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Docking protein 1 and free fatty acids are associated with insulin resistance in patients with type 2 diabetes mellitus

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Abstract

Objective: Insulin resistance (IR) is a key defect in type 2 diabetes mellitus (T2DM); therefore, effective means of ameliorating IR are sought.

Methods: We performed a retrospective cohort study of 154 patients with T2DM and 39 with pre-diabetes (pre-DM). The effects of IR and a high concentration of FFA on gene expression were determined using microarray analysis and quantitative reverse transcription polymerase chain reaction (RT-qPCR) in patients with T2DM or pre-DM.

Results: Serum FFA concentration and homeostasis model assessment of IR (HOMA-IR) were significantly higher in patients with T2DM but no obesity and in those with pre-DM than in controls. HOMA-IR was significantly associated with T2DM. RT-qPCR showed that the expression of FBJ murine osteosarcoma viral oncogene homolog (*FOS*) and AE binding protein 1 (*AEBP1*) was much lower in the circulation of participants with obesity and diabetes. RT-qPCR showed that the expression of docking protein 1 (*DOK1*) was significantly lower in the blood of participants with diabetes but no obesity and in those with pre-DM than in controls.

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Conclusions: FFA and DOK1 are associated with IR in patients with T2DM but no obesity or pre-DM. The downregulation of DOK1 might inhibit lipid synthesis and induce lipolysis, inducing or worsening IR.

Keywords
Docking protein 1, free fatty acid, type 2 diabetes mellitus, insulin resistance, polymerase chain reaction array analysis, obesity

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Introduction

Insulin resistance (IR) is a key metabolic defect that is present in the great majority of patients with type 2 diabetes mellitus (T2DM), the incidence of which has reached pandemic proportions.[1] A series of defects, including in insulin signalling, mitochondria, and the microvasculature, as well as inflammation, contribute to IR. In addition, the accumulation of lipid species, such as diacylglycerol (DAG) and/or ceramide, causes impairments in insulin signalling and results in IR.[2–4] High circulating concentrations of free fatty acids (FFA) are a facet of the disordered lipid metabolism that characterises obesity-induced IR, and can impair muscle insulin action, leading to IR and T2DM.[5,6] *In vitro* and *in vivo* animal studies have shown that exposure of muscle cells to the FFA palmitate or the high plasma lipid concentrations induced by high-fat diet-feeding results in IR.[7,8] Several biomarkers of the cardiovascular defects that are associated with IR and DM have been identified to help predict the development of such complications.[9–12]

Docking protein 1 (DOK1) is a docking protein that is expressed in adipose tissue and has been implicated in IR. It has been reported that the activation of DOK1 ameliorates the excessive lipolysis in insulin-resistant adipocytes.[13] However, the relationship between DOK1 and IR is unclear and few studies conducted in cells, animal models or humans have focused on the relationship between DOK1 and IR induced by high FFA concentrations. Therefore, in the present study, we aimed to characterise the relationship between the circulating FFA concentration and IR in patients with T2DM or pre-DM, and to identify differentially expressed genes that might play a role in the development of IR in patients with T2DM and represent potential molecular targets for use in the early diagnosis and/or treatment of T2DM and its complications.

Materials and methods

Participants

We performed a retrospective cohort study at our hospital in China. The reporting of this study conforms with the STROBE guidelines.[14] Patients with T2DM or pre-DM that had been diagnosed and who were being treated at the hospital were randomly selected. T2DM and pre-DM were defined using the American Diabetes Association (ADA) guidelines and obesity was defined according to the Guidelines for the Prevention of Overweight and

Obesity in Chinese adults.[15]According to these standards, the patients with T2DM were divided into two groups: those with obesity (the T2DM-O group) and those without (the T2DM-N group). We also divided the patients with pre-DM into two groups: an impaired fasting glucose (IFG) group and an impaired glucose tolerance (IGT) group. Healthy individuals who underwent a comprehensive health examination at the hospital were selected to comprise a control group (NC group). Blood samples were collected from a peripheral vein of all the participants and centrifuged at 12,500 × *g* for 20 minutes at 4°C to collect serum. The study was approved by the institutional review board of the People's Liberation Army Joint Service Support Unit 940 Hospital (approval no.: 2019KYLL078). Written informed consent was obtained from all the participants and all the data were de-identified for publication.

Clinical data

Body mass index (BMI) was calculated and is expressed in kg/m^2. Biochemical and endocrine parameters, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), fasting insulin (FINS), C-peptide, triglyceride (TG), haemoglobin A1C (HbA1C), total cholesterol (TCHO), high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol and FFA were measured using a Hitachi 7600 automatic biochemical analyser (Hitachi Medical Corp., Ibaraki, Japan). IR was estimated using the homeostasis model assessment-IR (HOMA-IR), which was calculated as serum insulin (μU/mL) × glucose (mmol/L)/22.5. The use of hypoglycaemic and/or hypolipidemic agents by the participants with T2DM was not recorded in this study; therefore it could not be determined whether these agents might affect the expression of DOK1.

Measurement of the expression of genes encoding insulin signalling pathway intermediates using a PCR Array

We used a quantitative reverse transcription polymerase chain reaction (RT-qPCR) array (Kangcheng Biological Co. Ltd., Shanghai, China) to identify differentially expressed genes encoding insulin signalling intermediates. RNA was extracted from blood samples using a Trizol RNA extraction kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, then 2 μg of each sample was reverse-transcribed to create first-strand cDNA using an RT2 First Strand Kit (Qiagen, Hilden, Germany). The quantity and quality of the RNA extracts were determined using a NanoDrop® ND-1000 Spectrophotometer (Thermo Scientific Inc, Waltham, MA, USA) and 260/280 nm absorbance ratios. Only high-quality RNA with a 260/280 nm ratio of 1.8 to 2.0 and a concentration >40 μg/mL was used for PCR analysis. Each array contained primers for 84 genes encoding signalling intermediates and five housekeeping genes; and seven wells contained reverse-transcription controls, positive PCR controls, and a genomic DNA contamination control. cDNA samples were mixed with an instrument-specific, ready-to-use RT2 SYBR Green qPCR Master Mix and each was then added to wells of a PCR array plate containing gene-specific primer sets, then PCR was performed. The threshold cycle (Ct) values for all the genes on each PCR array were calculated using the software provided and the fold-differences in gene expression were calculated using the $2^{-\triangle\triangle Ct}$ method. The array data were

analysed using R software (https://www.r-project.org/).

Quantitative reverse transcription PCR

RNA was extracted from blood samples using RNAiso Plus (TaKaRa, Tokyo, Japan) and then a PrimeScript II First strand cDNA synthesis kit was used to synthesise cDNA. The following primers were used: DOK1, 5′-ATGGACGGAGCAGTG ATGGA-3′ (Forward) and 5′-CCCAGG TCTTCCTCCACCTC-3′ (Reverse); AC TB, 5′-TGGCACCCAGCACAATGAA-3′ (Forward) and 5′-CTAAGTCATAGTCC GCCTAGAAGCA-3′ (Reverse). The cDNA was loaded into capillary tubes along with a SYBR Green II-containing master mix (Beijing Solabao Technology Co., Ltd., Beijing, China), and then real-time PCR analysis was performed in a LightCycler (Roche, Basel, Switzerland) with an initial denaturation step at 95°C for 10 s, followed by 40 cycles of 95°C for 5 s, 60°C for 10 s, and 72°C for 10 s. The expression of target genes was normalised to that of ACTB.

Statistical analysis

A database was established using EpiData 3.1 (https://www.epidata.dk/download.php), and statistical analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Continuous data are expressed as mean ± standard deviation (SD) if normally distributed or median (interquartile range) if not. Categorical variables are expressed as proportions and the χ^2 test was used to compare the groups. The paired *t*-test or one-way analysis of variance (ANOVA) with Bonferroni *post-hoc* testing or the Kruskal–Wallis H test was performed to compare continuous data between groups. Univariable relationships were evaluated by calculating Pearson correlation coefficients and multivariable stepwise regression analyses were performed to identify factors that were independently related to FFA concentration. $P < 0.05$ was considered to represent statistical significance.

Results

Serum FFA concentration is closely related to IR in participants with T2DM

One hundred and fifty-four patients with T2DM and 39 with pre-DM were studied, along with 33 controls. The T2DM-O group comprised 82 participants and the T2DM-N group comprised 72 participants.

As shown in Table 1, the serum FFA concentration and HOMA-IR were significantly higher in the T2DM group as a whole, the T2DM-N group and the T2DM-O group than in the NC group ($P < 0.05$). Moreover, HOMA-IR significantly correlated with FFA concentration in the T2DM-N group ($P < 0.05$), but not in the T2DM-O group or in the participants with T2DM as a whole (Table 2). Furthermore, HOMA-IR showed correlations with FFA concentration ($r = 0.963$, $p = 0.001$) (Figure 1a), TG concentration ($r = -0.251$, $p = 0.034$) and SBP ($r = 0.201$, $p = 0.047$) in the T2DM-N group after correcting for age, BMI, DBP, fasting C-peptide, TCHO, HDL-cholesterol, LDL-cholesterol and the other factors shown in Table 1. Thus, as SBP increased and TG decreased, both FFA and HOMA-IR tended to increase, and FFA is related to HOMA-IR in patients with T2DM.

Relationship between FFA and IR in participants with pre-DM

The serum FFA concentration and HOMA-IR were significantly higher in participants with pre-DM than controls,

Table 1. Clinical and biochemical characteristics of the NC, T2DM-N, T2DM-O and T2DM groups.

Parameter	NC	T2DM-N	T2DM-O	T2DM-all
Sex (male/female)	28/5	52/30	43/29	95/59
Age	45.72 ± 5.40	47.72 ± 6.26	47.46 ± 5.96	47.58 ± 6.05
BMI (kg/m^2)	21.33 ± 1.96	21.94 ± 2.16[c]	27.38 ± 1.97[a]	24.79 ± 3.42[a]
SBP (mmHg)	111.85 ± 9.22	128.92 ± 19.14[ad]	138.02 ± 18.02[a]	133.69 ± 19.03[a]
DBP (mmHg)	71.61 ± 6.82	81.90 ± 11.93[a]	84.57 ± 8.71[a]	83.30 ± 10.40[a]
FPG (mmol/L)	5.05 ± 0.28	10.06 ± 3.58[a]	8.97 ± 3.17[a]	9.49 ± 3.40[a]
FINS (mU/L)	6.90 (5.20–8.05)	6.9 (4.65–11.30)	11.6 (8.10–17.95)[a]	9.50 (5.85–14.15)[a]
C-peptide (pmol/mL)	466.39 ± 105.12	487.33 ± 244.05[c]	644.72 ± 264.41[a]	569.92 ± 265.68
FFA (mmol/L)	0.41 ± 0.10	0.51 ± 0.17[b]	0.51 ± 0.20[b]	0.51 ± 0.19[b]
HOMA-IR	1.50 (1.20–1.85)	2.75 (1.80–5.68)[a]	4.30 (2.50–7.35)[a]	3.70 (2.30–6.70)[a]
TG (mmol/L)	1.06 (0.86–1.27)	2.00 (1.34–3.02)[a]	2.17 (1.58–3.11)[a]	2.08 (1.49–3.11)[a]
HbA1C (%)	–	10.12 ± 2.97[d]	9.01 ± 2.10	9.60 ± 2.64
TCHO (mmol/L)	4.45 ± 0.56	4.48 ± 1.23	4.47 ± 1.17	4.48 ± 1.20
HDL (mmol/L)	1.40 ± 0.24	1.04 ± 0.20[a]	1.04 ± 0.36[a]	1.04 ± 0.29[a]
LDL (mmol/L)	2.27 ± 0.43	2.45 ± 0.73	2.34 ± 0.87	2.39 ± 0.80

Data are numbers, mean ± SD or median (interquartile range). N = 8 per group.
[a]$p < 0.01$, [b]$p < 0.05$ *vs.* the NC group; [c]$p < 0.01$, [d]$p < 0.05$ *vs.* the T2DM-O group.
T2DM: type 2 diabetes mellitus; O: obesity; N: no obesity; NC, control; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; FINS: fasting insulin; FFA: free fatty acids; HOMA-IR: homeostasis model assessment-insulin resistance; TG: triglyceride; HbA1C: haemoglobin A1C; TCHO: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 2. Pearson correlation coefficients for the relationships between HOMA-IR and clinical and biochemical parameters for the T2DM-all, T2DM-O and T2DM-N groups.

	T2DM-all		T2DM-O		T2DM-N	
Parameter	R	*p*	r	*p*	r	*p*
Age	−0.089	>0.05	−0.089	>0.05	−0.304	>0.05
BMI (kg/m^2)	0.165	>0.05	0.118	>0.05	0.141	>0.05
SBP (mmHg)	0.117	>0.05	0.120	>0.05	0.324	<0.05
DBP (mmHg)	0.037	>0.05	0.022	>0.05	0.372	<0.05
FPG (mmol/L)	0.147	>0.05	0.101	>0.05	0.319	<0.05
FINS (mU/L)	0.975	<0.01	0.983	<0.01	0.839	<0.05
C-peptide (pmol/mL)	0.178	>0.05	0.177	>0.05	0.204	>0.05
FFA (mmol/L)	0.149	>0.05	0.124	>0.05	0.898	<0.05
HbA1c (%)	−0.024	>0.05	0.022	>0.05	0.293	>0.05
TG (mmol/L)	0.172	>0.05	0.171	>0.05	0.403	<0.05
TCHO (mmol/L)	0.134	>0.05	0.149	>0.05	0.293	>0.05
HDL (mmol/L)	−0.095	>0.05	−0.123	>0.05	0.027	>0.05
LDL (mmol/L)	0.034	>0.05	0.03	>0.05	0.033	>0.05

N=8 per group.
HOMA-IR: homeostasis model assessment-insulin resistance; T2DM: type 2 diabetes mellitus; T2DM-all: the entire group of participants with T2DM; O: obesity; N, no obesity; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; FINS: fasting insulin; FFA: free fatty acids; TG: triglyceride;
HbA1C: haemoglobin A1C; TCHO: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

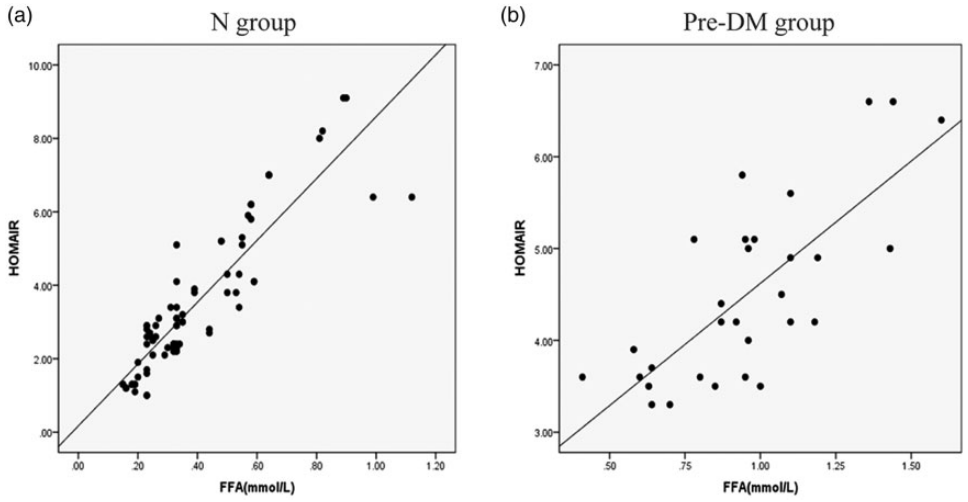

Figure 1. Relationship between homeostasis model assessment-insulin resistance (HOMAIR) and free fatty acid concentration (FFA) in the participants with types 2 diabetes but no obesity (N group) and in those with prediabetes (pre-DM group). Pearson correlation analysis showed a linear relationship with FFA in both the N ($r = 0.963$, $p = 0.000$) (a) and pre-DM ($r = 0.677$, $p = 0.003$) (b) groups after correcting for age, body mass index, diastolic blood pressure, fasting C-peptide, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein cholesterol and the other factors shown in Table 1. N = 3 per group.

including in both the IFG and IGT groups ($p < 0.05$ for all) (Table 3). HOMA-IR significantly correlated with the FINS, C-peptide, FFA and TG concentrations (all $p < 0.05$) in pre-DM group. Moreover, HOMA-IR correlated with FFA ($r = 0.677$, $p = 0.003$) (Figure 1b) and fasting C-peptide ($r = 0.392$, $p = 0.041$) after correcting for age, BMI, DBP, TG, SBP, TCHO, HDL-cholesterol, LDL-cholesterol and other factors. Thus, as C-peptide concentration increased, FFA concentration and HOMA-IR also increased, and FFA is related to HOMA-IR in patients with pre-DM.

Expression of genes encoding insulin signalling pathway intermediates

The expression of FBJ murine osteosarcoma viral oncogene homolog (*FOS*) was significantly lower in the T2DM-O group than in the NC group (fold difference 0.28, $p = 0.0242$) (Figure 2) on the PCR array. As shown in Figure 3, the expression of *FOS*, hexokinase 2 (*HK2*) and *DOK1* was significantly lower in the T2DM-N group than in the NC group (fold differences 0.09, $p = 0.0013$; 0.43, $p = 0.0038$; and 0.45, $p = 0.0085$; respectively). In addition, the expression of the AE binding protein 1 gene (*AEBP1*) was significantly lower in the T2DM-O group than in the T2DM-N group (fold difference 0.56, $p = 0.0410$) and those of *FOS* and the gene encoding mitogen-activated protein kinase 1 (*MAPK1*) were significantly higher (fold difference 2.72, $p = 0.0098$; and 1.77, $p = 0.0201$; respectively) (Figure 4).

Expression of DOK1 in the blood of participants with T2DM or pre-DM

As shown in Figure 5, the DOK1 mRNA expression was significantly lower in the T2DM-N and pre-DM groups than in

Table 3. Clinical and biochemical characteristics of the NC, IFG, IGT and Pre-DM groups.

Parameter	NC	IFG	IGT	Pre-DM
Sex (male/female)	28/5	29/1	8/1	37/2
Age	48.26 ± 4.89	48.00 ± 5.89	50.25 ± 8.88	48.32 ± 6.24
BMI (kg/m^2)	21.33 ± 1.96	28.00 ± 2.92[ad]	25.67 ± 2.60[a]	27.46 ± 3.00[a]
SBP (mmHg)	111.85 ± 9.22	132.50 ± 13.72[a]	118.78 ± 42.48[b]	129.33 ± 23.62[a]
DBP (mmHg)	71.61 ± 6.82	85.33 ± 9.00[a]	81.11 ± 12.44[b]	84.36 ± 9.88[a]
FPG (mmol/L)	5.05 ± 0.28	6.69 ± 0.54[ac]	9.72 ± 1.53[a]	7.39 ± 1.55[a]
FINS (mU/L)	6.90 (5.20–8.05)	13.55 (9.68–21.32)[a]	5.70 (5.15–19.75)	11.90 (8.90–21.00)[a]
C-peptide (pmol/mL)	466.39 ± 105.12	991.20 ± 374.56[ac]	534.78 ± 218.63	885.87 ± 393.80[a]
FFA (mmol/L)	0.41 ± 0.10	0.81 ± 0.26[ad]	0.97 ± 0.20[a]	0.84 ± 0.25[a]
HOMA-IR	1.50 (1.20–1.85)	3.95 (3.08–5.95)[a]	3.10 (2.15–7.70)[a]	3.90 (2.80–5.90)[a]
TG (mmol/L)	1.06 (0.86–1.27)	2.20 (1.57–3.93)[a]	1.87 (1.11–4.18)[a]	2.12 (1.52–3.93)[a]
HbA1c (%)	4.45 ± 0.56	4.99 ± 0.94[b]	4.27 ± 1.00	4.82 ± 0.99
TCHO (mmol/L)	1.40 ± 0.24	1.27 ± 0.41	1.21 ± 0.85[b]	1.25 ± 0.38
HDL (mmol/L)	2.31 (1.89–2.60)	2.59 (1.77–2.88)	2.11 (1.13–2.87)	2.56 (1.69–2.85)

Data are numbers, mean ± SD or median (interquartile range). N = 8 per group.
[a]$p < 0.01$, [b]$p < 0.05$ *vs.* the NC group; [c]$p < 0.01$, [d]$p < 0.05$ *vs.* the IGT group.
NC, control; Pre-DM, prediabetes; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; FINS: fasting insulin; FFA: free fatty acids; HOMA-IR: homeostasis model assessment-insulin resistance; TG: triglyceride; HbA1C: haemoglobin A1C; TCHO: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

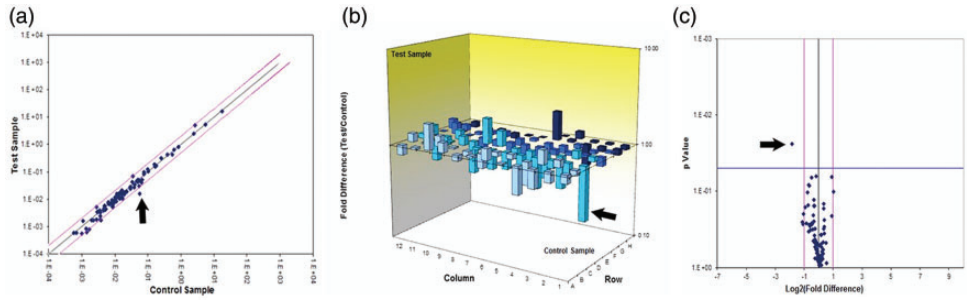

Figure 2. Results of PCR array analysis of gene expression in the participants with type 2 diabetes and obesity (T2DM-O group) and controls (NC group). The NC group provided the control samples and the T2DM-O group the test samples. (a) The black lines represent no difference and the pink lines represent differences of >1.5-fold. (b) The central horizontal plane includes columns representing the 84 genes assessed. Columns above the central plane represent genes with higher expression in the T2DM-O group and those below represent genes with lower expression. (c) The black lines represent no difference and the pink lines represent differences of >1.5-fold. The area above the blue horizontal line contains genes with statistically significant differences in expression ($p<0.05$). (A–C) The black arrow indicates the FOS gene.

the NC group ($z = -2.121$ and -2.40, respectively; both $P < 0.05$). In addition, DOK1 expression in the T2DM-O group tended to be lower than in the NC group, although this difference was not statistically significant ($z = -1.633$, $P = 0.13$), perhaps because of substantial variation between individuals and the small sample size.

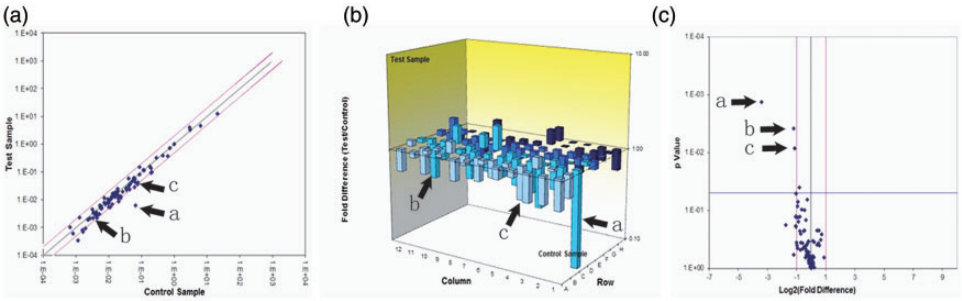

Figure 3. Results of PCR array analysis of gene expression in the participants with type 2 diabetes and no obesity (T2DM-N group) and controls (NC group). The NC group provided the control samples and the T2DM-N group the test samples. (a) The black lines represent no difference and the pink lines represent differences of >1.5-fold. (b) The central horizontal plane includes columns representing the 84 genes assessed. Columns above the central plane represent genes with higher expression in the T2DM-O group and those below represent genes with lower expression. (c) The black lines represent no difference and the pink lines represent differences of >1.5-fold. The area above the blue horizontal line contains genes with statistically significant differences in expression ($p < 0.05$). (A–C) The black arrows indicate the *FOS* gene(a), *HK2* gene(b) and *DOK1* (c).

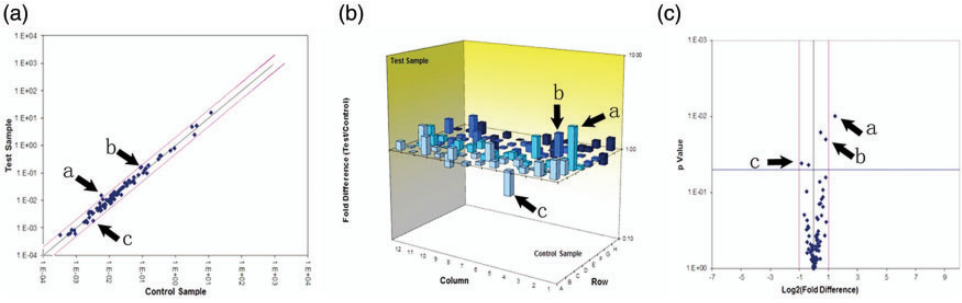

Figure 4. Results of PCR array analysis of gene expression in the participants with type 2 diabetes and no obesity (T2DM-N group) and those with diabetes and obesity (T2DM-O). (a) The black lines represent no difference and the pink lines represent differences of >1.5-fold. (b) The central horizontal plane includes columns representing the 84 genes assessed. Columns above the central plane represent genes with higher expression in the T2DM-O group and those below represent genes with lower expression. (c) The black lines represent no difference and the pink lines represent differences of >1.5-fold. The area above the blue horizontal line contains genes with statistically significant differences in expression ($p < 0.05$). (a–c) The black arrows indicate the *FOS* gene (a), *MAPK1* gene (b) and *AEBP1* (c).

Discussion

IR is a key defect contributing to T2DM, and abnormal lipid metabolism is considered to strongly predispose towards IR.[5,6] The principal lipid disturbances in patients with T2DM include hypertriglyceridemia and high serum FFA concentration. FFAs promote insulin secretion under physiological conditions; however, when the concentration of FFA is too high or remains high for a long period of time, it has harmful effects, which are referred to as lipotoxicity. For example, in patients with cerebral infarction and systemic lupus erythematosus, abnormally high FFA concentration is an independent risk factor for IR.[16,17] Many previous studies have shown

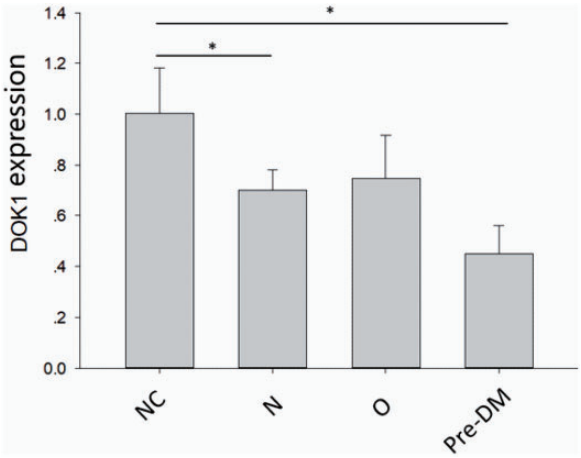

Figure 5. Expression of the *DOK1* gene (arbitrary units) in blood samples from participants in each group, measured using real-time PCR. *DOK1* expression was significantly lower in the type 2 diabetes and no obesity (N) and prediabetes (pre-DM) groups than in the control (NC) group, and tended to be lower in the type 2 diabetes and obesity (O) group. Data are mean ± SD. *$p < 0.05$ *vs.* the NC group, by ANOVA followed by Bonferroni's *post-hoc* test. N = 3 per group.

that high FFA concentration in patients with T2DM is significantly associated with IR and plays an important role in the pathogenesis of T2DM.[18,19] In the present study, we studied patients with T2DM that also had high blood pressure, abnormal circulating lipid profile, hyperglycaemia, high FFA concentration, and low HOMA-IR. In individuals with obesity, as in the present study, insulin secretion increases, but target tissues are less sensitive, which results in impaired glucose disposal. DM develops in obese and non-obese individuals when β-cell dysfunction manifests, resulting in a reduction in insulin secretion and an exacerbation of the abnormal glucose and lipid metabolism.[20,21]

Pearson correlation analysis showed that HOMA-IR positively correlated with SBP, DBP, FPG, FINS, FFA, and TG in the T2DM-N group, indicating that HOMA-IR and IR increase with increases in all these parameters in patients with T2DM but no obesity. In addition, multiple linear regression analysis showed that HOMA-IR increased alongside FFA, TG, and SBP after correcting for FPG, FINS, age, BMI, DBP, fasting C-peptide, TCHO, HDL-cholesterol, LDL-cholesterol and other factors. As shown in previous studies, when T2DM progresses to a certain stage, weight loss, abnormal lipid metabolism, high blood pressure and high FFA concentrations worsen IR and therefore glycemia.[4,22]

Pre-DM represents a metabolic state between those of normal glucose metabolism and diabetes, and can be classified as IFG, IGT, or IFG combined with IGT, which is characterised by both IR and defective insulin secretion. Previous studies have compared the gene expression in the blood of healthy individuals and patients with T2DM or pre-DM with the intention of identifying novel targets for the control or prevention of endothelial dysfunction and cardiovascular disease in such patients.[23,24] In the present study, we have shown that pre-DM is characterised by obesity, high blood pressure, abnormal glucose and lipid metabolism, high FFA concentrations and high HOMA-IR. Hypertension, obesity and hyperlipidaemia are important

features of T2DM, and their control in patients with pre-DM may help prevent the progression to T2DM. Pearson correlation analysis showed that HOMA-IR positively correlated with FFA, FINS, fasting C-peptide, and TG in the pre-DM group. In addition, multivariate linear regression analysis showed that HOMA-IR correlated with FFA and fasting C-peptide after correcting for FPG, FINS, age, BMI, SBP, DBP, TG, TCHO, HDL-cholesterol and LDL-cholesterol. Thus, as fasting C-peptide increases, FFA and HOMA-IR also increase. Furthermore, these data suggest that FFA is an important factor in the pathogenesis of IR in patients with pre-DM.

PCR arrays of genes encoding insulin signalling pathway intermediates and their targets, which are involved in carbohydrate, lipid and protein metabolism, can be used to screen for molecular markers that could be used in diagnosis, to provide a theoretical basis for the treatment of IR, and/or to delay the onset of T2DM.[25] In the present study, we used such an array to identify genes that are abnormally expressed in blood samples from patients with high FFA concentrations and IR ± T2DM. We found that genes of both the mitogen-activated protein kinase (MAPK) and phosphoinositol 3-kinase (PI3K) signalling pathways were abnormally expressed in the peripheral blood of such patients. The MAPK signalling pathway is a key regulator of various metabolic processes, and the PI3K signalling pathway is the most important pathway linking insulin with its metabolic effects.[26,27] In addition, we also found that with the development of diabetes, patients lose weight and the expression of AEBP1 significantly increases.

DOK1 is a major negative regulator of the mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) pathway, which is highly expressed in adipose tissue.[13,27] Previous studies have shown that DOK1 is a binding proteins for most receptor tyrosine kinases[28] and inhibits cell proliferation by inhibiting MAPK/ERK as a tumour suppressor.[29] It has also been shown that low-power laser irradiation (LPLI) inhibits excessive lipolysis in insulin-resistant adipocytes by activating a DOK1/ERK/peroxisome proliferator-activated receptor gamma (PPARγ) signal transduction pathway: LPLI inhibits the phosphorylation of ERK by activating DOK1 and reduces the phosphorylation of PPARγ. Unphosphorylated PPARγ promotes the expression of adipogenic genes, reduces circulating FFA concentrations and reduces lipolysis in insulin-resistant adipocytes.[13] In the present study, we have identified an association between *DOK1* and the progression of DM and that is associated with high FFA concentration and therefore abnormal lipid metabolism. Furthermore, the expression of *DOK1* correlates with IR in patients with pre-DM or T2DM. These results might suggest that DOK1 could be targeted therapeutically to slow the progression of pre-DM to T2DM.

There were some limitations to this study. First, the sample size was small, which may have introduced type II statistical errors into the results. For example, there was no correlation between FFA concentration and HOMA-IR in patients with obesity and T2DM in the present study. Therefore, a larger study should be performed to corroborate the present findings. Second, low expression of *DOK1* mRNA was present in patients with T2DM but no obesity and in those with pre-DM, but the DOK1 protein expression and the signalling mechanism whereby it might affect fat metabolism in such patients were not investigated. These deficiencies should be remedied in future studies.

In conclusion, patients with T2DM but no obesity and those with pre-DM have high serum FFA concentrations, and we have identified a gene, *DOK1*, the mRNA

expression of which is downregulated in the same groups of patients. Thus, FFA and DOK1 might be involved in the development of IR in non-obese individuals.

Author Contributions

XH is the guarantor of the integrity of the entire study, and was responsible for the study concept and design, and reviewing the manuscript. XC was responsible for the clinical and experimental studies. HC was responsible for the definition of intellectual content and literature searching. JL and BY were responsible for data acquisition. RJ was responsible for data analysis. XL was responsible for statistical analysis. BL was responsible for manuscript editing and review. YX was responsible for manuscript preparation. All the authors read and approved the final version of the manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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