JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Apolipoprotein M overexpression through adeno-associated virus gene transfer improves insulin secretion and insulin sensitivity in Goto-Kakizaki rats

Yang Yu[1], Jun Zhang[1], Shuang Yao[1], Lili Pan[1], Guanghua Luo[1]*, Ning Xu[2]*

[1]Comprehensive Laboratory, the Third Affiliated Hospital of Soochow University, Changzhou, China, and [2]Section of Clinical Chemistry and Pharmacology, Institute of Laboratory Medicine, Lunds University, Lund, Sweden

Keywords
Apolipoprotein M, Diabetes, Insulin sensitivity

***Correspondence**
Guanghua Luo
Tel.: +86-519-6887-0619
Fax: +86-519-8662-1235
E-mail address:
shineroar@163.com

Ning Xu
Tel.: +46-46-173487
Fax: +46-46-130064
E-mail address:
ning.xu@med.lu.se

J Diabetes Investig 2020; 11: 1150–1158

doi: 10.1111/jdi.13261

ABSTRACT

Aims/Objective: The development of type 2 diabetes is a result of insulin resistance in various tissues, including skeletal muscle and liver. Apolipoprotein M (ApoM) plays an important role in the function of high-density lipoprotein, and also affects hepatic lipid and glucose metabolism. In this study, we aimed to investigate whether ApoM overexpression modulates glucose metabolism and improves insulin sensitivity.

Materials and Methods: The Goto-Kakizaki (GK) rats were transfected with adeno-associated virus (AAV) encoding rat ApoM gene or control blank. The oral glucose tolerance test (OGTT) and hyperinsulinemic-euglycemic clamp (HEC) experiment were used to assess the insulin sensitivity of GK rats.

Results: The results show that ApoM messenger ribonucleic acid and protein were significantly overexpressed in the pancreatic tissues. Overexpression of ApoM decreased fasting blood glucose and random blood glucose, improved glucose tolerance, and increased bodyweight and insulin levels in GK rats. The glucose infusion rate of rats in the AAV encoding rat ApoM gene group during HEC test was 1.04-, 1.23- and 1.95-fold higher than that in the AAV control blank group at 1–3 weeks after injection of AAV, respectively. A Wes-ProteinSimple assay and quantification was carried out to assess phosphorylated protein kinase B/protein kinase B protein levels in the muscle tissues of ApoM-overexpressing GK rats, and they were found to be higher than those of the control group at the seventh week after AAV injection.

Conclusions: ApoM overexpression through adeno-associated virus gene transfer might improve insulin secretion and insulin sensitivity in GK rats.

INTRODUCTION

Apolipoprotein M (ApoM) is a member of the lipocalin superfamily and is expressed on high-density lipoprotein (HDL)[1]. ApoM is also the carrier of sphingosine-1-phosphate (S1P) in lipoproteins[2]. HDL in type 2 diabetes patients loses the ability to maintain proper endothelial function, possibly as a result of the loss of S1P, and might contribute to the development of diabetic complications[3]. Christoffersen *et al.*[4] showed that ApoM deficiency protects against diet-induced obesity and improves glucose tolerance. Furthermore, we reported that plasma ApoM was decreased by 70% in diabetic mice, ApoM messenger ribonucleic acid (mRNA) levels in the liver were decreased by 40%, and ApoM mRNA levels in the liver and kidneys were increased by the injection of exogenous insulin[5]. SNPs (rs707922 and T-778C) in the *ApoM* promoter are associated with susceptibility to type 2 diabetes and might increase the risk of type 2 diabetes[6,7]. In the present study, to investigate whether ApoM modulates glucose metabolism, ApoM was overexpressed in Goto-Kakizaki (GK) rats, a type 2 diabetes model, by injecting adeno-associated virus (AAV) vectors carrying the ApoM gene; this might help to further explore the effect of ApoM on the pathogenesis of diabetes, and to provide a new theoretical basis for the prevention and treatment of diabetes.

Received 5 July 2019; revised 1 March 2020; accepted 22 March 2020

METHODS

Animals

The present study was approved by the Animal Research Ethics Committees of the Third Affiliated Hospital of Soochow University and performed in accordance with their guidelines. Male GK rats aged 13–15 weeks and weighing 300–350 g (n = 36) were used in the study, and rats fed with a standard rodent chow were housed in a 12-h dark/light cycle and maintained at a controlled temperature (23 ± 2°C).

AAV vectors and transduction of GK rats with AAV vectors

Plasmids encoding AAV2 replication proteins and serotype-specific AAV8 capsid proteins were used. The GK rats were infected with AAV encoding rat ApoM (AAV-ApoM; pAAV-CAG-Apom-EGFP-3FLAG; n = 18; plasmid map and plasmid target gene sequence of rat ApoM is shown in Figure 1) or control blank (AAV-NC; pAAV-CAG-MCSEGFP-3FLAG; n = 18) AAV at a dose of 5×10^{11} vg/200 μL/animal through the tail vein. The expression of enhanced green fluorescent protein of AAV vector was visualized by fluorescence microscopy (Olympus IX73, Tokyo, Japan) in frozen tissue sections.

Animal experiments

Fasting blood glucose (Glucose Meter; Roche, Mannheim, Germany), oral glucose tolerance test (OGTT) and hyperinsulinemic-euglycemic clamp (HEC) experiments were measured 1 week before and 1–3 weeks after AAV transfection. Measurement of insulin levels (enzyme-linked immunosorbent assay kit; Saiweier, Wuhan, China) at the second and sixth weeks after AAV transfection.

OGTT

For the OGTT, overnight-fasted GK rats were challenged by loading glucose solution orally at a dose of 2.5 g/kg bodyweight. Blood samples were collected before glucose load (fasting blood glucose), and 30, 60 and 120 min after oral glucose load. Blood glucose level was measured by blood glucose meter at each time point. The definition of diabetes was determined as fasting plasma glucose (FPG) ≥7.0 mmol/L or 2-h postprandial plasma glucose ≥11.1 mmol/L, during OGTT, according to the 2003 American Diabetes Association criteria. The participants were defined as impaired fasting glucose (5.6 mmol/L ≤ FPG < 7.0 mmol/L) or impaired glucose tolerance (7.8 mmol/L ≤ 2-h postprandial plasma glucose < 11.1 mmol/L)[8].

Hyperinsulinemic-euglycemic clamp

The hyperinsulinemic-euglycemic clamp experiment was carried out by double caudal venipuncture without anesthesia. For the HEC, the GK rats were fasted for 6 h, and a 2-h HEC procedure was carried out. The rate of primed continuous infusion of insulin was 0.6 U/kg/h, and a variable infusion of 25% dextrose was used to adjust the plasma glucose concentration at the basal level. The steady-state clamp period was 80–120 min. The glucose infusion rate was used as a measure of insulin sensitivity.

Real-time polymerase chain reaction

The total RNA was extracted from the liver, muscle, adipose, pancreas, kidney and heart tissues using a total RNA purification kit, according to the manufacturer's instructions. The rat β-actin and ApoM primers and probes were designed using Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto,

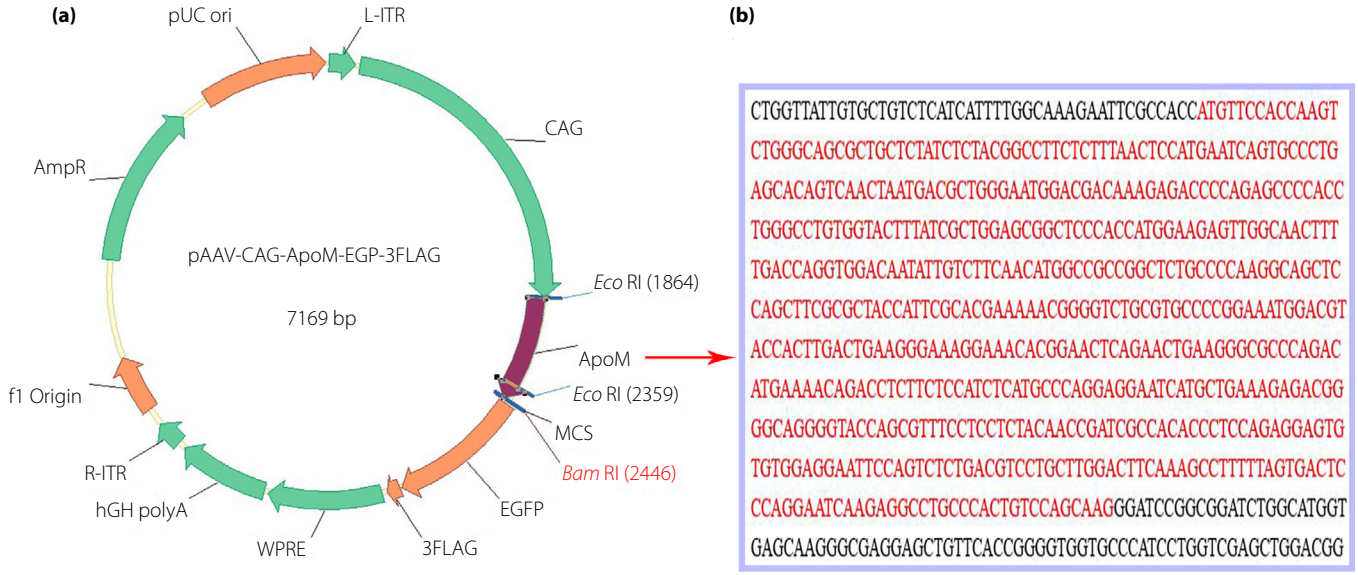

Figure 1 | (a) Plasmid map for the pAAV-CAG-Apom-EGFP-3FLAG vector. Plasmids encoding adeno-associated virus serotype 2 replication proteins and serotype-specific adeno-associated virus capsid proteins were used. (b) The size of the plasmid target gene of rat apolipoprotein M (ApoM) is approximately 570 bp. The red part is the plasmid target gene sequence of rat ApoM.

CA, USA) (Table 1). β-Actin was used as a reference gene. Quantification of ApoM mRNA levels was relative to β-actin mRNA levels and was carried out using the LightCycler 480 at a final volume of 25 μL. Optimum reaction conditions were obtained with 10× polymerase chain reaction buffer (2.5 μL), 25 mmol/L Mg^{2+} (2.5 μL), 10 mmol/L 4× deoxyribonucleotide triphosphates (0.5 μL), 5 U/μL Taq deoxyribonucleic acid polymerase (0.25 μL), the 100 μmol/L primers and probe were 0.04 μL each and 2 μL template complementary deoxyribonucleic acid. Finally, 17.13 μL ddH_2O was added to the reaction mixture. The following polymerase chain reaction procedure was used: 3 min at 95°C. Subsequently, a 40-cycle two-step polymerase chain reaction was carried out consisting of 5 s at 95°C and 10 s at 61°C (ApoM: 12 s at 58°C).

Protein was analyzed by western blotting using the Wes-ProteinSimple system

At the first (n = 6 for each group), 2nd (n = 3 for each group), third (n = 3 for each group) and seventh (n = 6 for each group) week after AAV transfection, the GK rats were injected intraperitoneally with insulin after fasting for 12 h. After 30 min of insulin injection, the serum, liver, muscle, adipose, pancreas, kidney and heart tissues were taken separately for protein kinase B (AKT) phosphorylation and protein detection. Total protein was extracted from the liver, muscle, epididymis adipose and heart tissues of GK rats using a total protein extraction kit (BestBio, Shanghai, China), in accordance with the manufacturer's instructions. The protein concentration was assayed using the BCA Protein Assay kit (BestBio). The Simple Western (Wes-ProteinSimple, San Jose, CA, USA) system was used to quantify the ApoM (anti-apoM; Abnova, Taipei, China), phosphorylated AKT (p-AKT) (anti-p-AKT, CST, Danvers, MA, USA), total AKT (anti-AKT; CST), β-actin (anti-β-actin, CST) and tubulin (anti-tubulin; Abcam, Cambridge, MA, USA) in the liver, muscle, epididymis adipose and heart tissues of GK rats.

Detection of related indexes in serum

We determined HDL cholesterol (Cholesterol HDL test kit; Jingyuan, Shanghai, China), glucose (Glucose test kit based on glucose oxidase method; Lindeman, Beijing, China), C-reactive protein (Immunoturbidimetric assay of C-Reactive Protein test kit; Jien, Shanghai, China) and triacylglycerol (Triglycerides kit based on GPO-PAP method; Kehua, Shanghai, China) in serum samples from GK rats at the seventh week after AAV injection (Beckman Coulter AU5800, Tokyo, Japan). The expression level of ApoM protein in serum at the seventh week after AAV injection was detected by Wes-ProteinSimple system.

Table 1 | Sequences of primers and probes

Genes	Primer/probe	Sequence (5′–3′)
Rat ApoM	Forward primer	ACAAAGAGACCCCAGAGCCC
	Reverse primer	TCCATGGTGGGAGCCG
	Probe	FAM-ACCTGGGCCTGTGGTACTTTATT GCTGG-TAMRA
Rat β-Actin	Forward primer	GCCACTGCCGCATCCTCT
	Reverse primer	CTGGAAGAGAGCCTCGGGG
	Probe	FAM-AGCTGCCTGACGGTCAGGTCA TCACTATC-TAMRA

ApoM, apolipoprotein M.

Statistical analysis

Data are expressed as the mean ± standard error of the mean and were analyzed using Student's t-test (GraphPad Prism 6.0 software; GraphPad Software Inc., La Jolla, CA, USA). Differences among more than two groups were assessed using a one-way ANOVA, followed by multiple comparison tests. Significance was established at a P-value <0.05.

RESULTS

ApoM gene transfer in different tissues between the two groups

At the second and third week after AVV injection, the liver, muscle, adipose, pancreatic, kidney and heart tissues of the AAV-apoM group GK rats were taken to prepare frozen sections. The results showed that strong enhanced green fluorescent protein green fluorescence was expressed in all the tissues, showing that the ApoM overexpression plasmid had been successfully transfected into the rats (Figure 2). At the third week after AAV transfection, ApoM mRNA (Figure 3a) and protein (Figure 3b) were overexpressed in the pancreatic tissue ($P < 0.05$). However, the ApoM mRNA and protein were not overexpressed in any other tissues, including liver, muscle, adipose tissue, kidney and heart.

ApoM overexpression in GK rats decreased blood glucose, but increased bodyweight and insulin levels

Fasting blood glucose levels of rats were significantly lower in the AAV-ApoM group than that in AAV-NC group at the third week after AAV injection (Figure 4a; $P < 0.01$). After AAV transfection, the random blood glucose levels of the AAV-ApoM group were significantly lower compared with those in the AAV-NC group in the second week and remained significantly decreased in the fourth and sixth weeks (Figure 4b; $P < 0.001$). Compared with the AAV-NC group, the bodyweight of the AAV-ApoM group at fifth and sixth weeks after AAV transfer was significantly increased (Figure 4c; $P < 0.01$), and the insulin levels in the AAV-ApoM group were also significantly increased in the sixth week (Figure 4d; $P < 0.01$).

Hyperinsulinemic-euglycemic clamp and oral glucose tolerance test

The result of OGTT test (Table 2) showed the blood glucose levels at the first week (at 60 and 120 min), second week (at 30, 60 and 120 min) and third week (at 0, 30, 60 and 120 min) after AAV transfection in the AAV-ApoM group were significantly lower than those in the AAV-NC group ($P < 0.05$). The glucose infusion rate of rats in the AAV-ApoM group during

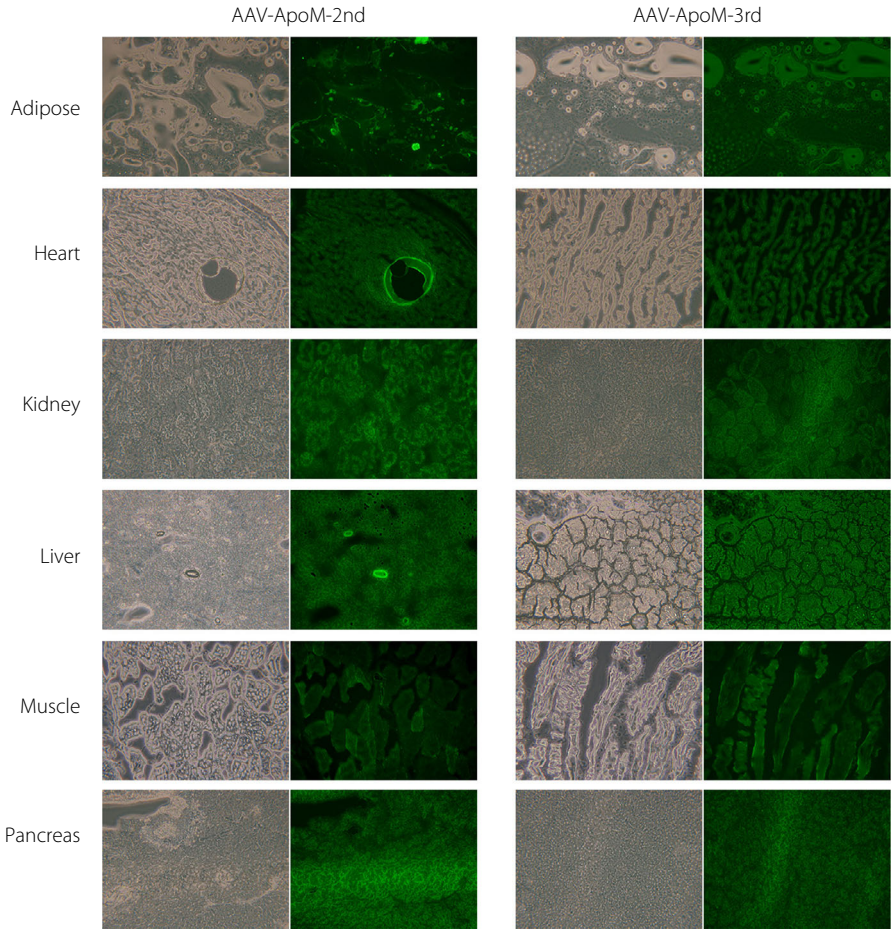

Figure 2 | At the second and third week after adeno-associated virus (AVV) injection, the liver, muscle, adipose, pancreatic, kidney and heart tissues of the AAV encoding rat apolipoprotein M (AAV-ApoM) group Goto-Kakizaki rats were taken to prepare frozen sections. The expression of enhanced green fluorescent protein of AAV vector was visualized by fluorescence microscopy (Olympus IX73) in frozen tissue sections.

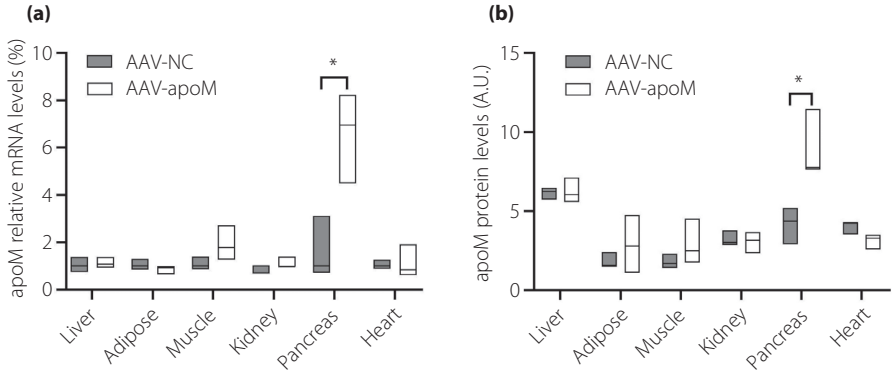

Figure 3 | The reverse transcription polymerase chain reaction results are shown for (a) apolipoprotein M (ApoM) messenger ribonucleic acid (mRNA), and (b) the Wes-ProteinSimple and its quantification are shown for ApoM in the liver, epididymis adipose, muscle, kidney, pancreatic and heart tissues of Goto-Kakizaki rats treated with adeno-associated virus (AAV) control blank (AAV-NC; $n = 3$) or AAV encoding rat ApoM (AAV-ApoM; $n = 3$) at a dose of 5×10^{11} vg/200 μL/rat for 3 weeks. *$P < 0.05$.

hyperinsulinemic-euglycemic clamp test was 1.04-, 1.23- and 1.95-fold higher than that in the AAV-NC group at 1–3 weeks after injection of AAV, respectively. ($P < 0.0001$; Figure 5b–d).

ApoM overexpression augmented the phosphorylation of AKT in GK rats

As shown in Figure 6, a representative Wes-ProteinSimple assay and quantification is shown for p-AKT and total AKT protein levels in the tissue of GK rats at the seventh week after the injection. The levels of p-AKT/AKT in the muscle were significantly higher in GK rats receiving AAV-ApoM vectors than in those receiving AAV-NC.

Expression of HDL, glucose, C-reactive protein, triacylglycerol and ApoM in serum from GK rats

The results showed that only glucose in the AAV-ApoM group was decreased by 1.6-fold compared with that in the AAV-NC group, and the expression level of ApoM protein in serum was significantly higher in GK rats receiving AAV-ApoM vectors than in those receiving AAV-NC at the seventh week after AAV injection (as shown in Figure 7; $P < 0.05$). There was no significant difference in serum ApoM expression between the two groups from the first week and the third week after AAV transfection (data not shown).

DISCUSSION

Diabetes is a serious chronic metabolic disease characterized by hyperglycemia, mainly caused by insulin deficiency or insulin resistance. Type 2 diabetes is the most common type, and its causes are relatively complex; in addition to insulin secretion, insulin resistance and other factors, the target organs of insulin, such as the liver, muscle and adipose tissue, might be involved[9,10]. Studies have shown that a decrease in the number and function of pancreatic β-cells is an important factor in the development of type 2 diabetes[11]. In addition, diabetic dyslipidemia is characterized by high levels of triglyceride-rich lipoproteins and low concentrations of HDL cholesterol. In addition, preβ-HDL in diabetes patients is less able to induce cellular cholesterol efflux[12]. Reportedly, in men with type 2 diabetes mellitus, elevated plasma S1P levels are associated with blood glucose levels and fat mass accumulation[13]. ApoM is mainly enriched in HDL. It plays an important role in the modulation of cholesterol efflux by influencing preβ-HDL formation. In HDL, S1P is bound to ApoM[14], which through its action on endothelial S1P1 receptors contributes to the vasculoprotective action of HDL[15]. ApoM augments insulin secretion by maintaining the S1P concentration under both *in vivo* and *in vitro* conditions[16]. Leptin and hyperglycemia downregulates serum ApoM and liver ApoM mRNA levels in rats[17,18], and

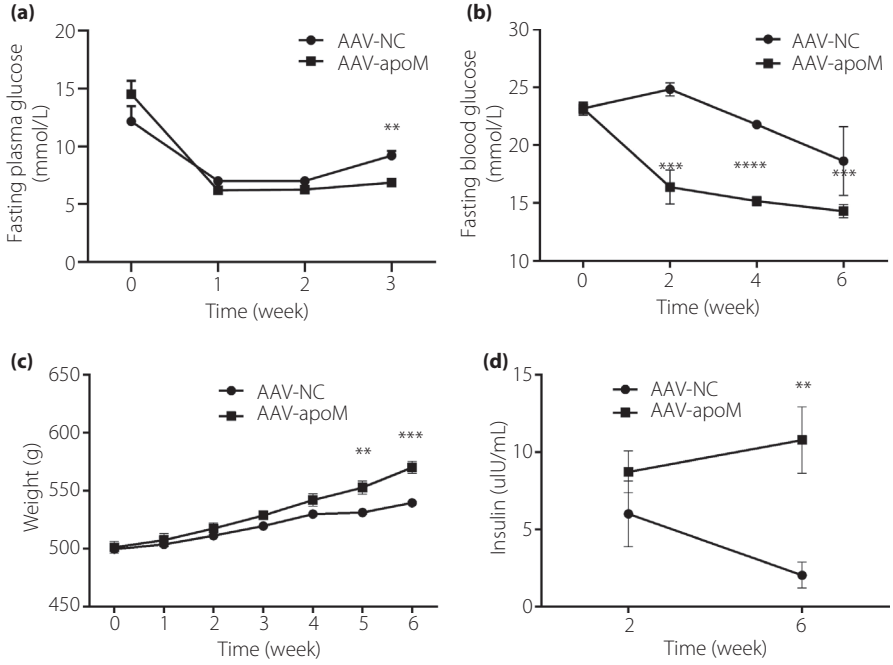

Figure 4 | After adeno-associated virus (AAV) transfection, (a) the fasting plasma glucose levels were detected in the first ($n = 6$ for each group), second ($n = 3$ for each group) and third ($n = 3$ for each group) week; (b) the random blood glucose levels were detected in the second, fourth and sixth weeks ($n = 6$ for each group). (c) The bodyweights were detected between weeks 0 and 6 ($n = 6$ for each group). (d) The insulin levels were detected in the second and sixth weeks ($n = 6$ for each group). $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. AAV-ApoM, adeno-associated virus encoding rat apolipoprotein M; AAV-NC, adeno-associated virus control blank.

the latter significantly decreases insulin sensitivity in rats, whereas rosiglitazone, an insulin sensitizer, significantly reverses this effect and enhances liver ApoM expression in rats[19]. Based on these findings, we speculate that ApoM might be involved in glucose metabolism, and that overexpression of ApoM might improve insulin sensitivity.

Table 2 | Results of oral glucose tolerance test of all groups

Group	0 min	30 min	60 min	120 min
Before injection of AAV				
NC	12.16 ± 1.33	25.94 ± 0.60	29.51 ± 0.65	31.18 ± 0.38
ApoM	14.50 ± 1.15	25.22 ± 0.52	28.03 ± 0.30	29.60 ± 0.81
1st week				
NC	7.01 ± 0.29	24.30 ± 0.90	26.85 ± 0.43	27.83 ± 0.51
ApoM	6.22 ± 0.30	22.38 ± 0.62	24.50 ± 0.60**	25.96 ± 0.55*
2nd week				
NC	7.00 ± 0.22	24.28 ± 0.71	26.80 ± 0.75	28.42 ± 0.58
ApoM	6.28 ± 0.34	21.07 ± 0.83*	23.53 ± 0.23**	25.08 ± 0.39***
3rd week				
NC	9.20 ± 0.40	23.60 ± 0.70	27.47 ± 0.66	26.97 ± 0.74
ApoM	6.87 ± 0.09**	20.90 ± 0.40*	23.83 ± 0.83*	22.47 ± 0.58**

Data are expressed as the mean ± standard error of the mean. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. AAV, adeno-associated virus; ApoM, apolipoprotein M; NC, control blank.

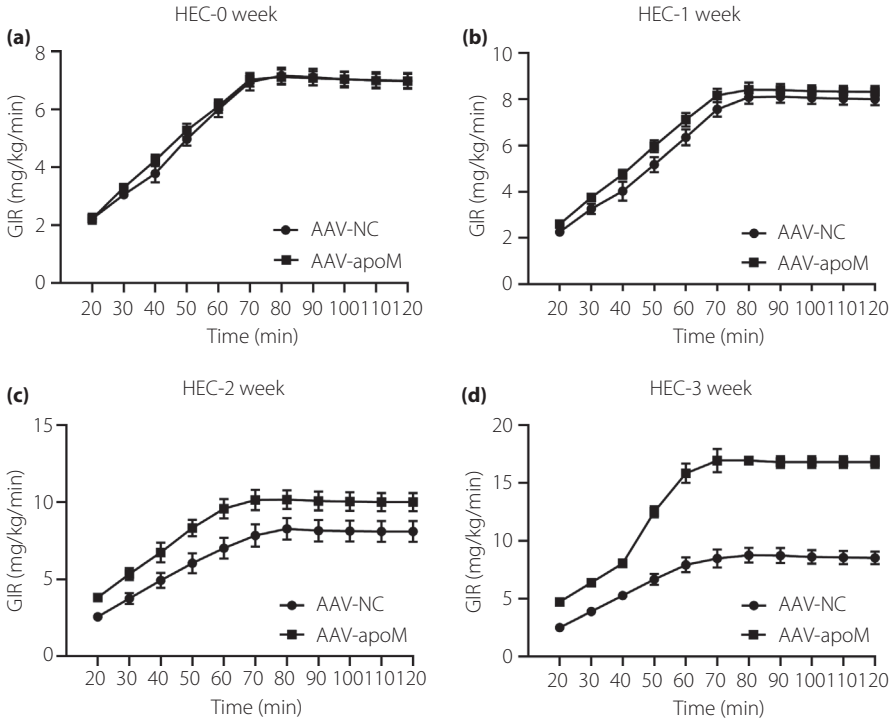

Figure 5 | For the hyperinsulinemic-euglycemic clamp (HEC), Goto-Kakizaki rats were fasted for 6 h, and a 2-h HEC procedure was carried out. The rate of primed continuous infusion of insulin was 0.6 U/kg/h, and a variable infusion of 25% dextrose was used to adjust the plasma glucose concentration at the basal level. The glucose infusion rate (GIR) was used as a measure of insulin sensitivity. (a) Before adeno-associated virus (AAV) injection ($n = 12$ for each group), (b) The first week after AAV injection ($n = 12$ for each group). (c) The second week after AAV injection ($n = 6$ for each group). (d) The third week after AAV injection ($n = 3$ for each group). AAV-ApoM, adeno-associated virus encoding rat apolipoprotein M; AAV-NC, adeno-associated virus control blank.

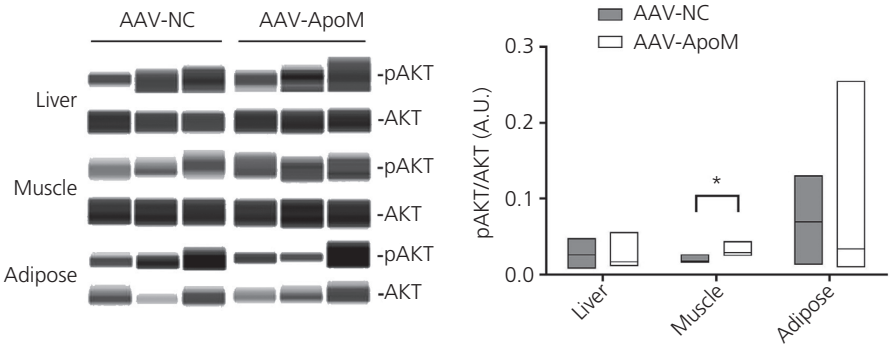

Figure 6 | Representative Wes-ProteinSimple assay and quantification is shown for phosphorylated protein kinase B (pAKT) and total protein kinase B (AKT) protein levels in the liver, muscle and epididymis adipose tissue of GK rats at the seventh week after the AAV injection ($n = 6$ for each group). *$P < 0.05$. AAV-ApoM, adeno-associated virus encoding rat apolipoprotein M; AAV-NC, adeno-associated virus control blank.

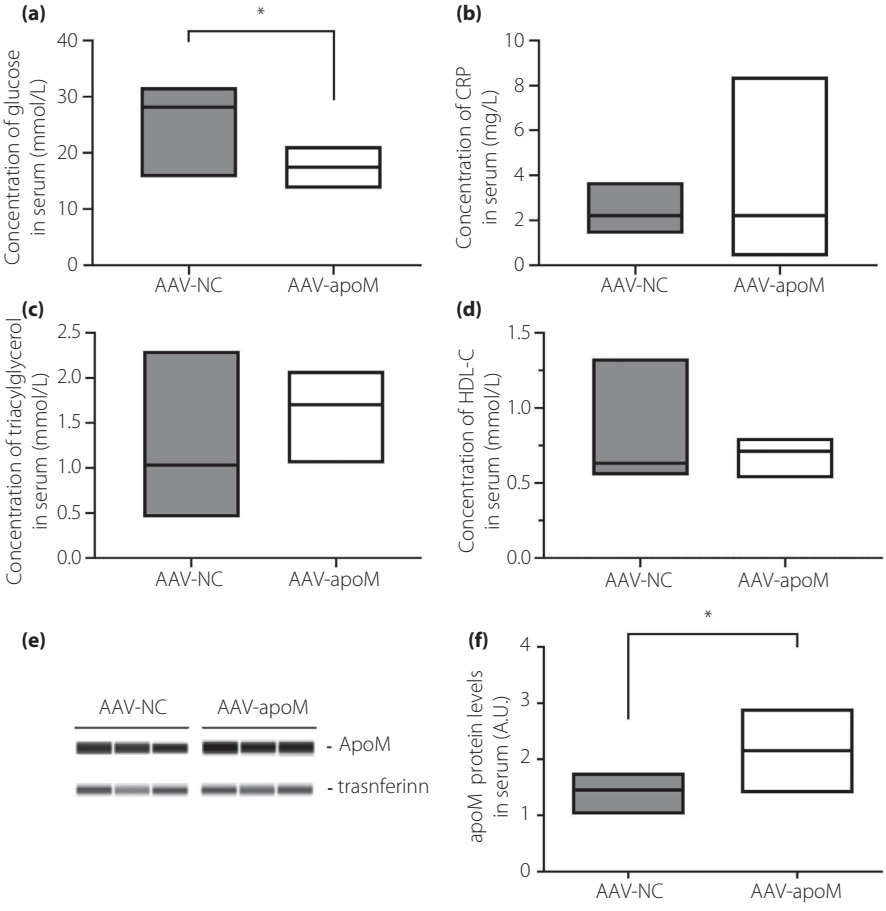

Figure 7 | (a) Serum glucose, (b) C-reactive protein (CRP), (c) triacylglycerol and (d) high-density lipoprotein cholesterol (HDL-C) concentration was measured by Beckman Coulter AU5800 after adeno-associated virus (AAV) injection at the seventh week in Goto-Kakizaki rats. (e) Representative Wes-ProteinSimple assay and quantification is shown for apolipoprotein M (ApoM) protein levels in the serum of Goto-Kakizaki rats at the seventh week after the AAV injection ($n = 6$ for each group). *$P < 0.05$. AAV-ApoM, adeno-associated virus encoding rat apolipoprotein M; AAV-NC, adeno-associated virus control blank.

In addition, it was shown that ApoM deficiency protects against diet-induced obesity and improves glucose tolerance. However, in the present study, female ApoM-knockout mice were used[4]. Previous studies have shown that estrogen upregulates the expression of ApoM through estrogen receptor α[20]. GK rats have been shown to be a reliable model for studying type 2 diabetes mellitus[21]. GK rats are a non-obese and spontaneous type 2 diabetes experimental animal model[21]; thus, in the present study, interfering factors, such as obesity, can be excluded. The model is similar to human type 2 diabetes patients in terms of insulin secretion defects and complications[22]. This study used an AAV2 vector genome pseudo-serotyped with the type 8 capsid (AAV2/8), which has recently shown exciting promise as an effective liver-directed gene transfer agent[23]. The expression of enhanced green fluorescent protein of AAV vector was readily visualized by fluorescence microscopy in frozen tissue sections. The results showed that ApoM overexpression plasmid had been successfully transfected into the rats. However, overexpression of ApoM mRNA and protein was only detected in pancreatic tissues at the third week after AAV transfection. This might be due to different AAV serotypes having different tropism or infective cell or tissue types. AAV serotype 8 showed better transduction efficiency in pancreatic tissue[24]. The endocrine pancreas is a key player in the regulation of blood glucose levels by secreting insulin, glucagon, somatostatin and pancreatic polypeptide into the blood[25]. As shown in Figure 4d, insulin levels also increased significantly after AAV transfection. Therefore, we hypothesized that the overexpression of ApoM in pancreatic tissue could increase insulin secretion and decrease blood glucose. In addition, at the third week after AAV transfection, there were significant differences in fasting blood glucose, OGTT and clamp test results when ApoM was overexpressed in pancreatic tissue. We can see from Figure 4a that the blood glucose levels of both groups decreased significantly in the first week after AAV injection. This might be because a week before the start of the formal experiment, GK rats were fed with a high-fat diet to induce diabetes. After the beginning of the experiment, all the rats were fed with ordinary basic feed, so the fasting blood glucose decreased sharply in the first week after the injection of the virus. However, the fasting blood glucose of GK rats in the AAV-ApoM group was maintained at the same low level at the first to third weeks, indicating that the increase of ApoM in pancreatic tissue can maintain the stability of fasting blood glucose in GK rats, and random blood glucose also verified this result. The OGTT is widely used to evaluate apparent insulin resistance in various clinical settings[26]. According to the analysis of the results, the FPG was ≥7.0 mmol/L and 2-h postprandial plasma glucose was ≥11.1 mmol/L in the rats of the NC group, which proved that the experimental animals were in line with the diabetic model. In the AAV-ApoM group, the FPG was <7.0 mmol/L and ≥5.6 mmol/L, GK rats changed from a diabetic state to an impaired fasting glucose state, indicating that the overexpression of ApoM significantly improved the glucose tolerance of GK rats.

In the present study, the bodyweights of rats in the AAV-ApoM group at the fifth and sixth weeks after AAV transfer were significantly higher than those in the AAV-NC group. GK rats showed a significant increase in bodyweight with age. However, studies have shown that GK rats are resistant to weight gain, showing 28% less weight gain when compared with Wistar rats of the same age[21]. After ApoM overexpression, the bodyweights and insulin levels of the GK rats increased significantly, and the blood glucose levels decreased significantly, which might be due to the fact that ApoM could improve insulin resistance and increase insulin sensitivity to alleviate weight loss caused by the body's inability to fully utilize glucose. In order to further evaluate the effect of ApoM on insulin sensitivity in GK rats, we used hyperinsulinemic-normal glucose clamp technology to study two groups of GK rats. HEC is the gold standard method of assessing insulin sensitivity[27]. It can also exclude the effect of endogenous insulin and glycemic factors. In the steady-state phase of the clamp, the glucose infusion rate of rats in the AAV-ApoM group was significantly higher than that in the AAV-NC group from the first week to the third week after AAV transfection, indicating that ApoM overexpression can increase the utilization of glucose in the peripheral tissues of GK rats, thereby increasing their sensitivity to insulin.

Hyperglycemia in type 2 diabetes is the result of insulin resistance in the liver and periphery (mainly muscle), as well as defects in insulin secretion. AKT is the downstream insulin signaling protein, a serine kinase that is activated by insulin and other growth factors[28]. Because decreased AKT phosphorylation results in impaired insulin receptor signal transduction, some catabolic pathways become active in muscle, eventually leading to protein degradation[29]. The present results suggest that the levels of p-AKT/AKT in the muscle tissue of rats receiving AAV-ApoM vectors were significantly higher than those of rats receiving AAV-NC. These results suggest that ApoM overexpression can decrease the level of blood glucose in GK rats, which in turn stimulates AKT phosphorylation in muscle tissue. We hypothesize that ApoM overexpression might improve the impaired insulin receptor pathway in GK rats, allowing insulin to bind to insulin receptors in muscle tissue, thereby facilitating the entry of glucose into the muscle and its full utilization. However, the specific mechanism requires further study.

These data suggest that *ApoM* might reduce blood glucose, improve weight loss and insulin resistance due to diabetes.

ACKNOWLEDGMENTS

The authors thank all participants for their cooperation and sample contribution. This research was supported by the Natural Science Foundation of Jiangsu province (BK20151179), the International Cooperation Foundation of Changzhou (CZ20150012) and the Changzhou High-Level Medical Talents Training Project (No: 2016ZCLJ002).

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Xu N, Dahlback B. A novel human apolipoprotein (apoM). *J Biol Chem* 1999; 274: 31286–31290.
2. Christoffersen C, Obinata H, Kumaraswamy SB, *et al*. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. *Proc Natl Acad Sci USA* 2011; 108: 9613–9618.
3. Vaisar T, Couzens E, Hwang A, *et al*. Type 2 diabetes is associated with loss of HDL endothelium protective functions. *PLoS ONE* 2018; 13: e0192616.
4. Christoffersen C, Federspiel CK, Borup A, *et al*. The Apolipoprotein M/S1P axis controls triglyceride metabolism and brown fat activity. *Cell Rep* 2018; 22: 175–188.
5. Xu N, Nilsson-Ehle P, Ahren B. Suppression of apolipoprotein M expression and secretion in alloxan-diabetic mouse: partial reversal by insulin. *Biochem Biophy Res Commun* 2006; 342: 1174–1177.
6. Zhou JW, Tsui SK, Ng MC, *et al*. Apolipoprotein M gene (APOM) polymorphism modifies metabolic and disease traits in type 2 diabetes. *PLoS ONE* 2011; 6: e17324.
7. Niu N, Zhu X, Liu Y, *et al*. Single nucleotide polymorphisms in the proximal promoter region of apolipoprotein M gene (apoM) confer the susceptibility to development of type 2 diabetes in Han Chinese. *Diabetes Metab Res Re* 2007; 23: 21–25.
8. Kim HJ, Choi EY, Park EW, *et al*. The utility of HbA1c as a diagnostic criterion of diabetes. *Korean J Fam Med* 2011; 32: 383–389.
9. Kovacs P, Stumvoll M. Fatty acids and insulin resistance in muscle and liver. *Best Pract Res Clin Endocrinol Metab* 2005; 19: 625–635.
10. Creely SJ, McTernan PG, Kusminski CM, *et al*. Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. *Am J Physiol Endocrinol Metab* 2007; 292: E740–E747.
11. Ashcroft FM, Rorsman P. Diabetes mellitus and the beta cell: the last ten years. *Cell* 2012; 148: 1160–1171.
12. Cavallero E, Brites F, Delfly B, *et al*. Abnormal reverse cholesterol transport in controlled type II diabetic patients. Studies on fasting and postprandial LpA-I particles. *Arterioscler Thromb Vasc Biol* 1995; 15: 2130–2135.
13. Tanaka S, Kanazawa I, Sugimoto T. Visceral fat accumulation is associated with increased plasma sphingosine-1-phosphate levels in type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2018; 143: 146–150.
14. Ruiz M, Okada H, Dahlback B. HDL-associated ApoM is anti-apoptotic by delivering sphingosine 1-phosphate to S1P1 & S1P3 receptors on vascular endothelium. *Lipids Health Dis* 2017; 16: 36.
15. Frej C, Mendez AJ, Ruiz M, *et al*. A shift in ApoM/S1P between HDL-particles in women with type 1 diabetes mellitus is associated with impaired anti-inflammatory effects of the ApoM/S1P Complex. *Arterioscler Thromb Vasc Biol* 2017; 37: 1194–1205.
16. Kurano M, Hara M, Tsuneyama K, *et al*. Induction of insulin secretion by apolipoprotein M, a carrier for sphingosine 1-phosphate. *Biochim Biophys Acta* 2014; 1841: 1217–1226.
17. Luo G, Hurtig M, Zhang X, *et al*. Leptin inhibits apolipoprotein M transcription and secretion in human hepatoma cell line, HepG2 cells. *Biochim Biophys Acta* 2005; 1734: 198–202.
18. Zhang X, Jiang B, Luo G, *et al*. Hyperglycemia down-regulates apolipoprotein M expression *in vivo* and *in vitro*. *Biochim Biophys Acta* 2007; 1771: 879–882.
19. Luo G, Feng Y, Zhang J, *et al*. Rosiglitazone enhances apolipoprotein M (Apom) expression in rat's liver. *Int J Med Sci* 2014; 11: 1015–1021.
20. Wei J, Shi Y, Zhang X, *et al*. Estrogen upregulates hepatic apolipoprotein M expression via the estrogen receptor. *Biochim Biophys Acta* 2011; 1811: 1146–1151.
21. Kuwabara WMT, Panveloski-Costa AC, Yokota CNF, *et al*. Comparison of Goto-Kakizaki rats and high fat diet-induced obese rats: Are they reliable models to study type 2 diabetes mellitus? *PLoS ONE* 2017; 12: e0189622.
22. Portha B, Giroix MH, Tourrel-Cuzin C, *et al*. The GK rat: a prototype for the study of non-overweight type 2 diabetes. *Methods Mol Biol* 2012; 933: 125–159.
23. Harding CO, Gillingham MB, Hamman K, *et al*. Complete correction of hyperphenylalaninemia following liver-directed, recombinant AAV2/8 vector-mediated gene therapy in murine phenylketonuria. *Gene Ther* 2006; 13: 457–462.
24. Jiang H, Lillicrap D, Patarroyo-White S, *et al*. Multiyear therapeutic benefit of AAV serotypes 2, 6, and 8 delivering factor VIII to hemophilia A mice and dogs. *Blood* 2006; 108: 107–115.
25. Moulle VS, Parnet P. Effects of nutrient intake during pregnancy and lactation on the endocrine pancreas of the offspring. *Nutrients* 2019; 11: 2708.
26. Stumvoll M, Mitrakou A, Pimenta W, *et al*. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 2000; 23: 295–301.
27. Kim JK. Hyperinsulinemic-euglycemic clamp to assess insulin sensitivity *in vivo*. *Methods Mol Biol* 2009; 560: 221–238.
28. Brozinick JT Jr, Birnbaum MJ. Insulin, but not contraction, activates Akt/PKB in isolated rat skeletal muscle. *J Biol Chem* 1998; 273: 14679–14682.
29. Workeneh B, Bajaj M. The regulation of muscle protein turnover in diabetes. *Int J Biochem Cell Biol* 2013; 45: 2239–2244.