 ± 18.5	45.0 ± 18.2	−4.8	NS	NS
SUB	47.8 ± 22.7	45.4 ± 19.9	−2.5		
Total IDL					
EGG	53.6 ± 36.9	47.5 ± 44.8	−6.1	<0.05	NS
SUB	62.7 ± 47.9[a]	39.3 ± 43.0[b]	−23.4		

Values are mean ± SD. $n = 20$ (EGG), $n = 17$ (SUB). *P*-values are for time effects and time x group interactions for all subjects analyzed by repeated measures ANOVA. Values with different superscripts in a row are significantly different, $P < 0.05$. *Indicates significant differences in EGG vs. SUB using independent samples *t* test, $P < 0.05$. NS, not significant ($P > 0.05$).

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

Table 3 – Total number of LDL, large, medium, and small LDL particles and plasma oxidized LDL for participants at baseline and after 12 weeks of the diet intervention.

Variable (*nmol/L*)	Baseline	Week 12	Change	*P*-value (time)	*P*-value (time × group)
Total LDL					
EGG	1266.6 ± 341.8	1157.8 ± 303.6	−108.9	NS	NS
SUB	1353.0 ± 393.3	1313.0 ± 387.9	−39.9		
Large LDL					
EGG	371.7 ± 149.1[a]	456.2 ± 170.6[b]	+84.5	<0.01	NS
SUB	401.9 ± 217.2	446.6 ± 192.4	+44.6		
Medium LDL					
EGG	173.4 ± 78.8[a]	132.4 ± 73.8[b]	−41.0	<0.05	NS
SUB	175.3 ± 86.4	166.0 ± 86.6	−9.3		
Small LDL					
EGG	668.1 ± 301.9[a]	521.1 ± 283.7[b]	−147.0	<0.05	NS
SUB	712.9 ± 351.7	661.2 ± 327.7	−51.7		
OxLDL[1]					
EGG	50.7 ± 11.6	48.2 ± 12.9	−2.6	<0.05	NS
SUB	51.9 ± 16.3[a]	47.3 ± 13.6[b]	−4.6		

Values are mean ± SD. $n = 20$ (EGG), $n = 17$ (SUB). *P*-values are for time effects and time x group interactions for all subjects analyzed by repeated measures ANOVA. Values with different superscripts in a row are significantly different, $P < 0.05$. NS, not significant ($P > 0.05$). [1]Expressed as arbitrary units/L.

by 30.4% in the EGG group ($P < 0.01$) and 10.3% in the SUB group ($P < 0.05$). In addition, there was a significantly greater increase in large HDL particles for the EGG group compared to the SUB group ($P < 0.05$). Increases in large HDL particles from baseline to week 12 were associated with increases in HDL-C for both the EGG group ($r = 0.923$, $P < 0.001$) and the SUB group ($r = 0.493$, $P < 0.05$). Regarding other HDL subclasses, medium HDL particles decreased significantly by 38.8% for all participants ($P < 0.01$), while there were no changes in the number of small HDL particles for either group.

3.9. *LCAT and CETP activities*

Plasma LCAT activity significantly increased from baseline to week 12 in the EGG group ($P < 0.05$), whereas it did not change over time for the SUB group (Fig. 3B). Plasma CETP activity did not change over time, and there were no differences between the EGG and SUB groups ($P > 0.5$, data not shown).

3.10. *Plasma apolipoproteins*

Plasma apolipoprotein concentrations at baseline and week 12 are presented in Table 5. There were no significant changes in plasma apoA-I over time for either group. In contrast, plasma apoA-II decreased over time for all participants ($P < 0.05$), with no differences between groups. Plasma apoB did not significantly change from baseline to week 12 for either group. However, reductions in apoB were associated with reductions in total VLDL particles ($r = 0.519$, $P < 0.01$), total LDL particles ($r = 0.458$, $P < 0.01$), and oxLDL ($r = 0.353$, $P < 0.05$). The plasma

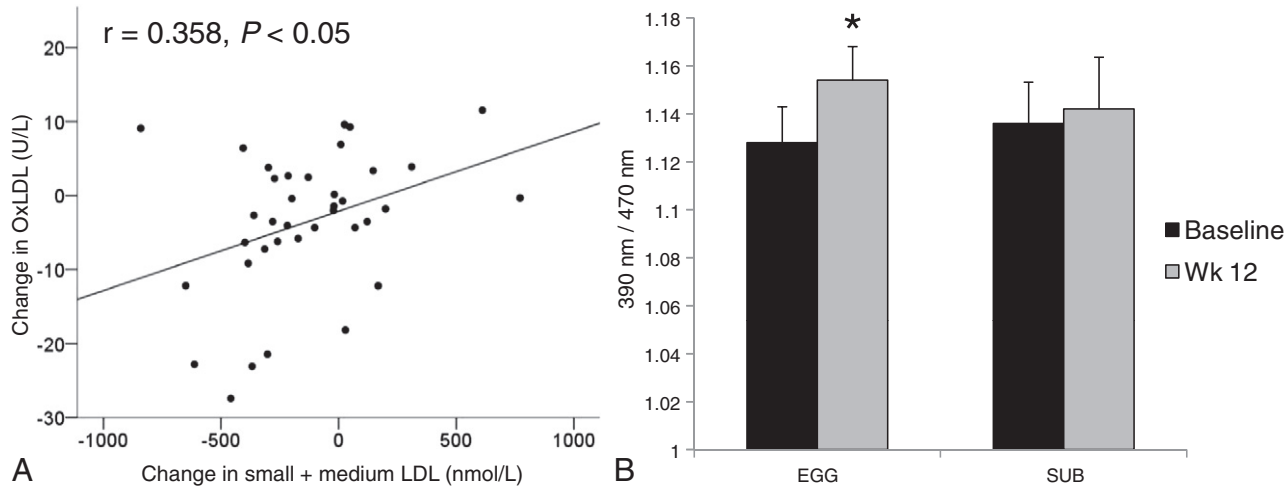

Fig. 3 – (A) Pearson correlation between the changes in the number of small + medium LDL particles and changes in oxidized LDL (oxLDL) from baseline to week 12. (B) Changes in LCAT activity from baseline to week 12 for both the EGG and SUB groups. Values are means ± SEM. *Indicates significantly different from baseline at $P < 0.05$.

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

Table 4 – Total number of HDL, large, medium, and small HDL particles for participants at baseline and after 12 weeks of the diet intervention.

Variable (μmol/L)	Baseline	Week 12	Change	P-value (time)	P-value (time × group)
Total HDL					
EGG	33.0 ± 5.3	33.2 ± 5.7	+0.2	NS	NS
SUB	31.0 ± 4.9	30.2 ± 5.8	−0.8		
Large HDL					
EGG	5.6 ± 2.7[a]	7.3 ± 3.2[b]	+1.7*	<0.001	<0.05
SUB	5.8 ± 3.0[a]	6.5 ± 3.4[b]	+0.6		
Medium HDL					
EGG	5.2 ± 3.4	3.4 ± 3.4	−1.8	<0.01	NS
SUB	5.0 ± 4.7[a]	2.8 ± 2.8[b]	−2.2		
Small HDL					
EGG	22.1 ± 4.2	22.5 ± 5.7	+0.3	NS	NS
SUB	20.1 ± 6.2	21.6 ± 6.8	+1.4		

Values are mean ± SD. $n = 20$ (EGG), $n = 17$ (SUB). P-values are for time effects and time x group interactions for all subjects analyzed by repeated measures ANOVA. Values with different superscripts in a row are significantly different, $P < 0.05$. *Indicates significant differences in EGG vs. SUB using independent samples t test, $P < 0.05$. NS, not significant ($P > 0.05$).

concentrations of apoC-II, apoC-III, and apoE significantly decreased over time for all participants ($P < 0.05$).

4. Discussion

The main findings reported in this study were that both moderate carbohydrate restriction and whole egg intake were able to improve atherogenic dyslipidemia and insulin resistance in men and women with MetS. Carbohydrate restriction and weight loss resulted in decreases in plasma TG, increases in HDL-C, and improvements in lipoprotein subclass distribution. All participants had reductions in VLDL particle size, atherogenic lipoprotein subclasses (small LDL, large VLDL, IDL), and oxLDL. Furthermore, there were increases in the number of large HDL particles in all participants, indicative of a healthier HDL phenotype. In addition, those consuming whole eggs had further improvements in lipoprotein metabolism, including larger HDL and LDL particle diameters, greater reductions in total VLDL and medium VLDL particles, and greater increases in large HDL particles, HDL-C, and LCAT activity compared to those consuming egg substitute.

The relationships between standard blood lipid measures, such as LDL-C and HDL-C, and CVD are well-established. However, despite their strengths in disease prediction, individuals with similar blood lipid values can often have very different relative risks for CVD [18]. In these circumstances, advanced lipoprotein testing is warranted [19]. NMR analysis enables the characterization of heterogeneous lipoprotein subclasses that may differ in their atherogenicity, thereby offering a tool to identify residual CVD risk among individuals with similar blood lipid values. Distinct lipoprotein subclasses,

Table 5 – Plasma apolipoproteins for participants at baseline and after 12 weeks of the diet intervention.

Variable (mg/L)	Baseline	Week 12	Change	P-value (time)	P-value (time × group)
ApoA-I					
EGG	1249.0 ± 196.8	1241.1 ± 181.5	−7.9	NS	NS
SUB	1162.2 ± 204.2	1136.0 ± 162.1	−26.2		
ApoA-II					
EGG	288.9 ± 62.4	267.5 ± 48.4	−21.4	<0.05	NS
SUB	256.5 ± 45.8	243.2 ± 50.3	−13.4		
ApoB					
EGG	1308.9 ± 536.7	1224.9 ± 386.5	−84.0	NS	NS
SUB	1243.0 ± 468.2	1229.0 ± 404.5	−14.0		
ApoC-II					
EGG	70.0 ± 19.9	63.1 ± 23.3	−7.0	<0.05	NS
SUB	68.2 ± 26.9	61.5 ± 25.9	−6.8		
ApoC-III					
EGG	182.6 ± 53.0	165.2 ± 54.5	−17.5	<0.05	NS
SUB	169.0 ± 57.9	151.0 ± 59.7	−18.0		
ApoE					
EGG	51.5 ± 10.2	48.2 ± 13.9	−3.3	<0.05	NS
SUB	53.9 ± 28.2	47.7 ± 25.7	−6.2		

Values are mean ± SD. $n = 20$ (EGG), $n = 17$ (SUB). P-values are for time effects and time x group interactions for all subjects analyzed by repeated measures ANOVA. NS, not significant ($P > 0.05$).

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

measured by NMR analysis, have been shown to be markers of cardiovascular disease severity [18], and predictive of risk for future CVD [6,7,20,21], and type 2 diabetes [22,23]. These associations are often independent of other common risk factors such as age and standard blood lipid measures.

Consistent with the known effects of carbohydrates in regulating VLDL–TG production [10], there were reductions in plasma TG, VLDL particle diameter and the number of large VLDL particles in all participants at the end of the study. Furthermore, reductions were seen in plasma apolipoproteins (C-III and E) associated with TG-rich large VLDL particles. ApoC-III and apoE are known to reduce VLDL–TG hydrolysis and catabolism by inhibiting LPL activity [24,25]. We observed greater decreases in total VLDL and medium VLDL particles for those consuming whole eggs vs. egg substitute. The number of total VLDL particles and medium VLDL particles has been shown to be positively associated with incident CVD [6] and type 2 diabetes [23]. The additional reductions in VLDL particles in the EGG group may be related to reduced hepatic production of VLDL, resulting from the improvements in plasma insulin and HOMA-IR observed post-intervention. Atherogenic dyslipidemia in MetS is driven by overproduction of large TG-rich VLDL, a consequence of hepatic insulin resistance [8]. Hepatic insulin resistance increases both lipid substrate and apoB availability for VLDL assembly [8].

Small LDL and medium LDL particles significantly decreased in all participants from baseline. In addition, there was a concomitant increase in the less atherogenic large LDL particles. In contrast to small LDL, larger LDL particles are generally not associated with risk for CVD [6]. Furthermore, we observed a significant increase in LDL particle size with whole egg feeding. Daily egg feeding has been previously shown to result in increases in large LDL subclasses and LDL diameter, measured by gel electrophoresis [26] and NMR methods [13]. Higher plasma concentrations of small LDL particles are related to CVD incidence [6]. Small LDL particles have been reported to be highly associated with low HDL-C and high plasma TG — constituting the major lipoprotein abnormalities of the atherogenic dyslipidemia seen in MetS [4]. The reductions in small LDL in this study are in accordance with parallel improvements in HDL-C, TG, and the TG/HDL-C ratio — the last of which has been used as a marker of insulin resistance [27]. Small LDLs are particularly atherogenic, due to their increased residence time and greater susceptibility for oxidation and subendothelial retention [2]. Consequently, we observed reductions in oxLDL in all participants, and this was significantly associated with reductions in both small and medium LDL particles [6]. Plasma apoB levels were unaffected by the intervention, in agreement with the lack of effect on the total number of LDL particles which contribute >90% of total plasma apoB [28]. Notable changes observed in the EGG group were the increases in large LDL and the decreases in smaller LDL subclasses. Importantly, these changes in LDL size and subclasses occurred independently of any changes in LDL-C.

There were significant improvements in both HDL particle size and the number of large HDL particles in those consuming whole eggs vs. egg substitute. Furthermore, increases in HDL size and large HDL were strongly correlated with increases in plasma HDL-C, consistent with other NMR studies [6,20]. Similar improvements in HDL particle characteristics with daily egg intake have been demonstrated in overweight men [13]. HDL particles differ in size, density, and charge — reflective of differences in composition such as protein and lipid content [29]. Small, dense HDL particles are considered less atheroprotective than larger subclasses, and are usually indicative of a phenotype characterized by atherogenic dyslipidemia [7]. HDL particle size measured by NMR was found to be significantly smaller in coronary heart disease (CHD) patients compared to controls [7]. Furthermore, large HDL particles are protective of CVD, while small HDL particles are associated with severity of CVD [6,18]. Some analysis has found no association between HDL particle size and risk of CHD after controlling for plasma apoB and triglyceride concentrations [7]. Nevertheless, therapies that result in larger HDL particles and HDL subclass profiles similar to healthy individuals may be indicative of improvements in cardiovascular risk [30].

We observed significant reductions in the number of medium HDL particles for all participants compared to baseline. This was expected, as we have consistently seen reductions in medium HDL particles in parallel with reductions in carbohydrate intake in other studies [13,31,32]. One case–control observational study in men with MetS found those with lower medium HDL (measured by NMR) particles had a greater incidence of CHD death [33]. However, other NMR-derived lipoprotein profile studies have found no association between medium HDL and CHD [6,7]. We observed significant reductions in apoA-II, which is predominately carried on particles of HDL_3 density and thus, likely decreased due to reductions in medium HDL particles [34]. ApoA-II has been associated with impaired LCAT activity, hypertriglyceridemia, and atherosclerosis in animal models [35]. Thus, the formation of larger HDL at the expense of medium HDL possibly reflects a more atheroprotective HDL subclass profile following egg consumption. Plasma apoA-I concentrations did not change, which is in agreement with no changes in total HDL particles, along with the reductions in medium HDL, which carry both apoA-I and apoA-II, and increases in large HDL, which carry predominately apoA-I [36].

LCAT activity increased from baseline in the EGG group only. This finding is consistent with other studies reporting that daily egg intake increases LCAT activity [13,14]. This could be indicative of an enhanced capacity for HDL maturation and may help explain the shift towards larger HDL particles seen with egg feeding [13]. LCAT is critically important in facilitating HDL particle stability and HDL maturation [37].Unlike LCAT, there were no changes in plasma CETP activity from baseline for either group. Therefore, increases in LCAT activity with egg consumption may be associated with enhancements in HDL-mediated reverse cholesterol transport.

The improvements in dyslipidemia with eggs observed in the current study are at least comparable to results using other whole foods to target and improve plasma lipid profiles. Consumption of 1.5 grapefruits per day for 6 weeks reduced plasma total cholesterol and LDL-C in overweight adults, but did not result in improvements in HDL-C and when compared to a control group [38]. In type 2 diabetics, 4 weeks of daily almond consumption (~60 g per day) resulted in 9.7% decreases in the LDL-C/HDL-C ratio compared to a control diet,

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

resulting from reductions in plasma LDL-C [39]. In the current study, egg consumption was associated with a 13.5% reduction in the LDL-C/HDL-C ratio from baseline, primarily attributed to 19.1% increases in plasma HDL-C. Compared to the yolk-free egg substitute, effects observed with whole egg consumption are likely due to the additional egg yolks. Egg yolks are a particularly rich source of exogenous phosphatidylcholine (PC) and other phospholipids [40]. Phospholipid feeding has been associated with increases in HDL-C in animal and human studies [41,42]. The additional phospholipid consumed by the EGG group may be partly responsible for the observed improvements in HDL-C and HDL particle profiles.

Among the strengths of the study is the practicality of the diet intervention used for managing MetS. The current study results demonstrate that even a moderate restriction of carbohydrates can result in global improvements in parameters of MetS. Such a dietary pattern may be easily incorporated and accepted by those with MetS. In addition, inclusion of whole eggs, which are low in carbohydrates, can enhance the beneficial effects of carbohydrate restriction on plasma lipids, lipoprotein profiles, and insulin resistance. One of the limitations of the study was that participants in the EGG group were more insulin resistant than those in the SUB at baseline, and therefore may have benefited more from a similar amount of weight loss. In addition, since the current study was only 12 weeks in duration, further studies may be needed to address more prolonged consumption of cholesterol and choline in individuals with MetS.

In summary, carbohydrate restriction and weight loss improved the lipoprotein profile of individuals with metabolic syndrome by reducing plasma oxLDL, and modifying the distribution of lipoprotein subclasses towards a less atherogenic phenotype. Further, the inclusion of whole eggs provides additional benefits compared to yolk-free egg substitute by increasing large HDL particles, HDL and LDL diameters, and LCAT activity, while reducing total VLDL and medium VLDL particles. Thus, results from this study suggest that incorporating daily egg intake into a moderately carbohydrate-restricted diet provides additional benefits for the atherogenic lipoprotein profile associated with MetS. Further studies need to be conducted to confirm these findings.

Author contributions

CNB conducted the research, analyzed data and wrote the paper; CJA conducted the research and provided input for the paper; JB participated in data collection and interpretation; JSV participated in interpretation and provided input; MLF designed research, analyzed data and had primary responsibility for final content. All authors read and approved the final manuscript.

Funding

This study was funded by the American Egg Board/Egg Nutrition Center.

Conflict of interest

MLF received funds to conduct the study. No other conflicts of interest exist for the remaining authors.

REFERENCES

[1] Grundy SM, Brewer Jr HB, Cleeman JI, et al. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Arterioscler Thromb Vasc Biol 2004;24(2):e13–8.
[2] Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 2002;43(9):1363–79.
[3] Holvoet P, Kritchevsky SB, Tracy RP, et al. The metabolic syndrome, circulating oxidized LDL, and risk of myocardial infarction in well-functioning elderly people in the health, aging, and body composition cohort. Diabetes 2004;53(4):1068–73.
[4] Kathiresan S, Otvos JD, Sullivan LM, et al. Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. Circulation 2006;113(1):20–9.
[5] Kontush A, Chapman MJ. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacol Rev 2006;58(3):342–74.
[6] Mora S, Otvos JD, Rifai N, et al. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation 2009;119(7):931–9.
[7] El Harchaoui K, Arsenault BJ, Franssen R, et al. High-density lipoprotein particle size and concentration and coronary risk. Ann Intern Med 2009;150(2):84–93.
[8] Adiels M, Olofsson SO, Taskinen MR, et al. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arterioscler Thromb Vasc Biol 2008;28(7):1225–36.
[9] Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285(19):2486–97.
[10] Volek JS, Fernandez ML, Feinman RD, et al. Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome. Prog Lipid Res 2008;47(5):307–18.
[11] Petersen KF, Dufour S, Savage DB, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc Natl Acad Sci U S A 2007;104(31):12587–94.
[12] Fernandez ML. Dietary cholesterol provided by eggs and plasma lipoproteins in healthy populations. Curr Opin Clin Nutr Metab Care 2006;9(1):8–12.
[13] Mutungi G, Waters D, Ratliff J, et al. Eggs distinctly modulate plasma carotenoid and lipoprotein subclasses in adult men following a carbohydrate-restricted diet. J Nutr Biochem 2010;21(4):261–7.
[14] Herron KL, Lofgren IE, Sharman M, et al. High intake of cholesterol results in less atherogenic low-density lipoprotein particles in men and women independent of response classification. Metabolism 2004;53(6):823–30.
[15] Djousse L, Gaziano JM. Egg consumption in relation to cardiovascular disease and mortality: the Physicians' Health Study. Am J Clin Nutr 2008;87(4):964–9.

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014

[16] 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.
[17] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412–9.
[18] Freedman DS, Otvos JD, Jeyarajah EJ, et al. Relation of lipoprotein subclasses as measured by proton nuclear magnetic resonance spectroscopy to coronary artery disease. Arteriosclerosis, thrombosis, and vascular biology 1998;18(7): 1046–53.
[19] Davidson MH, Ballantyne CM, Jacobson TA, et al. Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. J Clin Lipidol 2011;5(5):338–67.
[20] Arsenault BJ, Lemieux I, Despres JP, et al. Comparison between gradient gel electrophoresis and nuclear magnetic resonance spectroscopy in estimating coronary heart disease risk associated with LDL and HDL particle size. Clin Chem 2010;56(5):789–98.
[21] Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation 2006;113(12): 1556–63.
[22] Festa A, Williams K, Hanley AJ, et al. Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the Insulin Resistance Atherosclerosis Study. Circulation 2005;111(25):3465–72.
[23] Hodge AM, Jenkins AJ, English DR, et al. NMR-determined lipoprotein subclass profile predicts type 2 diabetes. Diabetes Res Clin Pract 2009;83(1):132–9.
[24] Rensen PC, van Berkel TJ. Apolipoprotein E effectively inhibits lipoprotein lipase-mediated lipolysis of chylomicron-like triglyceride-rich lipid emulsions in vitro and in vivo. J Biol Chem 1996;271(25):14791–9.
[25] Tomiyasu K, Walsh BW, Ikewaki K, et al. Differential metabolism of human VLDL according to content of ApoE and ApoC-III. Arterioscler Thromb Vasc Biol 2001;21(9): 1494–500.
[26] Ballesteros MN, Cabrera RM, Cabrera RM, Saucedo Mdel S, et al. 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] Gasevic D, Frohlich J, Mancini GB, et al. The association between triglyceride to high-density-lipoprotein cholesterol ratio and insulin resistance in a multiethnic primary prevention cohort. Metabolism 2012;61(4):583–9.
[28] Sniderman A, Vu H, Cianflone K. Effect of moderate hypertriglyceridemia on the relation of plasma total and LDL apo B levels. Atherosclerosis 1991;89(2–3):109–16.
[29] Asztalos BF, Tani M, Schaefer EJ. Metabolic and functional relevance of HDL subspecies. Curr Opin Lipidol 2011;22(3): 176–85.
[30] Asztalos BF, Batista M, Horvath KV, et al. Change in alpha1 HDL concentration predicts progression in coronary artery stenosis. Arterioscler Thromb Vasc Biol 2003;23(5):847–52.
[31] Jones JL, Comperatore M, Barona J, et al. A Mediterranean-style, low-glycemic-load diet decreases atherogenic lipoproteins and reduces lipoprotein (a) and oxidized low-density lipoprotein in women with metabolic syndrome. Metabolism 2012;61(3):366–72.
[32] Al-Sarraj T, Saadi H, Volek JS, et al. Carbohydrate restriction favorably alters lipoprotein metabolism in Emirati subjects classified with the metabolic syndrome. Nutr Metab Cardiovasc Dis 2010;20(10):720–6.
[33] Kuller LH, Grandits G, Cohen JD, et al. Lipoprotein particles, insulin, adiponectin, C-reactive protein and risk of coronary heart disease among men with metabolic syndrome. Atherosclerosis 2007;195(1):122–8.
[34] Cheung MC, Albers JJ. Distribution of high density lipoprotein particles with different apoprotein composition: particles with A-I and A-II and particles with A-I but no A-II. J Lipid Res 1982;23(5):747–53.
[35] Chan DC, Ng TW, Watts GF. Apolipoprotein A-II: evaluating its significance in dyslipidaemia, insulin resistance, and atherosclerosis. Ann Med 2012;44(4):313–24.
[36] Rothblat GH, Phillips MC. High-density lipoprotein heterogeneity and function in reverse cholesterol transport. Curr Opin Lipidol 2010;21(3):229–38.
[37] Rousset X, Shamburek R, Vaisman B, et al. Lecithin cholesterol acyltransferase: an anti- or pro-atherogenic factor? Curr Atheroscler Rep 2011;13(3):249–56.
[38] Dow CA, Going SB, Chow HH, et al. The effects of daily consumption of grapefruit on body weight, lipids, and blood pressure in healthy, overweight adults. Metabolism 2012;61(7):1026–35.
[39] Li SC, Liu YH, Liu JF, et al. Almond consumption improved glycemic control and lipid profiles in patients with type 2 diabetes mellitus. Metabolism 2011;60(4):474–9.
[40] Kovacs-Nolan J, Phillips M, Mine Y. Advances in the value of eggs and egg components for human health. J Agric Food Chem 2005;53(22):8421–31.
[41] O'Brien BC, Corrigan SM. Influence of dietary soybean and egg lecithins on lipid responses in cholesterol-fed guinea pigs. Lipids 1988;23(7):647–50.
[42] Burgess JW, Neville TA, Rouillard P, et al. Phosphatidylinositol increases HDL-C levels in humans. J Lipid Res 2005;46(2): 350–5.

Please cite this article as: Blesso CN, et al, Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in i..., Metabolism (2012), http://dx.doi.org/10.1016/j.metabol.2012.08.014