

# Decrease of chemerin by aerobic exercise improved glycolipid metabolism of diabetes through increasing key metabolism enzymes and protein mediated by PPAR $\gamma$

Xiaojing Lin

Shanghai University of Sport School of Kinesiology

Lijun Yin

Shanghai University of Sport School of Kinesiology

Jing Qu

Shanghai University of Sport School of Kinesiology

Xiaohui Wang (✉ [wangpan96@126.com](mailto:wangpan96@126.com))

Shanghai University of Sport <https://orcid.org/0000-0001-8355-3871>

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## Research Article

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# Abstract

**Background:** Our previous work indicated that exercise-induced reduction of chemerin (an adipokine and chemokine) played an important role in the improvement of glycolipid metabolism of diabetic rats, and this manuscript are to: (1) clarify peroxisome proliferator-activated receptor (PPAR $\gamma$ ) mediating the regulation of decreased chemerin on glycolipid metabolism key enzymes (including adipose triglyceride lipase (ATGL), lipoprotein lipase (LPL), glucose transporter 4 (GLUT4) and phosphoenolpyruvate carboxykinase (PEPCK)) in diabetic rats; (2) demonstrate the crucial role of decreased chemerin on exercise-induced improvement of glycolipid metabolism in diabetic mice by exogenous chemerin supplement (for only mice-resource chemerin available).

**Methods:** After establishing type 2 diabetes models, diabetic SD rats were randomly divided into 4 groups: diabetes (DM), exercised diabetes (EDM), EDM plus PPAR $\gamma$  agonist pioglitazone (EDP), and EDM plus PPAR $\gamma$  antagonist GW9662 (EDG), while diabetic ICR mice divided into 3 groups: DM, EDM and EDM plus exogenous chemerin supplementation (EDC). The exercised rodents participated in 4-week (rats) or 6-week (mice) moderate-intensity aerobic exercise, and at 30 min before exercise pioglitazone (10 mg/kg) and GW9662 (1 mg/kg) were intragastrically administered to EDP and EDG rats respectively, while recombinant mouse chemerin (8  $\mu$ g/kg) was intraperitoneally injected to C and EDC mice. Glycolipid metabolism indexes were determined. Serum chemerin and the protein levels of the above molecules in metabolic organs (liver, gastrocnemius and epididymal fat) were detected by ELISA and Western blot, respectively.

**Results:** (1) In diabetes rats, aerobic exercise-induced increases of ATGL and LPL (livers and gastrocnemius) as well as decrease of PEPCK (livers) were reversed by GW9662, and further strengthened by pioglitazones. (2) In diabetes mice, aerobic exercise also significantly increased the levels of PPAR $\gamma$ , ATGL, LPL and GLUT4 as well as decreased the level of PEPCK; furthermore, the changes of the above molecules and improvements of glycolipid metabolism and fatty liver were partly reversed by exogenous chemerin.

**Conclusion:** The decreased chemerin played important roles in aerobic exercise-induced improvements of glycolipid metabolism and fatty liver in diabetes, through increasing glycolipid metabolism key enzymes and protein (ATGL, LPL and GLUT4) mediated by PPAR $\gamma$ .

## Introduction

Chemerin, a novel adipokine and chemokine, is primarily produced in liver and adipocytes and exerts its multiply functions such as modulating adipogenesis, lipolysis, glycolipid metabolism, insulin resistance (IR) and inflammation mainly through its receptor chemokine-like receptor 1 (CMKLR1)[1]. Numerous clinical studies confirmed the greatly elevated serum chemerin in multiple metabolic and inflammatory diseases including obesity[2], type 2 diabetes[3], atherosclerosis[4], metabolic syndrome[5], cardiovascular disease[6] and nonalcoholic fatty liver disease[7], and chemerin level was positively

associated with the severity of these inflammatory diseases[8]. Besides serum chemerin, the increases of chemerin/CMKLR1 in liver, muscle and fat were likely to be closely related to glycolipid metabolism disorders and obesity-related chronic inflammation in the rats and mice with obesity or obesity related diseases, [9–11].

Exercise has positive effects on preventing and treating obesity and obesity related diseases[12, 13]. The benefits of exercise on improvement of glycolipid metabolism and suppression of chronic inflammation have been confirmed in patients with obesity[14, 15] and diabetes[16, 17], but the underlying mechanisms are not fully clarified. An increasing number of evidence has demonstrated that exercise induced decline of serum chemerin[18–20], and the decrease of serum chemerin is likely to be associated with the improvement of glycolipid metabolism and alleviation of inflammation not only in normal elderly[21], but also in obese[2, 22] and diabetic patients[23]. Our previous study further indicated that exercise-induced decreases of serum chemerin and the levels of chemerin/CMKLR1 in peripheral metabolic organs were associated with the improvement of glycolipid metabolism in obese and diabetic rats[10].

However, the mechanisms of exercise-induced lower of chemerin improved glycolipid metabolism of obesity and diabetes are still unknown. Glycolipid metabolism key enzymes and protein exert vital roles in glycolipid anabolism, catabolism and transportation. For example, adipose triglyceride lipase (ATGL) plays important roles in liver glycolipid metabolism and lipid decomposition by affecting lipogenesis, glycolysis and gluconeogenesis [24]; lipoprotein lipase (LPL) is a key speed-limiting enzyme for the breakdown of chylomicron and TG in blood as well as the produce of free fatty acids (FFA) for cellular uptake and utilization[25, 26]; phosphoenolpyruvate carboxykinase (PEPCK) is a key enzyme in gluconeogenesis by promoting the production of glucose from pyruvate in hepatic parenchymal cells[27]; and glucose transporter 4 (GLUT4), a principal glucose transporter protein, plays a key role in regulating whole body glucose homeostasis through mediating insulin-stimulated glucose transport into skeletal muscle thus decreasing blood glucose[28, 29].

Furthermore, ATGL and LPL are peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) target genes, and PEPCK and GLUT4 were also reported to be regulated by PPAR $\gamma$ . PPAR $\gamma$ , a ligand-activated nuclear receptor, has been identified as a therapeutic target for obesity, hyperlipidemia and diabetes for the dual functions of regulating glycolipid metabolism[30, 31] and inhibiting inflammation[32, 33]. Exercise increased PPAR $\gamma$  in the liver, muscle and circulating monocytes, which might be related to the decreases of IR and hepatic lipid content[34] and enhancement of glucose uptake, FFA oxidation and insulin sensitivity of skeletal muscle in obese rats[35], as well as prevention of type 2 diabetes in human[33]. It's worth mentioning that chemerin is considered as a novel PPAR $\gamma$  target gene in promoting mesenchymal stem cell adipogenesis *in vitro* [36], and PPAR $\gamma$  inhibited chemerin secretion from adipocytes by more than 80%[37]. But other literatures reported that *in vitro* chemerin promoted preadipocyte differentiation and maturation by up-regulating the levels of PPAR $\gamma$ [36, 38], indicated an interaction between chemerin and PPAR $\gamma$ .

Therefore, we speculate that the effects of exercise-induced decreased chemerin on improvements of glycolipid metabolism in diabetes may be fulfilled through PPAR $\gamma$ -mediated regulations on ATGL, LPL, PEPCK and GLUT4. To verify our hypothesis, the current study firstly determined the influences of exercise on the levels of PPAR $\gamma$ , ATGL, LPL and PEPCK in DM rats, then explored the indispensability of PPAR $\gamma$  on exercise-induced changes of the above enzymes and protein using PPAR $\gamma$  antagonist GW9662 and PPAR $\gamma$  agonist pioglitazone, lastly treated diabetes mice with exogenous chemerin (for only mouse commercial recombinant chemerin available) to demonstrate the roles of reduced chemerin in exercise-induced improvements of glycolipid metabolism in diabetes and to further explore the mechanisms: PPAR $\gamma$ -mediated regulations on ATGL, LPL, PEPCK and GLUT4?

## Material And Methods

### Animals

One hundred and two male Sprague-Dawley (SD) rats (190–210 g weight) were purchased from Beijing Vital River laboratory animal technology Co. Ltd., and 70 male ICR (30-35g weight) mice were purchased from Jiansu GemPharmatech Co. Ltd.. All the animals were housed under standard specific pathogen free (SPF) conditions with 12 h:12 h light and dark cycles and food and water were provided ad libitum.

### Establishment of type 2 diabetes rats and mice and grouping

Type 2 diabetes rats were established by high fat diet (HFD) feeding combined with STZ injection, and the details were shown in our previous article[10]. Forty successfully established diabetes rats were randomly divided into four groups of 10 rats each: diabetes mellitus group (DM), exercised diabetes mellitus group (EDM), EDM plus PPAR $\gamma$  agonist pioglitazone group (EDP), and EDM plus PPAR $\gamma$  antagonist GW9662 group (EDG) to clarify whether PPAR $\gamma$  mediated exercise-induced decrease of chemerin.

For demonstrating the effects of reduced chemerin in aerobic exercise-induced improvements of glycolipid metabolism in diabetes and exploring its mechanisms (glycolipid metabolism key enzymes and proteins), type 2 diabetes model mice were established by HFD plus STZ injection due to only mouse exogenous chemerin available. In detail, ICR mice were randomly divided into control group (n = 24) and high fat diet (HFD) group (n = 46) after acclimating to laboratory condition for 3 days, and were fed ad libitum by 6-week standard diet and high-fat 60 kcal% fat diet (PD6000, purchased from Changzhou SYSE Bio-Tec. Co., Ltd.), respectively. Then, the 46 mice from HFD group were injected intraperitoneally with STZ (Sigma, St. Louis, MO, USA) at a dose of 100 mg/kg body weight[39] to establish diabetes mice, and 27 mice showed fasting hyperglycemia (fasting blood glucose, FBG) > 11.1 mmol/L at 3 and 7 day post-injection were considered as diabetes mice. Twenty-two control mice were randomly divided into 3 groups: control group (Con, n = 8), exercised control group (E, n = 8) and exogenous chemerin group (C, n

= 8), while 27 diabetes mice were randomly divided into diabetes mellitus group (DM, n = 8), exercised DM group (EDM, n = 9) and EDM plus exogenous chemerin group (EDC, n = 10).

## **Exercise intervention, and supplementations of PPAR $\gamma$ agonist and antagonist as well as exogenous mouse chemerin**

The SD rats in the groups of Con, OB and DM kept sedentary life, while EOB, EDM, EDP and EDG rats participated in moderate-intensity aerobic exercise on a treadmill with gradually increased load, once a day and 6 days/week, 4 weeks in total, and at 30 min before exercise the rats in EDP and EDG groups were intragastrically administered 10 mg/kg body weight of PPAR $\gamma$  agonist pioglitazone and 1 mg/kg body weight of PPAR $\gamma$  antagonist GW9662 (both from MedChem Express, NJ, USA), respectively. During the 4-week intervention period, one rat in EDG group was accidentally squeezed to death in the gap between the runways of treadmill and six rats from the four groups died probably to be associated with diabetes, so finally 9, 8, 9 and 7 rats were involved in the analysis of results in DM, EDM, EDP and EDG rats groups, respectively.

The ICR mice in Con, C and DM groups kept sedentary life while E, EDM and EDC mice participated in moderate-intensity aerobic exercise on a treadmill with gradually increasing intensity and duration, once a day and 6 days per week, lasts for 6 weeks, and all the mice in C and EDC groups received intraperitoneal injection of recombinant mouse chemerin (R&D SYSTEMS, MN, USA) at 8  $\mu$ g/kg/day body weight prior 30 min to each exercise session from the third exercise intervention and lasting for 3-week until the end of exercise intervention.

## **Determination of glycolipid metabolism index**

Lipid metabolism index including serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) were detected by Nanjing Jiancheng Bioengineering Institute. Glucose metabolism index such as FBG and fasting insulin (FINS) were measured by glucose meter (Roche Accu-Chek Performa, Germany) and ELISA kit, respectively. The variance coefficient of the mouse insulin ELISA kit (Nanjing Jiancheng Bioengineering Institute, China) was <10% in intra-assay and <12% in inter-assay. IR was estimated by homeostasis model assessment of IR (HOMA-IR), calculating by the formula: = FBG (mmol/L)  $\times$  FINS ( $\mu$ U/ml) / 22.5.

## **Detection of serum chemerin by ELISA**

Serum levels of chemerin in the mice were measured by ELISA according to the manufacturer's instruction.

The variance coefficient of the mouse chemerin ELISA Kit (R&D System, MN, USA) was <10% in intra-assay and <10% in inter-assay.

## **Western blot**

About 50 mg of liver and gastrocnemius, and 100mg of epididymal fat were cut into pieces and homogenized with a homogenizer after adding 500  $\mu$ L of RIPA Lysis Buffer containing 1 mmol/L phenylmethanesulfonyl fluoride (PMSF) and 2 mmol/L protease and phosphatase inhibitor cocktail for mammalian (Beyotime Biotechnology, Shanghai, China) to extract total protein. The lysates were briefly sonicated on ice and centrifuged at 12000 rpm for 20 min, then the liquid supernatant was transfer to another EP tube and centrifuged at 12000 rpm for 20 min.

Supernatants were collected and protein concentration was measured using a BCA protein assay kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instruction.

Extracts of liver (40  $\mu$ g), gastrocnemius (50  $\mu$ g) and perirenal fat (60  $\mu$ g) were fractionated on 10% SDS-PAGE gels for detecting all the molecules except chemerin on 15% SDS-PAGE gel. The resolved protein were electrotransferred onto nitrocellulose membranes, blocked with 5% nonfat milk for 2 h, and then incubated overnight at 4  $^{\circ}$ C with primary antibodies against chemerin (1:2000), LPL and ATGL (1:1000, R&D System, MN, USA), CMKLR1 and PPAR $\gamma$  (1:1000, abcam Company, UK), GLUT4 and  $\beta$ -actin (1:1000, Cell Signaling Technology, MA, USA), PEPCK (1:1000, Santa cruz Biotechnology, CA, USA), and GAPDH (1:10000, Zen Bioscience, Chengdu, China). The blots were washed for four times, 5 min each time, with Tri-buffered saline with 0.1% Tween 20 (TBST) and incubated with horseradish peroxidase (HRP) conjugated secondary antibodies for 1.5 h at room temperature. The blots were washed again as described above, developed with Immobilon Western chemiluminescent HRP substrate (Millipore, MA, USA), and visualized by automatic chemiluminescence image analysis system (Tanon Biotechnology, Shanghai, China). The density of bands was determined using Imagem software and normalized against  $\beta$ -actin or GAPDH.

## Statistical analysis

Statistical analysis of data was performed using SPSS for Windows 21.0 software package (IBM Corporation, Armonk, NY, USA). All the data were expressed as mean  $\pm$  SD, and the levels of statistical significance was set as  $p < 0.05$ . Mean values in different groups with body weight and FBG were compared using two-way repeated measures analysis of variance (ANOVA). Other data were analyzed using one-way ANOVA and post hoc comparisons using least significant difference (LSD)-t test.

## Results

### Successful establishment of diabetes model rats and mice

Diabetes model rats were judged according to fasting hyperglycemia (FBG  $> 11.1$  mmol/L) at 3 and 7 day post-injection after HFD feeding in combination with STZ injection, and the details showed in our published article[10].

For mice, the mean FBG of DM model mice was increased by 13.4 mmol/L, higher than that of diabetes criteria of FBG  $> 11.1$  mmol/L ( $p < 0.01$ ). Furthermore, the DM mice had diabetes symptoms such as excessive appetite (daily intake: DM  $6.01 \pm 0.84$  g vs Con  $4.21 \pm 0.33$  g), weight loss (DM from baseline

38.69 ± 3.42 g to 6th week 37.81 ± 4.77 g vs Con from baseline 42.11 ± 2.31 g to 6th week 46.02 ± 2.85 g) and polyuria. These results indicated the successful establishment of DM models mice.

### **Aerobic exercise changed the protein levels of ATGL, LPL and PEPCK in the liver and gastrocnemius of diabetic rats, which were reversed by PPAR $\gamma$ antagonist and promoted by PPAR $\gamma$ agonist**

We found that the protein levels of ATGL and LPL were up-regulated in the liver and gastrocnemius while PEPCK was down-regulated in the liver of DM rats after 4-week aerobic exercise (Fig. 1). Actually, in our obesity model rats established by HFD feeding, similar results were found (Fig. 1).

Furthermore, in EDM rats, the exercise-induced increases of ATGL and LPL in the livers and gastrocnemius as well as decrease of PEPCK in the livers were reversed by PPAR $\gamma$  antagonist GW9662, and further strengthened by PPAR $\gamma$  agonist pioglitazone (Fig. 2).

### **Exogenous chemerin treatment partly reversed the exercise-induced change in body fat and improvements of glycolipid metabolism and fatty liver in diabetes mice**

The mean body weights of DM mice was significantly decreased compared to Con mice, and exogenous chemerin treatment and exercise had no influence on body weight in diabetic mice (Fig. 3); however, exogenous chemerin treatment (from the third week of exercise intervention and lasting for 3-week until the end of exercise intervention) partly reversed the 6-week exercise induced increase of body fat rate (body fat/body weight ratio) in DM mice (Table 1). In addition, no significant difference was found in daily food consumption between C and Con (4.41 ± 0.91g vs 4.77 ± 0.81g) as well as between EDC and EDM (3.85 ± 0.65g vs 4.48 ± 0.91g) mice, indicated no influence of chemerin on food intake of Con and DM mice.

Table 1  
Effects of exogenous chemerin treatment on body fat of exercised diabetes mice ( $\bar{x} \pm SD$ )

	Con (n = 8)	C (n = 8)	E (n = 8)	DM (n = 8)	EDM (n = 9)	EDC (n = 10)
Fat (g)	7.68 ± 1.98	7.62 ± 1.06	3.35 ± 1.06**	3.61 ± 0.89**	4.86 ± 0.70 <sup>#</sup>	4.02 ± 1.46
Fat/body weight (%)	16.57 ± 4.03	16.67 ± 2.06	7.38 ± 2.61**	9.00 ± 1.92**	11.00 ± 3.60 <sup>#</sup>	9.67 ± 2.95 <sup>△</sup>
Lean (g)	34.53 ± 2.52	34.20 ± 1.89	38.97 ± 2.63**	32.61 ± 3.65	31.43 ± 2.84	33.74 ± 1.44
Lean/body weight (%)	75.14 ± 4.01	75.00 ± 2.00	84.00 ± 2.07**	81.38 ± 2.20**	80.33 ± 3.42	81.89 ± 2.66
Perirenal fat (g)	0.55 ± 0.16	0.45 ± 0.08*	0.15 ± 0.06**	0.10 ± 0.03**	0.15 ± 0.05	0.09 ± 0.03
Perirenal fat/body weight (%)	1.20 ± 0.32	0.99 ± 0.18*	0.32 ± 0.15**	0.24 ± 0.07**	0.38 ± 0.16	0.23 ± 0.08
Epididymal fat (g)	1.46 ± 0.46	1.74 ± 0.31	0.79 ± 0.28**	0.65 ± 0.16**	0.84 ± 0.12	0.71 ± 0.37
Epididymal fat/body weight (%)	3.16 ± 0.94	3.83 ± 0.77	1.72 ± 0.65**	1.56 ± 0.39**	2.15 ± 0.22 <sup>#</sup>	1.68 ± 0.78
Note: Con: control; C: chemerin treatment; E: exercise; DM: diabetes mellitus; EDM: exercised DM; EDC: EDM + chemerin treatment. * $P < 0.05$ , ** $P < 0.01$ , vs Con; <sup>#</sup> $P < 0.05$ , <sup>##</sup> $P < 0.01$ , EDM vs DM; <sup>△</sup> $P < 0.05$ , <sup>△△</sup> $P < 0.01$ , EDC vs EDM.						

As shown in Fig. 4, aerobic exercise and exogenous chemerin treatment had no influence on the FBG levels of normal mice, but at 6th week of exercise intervention, the exercise-induced decrease of FBG in DM mice was totally reversed by exogenous chemerin treatment in EDM mice. Similarly, the exercise-induced improvements of glucose metabolism index (insulin and HOMA1R) and lipid metabolism index (TC and LDL) in EDM mice were totally reversed by exogenous chemerin treatment (Table 2). These results indicated that decreased chemerin mediated exercise-induced improvements of glycolipid metabolism in diabetic mice.

Table 2  
Effects of exogenous chemerin treatment on the levels of blood glucose and blood lipids of exercised diabetes mice ( $\bar{x} \pm SD$ )

	Con (n = 8)	C (n = 8)	E (n = 8)	DM (n = 8)	EDM (n = 9)	EDC (n = 10)
<b>Glucose metabolism index</b>						
FBG (mmol/L)	5.35 ± 0.89	5.84 ± 0.56	4.80 ± 0.77	18.21 ± 3.91**	15.12 ± 3.28#	21.18 ± 3.48△△
FINS(μIU/ml)	10.95 ± 2.10	12.32 ± 3.44	11.63 ± 2.42	20.77 ± 6.28**	13.83 ± 2.37##	20.04 ± 6.83△△
HOMA-IR	2.63 ± 0.84	3.21 ± 1.00	2.52 ± 0.78	16.56 ± 8.02**	9.22 ± 2.25##	18.42 ± 5.23△△
<b>Lipid metabolism index</b>						
TC (mmol/L)	2.44 ± 0.65	3.31 ± 1.16	1.80 ± 0.51#	4.67 ± 1.21**	3.64 ± 0.96#	4.90 ± 1.30△
TG (mmol/L)	0.50 ± 0.16	0.60 ± 0.14	0.34 ± 0.10*##	0.71 ± 0.07**	0.50 ± 0.13##	0.58 ± 0.15
LDL (mmol/L)	1.34 ± 0.24	1.89 ± 0.31	1.21 ± 0.38#	2.92 ± 0.59**	2.03 ± 0.64##	2.74 ± 0.76△
HDL (mmol/L)	1.30 ± 0.14	0.88 ± 0.11**	0.97 ± 0.06**	0.79 ± 0.13**	0.80 ± 0.18	0.77 ± 0.13
Note: Con: control; C: chemerin treatment; E: exercise; DM: diabetes mellitus; EDM: exercised DM; EDC: EDM + chemerin treatment. TG: triglyceride; TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol. ** $p < 0.01$ , DM vs Con; # $p < 0.05$ , ## $p < 0.01$ , EDM vs DM; △ $p < 0.05$ , △△ $p < 0.01$ , EDC vs EDM						

Figure 5A showed liver gross specimen of mice, we found that 6-week exercise and exogenous chemerin treatment had no influence on the liver of normal mice; but exercise significantly alleviated fatty liver of DM mice (DM mice appeared pathological characteristic of fatty liver: big, yellow and oil), which was reversed by exogenous chemerin treatment. In addition, exercise-induced reduction in the liver weight of DM mice was reversed by exogenous chemerin treatment (Fig. 5B). As for liver oil red staining, Fig. 5C showed that a lot of lipid drops appeared in the liver of DM mice, and exercise significant decreased the amounts of lipid drops in the liver of DM mice, which was partly reversed by exogenous chemerin treatment.

**Exogenous chemerin treatment reversed the exercise-induced reductions of serum chemerin and chemerin/CMKLR1 in the liver, gastrocnemius and fat of diabetes mice**

Not only serum chemerin but also the protein levels of chemerin in peripheral metabolic organs including liver, gastrocnemius and epididymal fat were increased significantly in DM mice (Fig. 6). After 6-week aerobic exercise, serum chemerin and the protein levels of chemerin in the metabolic organs (liver, gastrocnemius and epididymal fat) of EDM mice were decreased, which were reversed by exogenous chemerin treatment (but only a reversed trend in gastrocnemius). In Con mice, although exercise and exogenous chemerin treatment both had no obvious influence on serum chemerin and chemerin levels in the liver and gastrocnemius, exercise decreased and exogenous chemerin increased the level of chemerin in the epididymal fat.

For chemerin receptor CMKLR1, similar results were found in DM and Con mice. In DM mice, the protein levels of CMKLR1 in the liver, gastrocnemius and epididymal fat were increased, and 6-week aerobic exercise significantly reduced the levels of CMKLR1 in the liver, gastrocnemius and fat of DM mice, which were reversed by exogenous chemerin treatment (Fig. 6). In Con mice, exercise lowered and exogenous chemerin treatment enhanced CMKLR1 in epididymal fat, while no alteration was induced by the exercise or exogenous chemerin in the liver and gastrocnemius of Con mice.

### **Exogenous chemerin treatment reversed exercise-induced increases of ATGL, LPL, GLUT4 and decrease of PEPCK in diabetes mice**

In Con mice, 6-week aerobic exercise increased the levels of ATGL (liver, gastrocnemius and epididymal fat), LPL (liver) and GLUT4 (gastrocnemius and epididymal fat), and exogenous chemerin treatment could decrease ATGL (liver and epididymal fat) and GLUT4 (gastrocnemius) of Con mice. For diabetes mice, 6-week aerobic exercise significantly increased the levels of ATGL, LPL (both in liver, gastrocnemius and epididymal fat) and GLUT4 (in gastrocnemius and epididymal fat) as well as decreased the levels of PEPCK (in liver) of DM mice, which were reversed by exogenous chemerin supplementation except for the LPL in epididymal fat and PEPCK in liver (only a trend) (Fig. 7). These results indicated that exercised-induced changes of ATGL, LPL and GLUT4 in the peripheral metabolic organs of DM mice were mediated by the decreased chemerin.

### **Exogenous chemerin treatment reversed exercise-induced increases of PPAR $\gamma$ in the liver, gastrocnemius and epididymal fat of diabetes mice**

As shown in Fig. 8, 6-week of aerobic exercise significantly enhanced the protein levels of PPAR $\gamma$  in the liver, gastrocnemius and epididymal fat of EDM mice, although aerobic exercise and exogenous chemerin treatment had no obvious influences on Con mice. Furthermore, exogenous chemerin supplementations reversed exercise-induced increases of PPAR $\gamma$  in the liver and epididymal fat of EDM mice compared to EDM mice, while only a reversed trend of PPAR $\gamma$  in the gastrocnemius of EDM mice. These results indicated that exercised-induced increase of PPAR $\gamma$  in DM mice was mediated by the decreased chemerin.

## **Discussion**

# Role of increased chemerin in the glycolipid metabolism disorder of diabetes

Many clinical studies have clearly confirmed the important role of enhanced serum chemerin in the development of type 2 diabetes and the disorder of glucose metabolism in the patients with obesity and diabetes[40]. Furthermore, increased serum chemerin was positively correlated with multiple glucose metabolism parameters (including HbA1c, FBG, serum insulin and HOMA-IR) and lipid profile (including TC, TG and LDL) in the patients with obesity and obesity related diseases [41–44]. Besides serum chemerin, the increases of chemerin in the livers were demonstrated to exert a key role in glucose metabolism disorder of ob/ob mice and db/db mice, and exogenous chemerin administration exacerbated glucose intolerance and decreased tissue glucose uptake in obese/diabetic mice[11]. In addition, studies have reported a close link between enhanced chemerin and IR (vital factor in the development of obesity), for example, excessive chemerin inhibited glucose intake and steatolysis of muscle tissue and lead to IR [45], and reducing the levels of serum chemerin in overweight, obese and type 2 diabetic patients would increase insulin sensitivity[46]. The vital role of chemerin/CMKLR1 in impairment of glycolipid metabolism including IR was demonstrated by chemerin or CMKLR1 knockout mice[47, 48], and CMKLR1 gene silencing improved cardiac dysfunction in diabetic cardiomyopathy rats[49].

Our previous study found similar results in diabetes rats, that up-regulation of serum chemerin and chemerin/CMKLR1 in metabolic organs was likely to be associated with the disorder of glycolipid metabolism in diabetes rats[10], and the present study extended the relationship of enhanced chemerin/CMKLR1 and glycolipid metabolism disorder from diabetes rats to diabetes mice, confirming the important role of chemerin/CMKLR1 in glycolipid metabolism disorder. These results are benefit to clarify adipokine and inflammatory cytokines (including chemerin) related mechanisms of diabetes.

## Exercise-induced decrease of chemerin in diabetes was involved in the improvement of glycolipid metabolism

It has been widely accepted that aerobic exercise improved glycolipid metabolism of obesity and obesity related diseases[12, 13], but the mechanisms remain not fully clarified. An increasing evidences showed that serum chemerin in diabetes patients [2, 18–20] and chemerin/CMKLR1 in the peripheral metabolic organs of diabetes rats[10] were decreased by aerobic exercise, which was involved in exercise-induced improvements of glycolipid metabolism. In the present study, similar results were observed in diabetes mice, that 6-week aerobic exercise reduced serum chemerin concentration and the protein level of chemerin in peripheral metabolism organs (liver, gastrocnemius and epididymal fat), accompanied with the improvement of glycolipid metabolism. Furthermore, exogenous chemerin treatment (from the 3rd to 6th week of exercise intervention) reversed exercise-induced improvement of glycolipid metabolism in diabetes mice, which demonstrated the important role of decreased chemerin in exercise-induced improvement of glycolipid metabolism in diabetes mice.

It is deserved to mention that numerous studies have reported a beneficial effect of weight loss and/or exercise training on chemerin levels, and the present study found no changes in the body weight and food intake of exercised diabetic mice, which suggested exercise alone could reduce the levels of chemerin in serum and metabolic organs of diabetes mice (in a weight loss-independent way).

### **Decreased chemerin by exercise improved glycolipid metabolism of diabetes mice through up-regulating key enzymes and protein (ATGL, LPL and GLUT4) mediated by PPAR $\gamma$**

It has been reported that exercise up-regulated the protein levels of ATGL[50, 51] and LPL[26] in skeletal muscle. Besides, exercise also promoted the expression of ATGL in adipose tissue, which promoted fat metabolism and facilitated conversion of TG into FFA; whereas exercise-induced increases of expression and activity of LPL in skeletal muscle and plasma could reduce the levels of plasma insulin and blood glucose and improve lipid metabolism[52, 53]. In addition, exercise could decrease PEPCK protein level in the liver of HFD-induced obese mice, thus inhibiting endogenous glucose production and subsequently improving IR[54]; and aerobic exercise increased the level of GLUT4 in skeletal muscle of exercised rats, thus promoting glucose uptake in skeletal muscle and speeding up the clearance of glucose[55]. Similar results were found in the present study, that exercise increased the protein levels of ATGL and LPL in the liver and gastrocnemius while decreased PEPCK in diabetic rats and diabetic mice, and GLUT4 in the gastrocnemius and adipose of diabetes mice was detected and showed an increase by exercise, accompanied with exercise-induced improvement of glycolipid metabolism and lowered levels of chemerin in serum and peripheral metabolism organs. More importantly, exogenous chemerin treatment reversed the increases of ATGL, LPL and GLUT4 but not the decrease of PEPCK in diabetes mice, and simultaneously reversed partly exercise-induced improvement of glycolipid metabolism in diabetes mice. These results demonstrated that the effect of decreased chemerin on exercise-induced improvement of glycolipid metabolism in diabetes was fulfilled through increasing metabolism key enzymes (ATGL, LPL) and key protein GLUT4 in peripheral metabolic organs.

The specific links of ATGL, LPL, GLUT4 and PEPCK with chemerin remain unclear. Limit literatures mainly reported *in vitro* experiment results. For example, the expression of chemerin was decreased during lipolysis while LPL level was increased in cultured bovine mature adipocytes. Exogenous chemerin treatment decreased the mRNA level of LPL in mature adipocytes on 8th day post-intervention[38] and in goat intramuscular preadipocyte[56], and greatly changed the mRNA levels of ATGL in mature adipocytes[57]. The modulation of CMKLR1 on GLUT4 was reported in CMKLR1 knockout mice fed with HFD, representing reduced GLUT4 protein level compared to wild-type mice[48]. The present study extended the regulatory effects of chemerin on ATGL, LPL, PEPCK and GLUT4 from *in vitro* to *in vivo* level, and further indicated that the decreased chemerin by exercise exerted its improved role in glycolipid metabolism through increasing the levels of ATGL, LPL and GLUT4 in diabetes mice.

How decreased chemerin by exercise resulted in the increases of key metabolic enzymes and protein (such as ATGL, LPL, PEPCK and GLUT4) in the peripheral metabolic organs of diabetes mice? Until now, researches on this issue are rarely reported. Considering ATGL, LPL, PEPCK and GLUT4 are regulated by

PPAR $\gamma$ , and the important roles of PPAR $\gamma$  in regulating glycolipid metabolism[30, 31] as well as the interaction between chemerin and PPAR $\gamma$  *in vitro*[36–38], we speculated that the modulations of decreased chemerin on exercise-induced changes of ATGL, LPL, PEPCK and GLUT4 were mediated by up-regulating PPAR $\gamma$ . To verify our speculation, two main studies in diabetes rats and diabetes mice were completed, and we found that: (1) in diabetes rats, exercise-induced alterations of ATGL, LPL and PEPCK were reversed by PPAR $\gamma$  inhibitor GW9662 and further strengthened by PPAR $\gamma$  agonist pioglitazone; (2) in diabetes mice, exogenous chemerin treatment reversed exercise-induced alterations of PPAR $\gamma$ , ATGL, LPL and GLUT4 (but not PEPCK) in the liver, gastrocnemius and fat. These results indicated that the decrease of chemerin by exercise increased key metabolic enzymes and protein (ATGL, LPL and GLUT4) in diabetes through up-regulating PPAR $\gamma$ .

It is interesting about the relationship between chemerin and PPAR $\gamma$ . Firstly, chemerin was reported to be a target gene of PPAR $\gamma$  *in vitro* in promoting mesenchymal stem cell adipogenesis[36], and PPAR $\gamma$  agonist reduced chemerin expression in adipose tissue and inhibited chemerin secretion from adipocytes by more than 80%[37]. Our previous work also reported that *in vivo*, exercise-induced decrements of chemerin/CMKLR1 were mediated by PPAR $\gamma$  in diabetic rats[10]. Then, other literatures reported that *in vitro* chemerin promoted preadipocyte differentiation and maturation by up-regulating the levels of PPAR $\gamma$ [36, 38], and the present study indicated the regulation of chemerin on PPAR $\gamma$  in diabetes mice *in vivo*. The interaction between chemerin and PPAR $\gamma$  and its biological significances deserve further study in future.

## Conclusions

The present study demonstrated that the decreased chemerin played important roles in exercise-induced improvements of glycolipid metabolism and fatty liver in diabetes, through increasing glycolipid metabolism key enzymes and protein (ATGL, LPL and GLUT4) in peripheral metabolic organs mediated by PPAR $\gamma$ .

## Abbreviations

CMKLR1: chemerin receptor chemokine-like receptor; PPAR $\gamma$ : peroxisome proliferator activated receptor  $\gamma$ ; ATGL: adipose triglyceride lipase; LPL: lipoprotein lipase; GLUT4: glucose transporter 4; PEPCK: phosphoenolpyruvate carboxykinase; HFD: high fat diet; STZ: streptozotocin; FBG: fasting blood glucose; TG: triglyceride; TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; FINS: fasting insulin; HOMA-IR: homeostasis model assessment of insulin resistance.

## Declarations

### Ethics approval

The animal protocol was approved and the experiments were supervised by the Ethics Committee of Shanghai University of Sport (Approval number: 2018009).

### **Consent for publication**

No applicable

### **Availability of data and materials**

The datasets used during the present study are available from the corresponding author upon reasonable request.

### **Founding**

This work is supported by grants from the National Natural Science Foundation of China (No.31872801) and from Shanghai Key Lab of Human Performance (Shanghai University of Sport) (No. 11DZ2261100).

### **Authors' contributions**

Xiaohui Wang, design the experiments as well as revised the manuscript; Xiaojing Lin performed experiments, analyzed data, made picture and drafted manuscript; Lijun Yin, participate in intraperitoneally injection of chemerin and aerobic exercise intervention in mice; Jing Qu, participate in the detection of body composition and body weight of mice and aerobic exercise intervention in mice.

### **Acknowledgments**

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### **Competing interests**

The authors declare that they have no conflict of interest.

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## Figures

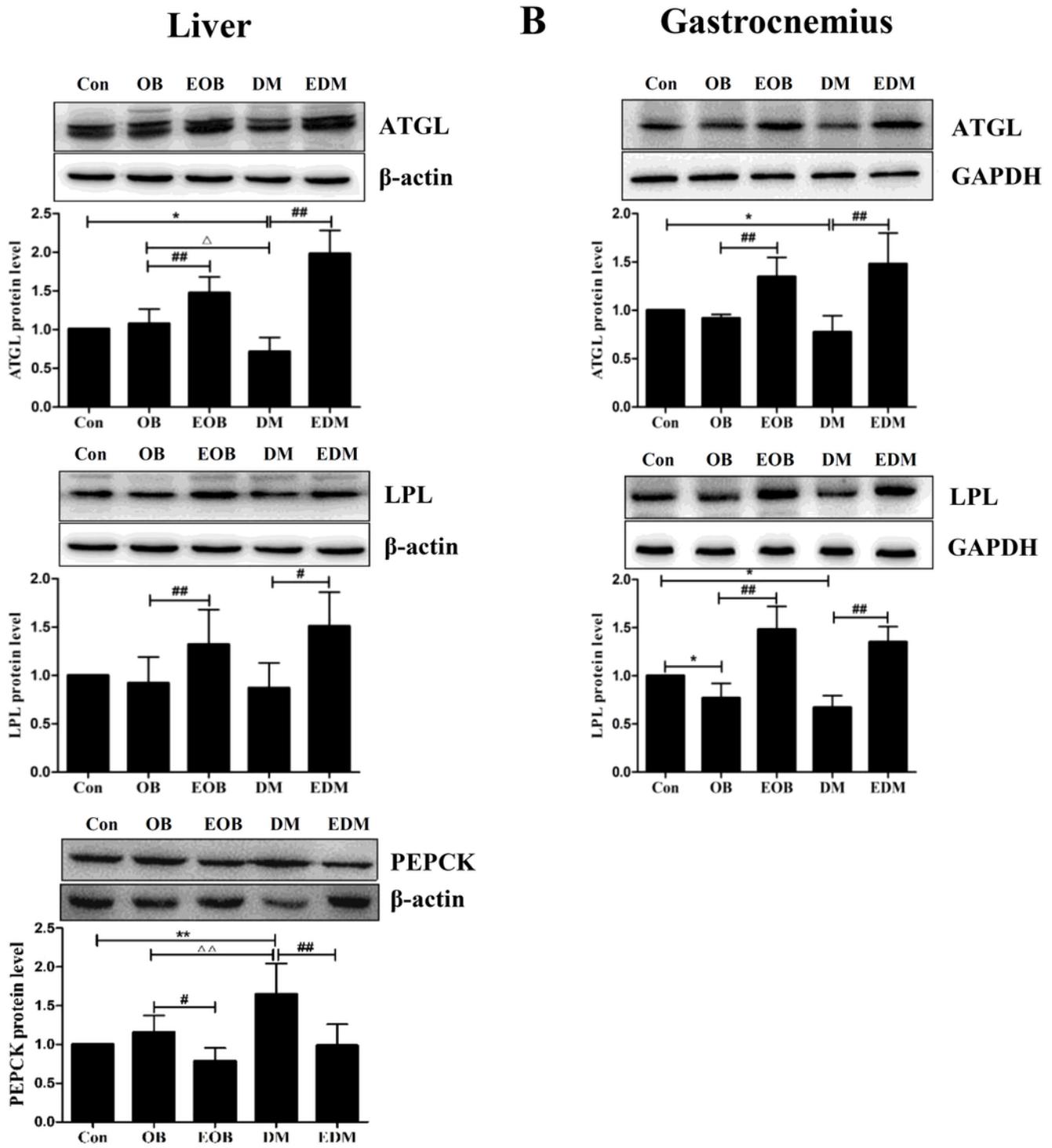
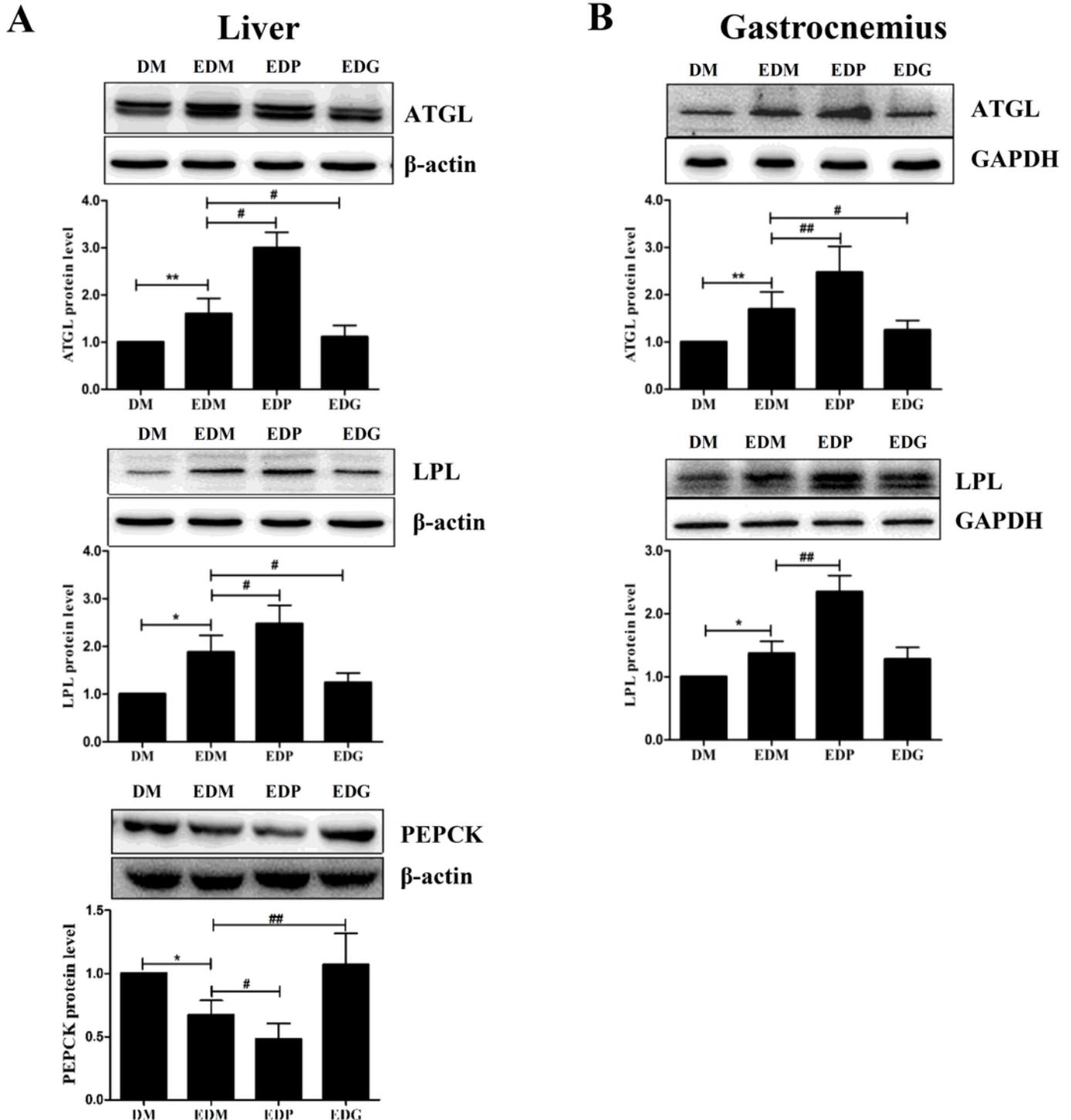


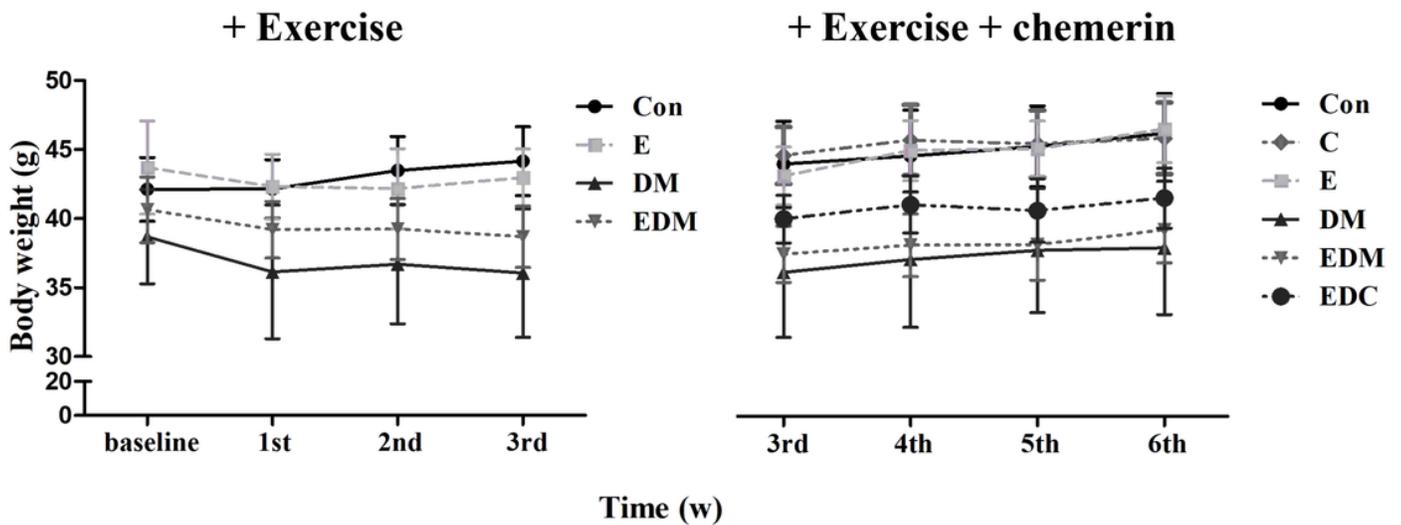
Figure 1

The protein levels of ATGL, LPL and PEPCK were significantly changed by 4-week exercise in the liver (A) and gastrocnemius (B) in diabetic rats. The levels of ATGL and LPL in the livers and gastrocnemius were up-regulated, and PEPCK in the livers was down-regulated by 4-week aerobic exercise in DM and OB rats. The blots of ATGL, LPL and PEPCK were quantified by Tanon software and normalized against  $\beta$ -actin or GAPDH, then the normalized numbers were compared between different groups. Con: control; OB: obesity; EOB: exercised OB; DM: diabetes mellitus; EDM: exercised DM. \* $P < 0.05$ , \*\* $P < 0.01$  vs Con; # $P < 0.05$ , ## $P < 0.01$  EOB vs OB or EDM vs DM;  $\Delta P < 0.05$  DM vs OB.



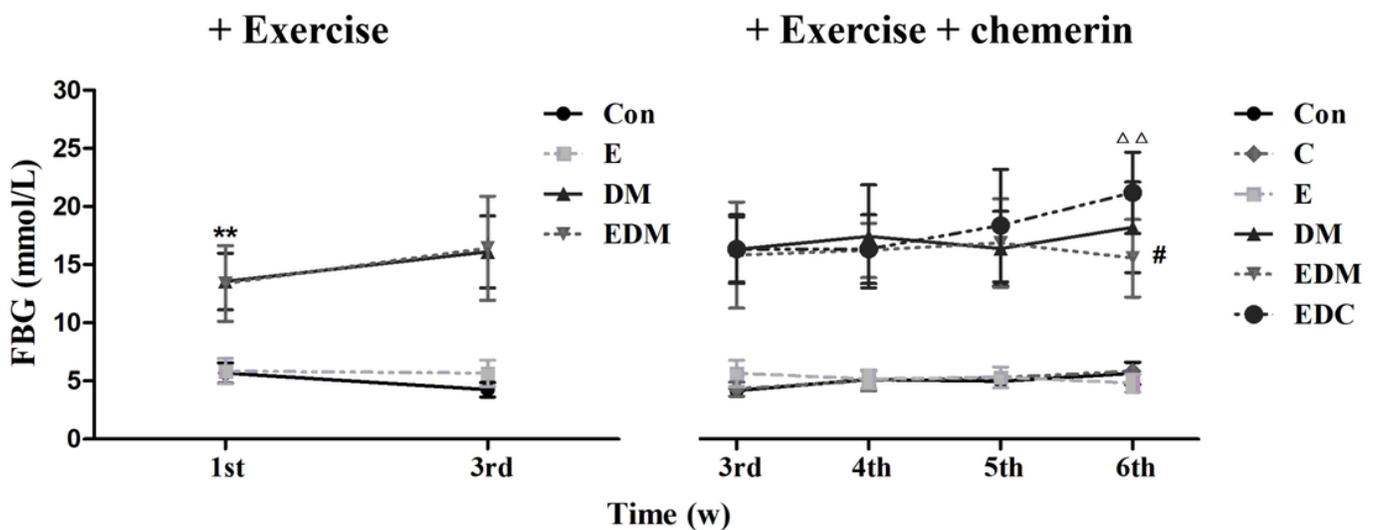
**Figure 2**

Changes of ATGL, LPL and PEPCK in the liver (A) and gastrocnemius (B) of exercise diabetes rats were reversed by PPAR $\gamma$  antagonist and further strengthened by PPAR $\gamma$  agonist. The exercise-induced changes of ATGL (livers and gastrocnemius), LPL (only livers) and PEPCK (livers) at protein levels in EDM rats were reversed by PPAR $\gamma$  antagonist GW9662, and further strengthened by PPAR $\gamma$  agonist pioglitazone. The blots of ATGL and LPL were quantified by Tanon software and normalized against  $\beta$ -actin or GAPDH, then the normalized numbers were compared between different groups. DM: diabetes mellitus; EDM: exercised DM; EDP: exercised DM + pioglitazone; EDG: exercised DM + GW9662. \* $P < 0.05$ , \*\* $P < 0.01$  vs DM. # $P < 0.05$ , ## $P < 0.01$  vs EDM.



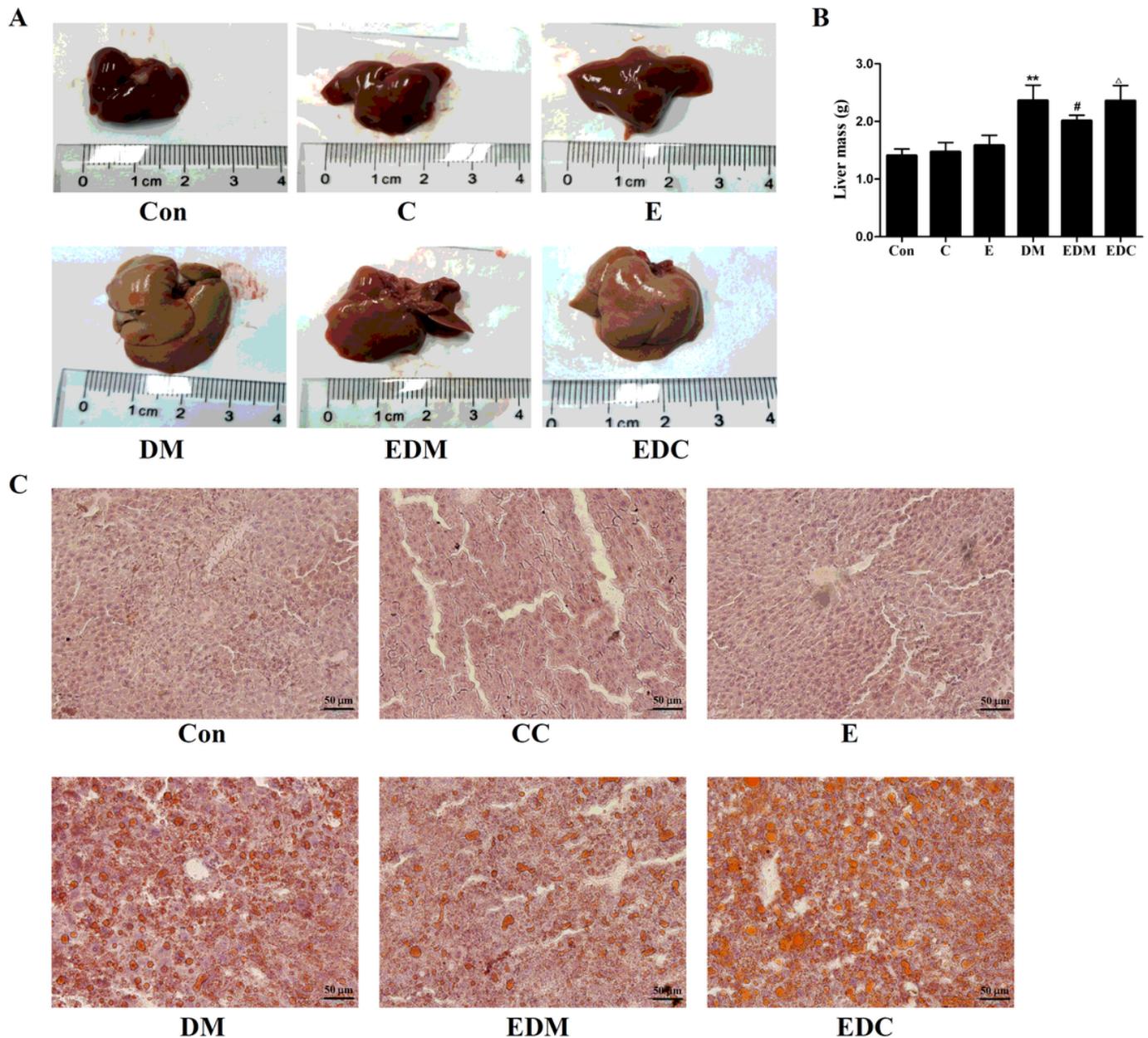
**Figure 3**

No influences of exogenous chemerin treatment on the body weight of exercised diabetic mice. Con: control; C: chemerin treatment; E: exercise; DM: diabetes mellitus; EDM: exercised DM; EDC: EDM + chemerin treatment.



**Figure 4**

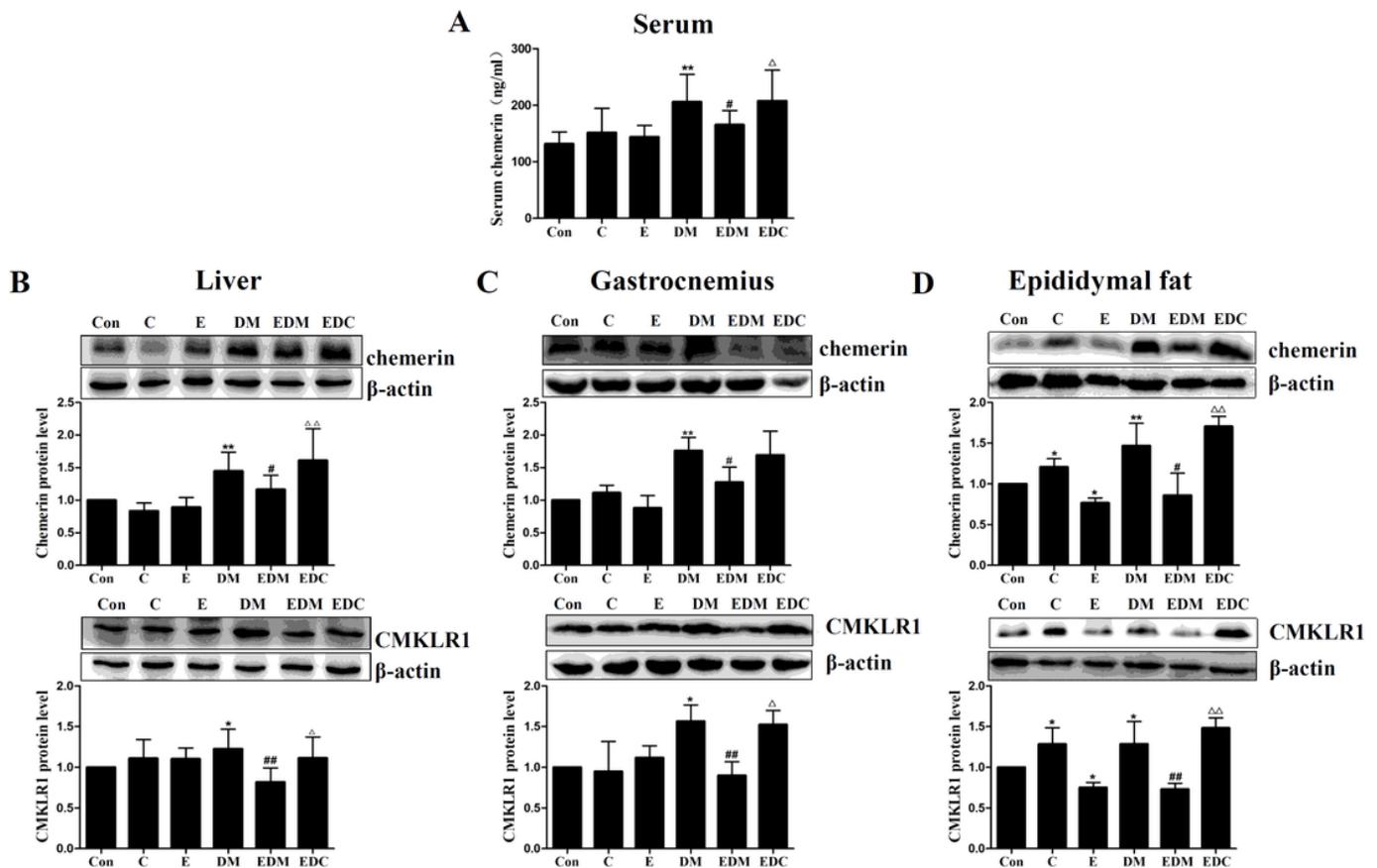
Effects of exogenous chemerin treatment on the levels of FBG of exercised diabetes mice. At 6th week of exercise intervention, exogenous chemerin treatment reversed the exercise-induced decrease of FBG in diabetic mice. Con: control; C: chemerin treatment; E: exercise; DM: diabetes mellitus; EDM: exercised DM; EDC: EDM + chemerin treatment. # $P < 0.05$ , EDM vs DM;  $\Delta\Delta P < 0.01$ , EDC vs EDM.



**Figure 5**

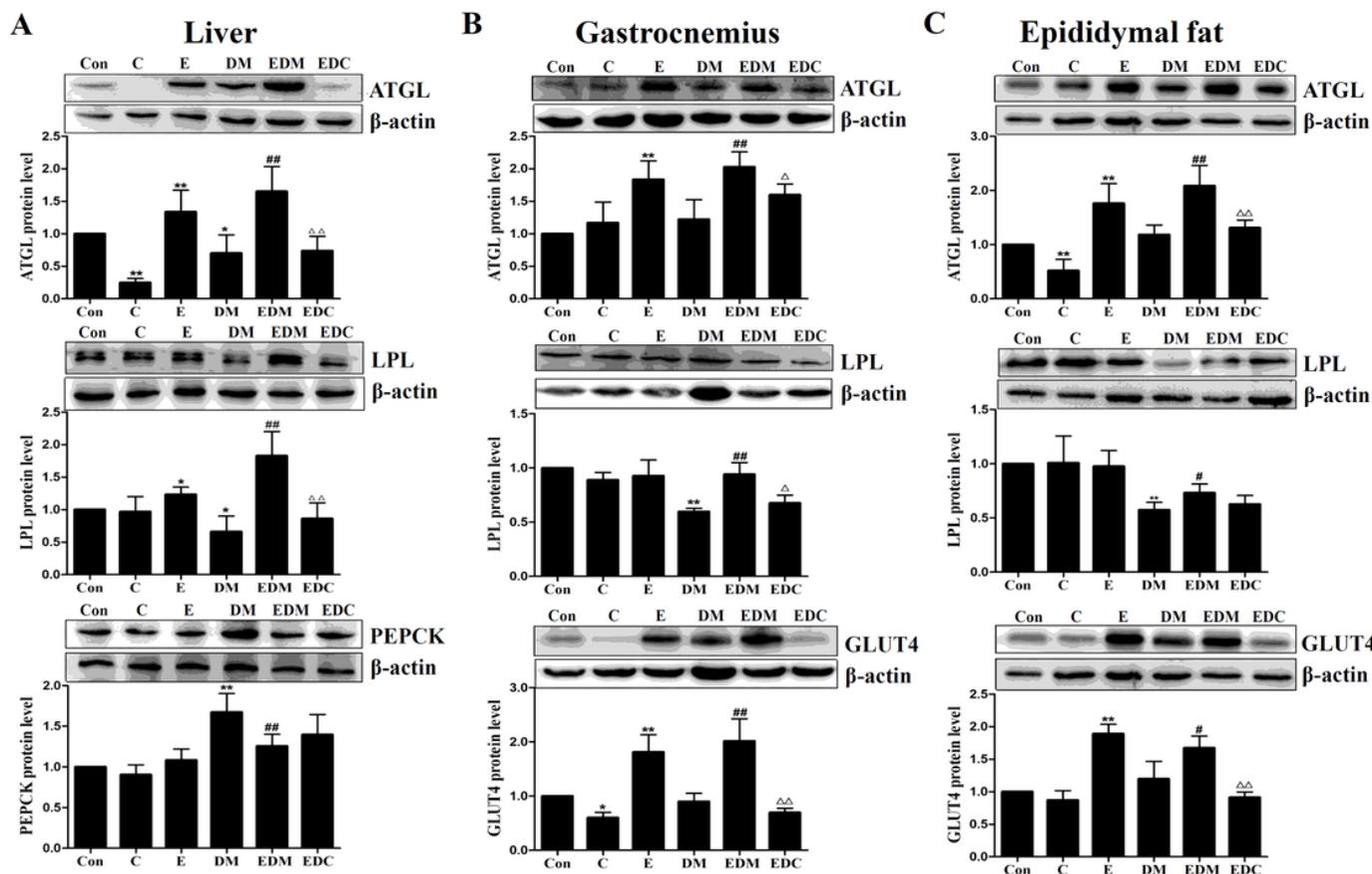
Effects of exogenous chemerin treatment on fatty liver from gross specimen (A), liver weights (B) and oil red staining (C) (400 $\times$ ) of exercised diabetes mice. Con: control; C: chemerin treatment; E: exercise; DM:

diabetes mellitus; EDM: exercised DM; EDC: EDM + chemerin treatment. \*\* $P < 0.01$ , vs Con; # $P < 0.05$ , EDM vs DM;  $\Delta P < 0.05$ , EDC vs EDM.



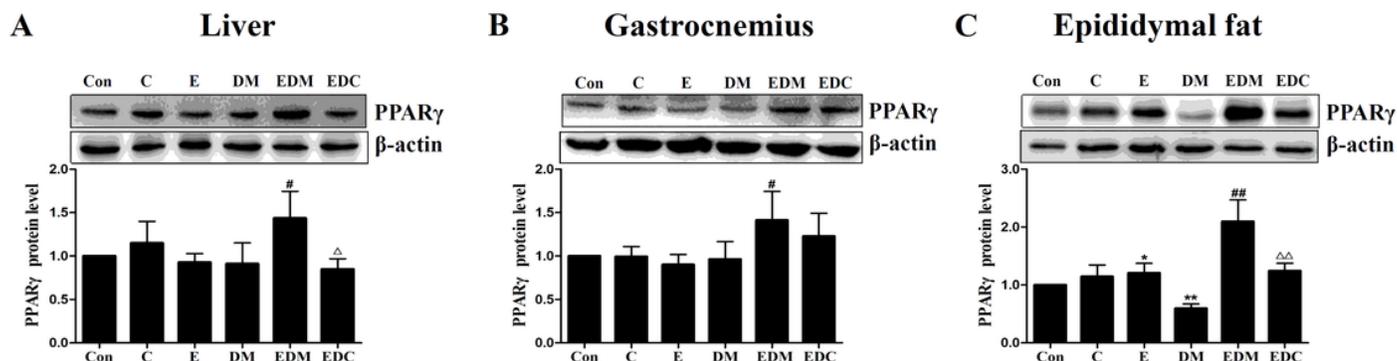
**Figure 6**

Effects of exogenous chemerin treatment on serum chemerin concentration (A) and the protein levels of chemerin and CMKLR1 in the liver (B), gastrocnemius (C) and epididymal fat (D) of exercised diabetes mice. The blots of chemerin and CMKLR1 were quantified by Tanon software and normalized against  $\beta$ -actin, then the normalized numbers were compared and statistically analyzed (bottom). CMKLR1: chemokine-like receptor 1; Con: control; C: chemerin treatment; E: exercise; DM: diabetes mellitus; EDM: exercised DM; EDC: EDM + chemerin treatment. \* $P < 0.05$ , \*\* $P < 0.01$ , vs Con; # $P < 0.05$ , ## $P < 0.01$ , EDM vs DM;  $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$ , EDC vs EDM.



**Figure 7**

Effects of exogenous chemerin treatment on the protein levels of ATGL, LPL, GLUT4 and PEPCK in liver (A), gastrocnemius (B) and perirenal fat (C) of exercised diabetes mice. The blots of ATGL, LPL, GLUT4 and PEPCK were quantified by Tanon software and normalized against  $\beta$ -actin, then the normalized numbers were compared and statistically analyzed (bottom). Con: control; C: chemerin treatment; E: exercise; DM: diabetes mellitus; EDM: exercised DM; EDC: EDM + chemerin treatment. \* $P < 0.05$ , \*\* $P < 0.01$ , vs Con; # $P < 0.05$ , ## $P < 0.01$ , EDM vs DM;  $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$ , EDC vs EDM.



## Figure 8

Effects of exogenous chemerin treatment on the protein levels of PPAR $\gamma$  in liver (A), gastrocnemius (B) and epididymal fat (C) of exercised diabetes mice. The blots of PPAR $\gamma$  were quantified by Tanon software and normalized against  $\beta$ -actin, then the normalized numbers were compared and statistically analyzed (bottom). Con: control; C: chemerin treatment; E: exercise; DM: diabetes mellitus; EDM: exercised DM; EDC: EDM + chemerin treatment. \* $P < 0.05$ , \*\* $P < 0.01$ , vs Con; # $P < 0.05$ , ## $P < 0.01$ , EDM vs DM;  $\Delta$   $P < 0.05$ ,  $\Delta\Delta$   $P < 0.01$ , EDC vs EDM.