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Carrageenan in meat: improvement in lipid metabolism due to Sirtuin1-mediated fatty acid oxidation and inhibited lipid bioavailability†

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Kappa-carrageenan (κ-CGN) is widely used in the meat industry. However, its impact on the host metabolism is less revealed. The current study investigated the effect of κ-CGN in pork-based diets on the lipid metabolism of male C57BL/6J mice. The κ-CGN supplement significantly suppressed the increase in body weight by 6.79 g on an average. Supplement of κ-CGN in high-fat diets significantly upregulated the genes and protein expression of Sirtuin1, which was accompanied by the increased gene expression of downstream fatty acids oxidation (*Cpt1a* and *Acadl*). The sirtuin1-mediated improvement of lipid metabolism was negatively associated with the levels of bile acids, especially for deoxycholic acid, 3β-cholic acid, glycodeoxycholic acid and glycolithocholic acid. Moreover, κ-CGN in high-fat diets inhibited lipid digestion and absorption, being associated with the decrease in lipid accumulation and improved serum lipid profile. These results highlighted the role of κ-CGN in alleviating diet-induced adiposity by promoting energy expenditure and suppressing the bioavailability of ingested lipids.

1 Introduction

Carrageenan (CGN) refers to a class of polysaccharides and soluble dietary fiber with high viscosity, which are mainly extracted from certain species of red algae.[1] κ-CGN is commonly used in the food industry, especially in meat processing,[2–4] due to its good thickening, gelling and stabilizing properties.[4] Recently, the high consumption of meat was shown to be associated with metabolic disease,[5,6] largely due to the high fat content in meat products. Traditional meat products usually contain 20% to over 30% fat to ensure a desirable texture and flavor.[2] The caloric density of fat is usually 2.25 higher than that of carbohydrate.[7] Consequently, overeating those meat products may trigger a positive energy balance and weight gain, leading to adiposity and finally causing lipid metabolic disorders such as nonalcoholic fatty liver disease and dyslipidemia.[8] Both academia and meat industry are finding proper methods to reduce the adverse effects of high fat contents in meat products.

As an important additive and fat analogue in the meat industry, the effect and mechanism of the κ-CGN supplement in meat-based diets on energy metabolism have not been studied in detail, but should be evaluated. κ-CGN was confirmed to improve the energy metabolism, whereas conflicting conclusions of κ-CGN on metabolic health were found in several studies.[9–11] Chin *et al.* and Wang *et al.* reported on the improving effect in glucose and lipid metabolism by κ-CGN in the high-fat diets. However, Zhou *et al.* discovered that the mice consuming standard diets developed glucose intolerance and insulin resistance by κ-CGN.[9–11] In addition, dietary κ-CGN was shown to inhibit lipid accumulation *via* different mechanisms, and seemed to depend on the dietary components.[7–9] Because of the low bioavailability and low fermentability of κ-CGN,[1,12] its digestion and absorption behavior in the upper gastrointestinal tract should also be very critical to the metabolism of the hosts. Some studies have shown that the food matrix may affect the bioavailability of macronutrients.[13,14] Moreover, the well-known protein-binding properties of κ-CGN[1] indicate the great potential of κ-CGN interacting with the food matrix and digestive fluid. However, it was neglected in the aforementioned studies. Bile acids (BAs) are essential for the digestion and absorption of lipids and lipophilic compounds, which can also regulate the metabolism through the interactions with their receptors, such as farnesoid X receptor (FXR).[15] As a key factor in regulating the energy metabolism, the activity of Sirtuin1 (SIRT1) is also affected by FXR in the liver.[16] Viscous polymers including CGN are well-known in blocking their motions.[17] Sokolova *et al.* proved that κ-CGN dose-dependently inhibited bile acid per-

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meation through the artificial phospholipid membranes. Therefore, it was presumed that BAs are key in glycolipid metabolism regulated by κ-CGN.

To verify these hypotheses, the dosage-dependent role of κ-CGN addition in pork-based diets in regulating the lipid metabolism of male C57BL/6J mice was investigated in this work. Especially, the role of BAs in lipid metabolism and related hepatic factors was analyzed. In addition, lipolysis affected by κ-CGN was investigated in *in vitro* digestion. This work could provide new insight into the possible mechanism of high-viscous non-fermentable soluble dietary fiber improving the lipid metabolism.

2 Materials and methods

2.1 Structure and molecular weight determination of κ-CGN

κ-CGN (Beijing Solarbio Science & Technology Co., Ltd, Cat No. C8833) with the properties of 50 mPa s and 1543 g cm^{-2} (20 °C, 1.5% in water) was employed. The structural characteristics of κ-CGN were measured as follows:

Nuclear magnetic resonance (NMR): κ-CGN (90 mg) were dissolved in 2 mL 80 °C D_2O, followed by the ^1H and ^{13}C NMR determination (accumulation times >1100) at 85 °C with a Bruker Ascend iii 400 MHz NMR spectrometer (Bruker Corporation, Germany) with a 400 MHz 5 mm broad band probe (iProbe) for structure analysis.

2.1.1. Gel permeation chromatography (GPC). The molecular weight was analyzed using high-performance GPC with an Agilent Technologies 1260 HPLC machine (Agilent Technologies, CA, USA), WYATT DAWN 8^+ light scattering detector (Wyatt Technology Corporation, CA, USA) and Shodex OHpak SB-805 HQ gel column. The mobile phase was 0.1 M $NaNO_3$ water solution, with a column temperature of 40 °C and a flow rate of 0.5 mL min^{-1}.

2.2 Diets and animals

A total of 72 four-week-old male mice purchased from Gempharmatech Co., Ltd were randomly divided into six groups (n = 12 in each group, 2 per cage) *ad libitum* to diets and water, in a specific pathogen-free animal facility (SYXK < Jiangsu > 2011-0037; temperature, 20 ± 2 °C; relative humidity, 45–60%; light cycle, 12 h/12 h). After one-week adaption, the mice are supplied with six different diets for 90 days, as shown in Table S1.† The diets and groups were named low-fat-carrageenan-free (LFNC), low-fat-low-carrageenan (LFLC), low-fat-high-carrageenan (LFHC), high-fat-carrageenan-free (HFNC), high-fat-low-carrageenan (HFLC) and high-fat-high-carrageenan (HFHC). κ-CGN was added by replacing the equal mass of cellulose to avoid the changes in energy density. The κ-CGN proportion was designed based on the common content (0.2%–2%) in meat processing.[18] The LFNC diet was formulated in reference to the AIN-93G standard. Diets with 45% calories from fat were adopted for high-fat groups, as the induction of obesity is more similar in humans.[19] Degreased and dehydrated pork gluteus muscle prepared with a previous method was selected as the dietary protein source.[20] Body weight and feed consumption were recorded every 3 days. All animal procedures were performed under the Guidelines of Care and Use of Laboratory Animals of Nanjing Agricultural University, and approved by the Animal Ethics Committee of Experimental Animal Center of Nanjing Agricultural University.

2.3 Oral glucose tolerance test (OGTT)

OGTT was conducted a week before sacrifice. Mice were given glucose (2 g per kg body weight) by oral gavage after a 16-hour-fasting. Blood was collected from the tail at 0, 30, 60, 90 and 120 min after the gavage, and the blood glucose was measured with a blood glucose meter (Sinocare Biosensing Co., Ltd, China).

2.4 Sample collection and recording

All mice (72 in total) were sacrificed after the dietary intervention immediately. Serums were collected by centrifuging blood at 3000 rpm and 4 °C for 30 min. After recording the intraabdominal morphology by photographing, the liver, epididymal white adipose tissue (EWAT), perirenal white adipose tissue (PWAT), subscapular brown adipose tissue (BAT) and intestinal content were weighed and stored at −80 °C.

2.5 Observation of histological sections

Hepatic and epididymal adipose tissues were fixed in 4% paraformaldehyde for a week, and embedded in paraffin wax for hematoxylin–eosin staining. Frozen hepatic tissue sections of 10 μm thickness were stained with oil red. Lipid droplets of epididymal adipose tissue were counted with AdipoCount software under the fields with equal size.

2.6 Sera lipid profile analyses

Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and total triglyceride (TG) were determined using commercial biochemical kits (Nanjing Jiancheng Bioengineering Institute Co., Ltd, Nanjing, China) following the instruction.

2.7 RNA extraction and real-time qPCR (RT-qPCR)

Total RNA extraction was implemented using the MiniBEST Universal RNA extraction kit (TaKaRa, Kusatsu, Shiga, Japan), and cDNA was synthesized from mRNA using the PrimeScript RT master mix (TaKaRa). RT-qPCR was performed in a QuantStudio 6 flex real-time PCR system (Applied Biosystems, Waltham, MA) with TB Green® Premix Ex Taq™ (Tli RNaseH Plus) kit (Takara, Japan). All operation steps were carried out in compliance with the instructions. The $2^{-\Delta\Delta Ct}$ method was chosen to analyze the relative mRNA expression with the *Gapdh* as a reference gene. All tested genes and their primers are listed in Table S2.†

2.8 Hepatic protein extraction and western blot

The protein was extracted with RIPA lysis buffer (strong) containing 1% (v/v) protease inhibitor cocktails (MCE) at a ratio of

1 : 10 (w/v) by homogenization (0–10 °C), and collected from supernatants by centrifugation (11 492*g*, 4 °C, 5 min). After the protein was quantified by BCA Protein Assay Kit (Thermo Fisher Scientific, Shanghai, China), 30 μg protein samples were subjected to 12% SDS-PAGE gel and transferred to 0.45 μm PVDF membrane. After the sealing (5% skim milk solution for 1 h) and incubation (12 h at 4 °C in the primary antibody diluents, β-actin and Sirt1, 1 : 3000; Cell Signaling Technology, Inc., Shanghai, China), the membrane was washed with 1× TBST buffer, followed by the incubation at room temperature for 1 h in the secondary antibody diluent. The protein expression was visualized by the chemiluminescence (PerkinElmer Co., Ltd, USA) method in a chemical imaging system (Image Quant LAS4000, General Electric Company), and quantified using Quantity One software (v4.6.2, Bio-Rad). β-Actin was used as the reference.

2.9 BAs extraction and analysis

BAs from the colonic content (50 mg) were extracted by vortexing, homogenization, ultrasound (30 min, room temperature) and centrifugation (12 000 rpm, 4 °C, 10 min) in 400 μL methanol (−20 °C). Then, 100 μL dilutions of the supernatant (1 : 2, v/v in deionized water) was added to 900 μL 30% methanol. A Shimadzu LC-30 liquid chromatography system coupled with an AB Sciex 6500 quadrupole time-of-flight mass spectrometer and Turbo Ionspray electrospray ionization source was used for the analysis of BAs with a previously described method.[21] All targeted BAs are listed in Table S3.†

2.10 Short-chain fatty acids (SCFAs) extraction and analysis

Cecal contents were dissolved in deionized water (1 : 5, w/v) by Precellys Evolution Super Homogenizer (Bertin Technologies, France) and centrifuged (13 500*g*, 10 min, 4 °C). Then, metaphosphoric acid (250 g L^{-1}) with 6.464 g L^{-1} crotonic acid (internal standard) was added to the supernatant (1 : 15, v/v). After standing overnight at −20 °C, the supernatant obtained by centrifugation (1200 rpm, 5 min, 4 °C) and filtration (0.22 μm membrane) was mixed with 200 μL diethyl ether. After lamination, 50 μL of the upper liquid was injected into a gas chromatography system (GC-2010 Plus Shimadzu, Japan) with an HP-INNO Wax capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, CA), as described previously.[22]

2.11. Static *in vitro* digestion

Diets from HFNC, HFLC and HFHC groups were subjected to static *in vitro* digestion according to INFOGEST 1.0.[23] Briefly, the ground diet (5 g) was digested with bovine bile (Beijing Solarbio Science & Technology Co., Ltd, Cat No. B8210), porcine pepsin (Sigma-Aldrich, Cat. no. P7012), and porcine pancreatin (Sigma-Aldrich, Cat. no. P7545) in a 100 mL screwed glass bottle approaching 40 mL during intestinal phase. Lipolysis and proteolysis were stopped by 5 μL mL^{-1} of 1 M 4-bromophenylboronic acid in methanol and 50 μL mL^{-1} of 0.1 M AEBSF in water, respectively. Later, the upper liquid and lower precipitate were obtained by standing for 30 min in a 50 ml vial at 37 °C.

2.12 Laser confocal microscopy (LSCM) observation

For the observation of lipid and protein, 25 μL Nile red (1 mg mL^{-1} in ethanol) and 20 μL Nile Blue (1 mg mL^{-1} in water) were vortexed with 1 mL of the mixture, precipitate, and upper layer liquid of the digesta. Then, 60 μL samples were observed on glass microslides by an LSCM (TCS SP8, Leica, Wetzlar, Germany) with 10× objective. Lasers with excitation wavelengths of 543 nm and a 633 nm were applied to excite the Nile Red and Nile Blue dyes, respectively.

2.13 Lipid extraction and thin layer chromatography (TLC)

Lipids from the ileal contents were digested with Folch's method and dissolved in chloroform (1 : 25, w/v).[24] Then, 10 μL sample was developed on the silica gel G plate with petroleum ether–diethyl ether–acetic acid (70 : 30 : 1) as the developing solvent. The lipids blots were photographed by a Gel Doc XR + Gel Documentation System (Bio-Rad Laboratories, Inc., CA, USA) after the coloration by iodine vapor.

2.14 Statistical analysis

Data were expressed as means ± standard error, and evaluated using two-way ANOVA, one-way ANOVA (feed intake and expression of Sirt1 protein level) and Spearman correlation analyses. The least-square means were compared by Tukey's *t*-test. A value of $p < 0.05$ was considered to indicate statistically significant differences between groups.

3. Results

3.1 Structural determination of κ-CGN

κ-Type was confirmed to be the predominant component in our CGN sample with the fraction of ι-type by ^{1}H NMR (Fig. S1A†) and ^{13}C NMR (Fig. S1B†) spectra, according to the previous results.[25,26] The average molecular weight (M_w) was 5.703×10^5 g mol^{-1} (±1.290%), with an average polydispersity index of M_w/M_n = 1.308 (Table S4†). Those features were matched with to the commercial products.

3.2 κ-CGN and whole body and visceral weight gain in high-fat diets

After 90 days of intervention, κ-CGN significantly inhibited the weight gain of mice fed with high-fat diets (Fig. 1A and B, $p < 0.05$). The heaviest weight occurred in the HFNC mice, followed by those in the HFLC and HFHC groups. The mice fed with the low-fat diets had lighter weights. Additionally, the variations in the weights of liver, visceral fat and subscapular BAT (Fig. 1C) and the intraabdominal morphologies (Fig. S2†) corresponded to those of the body weight among the groups (Fig. 1B). The above results indicated that the body weight loss was attributed to the reduction of lipid accumulation led by κ-CGN.

3.3 κ-CGN improved lipid metabolism in high-fat groups

κ-CGN in high-fat diets decreased the lipid accumulation in the liver and the extent of lipid droplets in epididymal adipo-

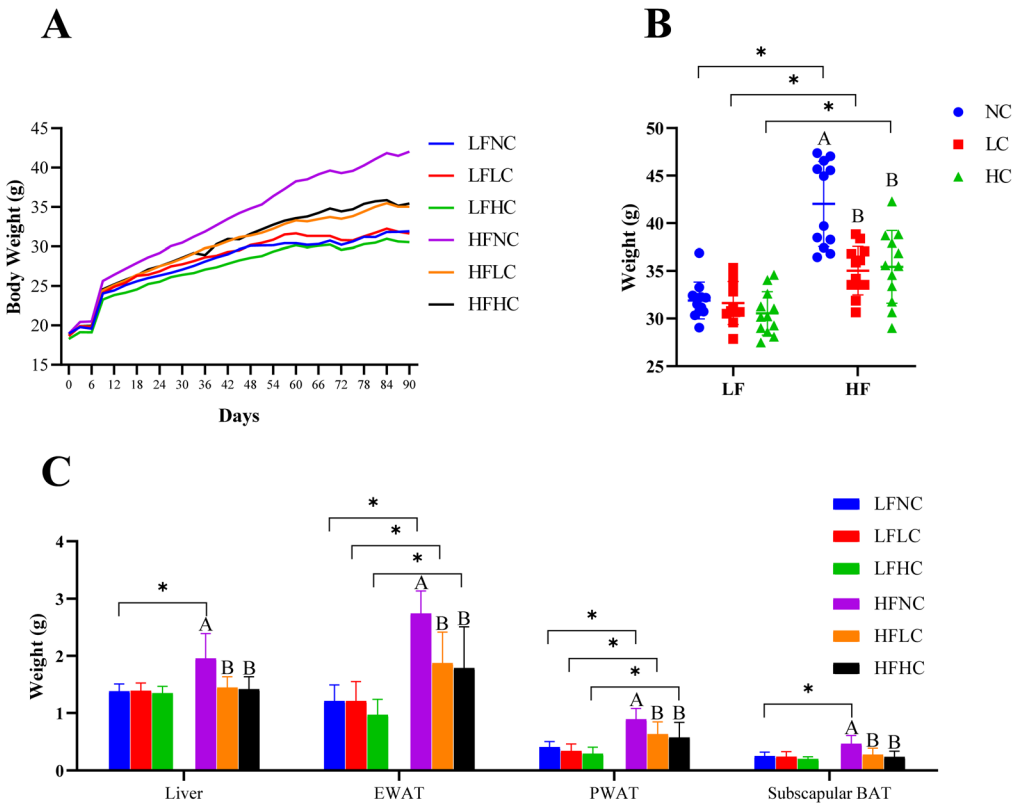

Fig. 1 The effects of diet on the body and visceral weight of C57BL/6J mice. (A) Body weights during the dietary intervention. (B) Final body weights. (C) Final liver and fat weights. Asterisks indicate significant differences between the low-fat diet groups and high-fat diet groups ($p < 0.05$); a and b indicate significant differences among the low-fat diet groups ($p < 0.05$); A and B indicate significant differences among the high-fat diet groups ($p < 0.05$).

cytes (Fig. 2A). The epididymal lipid droplets numbers (Fig. 2B) were significantly increased ($p < 0.05$) by κ-CGN in both high- and low-fat diets, indicating the remarkable effect of κ-CGN in regulating the lipid metabolism.

In serum, the HFNC diet led to a significant rise in TC, TG, LDL-C and HDL-C levels compared with the LFNC diets ($p < 0.05$, Fig. 2C and D). The levels of TC, HDL-C and LDL-C were dose-dependently reduced ($p < 0.05$) among groups, whereas the TG content was decreased ($p < 0.05$) merely in high-fat groups after the κ-CGN supplementation (Fig. 2C and D).

Mice fed with κ-CGN from high-fat diets did not exhibit any decrease in the blood glucose levels in OGTT. However, there was a significant decrease in their low-fat counterparts ($p < 0.05$, Fig. S3†). These results display the remarkable effects of κ-CGN on the *in vivo* glycolipid metabolism homeostasis in diets and improvement of lipid accumulation in high-fat diets.

3.4 κ-CGN enhanced fatty acid oxidation in liver

The dose-dependent decrease of the HDL-C and LDL-C levels in serum by κ-CGN indicated that the variation of lipid metabolism stemmed from the liver.[27] Therefore, the mRNA and protein expression regulating the lipid metabolism in the liver was determined. The β-oxidation mRNA levels of *Acadl* and *Cpt1a* (Fig. 3A) were significantly higher in the HFLC and HFHC groups than those in the HFNC group ($p < 0.05$).[28,29] Their upstream regulator Pparα mRNA expression (Fig. 3A) was merely significantly upregulated in HFHC (*versus* the HFNC group, $p < 0.05$). However, the expression of some Sirtuin family genes (*Sirt1*, *Sirt3* and *Sirt6*, Fig. 3A) was significantly upregulated. In particular, *Sirt1* (Fig. 3A) was significantly upregulated in high and low-fat groups with κ-CGN intervention. Furthermore, the protein levels were also significantly increased (Fig. 3C). As Sirtuin1 is a NADH-dependent regulator, the expression of the gene (*mt-Nd1*) predicted to enable NADH dehydrogenase[30] was investigated, and the results were correlated to the *Sirt1* mRNA expression. Considering the interplay between *Sirt1* and BAs,[31–34] the expression of *Cyp7a1* was measured as it encodes a key enzyme in the neutral BAs biosynthesis pathway.[15] The results (Fig. S4A†) revealed that κ-CGN significantly up-regulated *Cyp7a1* expression transcriptionally in high-fat diets, and slightly increased the mRNA levels in low-fat diets (no statistical significance).

All of the determined lipogenic genes in the HFLC and HFHC groups (Fig. 3D and Fig. S4B–F†) did not show any significant down-regulation compared with those in the HFNC group. However, *Xbp1* and *Cebpb* (Fig. 3D), which were reported to induce hepatic steatosis,[35–37] increased in the HFLC and HFHC groups. On the contrary, κ-CGN in low-fat diets signifi-

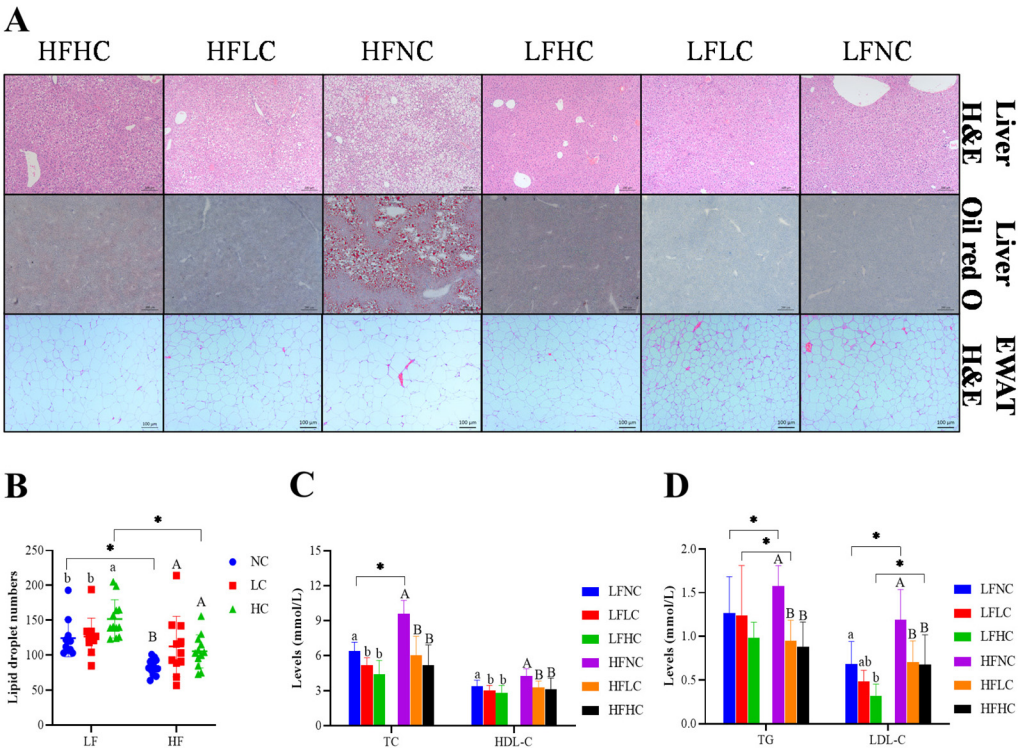

Fig. 2 κ-CGN reduced the lipid accumulation and serum lipid content. (A) Hepatic and EWAT histological sections. (B) Numbers of epididymal lipid droplets. (C and D) Contents of the serum TC, HDL-C, TG and LDL-C. Asterisks indicate significant differences between the low-fat diet groups and high-fat diet groups ($p < 0.05$); a and b indicate significant differences among the low-fat diet groups ($p < 0.05$); A and B indicate significant differences among the high-fat diet groups ($p < 0.05$).

cantly inhibited the mRNA expression of lipogenic genes, including *Fasn*, *Acaca*, *Dgat2*, *Elovl6*, *Mgat1* and *Cebpb* (Fig. 3D).

3.5 BAs and SCFAs concentrations were negatively associated with improved lipid metabolism

Because of the low incidence of κ-CGN crossing the intestinal barrier,[1] the interaction between *Sirt1* and BAs[31–34] and the significantly enhanced *Cyp7a1* expression by κ-CGN in high-fat diets (Fig. S4A†), the BAs in the colonic contents were measured. κ-CGN intervention dose-dependently reduced the BAs contents (Fig. 4A), except for NorDCA, 12-ketoLCA and UCA (Fig. S5†). In the high-fat diet groups, the intervention of κ-CGN reduced the content of bile acids by half on average.

The heatmap consisting of individuals with high-fat diets (Fig. 4B) implied that the rise of deoxycholic acid (DCA), 3β-cholic acid (βCA), hyodeoxycholic acid (HDCA), lithocholic acid (LCA) and several conjugated BAs was correlated with elevated LDL-C, TG and TC levels in the serums and more adipose weight. Furthermore, most of the BAs level were negatively associated with the hepatic *Sirt1* mRNA level in the high-fat diet groups (Fig. 4C). Notably, strong negative correlations occurred (correlation coefficient >0.6) in the relation of α-muricholic acid (α-MCA), glycodeoxycholic acid (GDCA) and taurocholic acid (TCA) with *Sirt1* expression.

SCFAs are critical substances in energy metabolism and usually transferred from undigested carbohydrates in large intestinal luminal by microbiota. These compounds has already been proved to profoundly regulate lipolysis, satiety, gluconeogenesis and fat accumulation.[38] After the dietary intervention, the acetate (Fig. 5A) and butyrate (Fig. 5B) levels were significantly elevated in the HFNC group compared to those in the LFNC group. Meanwhile, the contents of most SCFAs (acetate, isobutyrate, butyrate, 2-methylbutyrate and valerate, Fig. 5A–C) were significantly decreased by the κ-CGN supplement in high-fat diets ($p < 0.05$). Moreover, the concentrations of SCFAs were positively correlated to the lipid deposition (Fig. 5D). Positively strong correlations (correlation coefficient >0.6) appeared between certain SCFAs (isobutyrate and valerate) levels and EWAT weight, as well as the isobutyrate level and serum profiles (TC, TG and HDL-C). However, all SCFAs levels were negatively associated with the hepatic *Sirt1* mRNA level. These results suggested a potentially diminished supply of SCFAs as an energy source by κ-CGN.

3.6 κ-CGN impeded bioavailability of lipids in high-fat diets

Because low energy level was reported to up-regulate Sirtuin1,[39,40] we assumed that κ-CGN possibly changed the BAs and SCFAs distribution through the impeded digestion and absorption of macronutrients in the upper gastrointestinal tracts. Thus, the diet intake and lipids from the ileal contents were analyzed. Interestingly, κ-CGN lowered the total feed intake in high-fat diets, albeit not statistically significantly

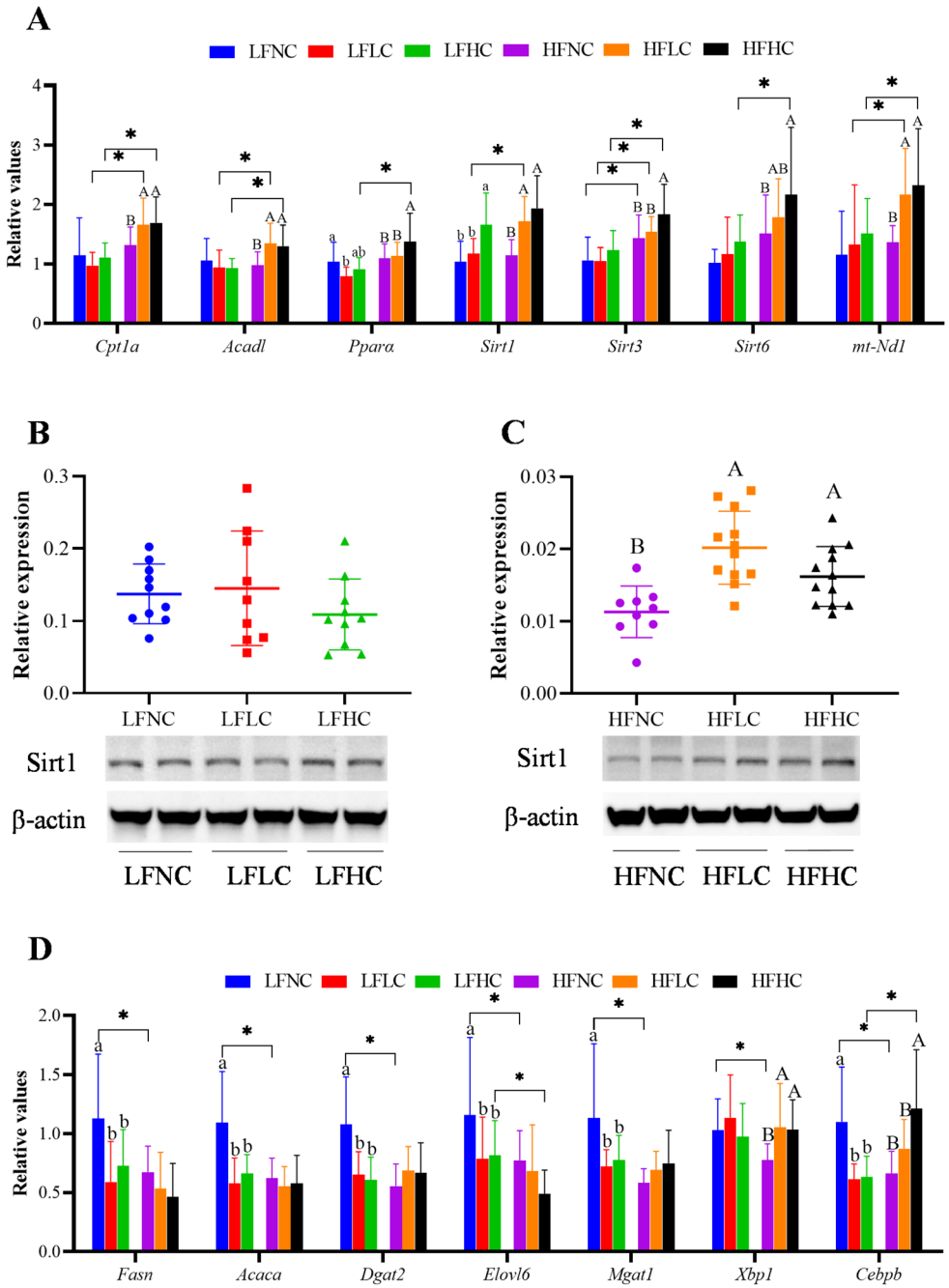

Fig. 3 κ-CGN enhanced hepatic fatty acid oxidation. (A) mRNA expressions of β-oxidation-promoting and mitochondrial genes relative to the LFNC group. (B and C) Hepatic Sirt1 protein levels. (D) mRNA expressions of the lipid synthesis genes relative to the LFNC group. Asterisks indicate significant differences between the low-fat and high-fat diet groups ($p < 0.05$); a and b indicate significant differences among the low-fat diet groups ($p < 0.05$); A and B indicate significant differences among the high-fat diet groups ($p < 0.05$).

(Fig. 6A and B). The dietary consumptions in the first 15 days were significantly reduced by κ-CGN in the high-fat diets ($p < 0.05$, Fig. 6C), as the dietary lipids are the key factors inducing obesity and hepatic steatosis[8] and most of the lipids were absorbed in jejunum.[7] The lipids from ileal contents were extracted and analyzed by TLC. The results revealed that κ-CGN in high-fat diets decreased the proportion of triglycerides (TAG), while increasing the proportion of free fatty acids (FFA) (Fig. S6†). Those results implied the ability of κ-CGN in regulating lipids digestion and absorption in diets.

High-fat diets were subjected to static *in vitro* digestion for further exploration. The blots on the TLC plates are triglycerides, free fatty acids, diglycerides (DAG), monoglycerides (MAG), and phospholipids (PL) from top to bottom (Fig. 6D). The TLC results of *in vitro* lipids digestion indicated that lipolysis was suppressed by κ-CGN because of the shrunk TAG and

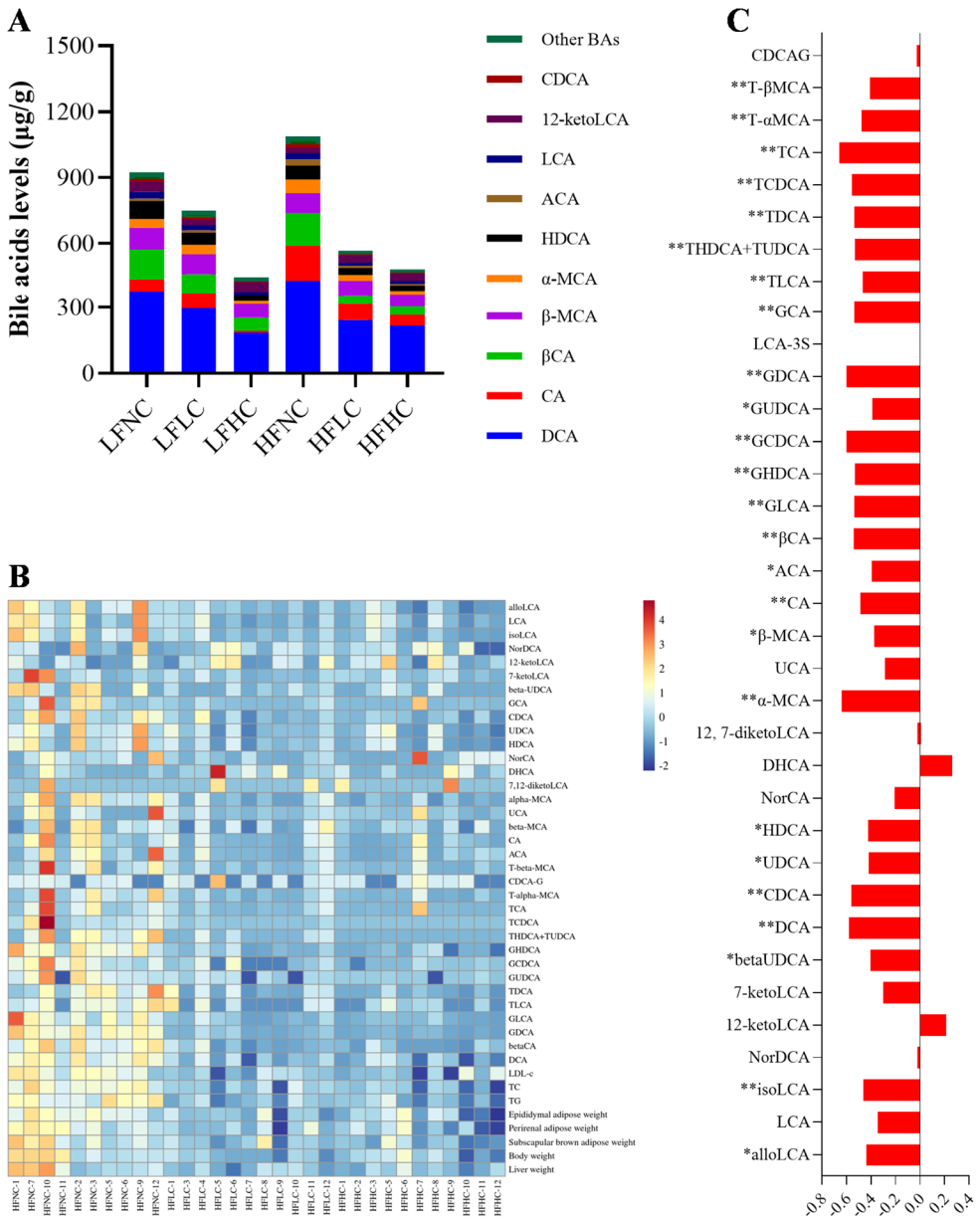

Fig. 4 The reduction of the BAs concentration was accompanied by the alleviated lipid metabolism. (A) BAs distribution. (B) The relationship between the BAs levels and lipid metabolism in the high-fat diet groups displayed by a heatmap with the statistics modified by the Z-score method. (C) The correlation coefficient between the BAs concentration and *Sirt1* mRNA expression in the high-fat diet groups. * and ** indicate the significance of the correlation ($p < 0.05$ and $p < 0.01$, respectively).

enlarged FFA blots led by the existence of κ-CGN on the plates (Fig. 6D). Interestingly, despite the similar lipid composition in the upper layer liquid among the diets exhibited by TLC, the larger and more abundant lipid droplets were observed from HFLC and HFHC digesta by LSCM (Fig. 6D and Fig. S7–S12†).

4. Discussion

Our earlier studies proved that excessive fat content in meat is the main cause of lipid metabolism disorders.[41–44] The high content of fat in meat diets was proved to cause obesity, fatty liver disease, elevated inflammation levels and oxidative stress.[41–44] κ-CGN is a very important indigestible hydrocolloid and carbohydrate polymer in the food industry with controversial effects on metabolic health.[9–11] Its safety needs to be carefully evaluated. In general, the molecular weight of CGN applied in the food industry ranges from 200 to 800 kDa.[1,18] Moreover, "pure" κ-CGN is rare owing to the inevitable occurrence of ι-CGN units.[26] Consequently, the κ-CGN with those properties were deliberately chosen in our work. Consistently, κ-CGN addition in high-fat-pork-based diets could also signifi-

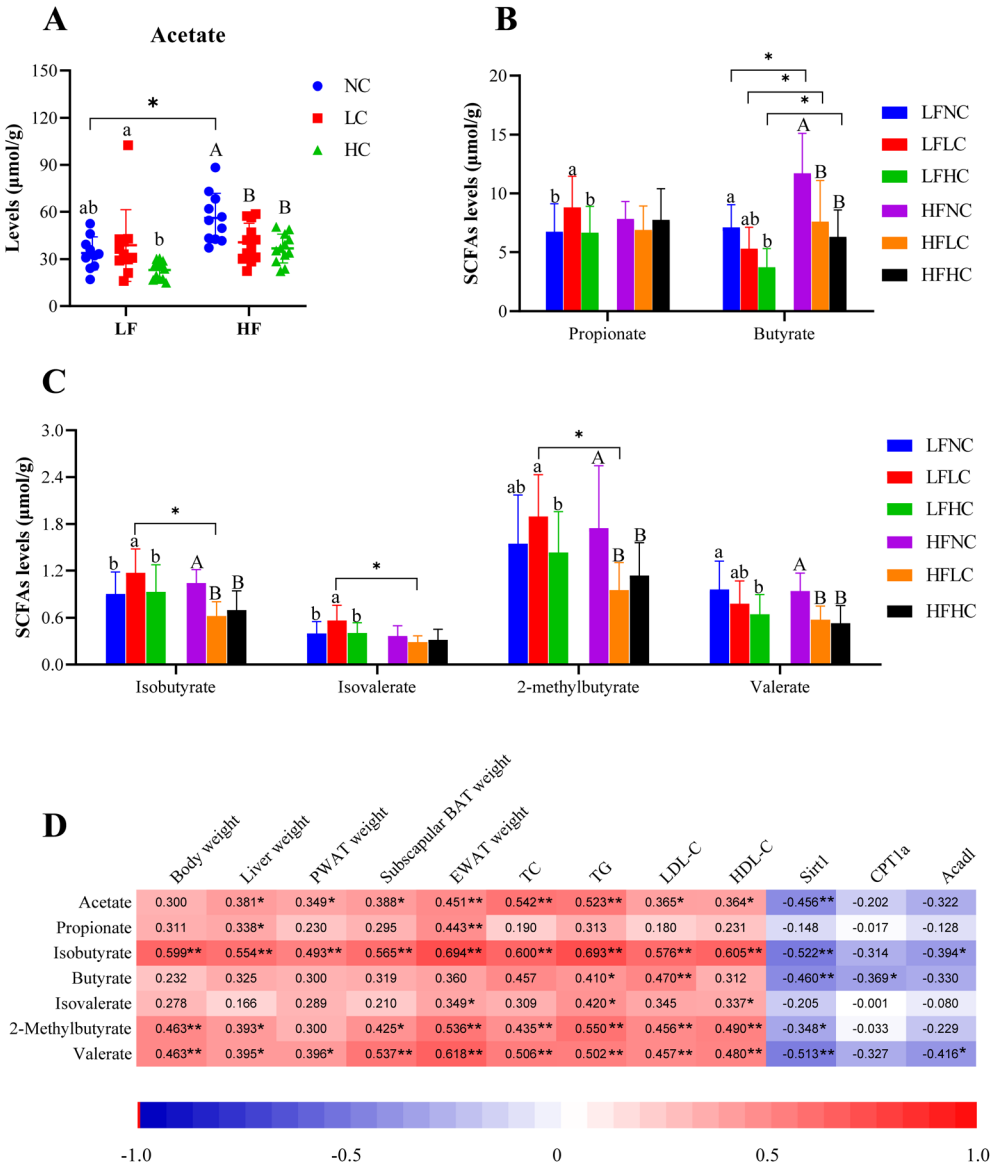

Fig. 5 The declined SCFAs concentration in cecal contents was associated with lipid metabolism. (A–C) The distribution of acetate, propionate, butyrate, isobutyrate, isovalerate, 2-methylbutyrate and valerate concentration in the cecal content. (D) Association between the SCFAs contents, body weight, visceral weight, serum lipid and hepatic mRNA expression levels. Asterisks indicate significant differences between the low-fat and high-fat diet groups or the significance of correlation (* $p < 0.05$, ** $p < 0.01$); a and b indicate significant differences among the low-fat diet groups ($p < 0.05$); A and B indicate significant differences among the high-fat diet groups ($p < 0.05$).

cantly restrain the body weight gain and fat mass rise, and alleviate the lipid profiles in blood. The lipid profiles (TC, TG, HDL-C and LDL-C) in the serums and tissue histological sections displayed the inhibited positive energy balance by κ-CGN in high-fat diets. Thus, we focused on hepatic lipid metabolism in our following study for the reason that low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are complexes originating from the liver, and play a fundamental role in lipids transportation.[27]

Our results suggested that κ-CGN decreased the lipid accumulation by enhancing hepatic fatty acid oxidation. *Cpt1a* and *Acadl* are protein coding genes, which directly encode carnitine palmitoyltransferase 1a and long-chain acyl-Coenzyme A dehydrogenase, respectively. These two proteins are directly involved in fatty acid β-oxidation.[28,29] In our study, mRNA levels of *Cpt1a* and *Acadl* were significantly promoted in both high-fat diet groups supplied with κ-CGN. Sirtuin expression is very important in exploring the mechanism of κ-CGN regulation in lipid metabolism. Several research studies discovered the promoted β-oxidation by activating Sirtuins.[16,45,46] In our study, the gene expressions of *Sirt1*, *Sirt3* and *Sirt6* in the liver were all significantly increased by κ-CGN in high-fat diets. However, we took the most interest in the expression of Sirtuin1 for several reasons. First, the protein levels were sig-

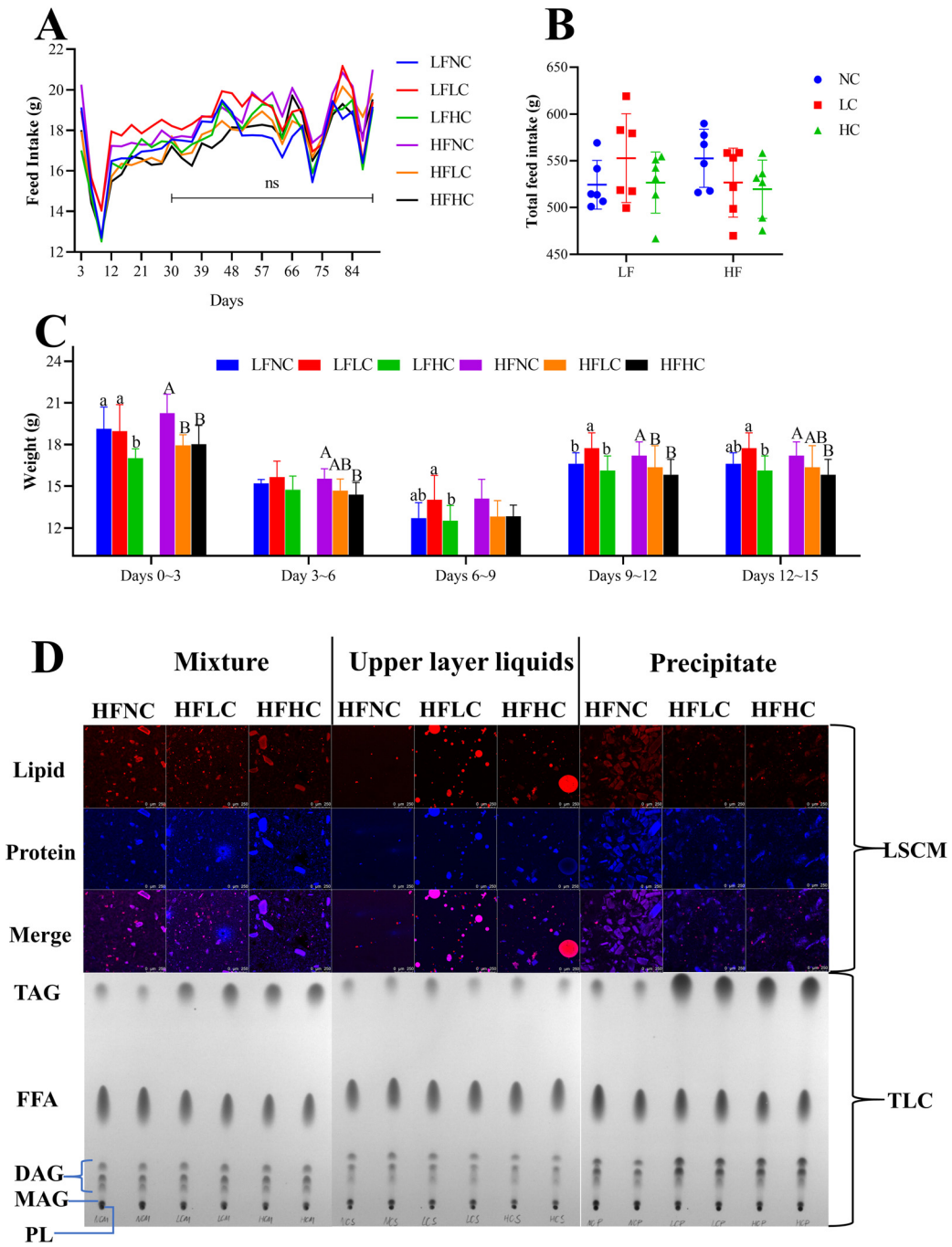

Fig. 6 κ-CGN inhibited the calorie uptake in diets. (A) Changes of the feed intake during the intervention for each cage. (B) Total average dietary intake for each cage. (C) The feed intake from day 0–15. (D) The *in vitro* digestion results of high-fat diets.

nificantly higher in the HFLC and HFHC groups compared with the HFNC group. Second, the *Sirt1* gene expression was also significantly upregulated by κ-CGN in the LFHC group, and the expressions in all groups seemed to be negatively correlated with the BAs concentration in the colonic contents. Third, *Cpt1a* and *Acadl* were both transcriptionally up-regulated in the HFLC and HFHC groups compared to those in the HFNC group, suggesting the possibility of the Sirtuin1-mediated promotion of fatty acid oxidation.[47]

BAs are well-known in the regulation of glycolipid metabolism. The total targeted BAs levels in colonic contents were positively associated with the serous lipid profiles in high-fat diets, and exhibited a remarkably negative correlation with hepatic *Sirt1* expression. The BAs receptor FXR manipulated the *miR-34a* expression and targeted *Sirt1* in hepatocytes.[16] Moreover, fibroblast growth factor 15/19 (FGF15/19) is an enteroendocrine hormone regulating BAs synthesis and composition, and is secreted by intestinal FXR activation.

Inhibiting the intestinal FXR/FGF15 signal pathway benefits glycolipid metabolism.[48,49] In the results of our work, κ-CGN radically lowered most of the targeted BAs, potentially resulting in intestinal FXR/FGF15 signal inhibition. Furthermore, hepatic fibroblast growth factor receptor 4 (FGFR4), a key in regulating bile acid synthesis and energy metabolism, was probably inactivated and thereby affected the BAs composition, leading to the regulated energy metabolism.[15]

Reducing the SCFAs production by κ-CGN in the intestinal tract is a possible mechanism to improve the lipid metabolism, as it was reported that only the 4.5 kDa CGN was able to be degraded and transferred to SCFAs by human intestinal microbes.[12] In line with the human study,[50] we found that the increase of the SCFAs concentration in the cecal contents was associated with lipid metabolism disorder and obesity. It could be explained as follows. Firstly, acetate promotes the metabolic syndrome through the microbiome-brain-β-cell axis.[51] Secondly, SCFAs can be exploited as energy by the host and aggravate the positive energy balance.[52,53] The low SCFAs levels may cause lower energy states, which can potentially elevate Sirtuin1 expression (such as fasting and calorie restriction).[39,40] Thus, hepatic Sirtuin1 upregulation may indicate the obstructed energy absorption.

The inhibited digestion and bioavailability of energy in the upper gastrointestinal tracts may also cause low energy states. Furthermore, the lipids digestion and absorption should be focused on primarily since it contributed most of the calories in our high-fat diets. Generally, the digested lipids should be removed from the lipid droplets to prevent the inhibition of lipolysis, and form micelles for the absorption.[7] In the upper layer liquids of the digesta, the existence of κ-CGN increased the size and number of lipid droplets without the changes in lipid composition. Consequently, our static *in vitro* digestion results suggested that κ-CGN had the potential to inhibit lipolysis and lipid absorption in certain dietary modes, which could partially explain the feed intake being decreased by κ-CGN. These phenomena might be attributed to the blunting effect on bile salts by κ-CGN,[54] which possibly affected the lipid uptake and BAs metabolism afterwards.

Xbp1 and *Cebpb* can be activated by endoplasmic reticulum stress, stimulating lipogenesis and gluconeogenesis.[46] In the present study, κ-CGN might control glycolipid metabolism through these two genes, which may have led to the inconsistent results with other studies. Other factors, such as SCFAs levels and hepatic *Sirt1* expression, may also contribute to the glycolipid metabolism in our study according to some literature studies.[46,55] Furthermore, their roles and mechanisms in the regulation of energy metabolism by κ-CGN still need to be answered in the future.

Our studies, as well as those of others,[9–11] stressed the complicated role of κ-CGN in glycolipid metabolism with different situations. We propose that future relative studies on κ-CGN should focus on the behavior of κ-CGN interacting with other dietary components and digestive fluids.

5. Conclusion

Our work demonstrated the beneficial effect of κ-CGN in the meat dietary patterns on energy homeostasis. The lipid accumulation was decreased by κ-CGN in high-fat diets, which was associated with Sirtuin1-participated hepatic fatty acid oxidation, inhibition of calorie intake, and reduced the BAs and SCFAs content in the large intestinal lumina.

Abbreviations

BAs	Bile acids
BAT	Brown adipose tissue
CGN	Carrageenan
EWAT	Epididymal white adipose tissue
FFA	Free fatty acid
FGF15/19	Fibroblast growth factor 15/19
FGFR4	Fibroblast growth factor receptor 4
GPC	Gel permeation chromatography
HDL-C	High-density lipoprotein cholesterol
HFHC	High-fat-high-carrageenan
HFLC	High-fat-low-carrageenan
HFNC	High-fat-carrageenan-free
LDL-C	Low-density lipoprotein cholesterol
LFNC	Low-fat-carrageenan-free
LSCM	Laser confocal microscopy
NMR	Nuclear magnetic resonance
OGTT	Oral glucose tolerance test
PWAT	Perirenal white adipose tissue
real-time qPCR	RT-qPCR
Sirtuin (Sirt)	Silent information regulator
SCFAs	Short chain fatty acids
TAG	Triglyceride
TC	Total cholesterol
TG	Total triglyceride
TLC	Thin layer chromatography
WAT	White adipose tissue

Author contributions

Chunbao Li contributed to the conceptualization, funding acquisition and review. Zhiji Huang contributed to the conceptualization, data curation, formal analysis and writing. Yafang Ma and Yunting Xie performed the experiments. Di Zhao contributed to reviewing the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

1 J. M. McKim, Food additive carrageenan: Part I: A critical review of carrageenan in vitro studies, potential pitfalls, and implications for human health and safety, *Crit. Rev. Toxicol.*, 2014, **44**, 211–243.
2 R. Chizzolini, E. Zanardi, V. Dorigoni and S. Ghidini, Calorific value and cholesterol content of normal and low-fat meat and meat products, *Trends Food Sci. Technol.*, 1999, **10**, 119–128.
3 X. D. Sun, Utilization of restructuring technology in the production of meat products: a review, *CyTA – J. Food*, 2009, **7**, 153–162.
4 A. Trius, J. G. Sebranek and T. Lanier, Carrageenans and their use in meat products, *Crit. Rev. Food Sci. Nutr.*, 1996, **36**, 69–85.
5 M. Noureddin, S. Zelber-Sagi, L. R. Wilkens, J. Porcel, C. J. Boushey, L. Le Marchand, H. R. Rosen and V. W. Setiawan, Diet associations with nonalcoholic fatty liver disease in an ethnically diverse population: The multiethnic cohort, *Hepatology*, 2020, **71**, 1940–1952.
6 S. Zelber-Sagi, D. Ivancovsky-Wajcman, N. F. Isakov, M. Webb, D. Orenstein, O. Shibolet and R. Kariv, High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance, *J. Hepatol.*, 2018, **68**, 1239–1246.
7 E. Bauer, S. Jakob and R. Mosenthin, Principles of physiology of lipid digestion, *Asian-Australas. J. Anim. Sci.*, 2005, **18**, 282–295.
8 S. B. Heymsfield and T. A. Wadden, Mechanisms, Pathophysiology, and Management of Obesity, *N. Engl. J. Med.*, 2017, **376**, 254–266.
9 Y. X. Chin, Y. Mi, W. X. Cao, P. E. Lim, C. H. Xue and Q. J. Tang, A pilot study on anti-obesity mechanisms of kappaphycus alvarezii: The role of native κ-carrageenan and the leftover aans-carrageenan fraction, *Nutrients*, 2019, **11**, 1133.
10 Q. Wang, L. Zhang, Y. He, L. Zeng, J. He, Y. Yang and T. Zhang, Effect of κ-carrageenan on glucolipid metabolism and gut microbiota in high-fat diet-fed mice, *J. Funct. Foods*, 2021, **86**, 104707.
11 J. Zhou, F. Wang, J. Chen, R. Yang, Y. Chen, D. Gu, T. Niu, Q. Luo, X. Yan, H. Chen and W. Wu, Long-term kappa-carrageenan consumption leads to moderate metabolic disorder by blocking insulin binding, *Pharmacol. Res.*, 2021, **165**, 105417.
12 Q. Shang, H. Jiang, C. Cai, J. Hao, G. Li and G. Yu, Gut microbiota fermentation of marine polysaccharides and its effects on intestinal ecology: An overview, *Carbohydr. Polym.*, 2018, **179**, 173–185.
13 M. Z. Ding, Z. X. Huang, Z. Y. Jin, C. Zhou, J. Q. Wu, D. Zhao, K. Shan, W. X. Ke, M. Zhang, Y. Q. Nian and C. B. Li, The effect of fat content in food matrix on the structure, rheological properties and digestive properties of protein, *Food Hydrocolloids*, 2022, **126**, 107464.
14 M. Ding, Z. Huang, Z. Huang, Z. Zhao, D. Zhao, K. Shan, W. Ke, M. Zhang, G. Zhou and C. Li, Proteins from different sources in a high-fat food matrix influence lipid hydrolysis through bolus coalescence and interactions with bile salts, *Food Hydrocolloids*, 2023, **141**, 108748.
15 S. Fiorucci, E. Distrutti, A. Carino, A. Zampella and M. Biagioli, Bile acids and their receptors in metabolic disorders, *Prog. Lipid Res.*, 2021, **82**, 101094.
16 J. Lee, A. Padhye, A. Sharma, G. Song, J. Miao, Y.-Y. Mo, L. Wang and J. K. Kemper, A pathway involving farnesoid X receptor and small heterodimer partner positively regulates hepatic sirtuin 1 levels via microRNA-34a inhibition, *J. Biol. Chem.*, 2010, **285**, 12604–12611.
17 S. Naumann, D. Haller, P. Eisner and U. Schweiggert-Weisz, Mechanisms of interactions between bile acids and plant compounds—a review, *Int. J. Mol. Sci.*, 2020, **21**, 6495.
18 F. Liu, P. Hou, H. Zhang, Q. Tang, C. Xue and R. W. Li, Food-grade carrageenans and their implications in health and disease, *Compr. Rev. Food Sci. Food Saf.*, 2021, **20**, 3918–3936.
19 M. Bastías-Pérez, D. Serra and L. Herrero, Dietary options for rodents in the study of obesity, *Nutrients*, 2020, **12**, 3234.
20 L. Yu, D. Zhao, Y. Nian and C. Li, Casein-fed mice showed faster recovery from DSS-induced colitis than chicken-protein-fed mice, *Food Funct.*, 2021, **12**, 5806–5820.
21 T. Yang, T. Shu, G. Liu, H. Mei, X. Zhu, X. Huang, L. Zhang and Z. Jiang, Quantitative profiling of 19 bile acids in rat plasma, liver, bile and different intestinal section contents to investigate bile acid homeostasis and the application of temporal variation of endogenous bile acids, *J. Steroid Biochem. Mol. Biol.*, 2017, **172**, 69–78.
22 X. Wang, S. Mao, J. Liu, L. Zhang, Y. Cheng, W. Jin and W. Zhu, Effect of the gynosaponin on methane production and microbe numbers in a fungus-methanogen co-culture, *J. Anim. Feed Sci.*, 2011, **20**, 272–284.
23 M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carrière, R. Boutrou, M. Corredig and D. Dupont, A standardised static in vitro digestion method suitable for food–an international consensus, *Food Funct.*, 2014, **5**, 1113–1124.
24 J. Folch, M. Lees and G. Sloanestanley, A simple method for the isolation and purification of total lipides from animal tissues, *J. Biol. Chem.*, 1957, **226**, 497–509.
25 V. L. Campo, D. F. Kawano, D. B. D. Silva and I. Carvalho, Carrageenans: Biological properties, chemical modifications and structural analysis – A review, *Carbohydr. Polym.*, 2009, **77**, 167–180.
26 F. van de Velde, S. H. Knutsen, A. I. Usov, H. S. Rollema and A. S. Cerezo, 1H and 13C high resolution NMR spec-

troscopy of carrageenans: application in research and industry, *Trends Food Sci. Technol.*, 2002, **13**, 73–92.
27 J. Heeren and L. Scheja, Metabolic-associated fatty liver disease and lipoprotein metabolism, *Mol. Metab.*, 2021, **50**, 101238.
28 O. Pougovkina, H. te Brinke, R. Ofman, A. G. van Cruchten, W. Kulik, R. J. A. Wanders, S. M. Houten and V. C. J. de Boer, Mitochondrial protein acetylation is driven by acetyl-CoA from fatty acid oxidation, *Hum. Mol. Genet.*, 2014, **23**, 3513–3522.
29 I. R. Schlaepfer and M. Joshi, CPT1A-mediated fat oxidation, mechanisms, and therapeutic potential, *Endocrinology*, 2020, **161**, bqz046.
30 J. Hirst, Mitochondrial Complex I, *Annu. Rev. Biochem.*, 2013, **82**, 551–575.
31 Q. Chen, X. Yang, H. Zhang, X. Kong, L. Yao, X. Cui, Y. Zou, F. Fang, J. Yang and Y. Chang, Metformin impairs systemic bile acid homeostasis through regulating SIRT1 protein levels, *Biochim. Biophys. Acta, Mol. Cell Res.*, 2017, **1864**, 101–112.
32 Z. D. Fu, J. Y. Cui and C. D. Klaassen, The role of sirt1 in bile acid regulation during calorie restriction in mice, *PLoS One*, 2015, **10**, e0138307.
33 C. Fernández-Hernando, Emerging role of microRNAs in the regulation of lipid metabolism, *Hepatology*, 2013, **57**, 432–434.
34 J. K. Kemper, S.-E. Choi and D. H. Kim, in *Vitam. Horm*, ed. G. Litwack, Academic Press, 2013, vol. 91, pp. 385–404.
35 A.-H. Lee, E. F. Scapa, D. E. Cohen and L. H. Glimcher, Regulation of hepatic lipogenesis by the transcription factor XBP1, *Science*, 2008, **320**, 1492–1496.
36 J. Ning, T. Hong, A. Ward, J. Pi, Z. Liu, H.-Y. Liu and W. Cao, Constitutive role for IRE1α-XBP1 signaling pathway in the insulin-mediated hepatic lipogenic program, *Endocrinology*, 2011, **152**, 2247–2255.
37 S. Oyadomari, H. P. Harding, Y. Zhang, M. Oyadomari and D. Ron, Dephosphorylation of Translation Initiation Factor 2α Enhances Glucose Tolerance and Attenuates Hepatosteatosis in Mice, *Cell Metab.*, 2008, **7**, 520–532.
38 A. Koh, F. De Vadder, P. Kovatcheva-Datchary and F. Bäckhed, From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites, *Cell*, 2016, **165**, 1332–1345.
39 J. G. Wood, B. Rogina, S. Lavu, K. Howitz, S. L. Helfand, M. Tatar and D. Sinclair, Sirtuin activators mimic caloric restriction and delay ageing in metazoans, *Nature*, 2004, **430**, 686–689.
40 A. Cordeiro, L. L. de Souza, L. S. Oliveira, L. C. Faustino, L. A. Santiago, F. F. Bloise, T. M. Ortiga-Carvalho, N. Almeida and C. C. Pazos-Moura, Thyroid hormone regulation of Sirtuin 1 expression and implications to integrated responses in fasted mice, *J. Endocrinol.*, 2013, **216**, 181–193.
41 M. I. Ahmad, M. U. Ijaz, M. Hussain, I. U. Haq, D. Zhao and C. Li, High-fat proteins drive dynamic changes in gut microbiota, hepatic metabolome, and endotoxemia-TLR-4-NFκB-mediated inflammation in mice, *J. Agric. Food Chem.*, 2020, **68**, 11710–11725.
42 M. I. Ahmad, M. U. Ijaz, M. Hussain, I. A. Khan, N. Mehmood, S. M. Siddiqi, C. Liu, D. Zhao, X. Xu and G. Zhou, High fat diet incorporated with meat proteins changes biomarkers of lipid metabolism, antioxidant activities, and the serum metabolomic profile in Glrx1−/− mice, *Food Funct.*, 2020, **11**, 236–252.
43 M. I. Ahmad, X. Zou, M. U. Ijaz, M. Hussain, C. Liu, X. Xu, G. Zhou and C. Li, Processed meat protein promoted inflammation and hepatic lipogenesis by upregulating Nrf2/Keap1 signaling pathway in Glrx-deficient mice, *J. Agric. Food Chem.*, 2019, **67**, 8794–8809.
44 M. U. Ijaz, M. I. Ahmad, M. Hussain, I. A. Khan, D. Zhao and C. Li, Meat protein in high-fat diet induces adipogenesis and dyslipidemia by altering gut microbiota and endocannabinoid dysregulation in the adipose tissue of mice, *J. Agric. Food Chem.*, 2020, **68**, 3933–3946.
45 V. Avilkina, C. Chauveau and O. G. Mhenni, Sirtuin function and metabolism: Role in pancreas, liver, and adipose tissue and their crosstalk impacting bone homeostasis, *Bone*, 2022, **154**, 116232.
46 L. Rui, Energy metabolism in the liver, *Compr. Physiol.*, 2014, **4**, 177.
47 A. Purushotham, T. T. Schug, Q. Xu, S. Surapureddi, X. M. Guo and X. L. Li, Hepatocyte-Specific Deletion of SIRT1 Alters Fatty Acid Metabolism and Results in Hepatic Steatosis and Inflammation, *Cell Metab.*, 2009, **9**, 327–338.
48 F. Li, C. Jiang, K. W. Krausz, Y. Li, I. Albert, H. Hao, K. M. Fabre, J. B. Mitchell, A. D. Patterson and F. J. Gonzalez, Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity, *Nat. Commun.*, 2013, **4**, 2384.
49 L. Sun, C. Xie, G. Wang, Y. Wu, Q. Wu, X. Wang, J. Liu, Y. Deng, J. Xia, B. Chen, S. Zhang, C. Yun, G. Lian, X. Zhang, H. Zhang, W. H. Bisson, J. Shi, X. Gao, P. Ge, C. Liu, K. W. Krausz, R. G. Nichols, J. Cai, B. Rimal, A. D. Patterson, X. Wang, F. J. Gonzalez and C. Jiang, Gut microbiota and intestinal FXR mediate the clinical benefits of metformin, *Nat. Med.*, 2018, **24**, 1919–1929.
50 J. De la Cuesta-Zuluaga, N. T. Mueller, R. Álvarez-Quintero, E. P. Velásquez-Mejía, J. A. Sierra, V. Corrales-Agudelo, J. A. Carmona, J. M. Abad and J. S. Escobar, Higher Fecal Short-Chain Fatty Acid Levels Are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors, *Nutrients*, 2018, **11**, 51.
51 R. J. Perry, L. Peng, N. A. Barry, G. W. Cline, D. Zhang, R. L. Cardone, K. F. Petersen, R. G. Kibbey, A. L. Goodman and G. I. Shulman, Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome, *Nature*, 2016, **534**, 213–217.
52 H. Liu, J. Wang, T. He, S. Becker, G. Zhang, D. Li and X. Ma, Butyrate: A double-edged sword for health?, *Adv. Nutr.*, 2018, **9**, 21–29.
53 S. Zhang, J. Zhao, F. Xie, H. He, L. J. Johnston, X. Dai, C. Wu and X. Ma, Dietary fiber-derived short-chain fatty

acids: A potential therapeutic target to alleviate obesity-related nonalcoholic fatty liver disease, *Obes. Rev.*, 2021, **22**, e13316.

54 E. V. Sokolova, A. O. Kravchenko, N. V. Sergeeva, V. N. Davydova, L. N. Bogdanovich and I. M. Yermak, Effect of carrageenans on some lipid metabolism components in vitro, *Carbohydr. Polym.*, 2020, **230**, 115629.

55 J. Hu, S. Lin, B. Zheng and P. C. K. Cheung, Short-chain fatty acids in control of energy metabolism, *Crit. Rev. Food Sci. Nutr.*, 2018, **58**, 1243–1249.