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ORIGINAL ARTICLE

Comparison of gene expression in cynomolgus monkeys with preclinical type II diabetes induced by different high energy diets

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Abstract

Background: Cynomolgus disease models that are similar to the preclinical stage of human type 2 diabetes mellitus (T2DM) were established by feeding middle-aged cynomolgus monkeys different high energy diets to study the differential expression of diabetes-related genes.

Methods: A total of 36 male monkeys were randomly divided into four groups and fed human diets with high sugar, high fat, double high sugar and fat, and a normal diet. The preclinical diabetes phase was determined by monitoring the metabolic characteristic indices and the results of oral glucose tolerance tests (OGTT). The mRNA expression of 45 diabetes-related genes in peripheral blood leukocytes was analyzed using real-time PCR.

Results: A total of 22, 25, and 21 genes were significantly up-regulated ($P < 0.05$) and 5, 7, and 5 genes were significantly down-regulated ($P < 0.05$) in the above three induced groups, respectively, compared with the control group. Of the 45 tested genes, the expression profiles of 21 genes were consistent. Most of the expression levels in the double high sugar-and-fat individuals were slightly lower than those in the high glucose and high fat groups, although the expression patterns of the three groups were essentially similar.

Conclusion: The different high energy diets all induced diabetes and shared some phenotypic properties with human T2DM. Most of the expression patterns of the related genes were identical. The gene expression profiles could be used as references for the study of early diagnostic indicators and T2DM pathogenesis.

KEYWORDS

cynomolgus monkey, gene expression, high energy diet, preclinical phase, type 2 diabetes

1 | INTRODUCTION

More than 90% of diabetes is type 2 diabetes (T2DM), which is also known as adult-onset diabetes. The main age at onset ranges between 35 and 40 years. T2DM is a multifactorial metabolic disease that results from the interaction of genetic and environmental factors and is characterized by high blood glucose. Investigations of diabetes epidemiology have indicated that the incidences of diabetes and pre-diabetes are 9.7% and 15.5%, respectively, in China.[1] Pre-diabetes can cause adverse consequences that put an individual at great risk for overt diabetes and cardiovascular, and certain races have a higher risk. Studying the preclinical prediagnostic factors for T2DM in human patients is difficult, but these factors can be closely examined in animal models. Many factors may give rise to differences in gene expression profiling, and different T2DM model animals may produce gene expression patterns that differ from the patterns observed in human diabetes patients. Furthermore, these models may have a different influence on the development process of diabetes and relevant research studies. The genetic characteristics, life span, and endocrine system of cynomolgus monkeys (*Macaca fascicularis*) are similar to those of humans.[2] Additionally, spontaneous diabetes has been reported in cynomolgus monkeys, and the pathogenesis and characteristics of diabetes in these monkeys are similar to human diabetes.[3,4] Therefore, studying gene expression profiles using a cynomolgus prediabetes model induced by a high energy diet, which simulates the human high-energy dietary constituent, is very important because the induced cynomolgus diabetes models share some phenotypic properties with human T2DM.

During the early onset of diabetes, abnormal metabolism or mutation in some genes involved in glucose and lipid metabolism or signal transduction lead to abnormal glucose tolerance, insulin resistance, and functional obstacles in pancreatic islet beta cells. Excessive dietary fat intake is associated with an increased risk of obesity, and obesity-related genes are also related to the onset of diabetes. Excessive *FTO* expression in mice can increase food intake and cause obesity.[5,6] Additionally, certain inflammatory factors play important roles in the development and progression of T2DM; these factors can cause insulin resistance and obstruction of beta cell structures and functions and lead to T2DM by interacting with endocrine adipose tissues, thereby inducing oxidative stress and an immune response.[7,8] The TNF-α level was significantly increased in T2DM patients, which decreased the expression and transport of GLUT-4 and reduced *PPARγ* levels by preventing *PPARγ* gene expression.[9,10] In our study, the expression patterns of genes related to glucolipid metabolism, signal transduction, and inflammation were analyzed in the preclinical phase in T2DM cynomolgus monkeys induced with different high energy diets. The study results will provide guidance and references for studies of the early diagnosis and treatment of human T2DM.

2 | MATERIALS AND METHODS

2.1 | Reagents

The TRIzol reagent was purchased from Invitrogen, USA. SYBR® Green and SYBR® Premix Ex Taq™ were purchased from TaKaRa, Japan. The EasyScript First-Strand cDNA Synthesis SuperMix was purchased from TransGen Biotech, China. Red Blood Cell Lysis Buffer was prepared in the lab (1.6 mmol/L EDTA, 10 mmol/L $KHCO_3$, and 153 mmol/L NH_4C1, pH 7.4). All other chemicals and reagents were obtained from other companies in China. The blood sugar test paper and glucose meter were purchased from Johnson & Johnson, USA.

2.2 | Animals and diets

A total of 36 slightly overweight middle-aged or aged male cynomolgus monkeys (all more than 8.5 kg and 9 years old) were supplied by Guangdong Landau Biotechnology Co., Ltd, Guangzhou, China, and bred in a conventional facility with one monkey per cage. The environmental temperature was between 18 and 26°C and the relative humidity was 60%-80% following the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines. Animal housing and all research protocols were approved by the Guangdong Laboratory Primate Institute Institutional Animal Care and Use Committee (IACUC). The protocols were approved by the Committee on the Ethics of Animal Experiments of the Guangdong Institute of Applied Biological Resources (Permit Number: 2005005).

All monkeys were divided into four groups and fed a high-sugar (G), high-fat (F), double high-sugar and -fat (D), or normal diet (control group; C) for 18 months in this study. The monkey numbers fed the different dietary formulations are shown in Table 1.

TABLE 1 Four feed formulas used for the diet-induced cynomolgus T2DM models

Groups	Number (n)	Fat (%)	Cholesterol (%)	Sugar (%)	Salt (%)	Protein (%)	Standard basic feed
Control group (C)	6	0	0	0	0	0	100
High-sugar group (G)	10	7	0.3	30	1	0	61.7
High-fat group (F)	10	24	0.3	5	1	15	54.7
Double high-sugar and -fat group (D)	10	15	0.3	30	1	0	54.7

2.3 | Body mass index calculation and detection of the serum biochemical indices

The body mass index (BMI) was calculated using the body weight divided by the square of the body length. The BMI of normal monkeys was 18.5-27 kg/m^2 and the BMI of the obese monkeys was greater than 27 kg/m^2, which were similar to the BMIs of normal humans.[11]

Whole blood was collected from the hind limb veins of the monkeys and centrifuged for 5 minutes at 3000 × g to extract blood serum for the detection of the serum biochemical indices, including glucose (GLU), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). An additional 5 mL of blood was collected for RNA extraction from white blood cells.

2.4 | Determination of the T2DM preclinical stage in cynomolgus monkeys

The preclinical phase of T2DM in the monkeys was determined according to the fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), glucose metabolism index, and urine test results, among other factors, based on the classification standard of the preclinical stages of human diabetes.

2.5 | Primer design

According to the human diabetes PCR array (SABiosciences, America) and recent reports, 46 diabetes-associated genes were screened in this study. The primers were designed according to the exon sequences of rhesus monkeys and human mRNA. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The lengths of the PCR products of all genes were between 180 and 250 bp. These genes could be classified into different categories according to their functions, such as metabolic enzymes (*ACE*, *ACLY*, *ENPP1*, *G6PC*, *GSK3B*, *IDE*, *ME1*, *PARP1*, *PRKAA1*, *PRKAG2*, *PRKCB1*, and *PYGL*), nuclear reporters (*PPARG*), reporters, transporters and channels (*ADRB3*, *AQP2*, *CCR2B*, *CEACAM1*, *CTLA4*, *GCGR*, *ICAM1*, *IL4R*, *NSF*, *RAB4A*, *SELL*, *SLC2A4*, *SNAP23*, *SNAP25*, *STXBP1*, *VAMP3*, and *VAPA*), secreted factors (*AGT*, *CCL5*, and *VEGF*), signal transduction (*DUSP4*, *IRS1*, *PIK3C2D*, *IKB*α and *IKB*β), transcription factors (*FOX*P3, *PDX1*, *NEUROD1*, *PPARG*C1, and *SREBF1*), and obesity-associated genes (*CDKN2B*, *IGF2BP2*, and *FTO*). *GAPDH* and *YWHAZ* were chosen as the housekeeping genes.[12]

2.6 | Total RNA extraction, cDNA preparation and quantitative real-time PCR (RT-qPCR)

For RNA extraction, 5 mL of blood was taken from the femoral vein of cynomolgus monkeys after fasting for 14-16 hours. White blood cells were extracted from the blood,[13] and total RNA was extracted using the TRIzol method.[14-16] Then, cDNA was synthesized from 2 μg of total RNA using the EasyScript First-Strand cDNA Bio-Synthesis SuperMix (TransGen Biotech) and oligo-dT primers (Invitrogen).

Each DNA sample was amplified by PCR amplification in a 20 μL volume containing 10 μL of SYBR® Premix Ex TaqTM (2 ×), 0.4 μL of the forward primer (10 μmmol/L), 0.4 μL of the reverse primer (10 μmmol/L), 2 μL of cDNA as the template DNA, and 7.2 μL of ddH_2O. The amplification and analysis were performed using an ABI StepOne™ Real-Time PCR System and the SDS 2.3 software package (Applied Biosystems). The PCR conditions were as follows: after the initial denaturation at 95°C for 5 minutes, we performed a denaturing step at 95°C for 5 seconds and an annealing step at 60°C for 34 seconds for 40 cycles. A dissociation stage followed the system default setting at 95°C for 15 seconds, 60°C for 1 minute and then 95°C for 15 seconds.

2.7 | Statistical analysis

After removing the reactions with no amplification, multiple Tm peaks, Ct < 8 or Ct > 35 and △Ct >13, the fold-changes in relative gene expression were analyzed using the $2^{-\triangle\triangle CT}$ method and normalized to *GAPDH* after adjusting for differences in the amplification efficiency.[17] The values were reported as the mean ± SE. The statistical analysis was performed with one-way ANOVA in the SPSS 17.0 software package. $P < 0.05$ was considered a significant difference.

3 | RESULTS

3.1 | Body conditions and serum biochemical indices

The inclusion criteria for the preclinical T2DM cynomolgus monkeys included a fasting plasma glucose (FPG) ≤5.6 mmol/L, impaired glucose tolerance (IGT, 6.1 < 2 h-PG < 10) and *slight* urine sugar.[18] According to the above inclusion criteria and human preclinical T2DM criteria, 14 monkeys were determined to be in the preclinical T2DM stage in this study. A total of 3, 7, 4, and 6 qualified monkeys were included in the G, F, D, and C groups, respectively. The BMI, TC, TG, circulating LDL (LDL-C), and circulating HDL (HDL-C) were measured and compared as shown in Table 2.

3.2 | Gene expression in the different induction groups

The relative mRNA expression levels of the T2DM-related genes in the cynomolgus monkeys in the preclinical T2DM phase under different dietary conditions are shown in Table 3. A total of 22, 26, and 20 and 4, 6, and 6 genes were significantly up- and down-regulated, compared with the controls ($P < 0.05$). Of the 46 tested genes, the expression profiles of 22 genes were consistent. *ACLY* was expressed in peripheral blood leukocytes in all of the normal and several pre-diabetic monkeys. The CT value of *IDE* was significantly higher than *GAPDH* in the F and D subjects, although it appeared to be expressed at a low level; thus, the relative quantification (RQ) of this gene is represented by "N/A". The

TABLE 2 BMI and metabolic characteristics in the preclinical stage of T2DM cynomolgus monkeys induced by different diets

Measurement item	Groups with different glycolipid ratios			
	C (n = 6)	G (n = 3)	F (n = 7)	D (n = 4)
BMI (kg/m^2)	43.72 ± 9.56	53.40 ± 7.40	46.92 ± 8.49	56.18 ± 5.0
FPG (mmol/L)	3.60 ± 0.46	4.06 ± 0.45	5.04 ± 0.71	3.80 ± 0.42
CHO (mmol/L)	2.42 ± 0.55	5.76 ± 3.48*	8.54 ± 4.75**	8.57 ± 5.28**
TG (mmol/L)	0.73 ± 0.22	3.14 ± 0.42*	0.69 ± 0.22	1.76 ± 0.61*
HDL (mmol/L)	1.48 ± 0.41	1.37 ± 0.42	2.09 ± 0.55	2.22 ± 0.61*
LDL (mmol/L)	0.90 ± 0.23	3.78 ± 3.33*	6.86 ± 4.27**	6.48 ± 5.40**
2 h-PG (mmol/L)	3.52 ± 0.36	11.6 ± 3.41**	8.6 ± 1.54*	6.87 ± 0.84*

C, control group; G, high-sugar group; F, high-fat group; D, double high-sugar and -fat group. BMI, body mass index; FPG, fasting plasma glucose; CHO, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; 2 h-PG, 2-h plasma glucose levels followed oral glucose tolerance test (OGTT).
Values are the means ± SE.
*and **indicate significant differences, with $P < 0.05$ and $P < 0.01$, respectively, compared with the C group.

IRS1 expression level was higher in the pre-diabetic groups (vs the controls).

The comparison of the preclinical-related gene expression levels among the different dietary groups is shown in Table 4. The *GSK3B* expression pattern was different among the three experimental groups; this gene was down-regulated in G and F (vs D, $P < 0.01$) and up-regulated in D. The mRNA RQs of *PRKAA1*, *SELL*, and *CTLA4* in G were higher than the mRNA RQs in D whereas the RQ of *IGF2BP2* was lower ($P < 0.05$). The *ADRB3* and *NSF* mRNA expression levels differed among the three groups ($P < 0.05$).

4 | DISCUSSION

According to the 2006 WHO preclinical diabetes criteria for humans and previous studies on high energy diet-induced T2DM in cynomolgus macaques, the preclinical phase of cynomolgus T2DM is defined as a blood glucose value less than 5.6 mmol/L with the presence of glucose tolerance abnormities.[18,19] According to the above conditions and referring to the diagnostic criteria of preclinical T2DM in humans, 14 cynomolgus monkeys qualified for the preclinical stage of T2DM in the three high-energy diet-induced groups, with 3, 7, and 4 qualified monkeys in the G, F, and D groups, respectively. These results showed that all three high-energy diets induced the early symptoms of diabetes in the monkeys but that the pre-diabetes symptoms appeared more commonly in the F group than in the other two groups. The G group needed a longer time to develop early symptoms of diabetes. Because of the balanced glycolipid ratio in the D group diet, fewer monkeys exhibited preclinical diabetes symptoms during the same induction period compared with the F group.

Several epidemiological studies showed that a high fat diet was closely related to elevated LDL levels. A significant positive correlation was detected between the saturated fatty acid and serum total cholesterol levels.[20] Insufficient insulin secretion leads to blood lipid metabolism abnormalities and an increase in the CHO, and blood TG and LDL levels, which eventually cause a series of complications, such as cardiovascular disease and atherosclerosis.[21] The data from this study indicated that a glycolipid metabolism disorder occurred in three monkeys. This disorder mainly presented as a significant increase in the CHO and LDL levels compared with those of the control group; additionally, the FPG level was higher than the level in the C group. All of the profile variations, such as dyslipidemia and IGT, could give rise to changes in related gene expression.[13] Because exploring gene changes in the preclinical stage of T2DM is difficult in human patients, cynomolgus monkeys were induced to establish a diabetes model that shared some phenotypic properties with the early symptoms of human T2DM.

Catabolism is strengthened and anabolism is weakened in diabetes patients. Therefore, we selected genes related to the synthesis and regulation of blood glucose, lipids and protein expression changes. The *GCGR* gene was highly expressed in the liver and kidneys but weakly expressed in other tissues. Glycogen can affect *GCGR* expression; this gene can contain nonsense mutations that may be relevant to T2DM. Additionally, repressing *GCGR* activity can relieve clinical symptoms in patients with hyperglycemia.[22,23] *ACLY* is the key enzyme involved in the biosynthesis of fatty acids, which can be activated by insulin. Patients with T2DM have higher or lower fatty acid concentrations than normal subjects, suggesting that *ACLY* activity is decreased. *PPARGC1A* participates in transcriptional activation through a nuclear receptor and the metabolic regulation of the serum cholesterol levels.[24,25] Our hypothesis was confirmed in this study. Genes that correlated with blood glucose metabolism such as *G6PC*, *PRKAA1*, *PYGL*, *GCCR*, and *PIK3C2B* were significantly up-regulated. Additionally, the RQs of *ACLY* and *PRKAG2*, which are involved in fat metabolism, were slightly decreased, whereas the *PPARGC1* expression level, which participates in cholesterol synthesis, was obviously increased compared with that of the control group. Different degrees of change occurred in the relative quantities of other gene mRNAs, which influenced islet development, insulin resistance, signal transduction, transcriptional regulation

TABLE 3 Relative mRNA expression of T2DM-related genes in the preclinical phase of cynomolgus monkeys under different dietary conditions

Gene name	GenBank accession No.	Groups with different glycolipid ratios			
		C (n = 6)	G (n = 3)	F (n = 7)	D (n = 4)
ACE	AY348177	0.48 ± 0.46	4.08 ± 1.86*↑	6.88 ± 1.44**↑	4.57 ± 2.22**↑
ACLY	DQ147961	1.38 ± 0.60	1.47	0.40	0.26
ENPP1	XM_001103359.3	0.42 ± 0.34	8.79 ± 2.35**↑	8.34 ± 4.68**↑	6.62 ± 3.49*↑
G6PC	XM_001100750.2	0.40 ± 0.38	5.84 ± 1.45*↑	7.80 ± 3.16**↑	7.18 ± 4.43*↑
GSK3B	XM_001110547	1.81 ± 0.16	0.63 ± 0.54*↓	0.08 ± 0.09*↓	3.10 ± 1.24*↑
IDE	XM_001090017	1.54 ± 0.16	3.80	N/A	N/A
ME1	XM_001084298	0.41 ± 0.40	9.39 ± 2.77**↑	10.37 ± 2.72**↑	6.65 ± 3.28**↑
PARP1	XM_001090628	2.92 ± 0.23	0.91 ± 0.36**↓	0.45 ± 0.34**↓	0.16 ± 0.11**↓
PRKAA1	CO581780.1	0.65 ± 0.35	2.02 ± 0.73**↑	1.74 ± 0.38*↑	1.21 ± 0.61
PRKAG2	AF087875	1.04 ± 0.38	2.20 ± 1.61	1.60 ± 0.70	0.40
PRKCB1	XM_001095880	5.16 ± 028	1.43 ± 1.36**↓	0.30 ± 0.24**↓	0.03 ± 0.02**↓
PYGL	XM_001102253	0.70 ± 0.14	1.16 ± 0.74	2.15 ± 0.80*↑	1.45 ± 1.00
PPARG	NM_001032860	0.18 ± 0.07	61.22 ± 16.74**↑	62.83 ± 29.39**↑	50.10 ± 26.00*↑
ADRB3	NM_001044730.1	0.44 ± 0.29	2.99 ± 0.06	10.06 ± 2.64**↑	2.76 ± 0.41
AQP2	XM_001110572	0.16 ± 0.01	6.32 ± 2.15*↑	8.36 ± 4.88**↑	6.89 ± 3.25*↑
CCR2B	AF013958	0.48 ± 0.22	4.37 ± 1.53**↑	4.98 ± 2.50**↑	3. 97 ± 1.79*↑
CEACAM1	NM_001712	0.60 ± 0.46	8.22 ± 2.03**↑	10.08 ± 4.80**↑	7.89 ± 3.50**↑
CTLA4	AF344846	0.52 ± 0.22	12.61 ± 1.61**↑	12.21 ± 3.70**↑	8.69 ± 3.77**↑
GCGR	XM_001111894	0.79 ± 0.01	4.57 ± 1.78	4.50 ± 2.74	5.61 ± 4.53
ICAM1	NM_001047135	0.54 ± 0.41	3.20 ± 1.47	3.29 ± 2.02	3.86 ± 2.77*↑
IL4R	XM001093763	1.95 ± 1.77	1.13 ± 0.97	0.36 ± 0.15*↓	0.37 ± 0.15*↓
NSF	XM_001105450	1.21 ± 0.11	37.65 ± 26.63**↑	14.39 ± 5.95	12.74 ± 6.00
RAB4A	XM_001082985	3.84 ± 1.84	4.19 ± 1.51	0.03 ± 0.01*↓	0.02 ± 0.01*↓
SELL	NM_001042763.1	1.40 ± 0.28	0.84 ± 0.31**	0.42 ± 0.08**	0.27 ± 0.11**
SLC2A4	XM_001107391.2	1.12 ± 1.06	6.32 ± 2.98	3.28 ± 1.43	3.08 ± 2.95
SNAP25	AB169798	0.41 ± 0.21	7.25 ± 1.81**↑	7.69 ± 2.32**↑	5.88 ± 2.81**↑
STXBP1	NM_001261163.1	0.43 ± 0.36	6.89 ± 2.26**↑	6.45 ± 2.59**↑	5.96 ± 2.34**↑
VAMP3	XM_001095950	0.91 ± 0.18	1.58 ± 0.36	1.60 ± 0.60	1.33 ± 0.74
VAPA	AB174175	6.67 ± 1.41	0.09 ± 0.06	0.05 ± 0.04	0.03
AGT	XM_001107315	0.25 ± 0.21	7.48 ± 3.15*↑	8.17 ± 5.04*↑	7.22 ± 2.37*↑
CCL5	NM001032850	1.35 ± 0.42	0.08 ± 0.06	0.11	0.01
VEGF	XM001089925	0.65 ± 0.38	2.60 ± 0.60	3.48 ± 1.70*↑	3.38 ± 1.66*↑
DUSP4	XM_001110903.3	0.49 ± 0.38	20.32 ± 1.71**↑	15.96 ± 6.24**↑	19.44 ± 7.87**↑
IRS1	XM_015111187.1	N/A	0.70	0.67 ± 0.24	3.08 ± 3.04
PIK3C2B	AB172565	0.21 ± 0.11	19.01 ± 7.14**↑	9.01 ± 4.83*↑	9.60 ± 4.06*↑
IKBα	NM_020529.2	6.10 ± 3.73	1.53 ± 1.26*↓	0.19 ± 0.14**↓	0.06 ± 0.06**↓
IKBβ	XM_001087248	0.78 ± 0.71	5.55 ± 1.48	6.40 ± 4.20*↑	3.70 ± 0.56
FOXP3	NM_001032918.1	0.43 ± 0.11	6.99 ± 2.51*↑	4.58 ± 2.83*↑	4.48 ± 1.91*↑
PDX1	XM_001096758.3	0.30	2.81 ± 0.97	2.81 ± 2.50	1.16 ± 0.21
NEUROD1	XM_001101024	0.17 ± 0.04	11.11 ± 4.20*↑	13.08 ± 8.73*↑	9.45 ± 5.38*↑
PPARGC1	XM_001105289	0.14 ± 0.01	11.05 ± 2.71*↑	21.18 ± 15.96**↑	9.93 ± 6.81
SREBF1	XM_001095392.1	1.33 ± 0.36	1.19 ± 0.36	1.30 ± 0.04	0.67 ± 0.32
CDKN2B	XM001107263.1	0.25 ± 0.01	6.72 ± 1.37*↑	5.16 ± 4.28*↑	3.31 ± 0.39

(Continues)

TABLE 3 (Continued)

Gene name	GenBank accession No.	Groups with different glycolipid ratios			
		C (n = 6)	G (n = 3)	F (n = 7)	D (n = 4)
IGF2BP2	XM_001087426	0.38 ± 0.29	8.96 ± 2.73*↑	8.47 ± 1.64*↑	15.01 ± 8.64**↑
FTO	XM_001092038.1	0.56 ± 0.23	5.41 ± 2.68*↑	4.03 ± 0.53*↑	4.21 ± 1.26*↑
GAPDH	XR_010863	00.99 ± 0.16	00.94 ± 0.11	00.98 ± 0.13	00.96 ± 0.12
YWHAZ	XM_001098275	0.64 ± 0.16	1.12 ± 1.46	1.25 ± 0.64	1.55 ± 0.26

C, control group; G, high-sugar group; F, high-fat group; D, double high-sugar and -fat group.
Values are the means ± SE.
* and ** over the bars indicate significant differences ($P < 0.05$) and extremely significant differences ($P < 0.01$), respectively, compared with the levels in the C group.
N/A indicates data that were not available or had low expression levels.
↑ and ↓ show up-regulated and down-regulated expression compared with the C group.

TABLE 4 Differential expression of preclinical-related genes among the different dietary groups

Gene name	GenBank accession No.	Groups with different glycolipid ratios		
		G	F	D
GSK3B	XM001110547	$0.63 \pm 0.54^{GD,**}$	$0.08 \pm 0.09^{FD,**}$	$3.10 \pm 1.24^{GD,FD,**}$
PRKAA1	CO581780.1	$2.02 \pm 0.73^{GD,*}$	1.74 ± 0.38	$1.21 \pm 0.61^{GD,*}$
ADRB3	NM_001044730.1	$2.99 \pm 0.06^{GF,GD,*}$	$10.06 \pm 2.64^{GF,*}$	$2.76 \pm 0.41^{GD,*}$
CTLA4	AF344846	$12.61 \pm 1.61^{GD,*}$	12.21 ± 3.70	$8.69 \pm 3.77^{GD,*}$
NSF	XM_001105450	$37.65 \pm 26.63^{GF,GD,*}$	$14.39 \pm 5.95^{GF,*}$	$12.74 \pm 6.00^{GD,*}$
SELL	NM_001042763	$0.84 \pm 0.31^{GD,*}$	0.42 ± 0.08	$0.27 \pm 0.11^{GD,*}$
IGF2BP2	XM_001087426	$8.96 \pm 2.73^{GD,*}$	8.47 ± 1.64	$15.01 \pm 8.64^{GD,*}$
GAPDH	XR_0010863	0.94 ± 0.11	0.98 ± 0.13	0.96 ± 0.12
YWHAZ	XM_001098275	1.12 ± 1.46	1.25 ± 0.64	1.55 ± 0.26

Values are the means ± SE; GD, FD and GF show comparisons between the high-sugar and double high-sugar and -fat, high-fat and double high-sugar and -fat, and high-sugar and high-fat groups, respectively.
G, high-sugar group; F, high-fat group; D, double high-sugar and -fat group.
* and ** over the bars indicate significant differences, with $P < 0.05$ and $P < 0.01$, respectively.

and the inflammatory response. For example, the participation of *PRKCB1* in cell signal transduction was demonstrated and was related to the development of diabetic complications. Mutations in this gene can increase the risk of T2DM and its complications five-fold.[26] *NSF* plays an important role in glucose transport and exhibited up-regulated expression. Of all 45 evaluated genes, 22, 25, and 21 genes were significantly up-regulated ($P < 0.05$) in the G, F and D induced groups, and 5, 7, and 5 genes were significantly down-regulated ($P < 0.05$) compared with group C. Among these genes, the expression profiles of 21 genes in the three high energy diet groups were consistent with regard to their significant up-regulation or down-regulation compared with the levels in the N group. Although the expression patterns of the three groups were basically similar, we also found that the expression level changes of most genes in the D group were slightly lower than the changes observed in the G and F groups. The results also suggest that the balanced glycolipid diet was slightly healthier than a single high sugar or high fat diet and delayed the progression of the illness. Additionally, the comparison of gene expression levels among the different dietary groups showed no obvious differences in most of the gene expression levels. Although several genes (except *GSK3B*) had expression levels that differed significantly between the G and D groups, no significant differences were observed between the F and D groups. This outcome could be an indication that the 7 genes in F group might have a close correlation with a high-sugar diet, glucose metabolism and the influence of lipid components. However, this hypothesis needs further investigation.

The diabetes PCR chip has been applied to clinical diagnosis, but its application in early risk warnings and diagnosis of diabetes needs to be further explored. We used a fluorescent quantitative polymerase chain reaction (RT-qPCR) to detect the expression patterns of diabetes-related genes in an early T2DM cynomolgus monkey model and expected to find genetic indicators that could be used for early warning, diagnosis and prognostic evaluation of T2DM. This study narrowed the range of genes for use in genetic screening. Because most of the genes ultimately played roles through proteins and interacted with other relative factors, the gene expression patterns at the protein level need to be confirmed in a future study.

Finally, these screened genes should be verified in preclinical diabetic patients to determine accurate gene indicators and to provide a more accurate and convenient detection method for the early prevention and treatment of T2DM.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

All listed authors meet the requirements for authorship. LSJ was in charge of the experiments and wrote the article. LBZ and JHR gave writing suggestions. FJ, YCZ, XFH assisted with the animal experiments. BLP and XML provided ideas for the article. YXS analyzed the results of the experimental results, participated in the writing and revision of the article, and was responsible for article submission, etc. All authors read and approved the final manuscript.

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