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Intramuscular adipocyte and fatty acid differences between high-fat and control rabbit groups subject to a restricted diet

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Abstract

Fatty acids of intramuscular fat (IMF) in rabbits can influence meat quality, but it is unclear which fatty acids benefit to human health. A rabbit model of weight gain and weight loss was constructed using two rabbit groups and two growth stages. Stage 1 included control group1 fed a commercial diet(CG1) and experimental group1 fed a high fat diet (EG1). Stage 2 include control group2(CG2) and experimental group2 (EG2) both fed a restricted commercial diet. We detected differences in blood biochemical indicators as well as changes in intramuscular adipose cells and intramuscular fatty acid content in control and experiment groups at two stages. High fat induction can make rabbits become obese, have higher concentrations of glucose (GLU), total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and free fatty acid (FFA), and lower concentrations of insulin (INS). In addition, a high-fat diet promotes hypertrophy of precursor adipocytes in femoral muscles. Conversely, a restricted diet causes weight loss, decreases the concentration of TG, FFA, and INS in CG2 and EG2, and increases the deposition of unsaturated fatty acids in the femoral muscle. The content of monounsaturated trans oleic acid (C18:1n-9T) in EG2 was significantly higher than in CG2, whereas oleic acid (C18:1n-9C) was significantly lower in EG2 than in CG2. The polyunsaturated fatty acids Linolenate (C18:3 n-3) and cis-5,8,11,14,17-Eicosapentaenoate (C20:5 n-3) increased in CG2 and EG2. The content of Linoleate (C18:2 n-6) and γ-Linolenic acid (C18:3 n-6) significantly increased in CG2. The content of cis-11,14-Eicosatrienoic acid (C20:2) decreased significantly in CG2, but increased significantly in EG2.Thus, a high-fat diet can increase the formation of unhealthy fatty acids. Conversely, weight loss due to a restricted diet leads to an increase in unsaturated fatty acids in the femoral muscle, indicating that it reduces obesity symptoms and it may improve meat quality in rabbit.

KEYWORDS
fatty acids, femoral muscle, morphology, rabbit, weight loss

1 | INTRODUCTION

With a constantly increasing level of meat consumption, more and more people have drawn attention to the intake of a healthy diet and put forward higher requirements for meat quality. Differences in intramuscular fat (IMF) content affect the grade of meat products in the market. Studies have determined that 2.5 to 3% of IMF is the most desirable percentage in pork (Cameron et al., 1999). The IMF content in bovine dorsiflexus muscle is about 3.25%, and related to the formation of beef marbling, one of the main beef quality traits used for grading beef (Chambaz et al., 2002). The IMF content in lambs and chicken is about 4.92 and 1.96% (Enser et al., 1996), respectively. However, the IMF content in rabbit muscle is about 1.63%, which is lower than other livestock (Zotte, 2002). Meanwhile, the formation of IMF is a complex physiological and biochemical process affected by individual genetics, gender, age and nutritional factors. It contains various types of fatty substances such as phospholipids, triacylglycerols,glycerol diester, cholesterol and free fatty acids. Diets of different composition can be an effective way to change the composition of fatty acids to help increase meat quality. Previous studies show that a low protein diet (12%) can increase IMF content, change the content of unsaturated fatty acids and reshape muscle fiber formation pattern, thus improving the meat quality of growing and finishing pigs (Li et al., 2018). Fat deposition in the dorsal muscle of adult Angus cattle can be changed by feeding with different fat sources (Acetoze & Rossow, 2014). Interestingly, IMF deposition in rabbits is rich in lecithin and a variety of unsaturated fatty acids (Martínez-Ivaro et al., 2018). However, it is not clear whether high fat can influence on intramuscular fatty acids and improve meat quality of rabbits.

Fatty acids are the basic components of IMF and its accumulation is closely related to lipid metabolism. Exogenous lipids can affect the proportions of saturated fatty acids (SFA) and unsaturated fatty acids (UFA), thus affecting tissue fat deposition. Previous studies indicated that the deposition of SFA can be induced by intake of high-fat and high-energy diets in humans, resulting in an increased risk of lipid metabolic diseases (Alarcon et al., 2018). Conversely, diet restriction and exercise can reduce fat deposition in various organs, thereby alleviating obesity symptoms (Axelrod et al., 2019). Studies on lipid omics have found that hundreds of fatty acids and arachidonic acids are involved in obesity-related complications, among them polyunsaturated fatty acids (PUFA) (n-3, eicosapentaenoic [EPA]) can effectively reduce the incidence of cardiovascular diseases (Satoh-Asahara, 2010). A higher ratio of n-3/n-6 UFAs can reduce the inflammatory response caused by obesity in high-fat mice (Nong et al., 2020). Although, intramuscular fat deposition in rabbits is rich in lecithin and a variety of unsaturated fatty acids (Martínez-Ivaro et al., 2018), it is unclear whether a high-fat diet can influence intramuscular fatty acids and meat quality in rabbits.

Increasing rabbit meat quality requires knowledge of the kinds of fatty acids that are affected by obesity due to a high-fat diet and by weight loss due to a restricted diet. Thus, the aim of this study was to increase our understanding of intramuscular adipocyte differences and fatty acid deposition in rabbit femoral muscle in response to the intake of high-fat diet, and to lay the foundation for further studies on underlying molecular mechanisms of intramuscular adipocytes.

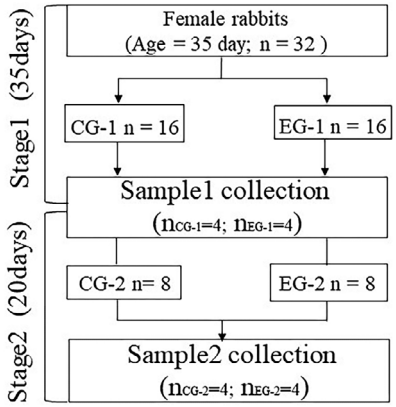

FIGURE 1 Experimental design. Stage1 = high fat induction stage; Stage2 = diet restriction stage

2 | MATERIALS AND METHODS

2.1 | Animals and experiment design

This study used 32 weaned (35 days of age) female rabbits from the teaching and research rabbit farm. Female rabbits were randomly divided into a control group (CG, n = 16) and an experimental group (EG, n = 16), and subjected to a two-stage feeding regimen. In stage 1 (35 days), the CG was fed a commercial diet (CG-1) whereas the EG received a high fat induction diet (EG-1). In stage 2 (20 days), the CG (CG-2) and the EG (EG-2) were fed a commercial diet. During feeding stage 1 (35 to 70 days of age), each of rabbits in EG-1 received averagely 120 g/day of a high-fat diet to induce fattening (a commercial rabbit diet mixed with 10% lard), while each of rabbits in CG-1 were averagely given 120 g/day of a commercial diet. During feeding stage 2 (70 to 90 days of age), eight rabbits from CG-1 and eight rabbits from EG-1 were fed a restricted commercial diet (20 g/d). Before starting the experiment, rabbits from the CG and EG groups went through a restricted diet adaptation period of 5 days where they were averagely fed 60 g/day of a commercial diet. During the two feeding stages, weak, disabled, and sick rabbits were weeded out. Table 1 shows the nutritional composition of the commercial and mixed diets, and Figure 1 presents the experimental design. The daily diet of each rabbit is shown in Table 2. At the end of the two feeding stages, four rabbits with visible high weight characteristics were chosen from each group for collection of femoral muscle tissue samples and blood samples. Tissue samples were stored at −20°C for fatty acid determination. Blood samples were stored at **4** and −**20** °C for different biochemical indexes determination, respectively.

TABLE 1 Nutritional composition of the commercial and mixed diets

Diet	CP	CF	CA	CF	Calcium/ Phosphorus	DE	Pork lard
Commercial diet	≥16.0	≥2.0	10	10.0–16.0	2:1	10.47 ≥ MJ/kg	0
Mixed diet	≥16.0	≥2.0	10	10.0-16.0	2:1	10.47 ≥ MJ/kg	10%

Note: The feed terms of Crude protein, Crude fat, Crude ash, Crude fiber, Digestive energy use the capital letters CP, CF, CA, CF and DE.

TABLE 2 Average daily feed intake of each rabbits in the two stages of the experiment (g/d)

Stage 1[1]								
Age, d	35	36	37	38	39	40	41	Mean
CG(n=16)	69.06	44.38	71.56	73.44	72.19	75.00	62.50	66.88
EG(n=16)	73.75	50.00	78.13	77.19	77.50	70.63	62.50	69.96
Age, d	42	43	44	45	46	47	48	Mean
CG(n=16)	109.69	100.00	78.13	100.00	100.00	71.88	100.00	94.24
EG(n=16)	105.63	96.25	74.06	97.19	93.44	61.56	100.00	89.73
Age, d	49	50	51	52	53	54	55	Mean
CG(n=16)	100.00	99.69	97.81	97.50	99.69	85.31	95.94	96.56
EG(n=16)	93.44	100.00	98.75	97.19	98.44	80.31	95.63	94.82
Age, d	56	57	58	59	60	61	62	Mean
CG(n=16)	101.56	84.06	97.81	100.31	95.63	107.50	102.81	98.53
EG(n=16)	123.33	65.42	98.33	128.75	133.75	138.75	121.25	115.65
Age, d	63	64	65	66	67	68	69	Mean
CG(n=12)	94.69	118.44	105.00	101.56	114.38	103.75	103.75	105.94
EG(n=12)	89.58	96.67	81.25	94.54	82.50	55.42	55.38	79.33
Stage 2[2]								
Age, d	70	71	72	73	74	75	76	Mean
CG(n=8)	113.44	60.00	60.00	60.00	60.00	60.00	20.00	61.92
EG(n=8)	61.67	60.00	60.00	60.00	60.00	60.00	20.00	54.52
Age, d	77	78	79	80	81	82	83	Mean
CG(n=8)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
EG(n=8)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Age, d	84	85	86	87	88	89	90	Mean
CG(n=8)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
EG(n=8)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00

[1]Stage 1. Four rabbits in each group were eliminated due to digestive tract diseases and weak body. Three rabbits were placed together for breeding and two rabbits were selected for feeding after 56 days of age in the CG and EG groups.
[2]Stage 2. Rabbits were raised in pairs. All rabbits had good body condition.

2.2 | Hematoxilin–Eosin stains

All femoral muscle tissue samples were fixed with 4% paraformaldehyde and embedded in paraffin wax. After slicing the fixed femoral muscle tissue samples and staining them with Hematoxilin–Eosin (HE), their physiological characteristics were observed and photographed under a microscope for subsequent analysis. The details of this three-step process (embedding, slicing, and staining with HE) were as follows. First, the fixed tissue samples were washed with sterile water for 30 min and dehydrated in a tissue embedding plastic basket using 75% ethanol for 6 h, 85% ethanol for 10 h, 95% ethanol for 4 h, anhydrous ethanol I for 2 h, anhydrous ethanol II for 2 h, through transparent (Xylene I 20 min, xylene II 15 min) and soaked with paraffin wax for 3 h, resulting in tissue blocks embedded in paraffin wax. Second, a Leica RM2235 slicer was used to cut the femoral muscle tissue samples into 5 μm-thick slices. After the tissue slices were flattened in warm water,

they were placed on slides and baked at 60°C for at least 2 h. Finally, the tissue slices were dewaxed with xylene and washed with water for 20 min. After staining the dewaxed tissue slices with hematoxylin for 30 min and with eosin for 5 min, the stained slices were sealed with a resin adhesive.

2.3 | Detection of blood biochemical indicators

After centrifugation with cryogenic refrigerated centrifuges, 400 μL of serum from rabbit blood samples were used to assess biochemical indicators. Four blood biochemical indicators (total cholesterol [TC], triglyceride [TG], low density lipoprotein cholesterol [LDL], and glucose [Glu]) were measured using a Veterinary Biochemical Analyzer (BS-240VET, Mindray Biomedical Electronics Co. Ltd, Shenzhen, China). The Rabbit free fatty acid (FFA) Elisa Kit (ZC-52747), Rabbit INS Elisa Kit (ZC-52732) from Shanghai Zhuochai Biological Technology Co. Ltd., were used to measure serum FFA and INS, respectively.

2.4 | Composition of fatty acids

2.4.1 | Preparation of standard solution

The standard substance contained a mixed fatty acid methyl ester standard substance (1 mg/mL). One millilitre of mother liquor (1 mg/mL) was diluted into 10 mL *n*-hexane and 10 μL BHT methanol solution with a mass ratio of 4 mg/mL was added to prepare 100 g/mL solution. Solutions of 0.05, 0.1, 0.2, 0.5, 1, and 2 mL were absorbed into 10 mL volumetric flasks to obtain concentrations of 0.5, 1, 2, 5, 10 and 10 g/mL, respectively.

2.4.2 | Preprocessing

After samples were ground with liquid nitrogen, 0.2 g (accurate to 0.1 mg) uniform samples were transferred into 15 mL centrifuge tubes, and 2 mL of a concentrated sulfuric acid/methanol solution with a volume ratio of 5% was added. Subsequently, 10 μL of a BHT methanol solution with a mass ratio of 4 mg/mL was added, mixed in a vortex for 1 min, heated at 90°C for 30 min, taken out and placed at room temperature, 2 mL of n-hexane and 2 mL of saturated sodium chloride solution were added, mixed in a vortex for 1 min, and the supernatant was taken for analysis after 15 min.

2.4.3 | Instrument conditions

Gas chromatograph (Agilent 7890B-5977) analysis used a capillary column (hP-5 capillary column [30 m (length) × 0.25 mm (inside diameter) × 0.25 mm (film thickness)]). The carrier gas was helium and the flow rate was 1.0 mL/min, injected in sample volumes of 1.0 μL. The inlet temperature was 270°C and the split ratio was 100 : 1.

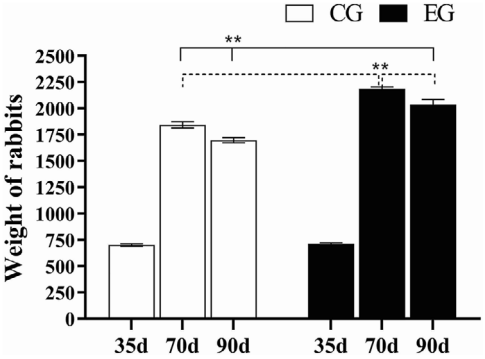

FIGURE 2 Rabbit weights at the beginning of the trial (35 days of age), at the end of the high fat induction stage (70 days of age), and at the end of the diet restriction stage (90 days of age) in the control group (CG) and the experimental group (EG); $^{**}p < 0.01$; $^{*}p < 0.05$

2.4.4 | Heating procedure

The initial temperature was kept at 50°C for 1 min, then increased to 205°C at 5 °C/min increments, and kept for 5 min. Then, the temperature was risen to 240°C at 5°C/min increments, and maintained for 10 min. The ion source temperature was 230°C, the four-stage bar temperature was 150°C, the solvent was delayed for 3 min, the detection range was 30 to 500 DA, the SIM ions were 74, 55, 67, 79, 87, 69 and105, and the dwell time was 25 ms.

2.5 | Data analysis

Image-Pro Plus 6.0 software was used to determine the quantity, diameter and area of adipose cells in the HE stained pictures (200×). Software package SPSS 22.0 was used for data processing and analysis, and GraphPad Prism 6.0 software was used for data analysis and mapping.

Means and standard errors of the mean (SEM) were computed for all blood biochemical indicators, morphological characteristics of adipocytes and fatty acid profile measurements in the two female rabbit groups in each of the two feeding stages (CG-1, EG-1, CG-2, EG-2). Rabbit blood biochemical indicator, adipocyte morphological characteristic and fatty acid profile differences between CG-1 and CG-2, EG-1 and EG-2, CG-1 and EG-1, and CG-2 and EG-2 were compared using One-Way ANOVA with SPSS 22.0 software. Levels of significance were $p < 0.05$ and < 0.01.

3 | RESULTS

3.1 | Body weight differences

Body weights measured at the beginning of the trial and at the end of the two feeding stages (35 d, 70 d and 90 d of age) for the CG and EG groups were compared using *t*-tests (Figure 2). At the end of the first stage, the body weight of rabbits in EG was significantly higher than

TABLE 3 Blood biochemical indicators in the control and experimental rabbit groups at the end of the high-fat induction and the diet restriction feeding stages[1]

	Stage1		Stage2			
Biochemical indicator[2]	CG-1	EG-1	CG-2	EG-2	SEM	*p*-value
GLU (mmol/L)	7.34	8.53^{b}	7.22	8.67^{B}	0.15	<0.01
TC (mmol/L)	1.30	2.17^{b}	3.57^{a}	4.21^{A}	0.25	<0.01
TG (mmol/L)	0.82^{A}	1.44^{ab}	0.42	0.61	0.10	<0.01
LDL-C (mmol/L)	0.49	0.67^{b}	2.51^{A}	3.49^{Ab}	0.21	<0.01
FFA (μmol/L)	111.22	637.92^{AB}	112.49	212.43^{B}	3.35	<0.01
INS (mU/L)	28.59^{AB}	2.64^{A}	0.94	0.99	0.16	<0.01

[1]CG-1, Control group, high-fat induction stage; EG-1, Experimental group, high fat induction stage; CG-2, Control group, diet restriction stage; EG-2, Experimental group, diet restriction stage. Significance of differences between the two feeding stages within the control and experimental groups (A = $p < 0.01$; a = $p < 0.05$). Significance of differences between the control and experimental groups within feeding stages (B = $p < 0.01$; b = $p < 0.05$).
[2]GLU, TC, TG, LDL-C, FFA and INS represent glucose, cholesterol, triglyceride, low density lipoprotein—cholesterol, free fatty acid, insulin.

that in CG ($p < 0.01$). At the end of the second stage, the weight of rabbits in the two groups were significantly lower than the weight before weight loss, and the weight of rabbits in CG was significantly lower than that in EG. This indicates that diet restriction in the second feeding stage resulted in significant weight loss in both groups, and that the weight loss was higher in CG.

3.2 | Blood biochemical indicators

There were significant differences in blood biochemical indicators between CG and EG at the end of the two feeding stages (Table 3). Although the concentrations of GLU, TC, TG, LDL-C and FFA in EG-1 were higher than that in CG-1, only the concentrations of TG and FFA in EG-1 were significantly higher than those in CG-1 ($p < 0.05$). The concentrations of INS decreased between the end of feeding stage 1 and the end of feeding stage 2 in the CG and EG groups indicating that the metabolism of rabbits changed when they became obese. After diet restriction in feeding stage 2, the concentration of GLU in EG1 became significantly higher than in CG1 ($p < 0.05$), and it was significantly higher than that in EG-2 ($p < 0.01$). The concentrations of TC and LDL-C increased significantly during the diet restriction stage in the CG and EG groups ($p < 0.01$), and their concentrations in EG-2 were significantly higher than those in EG-1. The concentration of TG significantly decreased between feeding stages 1 and 2 in both rabbit groups ($p < 0.01$). The concentration of FFA and INS decreased during the diet restriction stage, and the FFA concentration in EG-2 was significantly higher than that in CG-2. These results showed that most blood biochemical indicators changed significantly after the diet restriction period, and that a restricted diet could significantly alleviate obesity symptoms in rabbits.

TABLE 4 Morphological characteristics of muscle adipocytes in the control and experimental rabbit groups at the end of the high-fat induction and the diet restriction feeding stages[1]

	Stage1		Stage2			
Group[2]	CG1	EG1	CG2	EG2	SEM	*p* value
Area (μm^2)	483.96^{A}	506.54	374.79	625.90^{aB}	14.78	<0.01
Diameter (μm)	22.68^{a}	24.83^{ab}	21.52	23.19^{b}	0.37	<0.01
Number	26.67^{B}	20.67	40.67^{Ab}	24.00	1.35	<0.01
Density (per/mm^2)	299.93^{B}	232.45^{A}	458.90^{AB}	135.41	11.73	<0.01

[1]Measurement area = 88 908.17 μm^2.
[2]CG-1, control group, high-fat induction stage; EG-1, experimental group, high-fat induction stage; CG-2, control group, diet restriction stage; EG-2, experimental group, diet restriction stage. Significance of differences between the two feeding stages within the control and experimental groups (A = $p < 0.01$; a = $p < 0.05$). Significance of differences between the control and experimental groups within feeding stages (B = $p < 0.01$; b = $p < 0.05$).

3.3 | Muscle adipocyte morphology

The morphology of the adipocytes in the femoral muscle (*vastus intermedius*) in rabbits from the control group, high-fat induction stage (CG-1), the experimental group, high-fat induction stage (EG-1), the control group, diet restriction stage (CG-2), and the experimental group, diet restriction stage (EG-2) is shown in Figure 3. The adipose cells in this figure appear as white rings between the muscle fascicles. The areas of IMF in EG-1 were significantly larger than those in CG-1. After the diet restriction stage, the IMF in the femoral muscle of EG-2 rabbits exhibited irregular circular shapes between muscle bundles. This suggests that diet restriction could improve the phenomenon of excessive fat deposition.

The area, diameter, number, and density of adipose cells (Area = 88 908.17 μm^2) in femoral muscles were significantly different after diet restriction in CG and EG (Table 4). The area of adipose cells in EG was larger than that of CG ($p < 0.05$). Further, the area of adipose cells in EG-2 rabbits was significantly larger than that in CG-2 rabbits ($p < 0.01$). In addition, the diameter, number and density of adipose cells increased in EG and CG after diet restriction. The number and density of adipose cells in the femoral muscle of rabbits in CG-2 were significantly higher than those in CG-1 ($p < 0.05$) and EG-2 ($p < 0.01$). This indicated that a high-fat diet can induce hypertrophy of fat cells in muscle, and obesity symptoms can only be alleviated after weight loss. Similarly, the fat area in muscle from the control group can be significantly reduced after weight loss.

3.4 | Muscle fatty acid composition

The types of SFA and UFA differed before and after weight loss (Table 5). The content of the saturated stearic acid (C18:0) and arachidate (C20:0) in the femoral muscle of EG-1 rabbits was significantly reduced, whereas the contents of the SFAs C16:0 and C21:0 were significantly increased between CG-2 and EG-1. Further, the contents of

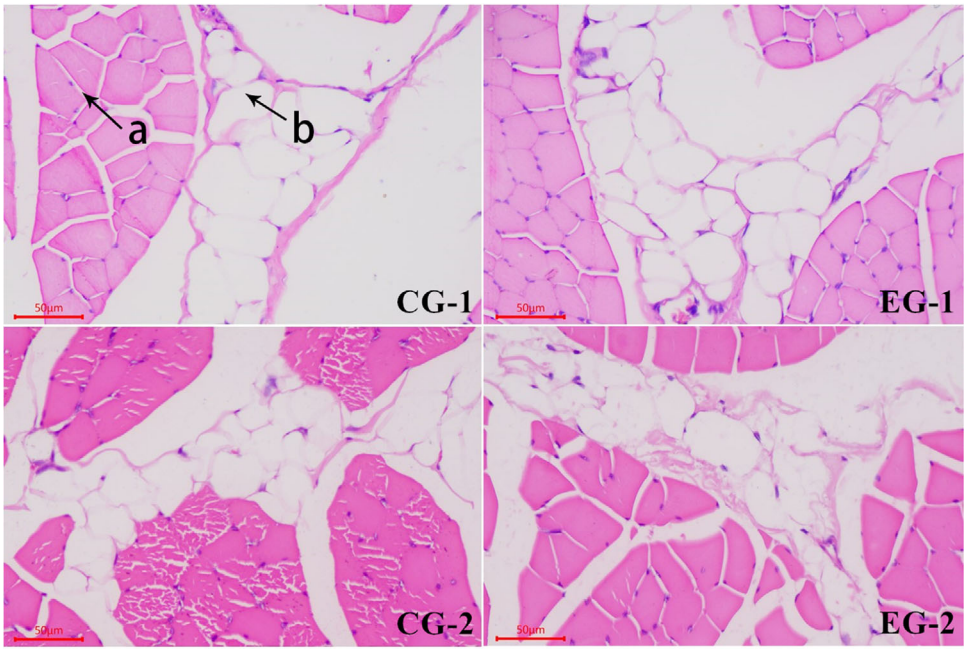

FIGURE 3 Morphology of adipocytes in the femoral muscle (*vastus intermedius*) of rabbits from the control group, high fat induction stage (CG-1), the experimental group, high fat induction stage (EG-1), the control group, diet restriction stage (CG-2), and the experimental group, diet restriction stage (EG-2). Both a and b in the figure represent muscle nucleus and adipocyte, respectively

C14:0 and C15:0 in the femoral muscle significantly decreased, and the contents of C16:0 and C21:0 significantly increased in the CG group during the diet restriction period.

The content of monounsaturated fatty acids (MUFA) in EG rabbits was significantly higher than in CG rabbits. The content of elaidic acid (C18: 1n-9T) in EG was significantly higher than in CG. Conversely, the content of oleic acid (C18: 1n-9C) in CG-2 was significantly higher than in CG-1, and significantly higher than that in EG-2. Changes in PUFA content indicated that the content of n-3 and n-6 UFA in the femoral muscle from both rabbit groups increased after diet restriction, the content of n-3 UFA significantly increased in CG-2 and EG-2, and the content of N-6 UFA significantly increased in CG-2. The content of PUFAs significantly increased in EG-2 and decreased in CG-2. The content of α-linolenic acid (C18:3 n-3) and cis-5,8,11,14,17-EPA acid (C20:5 n-3) significantly increased in CG-2 and EG-2. This fatty acid, also called EPA, plays a key role in the regulation of body growth. The content of linoleate (C18:2 n-6) and γ-linolenic acid (C18:3 n-6) significantly increased in CG-2 but there was little difference between EG-1 and EG-2. The content of cis-11,14-eicosadienoic acid (C20:2) significantly decreased in CG-2, but significantly increased in EG-2. This indicated that a high-fat diet caused in normal rabbits significant differences in SFAs. Conversely, UFAs play a vital role in the metabolism of diet restricted rabbits.

4 | DISCUSSION

Rabbits showed physical and behavioral changes during the stages of fat gain and diet restriction. The wool fleece in EG-1 was scattered and lackluster, their feed intake decreased, their feces were light black, and their urine was deep yellow. Diet restriction is an ideal state for the human body to lose weight because it both maintains nutritional requirements and keeps the body active and vigorous (Damon L Swift et al., 2018). During the diet restriction stage, rabbits in EG-2 gradually showed a strong sense of hunger similar to CG-2 rabbits. The intake of water and the output of urine of rabbits in both groups increased, and the color of urine became pale yellow. In addition, the wool fleece of rabbits in both groups had low gloss and sparsity. All rabbits showed similar physical characteristics, indicating weight loss due to diet restriction achieved visible results in the control and the experimental groups. However, the majority of obese people fail to lose weight and have low energy, which causes serious metabolic problems. We also found that weight gain in rabbits may become a metabolic burden, resulting in abnormal urine, wool fleece, and body posture, whereas weight loss can alleviate these symptoms.

Age is one of the most important factors affecting fat deposition. Increasing the accumulation of abdominal fat in obese patients can indirectly increase the content of TGs in muscles, thus, reducing the oxidative metabolism capacity of muscles and the sensitivity to insulin (Blaak, 2004). As obese patients grow older, muscle mitochondrial metabolism decreases and the risk of type II diabetes increases (Karakelides et al., 2010). It can be speculated that the best age to lose weight is when young. Research has found that the fat deposition stage in medium-sized meat rabbits occurs between 70 and 90 days of age (Duprat et al., 2016). To better understand fatty acid differences in skeletal muscle of rabbits, weaned medium-sized meat rabbits (Tianfu black) were selected for constructing a two-stage experiment for fatty acid synthesis and degradation. The high-fat induced and

TABLE 5 Fatty acid profiles in the control and experimental rabbit groups at the end of the high-fat induction and the diet restriction feeding stages[1]

	Stage 1		Stage 2			
Fatty acid[2]	CG1 (n=4)	EG1 (n=4)	CG2 (n=4)	EG2 (n=4)	SEM	*p* value
C14:0	9.80^{AB}	5.09	5.54^{b}	4.79	0.18	<0.01
C15:0	18.08^{AB}	6.51	11.84^{b}	8.00^{a}	0.40	<0.01
C16:0	106.76	106.56	154.77^{Ab}	117.08	1.88	<0.01
C18:0	41.26^{aB}	35.60	31.05	28.64	0.67	<0.01
C20:0	218.53^{b}	193.96^{A}	214.53^{B}	167.25	1.44	<0.01
C21:0	244.65	291.26^{B}	289.17^{A}	356.7^{AB}	2.43	<0.01
ΣSFA	639.11	639.01	706.92^{AB}	682.49^{A}	4.26	<0.01
C14:1	0.14^{AB}	0.06	0.07	0.07	0.00	<0.01
C15:1	0.23	0.22	0.28	0.34	0.02	<0.01
C16:1	0.42^{B}	0.13	0.21^{b}	0.16	0.01	<0.01
C17:1	0.16^{B}	0.03	0.15^{B}	0.02	0.01	<0.01
C18:1n-9T	13.39^{A}	17.66^{B}	1.20	12.86^{B}	0.16	<0.01
C18:1n-9C	11.08	11.16	18.05^{AB}	12.07	0.41	<0.01
C20:1	0.19	0.21	0.25	0.19	0.01	<0.01
ΣMUFA	25.65^{A}	29.51^{B}	20.23	25.74^{b}	0.46	<0.01
C18:3 n-3	5.05	5.93^{b}	9.53^{A}	7.84^{a}	0.36	<0.01
C20:3 n-3	0.06	0.06	0.08	0.05	0.01	<0.05
C20:5 n-3	8.24	9.59	15.92^{A}	13.72^{a}	0.21	<0.01
C22:6 n-3	1.34	1.46	2.74^{Ab}	2.24^{A}	0.10	<0.01
Σn-3	14.71	17.06^{B}	28.29^{A}	23.87^{a}	0.59	<0.01
C18:2 n-6	3.09	3.81	6.05^{A}	5.02	0.26	<0.01
C18:3 n-6	10.57	9.82	14.26^{ab}	10.45	0.44	<0.01
C20:3 n-6	0.12	0.10	0.13	0.12	0.01	ns
C20:4 n-6	0.50	0.81	1.00	0.55	0.25	ns
Σn-6	14.28	14.56	21.44^{aB}	16.17	0.70	<0.01
C20:2	7.57^{AB}	5.91	6.09	6.36^{a}	0.13	<0.01
C22:1 n-9	0.01	0.03	0.05	0.05	0.00	<0.01
C22:2	0.05	0.03	0.02	0.05	0.00	<0.01
ΣPUFA	7.65^{aB}	5.98	6.17	6.45	0.13	<0.01

[1]Unit of measurement, mg/100 g of total fat; Sample size = 0.2 g; CG-1, Control group, high-fat induction stage; EG-1, experimental group, high-fat induction stage; CG-2, control group, diet restriction stage; EG-2, experimental group, diet restriction stage. Significance of differences between the two feeding stages within the control and experimental groups (A = $p < 0.01$; a = $p < 0.05$).
Significance of differences between the control and experimental groups within feeding stages (B = $p < 0.01$; b = $p < 0.05$).
[2] SFA = saturated fatty acids; MUFA = monounsaturatedfatty acids; PUFA = polyunsaturated fatty acids.

control rabbit groups showed clear weight differences at the end of the weight gain and weight loss feeding stages. This demonstrated that the rate of weight loss in obese rabbits was slower than that in control rabbits. A long-term high-fat diet increases the risk of obesity and energy-metabolism disorders. A high-fat diet in the nonalcoholic fat model of rats can cause increasing levels of body blood lipid indicators, especially levels of FFA, which will directly lead to the accumulation of TGs and insulin sensibility (Wang et al., 2011; Hernández et al., 2004. In addition, a long-term high-fat diet will cause fat cells in adipose tissues to become hypertrophic, and insulin resistance will be reduced, increasing the concentration of a variety of blood lipids, and causing hyperlipidemia, cardiovascular disease and obesity (Wang et al., 2012). In this study, we also found that after a high-fat diet period, the levels of GLU, TC, TG, LDL-C and FFA in the blood serum from rabbits in the EG1 increased, and the concentration of insulin decreased, which was consistent with previous studies (Khosravi et al., 2018; Wang et al., 2017; Cabioglu et al., 2006). This indicated that a high-fat diet caused obesity in rabbits. However, although the blood serum biochemical indicators of high-fat diet rabbits decreased significantly, their concentrations were still higher than those of the control group, indicating

that the diet restriction could alleviate the obesity symptoms caused by the high-fat diet.

Morphological observations directly reflect the results of biogenesis, thus, they can help understand physiological states of tissues. Obesity produces different microscopic changes in many tissues. Morphological studies shows that obesity can make visceral adipose cells to produce an oxidative stress response, fat cells apoptosis, a large number of inflammatory cells aggregations and directly induce insulin resistance response, thus, causing inflammation and metabolic disorders in visceral adipose tissue (Revelo et al., 2014). Similarly, the accumulation of fat in the muscles can lead to a decrease in mitochondrial enzyme activity in obese patients or in patients with type 2 diabetes mellitus (Giovannelli et al., 2018). However, muscle lipid oxidation and glucose metabolism will be improved with regular exercise or weight loss (Axelrod et al., 2019). The above studies have shown that excessive accumulation of fat will cause metabolic dysfunction in body tissues including large differences in oxidative metabolism in the muscles. In this study, there were significant changes in fat areas and morphology of adipocytes in the femoral muscle, which indicate different metabolic states in rabbits from the two weight loss groups. Rabbits in EG2 would likely fail to restore normal femoral muscle function because of the large area of adipose cells after the diet restriction period compared to rabbits in the control weight loss group.

The composition of fatty acids is also closely associated with insulin resistance and metabolic disorders during the information of obesity. The monounsaturated palmitoleic acid (C16:1 n-7) and oleic acid (C18:1 n-9) increased whereas the polyunsaturated linoleic acid (C18:2n-6) and arachidonic acid (C20:4 n-6) decreased in blood and tissues, particularly in muscle and liver (Fukuchi et al., 2004). It shows that elevated levels of glucose and insulin in blood are linked to the types of fatty acids in muscle tissues. Other studies showed that EPA and DHA of n-3 PUFAS in fish oil could improve insulin sensitivity in rats, and that metabolites of n-6 PUFAS could improve the symptoms of metabolic syndrome through anti-inflammatory and anti-oxidant effects (Alfonso et al., 2011). However, none of the above results can properly explain which fatty acids are caused by high fat levels. Unsaturated fatty acids in the femoral muscles of rabbits in this study were significantly different after weight loss. Among MUFA, oleic acid (C18: 1n-9C) significantly increased in control rabbits after weight loss, whereas elaidic acid (C18: 1n-9T) significantly increased in the high-fat-induced rabbit weight loss group. Previous studies showed that excessive intake of trans fatty acids would impair their insulin sensitivity of female C57/BL6 mice, resulting in an imbalance of glucose metabolism regulation (Kylie et al., 2010). High intake of industrial trans fatty acids would increase the risk of obesity, cardiovascular disease and type II diabetes (Iwata et al., 2011). Conversely, oleic acid can affect the formation of fat, reduce the risk of obesity and protect the cardiovascular system (Hashem and Koohi, 2018; Tutunchi et al., 2020). Thus, elaidic acid may not help the recovery of oxidative metabolism function in femoral muscles after a weight loss.

Polyunsaturated fatty acids containing two or more nonconjugated cis double bonds form a straight chain of fatty acids 16 to 22 carbon atoms in length. The higher the number of double bonds, the higher the degree of unsaturation. PUFAs have numerous physiological functions such as regulation of lipid metabolism, prevention of cardiovascular and cerebrovascular diseases, stimulation of growth and development, immune regulation, aging delay, and weight loss. Currently, α-linolenic acid, docosapentaenoic acid, linoleic acid, γ linolenic acid and other long-chain PUFAs are considered to be closely related to the prevention of metabolic diseases such as fat formation, insulin resistance, diabetes and obesity (Ludwig et al., 2013). The type of n-3 long-chain UFAs, which has anti-inflammatory and promote T-cell immunosuppression function, can effectively alleviate the symptoms of non-alcoholic fatty liver disease (Jump et al., 2018). Results in our study show that linolenic acid (C18:3 n-3) and EPA of n-3 UFA and n-6 UFA significantly increased in the two types of weight loss groups. γ-linolenic acid (C18:3 n-6) significantly increased in normal weight loss rabbits. The content of cis-11,14-eicosadienoic acid (C20:2) among PUFA was the opposite between the two types of weight loss groups, suggesting that it may play an important role in the weight loss process.

In conclusion, weight gain and weight loss models in control and experimental groups were established by feeding rabbits with a high-fat diet and a commercial diet in two feeding stages. High-fat induction made rabbits become obese, have high concentrations of TC, TG, FFA, and promoted hypertrophy of precursor adipocytes in femoral muscles. Conversely, diet restriction caused weight loss, decreased the concentration of TG, FFA and INS, and increased the deposition level of different UFAs in femoral muscles, which had a positive effect on reducing obesity symptoms and likely improved the nutritional value of rabbit meat.

AUTHOR CONTRIBUTIONS

Jie Wang and Songjia Lai have made substantial contributions to conception; Xianbo Jia, Mingchuan Gan, Tao Tang, Jiahao Shao, Tianfu Lai and Yuan Ma provided technical help and collation of data; Yanhong Li wrote the original manuscript and design this study; Mauricio A. Elzo and Shenqiang Hu revised the manuscript critically for important intellectual content.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interest in this work.

ANIMAL WELFARE STATEMENT

This study was carried out in accordance with the ethical standards of and approved by the Institutional Animal Care and Use Committee of the College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, China (DKY-B20141401).

PEER REVIEW

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