Comparison of long-term effects of egg yolk consumption under normal and high fat diet on lipid metabolism and fatty acids profile in mice

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Abstract This study compared the long-term effects of EY consumption under two diet conditions: normal (ND + EY) and high fat diet (HFD + EY), on lipid metabolism in mice. ND + EY did not increase serum triglycerides, total cholesterol hepatic triglyceride concentrations, adipose tissue accumulation and glucose impairment, not leading to fatty liver. HFD + EY markedly decreased adipose tissue accumulation, the triglyceride and total cholesterol, and improved serum HDL-C and blood glucose impairment compared with HFD. PLS-DA analyzes showed both ND + EY and HFD + EY could decrease serum C18:1 and MUFA. HFD + EY could further decrease hepatic C18:2 and PUFA and increase C18:1 and MUFA excretion, which were associated with lower expression of Elov16 and higher expression of Scd1 in liver. These results suggest that HFD + EY significantly improved dyslipidemia caused by HFD through modifying lipid metabolism, and ND + EY did not adversely affect the biomarkers

associated with dyslipidemia risk, but showed less obvious regulation of lipid metabolism than HFD + EY.

Keywords Egg yolk · Normal diet · High fat diet · Lipid profile · Fatty acid biomarker

Introduction

Metabolic syndrome (MetS) is a commonly associated with obesity-related metabolic diseases and the risk of developing of cardiovascular disease (CVD) (Camargo et al., 2014). High cholesterol diets have drawn widespread attention due to the increasing risk of CVD worldwide (Blesso and Fernandez, 2018). It is well known that egg is one of the richest sources of dietary cholesterol (Palacios and Wang, 2005). In the past, egg especially egg yolk (EY) consumption was controversial and not advised for its high contents of cholesterol, which was regarded as a risk of increasing CVD. According to the Dietary Guidelines for Americans, there is no limit recommendation of dietary cholesterol intake to no more than 300 mg/d since 2015 (Nissen, 2016). Moreover, numerous studies failed to find the positive association between dietary cholesterol and increased biomarkers associated with the risk of CVD (Anderson et al., 2015; Lemos et al., 2018). These results have led to a reconsideration and evaluation of the relationship between eggs, especially EY and the risk of increasing CVD.

Several research and reviews aimed at the relation of EY consumption and the risk for CVD have been published in the past years. In recent years, more and more studies have revealed EY consumption could reduce the risk of CVD and prevent the development of related metabolic diseases. In randomized clinical trials, 3 whole eggs/day

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consumption along with carbohydrate restriction resulted in greater reduction in plasma triglycerides, low density lipoproteins (LDL) and very low density lipoproteins (VLDL) particle diameter and greater increases in high density lipoproteins cholesterol (HDL-C), large LDL and large HDL particles than EY-free egg substitute in MetS (Blesso et al., 2013a). Additionally, three whole eggs per day intake for 12 weeks could distinctly increase the enrichment of carotenoids and zeaxanthin in lipoprotein subclasses in adult men following a carbohydrate-restricted diet, which favorably altered the ability of HDL transporter (Blesso et al., 2013b). Another study reported that an EY-enriched diet had lower plasma triglycerides and improved the fecal neutral sterol and bile acid concentrations than those fed a plain cholesterol diet (Yang et al., 2012). Other research showed that EY consumption studies have not only resulted in the improvements of hyperlipidemia associated with CVD risk but also amelioration in of chronic inflammation, glucose intolerance, and insulin resistance in MetS (Missimer et al., 2017; Ratliff et al., 2008). Notably, these favorable effects were tended to be observed in carbohydrate restriction, which can independently regulate the lipid metabolism. Therefore, whether these favorable effects still exist in normal diets or high fat diets are still controversial and un-known. However, few experiments have been conducted to investigate the roles of EY intakes on lipid especially fatty acids metabolism in normal and high fat diets. Moreover, whether EY intake will accelerate the hyperlipidemia caused by high fat diet was controversial and needed further discussion. Therefore, this study focused on the overall effect of EY on the lipid metabolism in mice fed with normal and high fat diet.

The effect of a long-term consumption of EY under normal and high fat diet on plasma, hepatic and fecal lipid levels, and pathological changes was investigated in the liver and adipose tissue of KM mice. In order to evaluate the effects on the lipid metabolism, the fatty acids profile in serum, liver and feces and hepatic mRNA expression involved in fatty acids metabolism were further evaluated by GC-MS and qRT-qPCR. Additionally, the dose of EY was 3.75 mg/g mice, which was the equivalent dose of the consumption of 3 eggs per day for human, would experience greater overall effects of EY.

Materials and methods

Animals and experimental design

Three-week-old KM male mice weighing 18–22 g ($n = 80$) were purchased from the College of Veterinary Medicine Huazhong Agricultural University (Wuhan, China). Mice were housed in cages each containing five animals in a

temperature-controlled room (21 ± 2 °C), relative humidity ($60 \pm 5\%$) and light cycle (12 h light/dark) with free access to food and water. After 7 days of acclimation on laboratory chow, all mice were given normal diet ($n = 40$, ND, D12450B, Research Diets, New Brunswick, NJ, USA) and high fat diet ($n = 40$, HFD, D12492, Research Diet). The ingredients and fatty acids composition of normal and high fat diet could be seen in Table S1 and S2. All mice received isometric vehicle (3.75 mg/g mice EY dissolved in PBS in ND + EY and HFD + EY; the equivalent amount of PBS in ND and HFD), via orogastric gavage through a 50 mm Gauge feeding needle (Feiyang biotechnology Co. Ltd., Wu Han, China) for a total of 100 days. The EY dose (3.75 mg/g mice) was converted from a human equivalent dose based on body surface area by the following formula from pharmacological experiment methodology: assuming a human weight of 70 kg, the human equivalent dose of EY was $28.84 \text{ (g)}/70 \text{ (kg)}$ (3 eggs per day). Mice dose (mg/g) = $28.84 \text{ (g)}/70 \text{ (kg)} \times 9.1 = 3.75 \text{ mg/g mice}$; the conversion coefficient 9.1 was used to account for differences in body surface area between mice and human (Reagan-Shaw et al., 2008). Body weight and food intake were recorded once a week. After whole experimental period of dietary intervene, mice were euthanized and sacrificed by decapitation following a 12-h fasting period. The main internal tissues were weighed and stored at -80 °C for further analysis. The internal organs, including liver, kidney, spleen, perirenal adipose, perisplenic adipose, and epididymal adipose were removed for weight and all organ indexes calculations using the following formula: $\text{Index (\%)} = \text{organ weight (g)}/\text{body weight (g)} \times 100$.

Determination of lipids in serum, liver, and feces

Blood samples were harvested from eyepit and serum level of HDL-C, low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and total cholesterol (TC) were measured by using commercial kits (BioAssay Systems Inc, CA, USA). Total lipids were extracted from the liver and feces according to the method of Folch et al. (1957) and the level of TC and TG were measured as above. For fecal samples, the fecal samples (4 pellets of feces per mouse) in each group were collected during last 3 days period to satisfy experimental need.

Glucose tolerance test (GTT), insulin tolerance test (ITT) and histology

Before GTT and ITT, half of the mice in each group were fasted for 8 h in advance. Fasting blood glucose from the tail vein was measured by using an Accu-Chek blood glucose monitor (Roche Inc, Basel, Switzerland). The

blood glucose was measured at the time of 15, 30, 60 and 120 min after gavaging with a standardized glucose dose (2 mg/g body weight) and at the time of 30, 60, 90 and 120 min after injecting intraperitoneally with insulin (0.75 mU/g). Both GTT and ITT curves were plotted. The area under the blood glucose curve (AUC) was calculated according to the formula (Zhang et al., 2014): $AUC = 0.5 \times (BG_{0 \text{ min}} + BG_{30 \text{ min}})/2 + 0.5 \times (BG_{30 \text{ min}} + BG_{60 \text{ min}})/2 + 1 \times (BG_{60 \text{ min}} + BG_{120 \text{ min}})/2$, where BG is blood glucose.

For pathological analysis, the paraformaldehyde-fixed liver and adipose tissue samples were dehydrated and embedded in paraffin wax blocks, slicing into 5 μm thickness for morphological and pathological evaluations. Tissue sections were stained with hematoxylin and eosin (H&E) and photographed by bright field microscopy (Nikon Eclipse CI, Nikon, Tokyo, Japan).

Fatty acid composition of serum, liver and feces

Lipids extracted from the serum (50 μL), liver (50 mg) and feces (50 mg) were prepared by methylation with NaOH–MeOH, and the fatty acid composition was analyzed by GC–MS. The analyses were performed on an Agilent 7890B GC System interfaced to an Agilent 5977A mass spectrometry detector (Agilent Technologies, Palo Alto, CA, USA) with the module of 7693 autosampler. The DB-23 column (60 m $\times$ 250 μm $\times$ 0.25 μm , Supelco, Bellefonte, PA, USA) was used in the gas chromatography system. The temperatures of the detector and the injector were kept constant at 280 $^{\circ}\text{C}$ and 230 $^{\circ}\text{C}$, respectively, and the column temperature was increased from 130 to 170 $^{\circ}\text{C}$ at the rate of 6.5 $^{\circ}\text{C}/\text{min}$, then to 215 $^{\circ}\text{C}$ at the rate of 2.75 $^{\circ}\text{C}/\text{min}$, finally to 230 $^{\circ}\text{C}$ at the rate of 4 $^{\circ}\text{C}/\text{min}$, and held at 230 $^{\circ}\text{C}$ for 3 min. Nitrogen was used as a carrier gas at the flow rate of 1.0 mL/min and the injection volume was 1 μL . The identification and quantitative analysis were detected by comparing the retention time of 37 fatty acid methyl ester standards (Supelco 37 Component FAME Mix, Supelco).

Hepatic mRNA expression levels by real-time quantitative PCR analysis

Total RNA was extracted by animal total RNA isolation kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The reverse transcription reaction was conducted using a RevertAid first strand cDNA synthesis kit (Thermo Scientific). The qPCR was conducted with the SYBR[®] Premix Ex Taq[™] II (Tli RNaseH Plus, Takara, Dalian, China) in a StepOnePlus[™] Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The reaction was performed in triplicate with a

total volume of 25 μL containing 5 μL 10% cDNA, 12.5 μL SYBR Premix buffer (TaKaRa), 0.75 μL forward/reverse (10 μM) and 6 μL nuclease-free water (TaKaRa). The PCR program was initial denaturated at 95 $^{\circ}\text{C}$ for 10 min, followed by 45 cycles comprising 95 $^{\circ}\text{C}$ for 15 s, 60 $^{\circ}\text{C}$ for 10 s, and 72 $^{\circ}\text{C}$ for 45 s. The fold changes of the target gene expression of SREBP-1c, Acaca, Fasn, Scd1, Elov16, Dgat1, Dgat2, GPAT, CD36, Cpt1a, Fgf21 and MTP were calculated by the $2^{-\Delta\Delta\text{Ct}}$ method and GAPDH served as the internal control. The sequences of primers were based on sequences published in GenBank (Available online: <http://www.ncbi.nlm.nih.gov/>) and were listed in Supporting Information Table S3.

Data analysis

All data are expressed and analyzed using the SPSS Statistics 17.0 (IBM, Armonk, NY, USA), as mean $\pm$ standard error of the mean (SEM). Comparisons between groups were performed with One-way analysis of variance (ANOVA) followed by Dunnett's analysis methods. PLS-DA analyzes and heatmap were performed using MetaboAnalyst 4.0 (<http://www.metaboanalyst.ca/faces/home.xhtml>). Differences were considered significant when the p value was < 0.05 and very significantly when the p value was < 0.01 .

Results and discussion

Effect of egg yolk under ND and HFD on body weight and organ index

Body weights of mice in all groups increased constantly during whole dietary intervention period and were significantly higher in HFD and HFD + EY compared with mice in ND and ND + EY ($p < 0.01$). No differences were observed in weight gain between the control group and EY group in both ND and HFD diet (Fig. 1A). Comparing with the daily food and energy intake of each group, the HFD and HFD + EY group mice consumed significantly less food than ND group after 5-week experiment ($p < 0.01$). No differences were observed in daily food intake between the EY groups and control groups in both diets (Fig. 1B).

After 100 days of experiment, the liver, perirenal and epididymal adipose tissue index in the HFD groups mice were significantly increased compared with that in the ND group mice ($p < 0.01$). However, the kidney, spleen, perisplenic adipose tissue index were lower in HFD groups. There was no significant difference of perirenal and perisplenic adipose tissue index between ND and ND + EY group, while the epididymal adipose index (4.98 ± 0.09) was highest in HFD + EY group.

Fig. 1 General effect of EY consumption under ND and HFD. **A** Body weight. **B** Food consumption. **C** Morphological changes of liver (HE staining, $\times 200$). **D**, Morphological changes of adipose tissues (HE staining, $\times 200$). **E**, The area of adipocytes cells. The

area of adipocytes cells were calculated using the image J analysis software. Data are expressed as the mean $\pm$ SEM ($n = 10$). a, b, c stands for significant difference among groups (ANOVA, $p < 0.05$)

Effect of egg yolk under ND and HFD on hepatic and adipose tissue pathology

For liver morphology of mice in each group, the mice in both ND and ND + EY had normal liver appearance with soft texture, fresh red color and shiny surface, while the liver appearance of mice in both HFD and HFD + EY were slightly rough texture and with yellow dense fat particles on the surface, indicating the initial signs of fatty livers. Histopathological analysis from mice in ND and ND + EY showed a normal morphological appearance, with clear hepatic cord and sinusoid and no signs of hepatic steatosis on day 60 and 100 (Fig. 1C). In the HFD-fed group, the morphology of fatty liver, with hepatocytes comprising many different sizes of microvesicular steatosis on day 60 and even severe on day 100, were detected in

HFD and HFD + EY groups. In the meantime, as can be seen in Fig. 1D, E, the adipocyte sizes in HFD and HFD + EY were significantly larger than in ND and ND + EY ($p < 0.05$). The adipocyte sizes in HFD + EY were markedly larger than in other groups, while there was no significant difference between ND and ND + EY on day 100 ($p > 0.05$). These results indicate that ND + EY did not induce hyperplasia of subcutaneous adipose tissues, further leading to obesity (also can be seen in Fig. 1A). Although adipose size in HFD + EY increased almost 1.5-fold than HFD, which was mainly caused by higher additional energy intake contained in EY. However, HFD + EY did not increased the accumulation of perirenal and perisplenic adipose, which also can be in previous study (Acosta et al., 2016). Although previous studies demonstrated that adipose size may increase in cardiometabolic

risk related to obesity (Laforest et al., 2015), recent research confirmed that dysfunctional adipose tissue, including fatty acids contents and inflammatory response also can accelerate the pathogenesis of obesity (van Kruijsdijk et al., 2009). Hence, involved mechanism needed to further investigated by the analysis of lipid levels (Table 1).

Effect of egg yolk under ND and HFD on lipid levels in serum, liver and feces

In recent years, the main therapies for CVD protection aimed at lowering the level of LDL-C, TG and TC, and increasing the level of HDL-C (Taskinen and Boren, 2015). The results of lipid levels in serum, liver and feces are shown in Table 2. Consistent with previous studies, HFD significantly increased in TG, TC in serum and liver. After 60 and 100 days of diet feeding treatment, the TG (3.17 ± 0.01 vs 2.75 ± 0.02 mmol/L) and TC (153.30 ± 0.77 vs 143.81 ± 3.21 mg/dl) levels in the serum of HFD were significantly higher than in ND groups ($p < 0.01$). The serum TG levels had no significant difference between ND + EY and ND group ($p > 0.05$), while the serum TG (2.22 ± 0.06 vs 3.17 ± 0.01 mmol/L) and TC (144.97 ± 1.36 vs 153.30 ± 0.77 mg/dl) levels in the HFD + EY group were significantly lower than those in the HFD group ($p < 0.05$). In addition, the serum HDL-C concentrations in ND + EY and HFD + EY were 152.3 ± 1.08 and 155.93 ± 0.88 mg/dl, respectively, which markedly higher than those in ND group (128.78 ± 3.65 mg/dl) ($p < 0.05$). Although ND + EY markedly increased the level of LDL-C compared with ND, the difference of LDL-C level between HFD and HFD + EY were not significant ($p > 0.05$) Possibly, the HFD + EY alleviated the accumulation and transformation of TG and cholesterol by improving the cholesterol-accepting capacity of serum HDL and repairing the damage to LDL-cholesterol described in previous studies. Andersen et al. studied the effects of EY on HDL composition and function in MetS. That study demonstrated that EY intake increased HDL phosphatidylethanolamine composition and macrophage cholesterol efflux capacity of subject serum, which was consistent with increased HDL-associated lipid transporter ABCA1 mRNA expression and reduced insulin resistance (HOMA-IR) (Andersen et al., 2014). Other research also found the consistent results in the modification of HDL and LDL composition and capability by EY (Richard et al., 2017). All these favorable effects of EY on lipoproteins were conducted in MetS, which is often caused by high fat diet. These favorable effects were closely related to the abundant contents of lipoprotein of EY or other functional lipids (Eftekhar et al., 2015).

Table 1 The changes of tissue parameters after EY consumption under ND and HFD on 60 days and 100 days

Item	Experimental intervention time							
	60 days				100 days			
	ND	ND + EY	HFD	HFD + EY	ND	ND + EY	HFD	HFD + EY
Liver (%)	3.59 ± 0.16^{bc}	3.49 ± 0.08^{cd}	3.57 ± 0.13^c	3.34 ± 0.04^d	3.65 ± 0.15^{ab}	3.56 ± 0.06^e	3.81 ± 0.03^a	3.61 ± 0.10^{bc}
Kidney (%)	0.39 ± 0.05^b	0.4 ± 0.01^b	0.47 ± 0.03^a	0.38 ± 0.00^{bc}	0.46 ± 0.03^a	0.40 ± 0.02^b	0.34 ± 0.02^c	0.34 ± 0.02^c
Spleen (%)	1.34 ± 0.11^{ab}	1.28 ± 0.04^{ab}	1.37 ± 0.03^a	1.25 ± 0.02^{abc}	1.23 ± 0.01^{bc}	1.26 ± 0.04^{abc}	1.14 ± 0.08^{cd}	1.05 ± 0.10^d
Perirenal adipose (%)	0.16 ± 0.03^d	0.17 ± 0.03^d	0.26 ± 0.01^b	0.28 ± 0.02^b	0.20 ± 0.02^c	0.25 ± 0.00^b	0.45 ± 0.03^a	0.47 ± 0.02^a
Perisplenic adipose (%)	0.48 ± 0.03^a	0.39 ± 0.01^b	0.34 ± 0.01^{cd}	0.34 ± 0.03^d	0.44 ± 0.02^a	0.43 ± 0.02^a	0.31 ± 0.02^d	0.37 ± 0.02^{bc}
Epididymal adipose (%)	1.96 ± 0.04^e	1.89 ± 0.41^e	3.28 ± 0.15^c	3.30 ± 0.24^c	2.51 ± 0.12^d	2.61 ± 0.38^d	4.23 ± 0.11^b	4.98 ± 0.09^a

The results were expressed as mean $\pm$ SEM (n = 10). Different superscript letters indicate significant differences at $p < 0.05$

Table 2 The changes of lipid concentrations in serum, liver and feces after EY consumption under ND and HFD on 60 days and 100 days

Item	Experimental intervention time					
	60 d			100 d		
	ND	ND + EY	HFD	HFD + EY	ND	HFD
Serum lipid						
HDL-C (mg/dl)	113.69 ± 0.85 ^d	103.11 ± 5.89 ^e	129.14 ± 5.53 ^c	139.31 ± 7.02 ^b	128.78 ± 3.65 ^c	150.61 ± 3.19 ^a
LDL-C (mg/dl)	41.17 ± 2.15 ^e	53.88 ± 3.31 ^c	49.13 ± 0.98 ^d	60.37 ± 4.28 ^a	34.58 ± 0.66 ^f	57.21 ± 3.19 ^{abc}
TG (mmol/L)	2.92 ± 0.20 ^b	2.39 ± 0.10 ^d	2.52 ± 0.08 ^d	1.89 ± 0.01 ^f	2.75 ± 0.02 ^c	3.17 ± 0.01 ^a
TC (mg/dl)	140.3 ± 2.09 ^c	142.17 ± 1.87 ^c	146.78 ± 3.56 ^{bc}	132.76 ± 6.84 ^d	143.81 ± 3.21 ^c	153.3 ± 0.77 ^a
Liver lipid (μmol/g dry liver)						
TG	40.8 ± 3.75 ^c	46.99 ± 4.84 ^{de}	83.9 ± 6.45 ^b	103.38 ± 5.38 ^a	46.23 ± 4.18 ^{de}	82.09 ± 5.56 ^{bc}
TC	6.13 ± 0.67 ^c	6.65 ± 0.95 ^{de}	15.67 ± 0.66 ^b	15.71 ± 1.00 ^b	7.75 ± 0.58 ^d	17.84 ± 0.70 ^a
Fecal lipids (μmol/g dry feces)						
TG	6.27 ± 0.55 ^c	7.05 ± 0.65 ^{bc}	16.44 ± 0.83 ^a	17.34 ± 1.45 ^a	7.88 ± 0.26 ^b	16.44 ± 0.61 ^a
TC	3.63 ± 0.26 ^c	4.17 ± 0.31 ^c	6.39 ± 0.69 ^{bc}	7.47 ± 0.85 ^a	5.22 ± 0.56 ^d	7.06 ± 0.45 ^{ab}

The results were expressed as mean ± SEM (n = 10). Different superscript letters indicate significant differences at $p < 0.05$

The TG (73.89 ± 2.68 vs 82.09 ± 5.56 μmol/g dry liver) and TC (16.06 ± 0.75 vs 17.84 ± 0.70 μmol/g dry liver, $p < 0.05$) level in the liver of HFD + EY were lower than in the HFD on 100 days. Despite ND + EY increased the TC level in the liver than ND diet, no statistically significant difference was observed in the level of TG between the ND group and ND + EY group ($p > 0.05$). These results revealed that there was a stronger regulation effect of EY under dyslipidemia caused by HFD than normal condition. The favorable effect of EY on lipid profile is possibly due to the high contents of functional components in EY that can regulates the lipid metabolism, described in previous studies (Missimer et al., 2017). Fecal TG (16.44 ± 0.61 vs 7.88 ± 0.26 μmol/g dry feces) and TC (7.06 ± 0.45 vs 5.22 ± 0.56 μmol/g dry feces) contents were significantly higher in HFD group than ND group ($p < 0.01$). Fecal TC levels excreted in the ND, ND + EY, and HFD, HFD + EY were 5.22 ± 0.56 vs 5.85 ± 0.53 μmol/g dry feces ($p > 0.05$), 7.06 ± 0.45 vs 6.29 ± 0.61 μmol/g dry feces ($p > 0.05$), respectively, indicating EY intake did not affect the TC excretions in both ND and HFD diets. There were many anti-hyperlipemia and anti-hyperlipoidemia bioactive components in EY. Previous studies have confirmed some EY components such as phospholipids, active proteins and functional fatty acids have TG and cholesterol-lowering activity and regulate the TG absorption, metabolism and excretion (Anton, 2013). Previous studies in our lab indicated that EY-enriched diet could significantly reduce cholesterol absorption, restrain de novo cholesterol synthesis and excretion in feces by activating bile acid synthesis (Yang et al., 2012). In addition, in vitro studies showed that functional fatty acids such as EPA and DHA, inhibited the intestinal uptake of cholesterol by down-regulating NPC1L1 and SREBP-1/-2 mRNA (Yang et al., 2018). Therefore, these results revealed that ND + EY had no negative effect on TG contents in serum and liver, despite higher TC contents were observed in serum and liver. In contrast, HFD + EY significantly decreased the level of TC and TG in serum and liver than HFD.

Effect of egg yolk under ND and HFD on GTT and ITT

To investigate whether EY can affect glucose tolerance and exacerbate HFD-induced glucose impairment, GTT and ITT were conducted on 60 day and 100 day. GTT and ITT results showed that EY-fed mice in ND and HFD did not aggravate the impaired glucose tolerance and insulin resistance, while an improvement was observed instead. As shown in Fig. S1A and B, there was an increase in the blood glucose level of HFD mice compared with that in ND mice, which indicates HFD could cause blood glucose

impairment to some extent. Mice on ND + EY diet did not have a significant change in glucose tolerance on 60 days (AUC = 18.79 vs. 20.27, $p > 0.05$) and on 100 days (AUC = 18.20 vs. 19.34, $p > 0.05$) compared to ND mice (Fig. S1C). In HFD groups, there was an increase in the glucose tolerance of HFD + EY mice compared with that in HFD mice. In ITT experiment (Fig. S1D and E), although ND + EY decreased glucose tolerance compared with ND, there was no markedly difference in insulin sensitivity between HFD and HFD + EY (Fig. S1F), which indicates HFD + EY did not further promote blood glucose impairment.

Researchers from the Wallin study reported there is a positive association between egg consumption and fasting glucose and risk of type 2 diabetes (Wallin et al., 2016). Furthermore, a prospective study of 2332 middle-aged and older men from eastern Finland suggested higher egg intake was associated with a lower risk of type 2 diabetes (Virtanen et al., 2015). Our results were consistent with the previous studies that dietary EY in both ND and HFD could not induce impaired glucose tolerance and insulin resistance as an independent factor.

Effect of egg yolk under ND and HFD on fatty acids profile in liver, serum and fecal samples

In order to further identify the differential effects of EY under ND and HFD on the lipid metabolism, we compared the fatty acids profiles of liver, serum and fecal samples on day 60 and 100 based on GC-MS. There was a total of 9, 15 and 19 kinds of fatty acids species detected in serum, liver and fecal samples, respectively (Table S4–6). Moreover, comparison of each group was performed using the PLS-DA model to reveal the changes of fatty acids. In Fig. 2, score plots indicated significant differences between the HFD and ND groups with good model quality (R2X of 96.1% in serum, R2X of 94.6% in liver and R2X of 96.6% in feces, respectively). In the score plot, the fatty acids profiles of serum, liver and feces of HFD + EY and HFD on day 100 were well differentiated, which indicated HFD + EY significantly affected the fatty acids metabolism. However, ND and ND + EYHDL were not well separated in PLS-DA model. In order to select the specific fatty acid contributor, we compared the variable importance in projection (VIP) in PLS-DA (Fig. S2). The boxes on the right indicate the relative concentrations of the corresponding metabolite in each group. Based on the criterion of $VIP > 1.5$ and $p < 0.05$, the most contributors were selected for the discrimination of all groups: C18:1 and MUFA in serum (Fig. S2A), MUFA, C18:1, PUFA and C18:2 in liver (Fig. S2B), and MUFA, C18:1, C16:0 and SFA in feces (Fig. S2C).

The changes of these potential fatty acid biomarkers can be further seen in Fig. 3. In serum, C18:1 and MUFA were markedly elevated in HFD fed mice (Fig. 3A) compared with ND fed mice, and there was no significant difference of C18:1 and MUFA between ND + EY and HFD + EY, which were markedly lower than HFD. It may be assumed that the reduction in plasma MUFA especially C18:1 could be a beneficial effect, since these fatty acids are considered to increase cardiovascular disease risk by increasing levels of LDL-cholesterol and TC (Kang et al., 2017). Analysis of single fatty acids species changes also showed that the SFA mainly C16:0, C18:0 and C21:0 in serum increased significantly in HFD compared with ND ($p < 0.01$), while EY intake significantly decreased the level of SFA in serum in both ND and HFD groups (Table S4). Notably, EY intake under ND and HFD did not alter the TG in serum while decreased significantly the level of the majority of SFA and MUFA, which further inhibited the cholesterol accumulation in serum. Previous studies found that SFA such as 14:0, 16:0 and MUFA serum lipids were correlated positively with total cholesterol/HDL-C ratio and high sensitivity C-reactive protein (hsCRP) (Kaska et al., 2014). On the other hand, the content of MUFA especially C18:1 in serum in men aged 30–49 years decreased under recommended of Japan diet (more fish, soybeans and soy products) for 6 weeks, a finding reported by other authors as an anti-atherosclerotic diet intervene (Shijo et al., 2019).

In liver samples, these four detected fatty acids biomarkers MUFA, C18:1, PUFA and C18:2 were markedly elevated in HFD groups (Fig. 3B). Of note, there was no markedly difference in these contributors between ND and ND + EY ($p > 0.05$), while C18:2 (39.99 ± 3.13 vs 55.27 ± 7.32) and PUFA (75.07 ± 6.97 vs 89.05 ± 12.06 $\mu\text{g/g}$ dry liver) decreased significantly in HFD + EY than HFD. Consistent with serum results, the higher level of SFA especially C16:0 and C18:0 induced by HFD was significantly decreased in HFD + EY, and also decreased in ND + EY compared with ND (Table S5). This was similar to results from other research in previous high fat fed mice. In previous studies, the increase in C18:0 and MUFA in mouse liver showed a significant positive correlation with the variation in corresponding liver inflammation and non-alcoholic fatty liver disease (Wang et al., 2016). Notably, the level of C18:0, an indicator for predicting advanced fatty livers (Murase et al., 2011), decreased significantly in HFD + EY than HFD. Moreover, these results are consistent with those of a study in humans (Cohen et al., 2011). Notably, C18:1, the major detected MUFA, involves in apoptosis and further leads to fatty liver diseases (Barreyro et al., 2007). No significant changes were observed in C18:1 between ND + EY and HFD + EY, consistent with TG and hepatic pathology, not leading to fatty liver. HFD + EY did not induce higher

Fig. 2 Partial least square discriminant analysis (PLS-DA) score plots of serum (A), liver (B) and fecal (C) samples. Each point represents an individual serum, liver and fecal sample from ND, ND + EY, HFD and HFD + EY

Fig. 3 Box plots of the time-dependent fatty acids biomarkers changes of serum (A), liver (B) and fecal (C) samples after EY consumption under ND and HFD. a, b, c stands for significant difference among groups (ANOVA, $p < 0.05$)

level of SFA, while decreased the level of TG and total cholesterol, which showed the less accumulation of cholesterol and TG was promoted by HFD + EY.

In feces, EY intake significantly decreased the levels of C16:0 and SFA under both ND and HFD on 100 day. The levels of C18:1 (421.72 ± 21.35 vs 650.19 ± 27.23 $\mu\text{g/g}$ dry feces) and MUFA (578.59 ± 29.42 vs 881.42 ± 32.92 $\mu\text{g/g}$ dry feces) in feces were significantly diminished in ND + EY than ND group, while markedly elevated in HFD + EY than HFD group. In feces, higher contents of MUFA were detected in HFD + EY, which indicates excess MUFA excretion was promoted by EY under HFD diet. It is unclear whether the higher level of MUFA in feces will cause beneficial effect in obese mice caused by HFD. We speculated that higher MUFA may beneficially modify the bacterial population in the intestine by increasing the populations of commensal bacteria (Patterson et al., 2014). The level of SFA had no markedly difference between ND + EY and HFD + EY, which was markedly lower than that in ND and HFD (Fig. 3C). Of note, PUFA especially C22:6, C20:3 and C18:3 tended to be significantly diminished in HFD ($p < 0.05$, Table S6),

which indicated HFD inhibited the secretion of PUFA. EY intake under ND and HFD inhibited the secretion of PUFA (87.77 ± 6.39 vs 111.93 ± 4.14 $\mu\text{g/g}$ dry feces; 78.02 ± 2.28 vs 90.61 ± 3.04 $\mu\text{g/g}$ dry feces, respectively) in feces in comparison with control groups.

Effect of egg yolk under ND and HFD on hepatic mRNA expressions associated with fatty acids metabolism

To investigate the molecular mechanism involved in fatty acids metabolism induced by EY intake in ND and HFD in mice, the expressions of hepatic genes were investigated. SREBP-1c is an important nuclear transcription factor that regulates the expression of fatty acids metabolism genes in liver. Studies have shown that overexpression of SREBP-1c may lead to disorders of lipid metabolism, causing lipid accumulation and fatty liver (Li et al., 2015). Its mRNA expression levels in the ND, ND + EY and HFD + EY mice were 2.32, 4.66 and 5.60-fold lower than in the HFD mice at the end of 100 days, respectively ($p < 0.01$; Fig. 4B). Acaca (acetyl-CoA carboxylase α) is an

Fig. 4 Changes of hepatic mRNA level of lipid-related metabolic enzymes after EY consumption under ND and HFD for 60 days (A) and 100 days (B). The graph represents the fold changes of the target gene expression compared with the internal control gene, GAPDH. Data are expressed as the mean $\pm$ SEM ($n = 10$). a, b, c stands for extremely significant difference among groups (ANOVA, $p < 0.05$)

important coenzyme in long chain fatty acid biosynthesis (Tao et al., 2015). The *Acaca* mRNA expression level in the ND + EY was 2.0-fold higher than in the ND at the end of 100 days, while they were 1.62-fold lower than in the HFD + EY than HFD ($p < 0.01$). *Elovl-6*, a target of SREBP-1c, catalyzes the chain elongation of palmitate to stearate and upregulates in obesity, hepatic steatosis and insulin resistance (Jump, 2008). Its mRNA expression levels in the HFD were 1.66-fold higher than in the ND, while they were 3.12-fold lower in HFD + EY than in the HFD at the end of 100 days. The levels of *Elovl-6* were not significantly altered between ND and ND + EY ($p > 0.05$). We speculate that the lower level of C18:0 in liver was ascribed to the lower expression of *Elovl6*, catalyzing palmitic acid (C16:0) into stearic acid (C18:0), and higher expression of *Scd1*, converting C18:0–C18:1 (Doria et al., 2014; Mason et al., 2012).

Dgat1 and *Dgat2*, known as specific enzymes that catalyze the final step of TAG synthesis (Jump, 2011). Their mRNA expression levels in the ND + EY were 1.26-fold and 3.97-fold higher than in the ND, respectively, while they were 2.80-fold and 2.02-fold lower in HFD + EY than in the HFD at the end of 100 days. Combined with lower TG and total cholesterol in HFD + EY, EY intake did attenuate higher TAG and cholesterol in liver caused by

HFD. We speculated this is related with the lower expression of *Dgat1* and *Dgat2* (Nakamura et al., 2014). *Fasn*, *GPAT* and *MTP* are the crucial rate-limiting genes in the fatty acid and TG biosynthesis pathway (Iqbal et al., 2014; Morgan-Bathke et al., 2016), its mRNA expression was the highest in the HFD, while the lowest in HFD + EY than in the other groups ($p < 0.01$). Notably, the hepatic *Cptla* mRNA, the rate-limiting enzyme of mitochondrial fatty acid oxidation in cells (Long et al., 2016), was induced by HFD to 17.75-fold of ND, and there were no significant differences in its mRNA expression levels in HFD and HFD + EY groups. In the meantime, the mRNA expression levels of *Fgf21* in the ND + EY, HFD and HFD + EY were 1.74-, 2.57- and 3.51-fold higher than in the ND at the end of 100 days, respectively ($p < 0.01$).

In summary, although numerous studies demonstrated beneficial effects of daily EY consumption, the association between EY consumption and risk of CVD still remain controversial and is challenging for researchers. The purpose of this study was to evaluate the overall effects of EY consumption on lipid especially fatty acids metabolism in mice fed with ND and HFD. Our results demonstrated that ND + EY did not induce higher lipid accumulation and fatty liver than ND, whereas HFD + EY further attenuates dyslipidemia by modifying the lipid profile in serum, liver

and feces, and thus decrease the risks related to CVD. Additionally, EY further regulated the biosynthesis of fatty acids by the down-regulation of SREBP-1c, Elovl-6 and Fasn. However, the mechanisms by which bioactive component in EY need to be further identified and characterized.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval The animal use protocol has been reviewed and approved by the Animal Ethical and Welfare Committee (AEWC) of Huazhong Agricultural University (No. HZAUMO-2016-045).

References

- Acosta JR, Douagi I, Andersson DP, Bäckdahl J, Rydén M, Arner P, Laurencikiene J. Increased fat cell size: a major phenotype of subcutaneous white adipose tissue in non-obese individuals with type 2 diabetes. *Diabetologia*. 59: 560–570 (2016)
- Andersen CJ, Lee JY, Blesso CN, Carr TP, Fernandez ML. Egg intake during carbohydrate restriction alters peripheral blood mononuclear cell inflammation and cholesterol homeostasis in metabolic syndrome. *Nutrients*. 6: 2650–2667 (2014)
- Anderson TJ, Mancini GB, Genest J, Jr., Gregoire J, Lonn EM, Hegele RA. The new dyslipidemia guidelines: what is the debate? *Can. J. Cardiol*. 31: 605–612 (2015)
- Anton M. Egg yolk: structures, functionalities and processes. *J. Sci. Food Agric*. 93: 2871–2880 (2013)
- Barreiro FJ, Kobayashi S, Bronk SF, Werneburg NW, Malhi H, Gores GJ. Transcriptional regulation of Bim by FoxO3A mediates hepatocyte lipoapoptosis. *J. Biol. Chem*. 282: 27141–27154 (2007)
- Blesso CN, Andersen CJ, Barona J, Volek JS, Fernandez ML. Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in individuals with metabolic syndrome. *Metabolism*. 62: 400–410 (2013a)
- Blesso CN, Andersen CJ, Bolling BW, Fernandez ML. Egg intake improves carotenoid status by increasing plasma HDL cholesterol in adults with metabolic syndrome. *Food Funct*. 4: 213–221 (2013b)
- Blesso CN, Fernandez ML. Dietary cholesterol, serum lipids, and heart disease: are eggs working for or against you? *Nutrients*. 10: 426 (2018)
- Camargo A, Meneses ME, Perez-Martinez P, Delgado-Lista J, Rangel-Zuniga OA, Marin C, Almaden Y, Yubero-Serrano EM, Gonzalez-Guardia L, Fuentes F, Tinahones FJ, Roche HM, Malagon MM, Perez-Jimenez F, Lopez-Miranda J. Dietary fat modifies lipid metabolism in the adipose tissue of metabolic syndrome patients. *Genes Nutr*. 9: 409 (2014)
- Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science*. 332: 1519–1523 (2011)
- Doria ML, Ribeiro AS, Wang J, Cotrim CZ, Domingues P, Williams C, Domingues MR, Helguero LA. Fatty acid and phospholipid biosynthetic pathways are regulated throughout mammary epithelial cell differentiation and correlate to breast cancer survival. *FASEB J*. 28: 4247–4264 (2014)
- Eftekhari S, Parsaei H, Keshavarzi Z, Yazdi AT, Hadjzadeh MAR, Rajabzadeh A, Malayeri SO. The prevention and treatment effects of egg yolk high density lipoprotein on the formation of atherosclerosis plaque in rabbits. *Iran J. Basic Med. Sci*. 18: 343 (2015)
- Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 226: 497–509 (1957)
- Iqbal J, Boutjdir M, Rudel LL, Hussain MM. Intestine-specific MTP and global ACAT2 deficiency lowers acute cholesterol absorption with chylomicrons and HDLs. *J. Lipid Res*. 55: 2261–75 (2014)
- Jump DB. N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. *Curr Opin Lipidol*. 19: 242–247 (2008)
- Jump DB. Fatty acid regulation of hepatic lipid metabolism. *Curr Opin. Clin. Nutr. Metab. Care*. 14: 115–20 (2011)
- Kang M, Lee A, Yoo HJ, Kim M, Kim M, Shin DY, Lee JH. Association between increased visceral fat area and alterations in plasma fatty acid profile in overweight subjects: a cross-sectional study. *Lipids Health Dis*. 16: 248 (2017)
- Kaska L, Mika A, Stepnowski P, Proczko M, Ratnicki-Sklucki K, Sledzinski T, Goyke E, Swierczynski J. The relationship between specific Fatty acids of serum lipids and serum high sensitivity C-reactive protein levels in morbidly obese women. *Cell Physiol. Biochem*. 34: 1101–1108 (2014)
- Laforest S, Labrecque J, Michaud A, Cianflone K, Tchernof A. Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction. *Crit. Rev. Clin. Lab. Sci*. 52: 301–13 (2015)
- Lemos BS, Medina-Vera I, Blesso CN, Fernandez ML. Intake of 3 Eggs per day when compared to a choline bitartrate supplement, downregulates cholesterol synthesis without changing the LDL/HDL ratio. *Nutrients*. 10: 258 (2018)
- Li X, Huang W, Gu J, Du X, Lei L, Yuan X, Sun G, Wang Z, Li X, Liu G. SREBP-1c overactivates ROS-mediated hepatic NF-kappaB inflammatory pathway in dairy cows with fatty liver. *Cell Signal*. 27: 2099–2109 (2015)
- Long B, Muhamad R, Yan G, Yu J, Fan Q, Wang Z, Li X, Purnomoadi A, Achmadi J, Yan X. Quantitative proteomics analysis reveals glutamine deprivation activates fatty acid beta-oxidation pathway in HepG2 cells. *Amino Acids*. 48: 1297–1307 (2016)
- Mason P, Liang B, Li L, Fremgen T, Murphy E, Quinn A, Madden SL, Biemann HP, Wang B, Cohen A, Komarnitsky S, Jancsics K, Hirth B, Cooper CG, Lee E, Wilson S, Krumbholz R, Schmid S, Xiang Y, Booker M, Lillie J, Carter K. SCD1 inhibition causes cancer cell death by depleting mono-unsaturated fatty acids. *PLoS One*. 7: e33823 (2012)
- Missimer A, DiMarco DM, Andersen CJ, Murillo AG, Vergara-Jimenez M, Fernandez ML. Consuming two eggs per day, as compared to an oatmeal breakfast, decreases plasma ghrelin while maintaining the LDL/HDL ratio. *Nutrients*. 9: 89 (2017)
- Morgan-Bathke M, Chen L, Oberschneider E, Harteneck D, Jensen MD. More insights into a human adipose tissue GPAT activity assay. *Adipocyte*. 5: 93–96 (2016)
- Murase T, Misawa K, Minegishi Y, Aoki M, Ominami H, Suzuki Y, Shibuya Y, Hase T. Coffee polyphenols suppress diet-induced body fat accumulation by downregulating SREBP-1c and related molecules in C57BL/6J mice. *Am. J. Physiol. Endocrinol. Metab*. 300: E122–E133 (2011)

- Nakamura MT, Yudell BE, Loor JJ. Regulation of energy metabolism by long-chain fatty acids. *Prog. Lipids Res.* 53: 124–44 (2014)
- Nissen SE. U.S. dietary guidelines: an evidence-free zone. *Ann. Intern. Med.* 164: 558–559 (2016)
- Palacios LE, Wang T. Egg-yolk lipid fractionation and lecithin characterization. *J. Am. Oil Chem. Soc.* 82: 571–578 (2005)
- Patterson E, O'Doherty RM, Murphy EF, Wall R, O'Sullivan O, Nilaweera K, Fitzgerald GF, Cotter PD, Ross RP, Stanton C. Impact of dietary fatty acids on metabolic activity and host intestinal microbiota composition in C57BL/6J mice. *Br. J. Nutr.* 111: 1905–1917 (2014)
- Ratliff JC, Mutungi G, Puglisi MJ, Volek JS, Fernandez ML. Eggs modulate the inflammatory response to carbohydrate restricted diets in overweight men. *Nutr. Metab. (Lond.)* 5: 6 (2008)
- Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J.* 22: 659–661 (2008)
- Richard C, Cristall L, Fleming E, Lewis ED, Ricupero M, Jacobs RL, Field CJ. Impact of egg consumption on cardiovascular risk factors in individuals with type 2 diabetes and at risk for developing diabetes: a systematic review of randomized nutritional intervention studies. *Can. J. Diabetes.* 41: 453–463 (2017)
- Shijo Y, Maruyama C, Nakamura E, Nakano R, Shima M, Mae A, Okabe Y, Park S, Kameyama N, Hirai S. Japan diet intake changes serum phospholipid fatty acid compositions in middle-aged men: a pilot study. *J. Atheroscler. Thromb.* 26: 3–13 (2019)
- Tao H, Chang G, Xu T, Zhao H, Zhang K, Shen X. Feeding a high concentrate diet down-regulates expression of ACACA, LPL and SCD and modifies milk composition in lactating goats. *PLoS One.* 10: e0130525 (2015)
- Taskinen MR, Boren J. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. *Atherosclerosis.* 239: 483–495 (2015)
- van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. *Cancer Epidemiol. Biomark. Prev.* 18: 2569–2578 (2009)
- Virtanen JK, Mursu J, Tuomainen TP, Virtanen HE, Voutilainen S. Egg consumption and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am. J. Clin. Nutr.* 101: 1088–1096 (2015)
- Wallin A, Forouhi NG, Wolk A, Larsson SC. Egg consumption and risk of type 2 diabetes: a prospective study and dose-response meta-analysis. *Diabetologia.* 59: 1204–1213 (2016)
- Wang XH, Li CY, Muhammad I, Zhang XY. Fatty acid composition in serum correlates with that in the liver and non-alcoholic fatty liver disease activity scores in mice fed a high-fat diet. *Environ. Toxicol. Pharmacol.* 44: 140–150 (2016)
- Yang F, Chen G, Ma M, Qiu N, Zhu L, Li J. Fatty acids modulate the expression levels of key proteins for cholesterol absorption in Caco-2 monolayer. *Lipids Health Dis.* 17: 32 (2018)
- Yang F, Ma M, Xu J, Yu X, Qiu N. An egg-enriched diet attenuates plasma lipids and mediates cholesterol metabolism of high-cholesterol fed rats. *Lipids.* 47: 269–77 (2012)
- Zhang Q, Xiao X, Li M, Li W, Yu M, Zhang H, Zheng J. Berberine moderates glucose metabolism through the GnRH-GLP-1 and MAPK pathways in the intestine. *BMC Complement Altern. Med.* 14: 188 (2014)

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