ORIGINAL

C1qTNF-related protein 1 improve insulin resistance by reducing phosphorylation of serine 1101 in insulin receptor substrate 1

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Abstract. C1qTNF-related protein 1 (CTRP1) is independently associated with type 2 diabetes. However, the relationship between CTRP1 and insulin resistance is still not established. This study aimed to explore the role of CTRP1 under the situation of insulin resistance in adipose tissue. Plasma CTRP1 level was investigated in type 2 diabetic subjects (n = 35) and non-diabetic subjects (n = 35). The relationship between CTRP1 and phosphorylation of multi insulin receptor substrate 1 (IRS-1) serine (Ser) sites was further explored. Our data showed that Plasma CTRP1 was higher and negative correlation with insulin resistance in diabetic subjects ($r = -0.283$, $p = 0.018$). Glucose utilisation test revealed that the glucose utilisation rate of mature adipocytes was improved by CTRP1 in the presence of insulin. CTRP1 was not only related to IRS-1 protein, but also negatively correlated with IRS-1 Ser1101 phosphorylation ($r = -0.398$, $p = 0.031$). Furthermore, Phosphorylation levels of IRS-1 Ser1101 were significantly lower after incubation with 40 ng/mL CTRP1 in mature adipocytes than those with no intervention ($p < 0.05$). There was no significant correlation between CTRP1 and other IRS-1 serine sites (Ser302, Ser307, Ser612, Ser636/639, and Ser789). Collectively, our results suggested that CTRP1 might improve insulin resistance by reducing the phosphorylation of IRS-1 Ser1101, induced in the situation of insulin resistance as a feedback adipokine.

Key words: CTRP1, Insulin resistance, Adipose tissue, IRS-1 Ser1101

TYPE 2 DIABETES is a metabolic disease caused by many factors, including environment, heredity, immunity, and so on. Disorder of glucose metabolism is an important pathological feature of this disease. Insulin resistance of adipose tissue plays the important role of glucose metabolism disorders in type 2 diabetes. Glucose utilisation rate was significantly decreased in adipose tissue because of blockage in the insulin signalling pathway. Accordingly, the mechanism of insulin resistance in adipose tissue has important value.

Adipose tissue is an important endocrine organ in the human body. It regulates the metabolism of the

body by secreting a variety of cytokines. Adipokine-complement C1qTNF-related protein 1(CTRP1) is a secreted glycoprotein, which is composed of 281 amino acids. It is mainly produced by adipose-derived stromal cells and adipocytes, such as preadipocytes, fibroblasts, mature endothelial cells, and smooth muscle cells [1]. Related studies showed that hypoglycemic effect of CTRP1 was similar with adiponectin, and CTRP1 also was involved in the regulation of insulin sensitivity [2-5]. For example, CTRP1 overexpression improved insulin resistance to decrease blood glucose in adiponectin knockout mice. In addition, CTRP1 mRNA was increased after intervention with insulin-sensitising drugs (e.g., rosiglitazone) in mice with abnormal glucose metabolism [6]. However, the functional mechanism of CTRP1 is still in its infancy.

Our previous study also showed that CTRP1 levels were significantly increased in type 2 diabetes [3]. It

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was suggested that CTRP1 played the important role in the pathogenesis of type 2 diabetes. However, the relationship between CTRP1 and insulin resistance in adipose tissue is still not established. Therefore, in the present study, we explore the role of CTRP1 in the disorder of glucose metabolism, and the action mechanism of CTRP1 under the situation of insulin resistance in adipose tissue through experiments of clinical sample and *in vitro* cell model.

Materials and Methods

Subjects and selection criteria

A total of 70 subjects scheduled for elective abdominal operation were recruited from the Second Affiliated Hospital of Zhengzhou University between January 2012 and December 2013. They were allocated into non-diabetic group (35 subjects; median age, 51 years; range, 26–67 years) and type 2 diabetic group (35 subjects; median age, 54 years; range, 31–63 years) according to diagnostic criteria for type 2 diabetes (WHO 1999). Subjects with inflammatory disease were excluded, such as malignancy, severe trauma, *etc.* Type 2 diabetic subjects might be treated with diabetes drugs, including sulphonylurea, metformin, and insulin, but those using thiazolidinediones were eliminated. All of the subjects were required to carry out clinical examination, including height, weight, body mass index (BMI), blood pressure, *etc.* Eligible subjects were informed of related research contents, and they signed informed consent. The present study was conducted with the approval from the Research Ethics Committee of the Second Affiliated Hospital of Zhengzhou University and was conducted according to the approved guideline.

Primary preadipocyte culture

Subcutaneous adipose tissues were obtained from five non-diabetic subjects and five diabetic subjects during the operation. Preadipocytes were separated and cultured in DMEM/F12 (GIBCO, Carlsbad, CA, USA) medium supplemented with 10% fetal calf serum and placed in 37 °C, 5% CO₂ in cubator. Cells were plated in 60-mm culture dishes at 70–80% confluence and then cultured in differentiation-inducing medium (DMEM/F12 mix supplemented with 0.5 mM IBMX, 33 µM biotin, 0.2 nM T3, 0.5 µM insulin, 10 µg/mL transferin, 0.1 µM hydrocortisone, 100 U/mL streptomycin and 100 U/mL penicillin). After being incubated for 2

days, medium was replaced with differentiation-inducing medium without IBMX, and changed the medium every 2 days. Differentiation of adipocytes was determined by Oil Red O staining, and purity of mature adipocytes could reach 95%. Mature adipocytes were classed into two groups by origin: mature adipocytes coming from diabetic adipose tissues (MADAT) and mature adipocytes coming from non-diabetic adipose tissues (MANDAT). Meanwhile, mature adipocytes were separated into two groups according to different intervention methods: control group, mature adipocytes without intervention; CTRP1 group, mature adipocytes after incubation with 40 ng/mL CTRP1 (Abcam, Cambridge, MA, USA) for 12 hours.

ELISA analysis

A total of 3 mL fasting blood anticoagulated with EDTA-Na₂ was collected to centrifuge at 1,000 rcf for 10 min. Plasma was separated to store at –80 °C in deep freezer. CTRP1 levels were detected using CTRP1 human enzyme-linked immunosorbent assay (ELISA) Kit (Biovendor, Brno, AL, Czech). All operations were carried out exactly as described in the kit instructions.

Real-time PCR analysis

Total RNA was purified using Trizol (Invitrogen, Carlsbad, CA, USA) and the quality and concentration of RNA were analysed with Nanodrop 2000 (Eppendorf, Hamburg, Germany). A total of 1 µg total RNA was used to synthesise cDNA with RevertAid Reverse Transcriptase (Thermo Scientific, Waltham, MA, USA). CTRP1 mRNA (NM_153372.1) and insulin receptor substrate 1 (IRS-1) mRNA (NM_005544.2) were detected by SYBR Green I-based quantitative polymerase chain reaction (PCR) using ABI 7500Fast (Applied Biosystems, San Francisco, CA, USA). Related primers were as follows: CTRP1-F: 5'-GGCATTGTGCTTGACCCCTC-3'; CTRP1-R: 5'-AGCAAGAGTCCCTGTCCACG-3'; IRS-1-F: 5'-TGGGGACTACAAGGTAGGGG-3'; IRS-1-R: 5'-AACAGTAACACGGGCCTCAG-3'. PCR reaction was performed in a total volume of 25 µL containing 100 ng of cDNA, 10 mM of each primer, and 12.5 µL 2 × PCR buffer (containing MgCl₂, dNTP mix, and Taq polymerase) (Tiangen, Beijing, China). PCR reaction was carried out as follows: denaturation at 95 °C for 5 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min for 40 cycles. PCR products were 142 bp (CTRP1) and 144 bp (IRS-1) in size, separately. We

evaluated relative expression levels of CTRP1 mRNA and IRS-1 mRNA using Delta-delta Ct method, and GAPDH was as internal standard.

Western blot analysis

Total protein was extracted from adipose tissue or mature adipocytes using NE-PER nuclear and cytoplasmic extraction reagents (Thermo Scientific, Waltham, MA, USA). Equal amount of protein was separated on 10% SDS–polyacrylamide gel, and transferred to PVDF membrane. The membrane was blocked using 5% skim milk for 1 h, and then incubated with respective antibodies for 2 h. Primary polyclonal antibodies were purchased from Abcam (Cambridge, MA, USA) and Cell Signaling Technology (Danvers, MA, USA) including CTRP1 (ab25973), IRS-1 (ab52167), AKT (ab18785), GLUT4 (ab33780), GAPDH (ab9485), p-IRS-1 (Ser302) (2384S), p-IRS-1 (Ser307) (2381S), p-IRS-1 (Ser612) (2386S), p-IRS-1 (Ser636/639) (2388S), p-IRS-1 (Ser789) (2389S) and p-IRS-1 (Ser1101) (2385S). The secondary antibodies were anti-mouse or anti-rabbit immunoglobulin (Ig) G antibody that was purchased from Abcam. The blots were determined using ECL reagent (Millipore, Billerica, MA, USA).

Immunoprecipitation

A total of 500 µg total protein was incubated with 25 µL protein A-agarose beads (SC-2001, Santa Cruz, CA, USA) at 4 °C for 1 h. The mixture was subsequently centrifuged at 18,000 rcf for 5 min at 4 °C. Supernatant was removed and incubated with 2 µg primary antibodies against phospho-IRS-1 at 4 °C overnight. Then the mixture was incubated with 30 µL Protein A-agarose beads for 3 h at 4 °C and then centrifuged at 18,000 rcf for 5 min at 4 °C. The precipitation was recovered and washed with ice-cold PBS buffer three times. Finally, the pellet was dissolved in 40 µL of 1× electrophoresis sample buffer and boiled for 5 min. Each sample was analysed by Western blot.

Glucose utilisation test

Mature adipocytes were washed with KRPD buffer (Krebs-Ringer phosphate buffer) (Panera, Guangzhou, Guangdong, China) three times and then incubated with KRPD buffer containing or not containing 100 nM insulin for 30 min at 37 °C. Subsequently, adipocytes were incubated with 1 mL KRPD buffer containing 0.5 µCi/mL [3H]-2-deoxy-D-glucose and 0.1 mmol/L 2-deoxy-glucose (Sigma, Louis, MO, USA)

for 10 min at 37 °C. Glucose utilisation of adipocyte was detected using liquid scintillation counter; 10 mmol/L cytochalasin B was as the control of nonspecific utilisation of adipocyte. The reaction was terminated by washing three times using PBS buffer containing 10 mmol/L glucose.

Statistical analysis

Results were described by mean ± standard deviation. Chi-square test was used to analyse different proportions among groups. *T*-test was used to analyse intergroup comparisons in the chosen parameters. The relationship among different parameters was analysed using Pearson's correlation analysis. Logistic regression analysis was used to analyse multiple variables; 95% confidence intervals were calculated for the odds ratios in logistic regression analysis. The value $p < 0.05$ was considered to be statistically different. SPSS 13.0 statistical software was used in statistical analysis.

Results

Plasma CTRP1 negatively related with insulin resistance

As showed in Table 1, plasma levels of CTRP1 in type 2 diabetes were significantly higher than those in the non-diabetic group. Type 2 diabetic subjects also exhibited higher BMI index, fasting insulin, glycosylated hemoglobin and insulin resistance index than those in the control group. Because of treatment with diabetes drugs, fasting blood glucose levels showed no significant difference. There also were no significant differences in age and sex. We performed Pearson correlation analysis to examine the correlation between plasma CTRP1 and insulin resistance. In the diabetic group, plasma CTRP1 levels showed negative correlation with insulin resistance ($r = -0.283$, $p = 0.018$). However, there was no correlation between CTRP1 and insulin resistance in the non-diabetic group ($r = 0.108$, $p = 0.294$) (Fig. 1).

CTRP1 improved glucose utilisation rate of adipocytes mediated by insulin

To further explore the correlation of CTRP1 and insulin sensitivity, we used mature adipocytes coming from primary preadipocyte as *in vitro* cell model. In the presence of insulin, the glucose utilisation rate was increased after incubation with 40 ng/mL CTRP1 in MADAT and MANDAT. However, glucose utilisation

Table 1 Differences of CTRP1 and clinical data between the two study groups

	Diabetic group (n=35)	Non-diabetic group (n=35)	<i>p</i>
Median age, y (range)	54 (31-63)	51 (26-67)	0.317
Sex			
Male, n (%)	23 (65.7)	21 (60)	0.231
Female, n (%)	12 (34.3)	14 (40)	
BMI (kg/m ²)	29.3 ± 2.7	24.7 ± 1.5	0.023
Fasting plasma glucose (mmol/L)	6.0 ± 2.6	5.2 ± 0.58	0.062
Fasting insulin* (mU/L)	9.56 ± 1.91	7.26 ± 0.93	0.038
HbA1c (%)	8.1 ± 1.6	5.2 ± 0.69	0.004
HOMA-IR	5.8 ± 1.3	1.6 ± 1.3	0.000
CTRP1 (ng/mL)	712.3 ± 98.7	548.5 ± 121.9	0.030

Data are mean ± SD or median ± SD; *, fasting insulin of subjects receiving insulin treatment was excluded; BMI, body mass index; HbA1c, glycated haemoglobin; HOMA-IR, homeostasis model of assessment for insulin resistance index; CTRP1, Complement C1q tumor necrosis factor-related protein 1.

Fig. 1 The correlation between plasma CTRP1 and insulin resistance

The correlation between plasma CTRP1 and insulin resistance was examined by Pearson correlation analysis. (A) Plasma CTRP1 was negative correlated with insulin resistance in the diabetic group ($r = -0.283$, $p = 0.018$), $n = 35$; (B) Plasma CTRP1 was not correlated with insulin resistance in the non-diabetic group ($r = 0.108$, $p = 0.294$), $n = 35$.

rate showed no significant difference between MADAT and MANDAT after incubation with CTRP1 in the absence of insulin (Fig. 2).

CTRP1 closely related with IRS-1

IRS-1 is a major insulin receptor substrate, which play an important role in transmitting insulin signaling. However, high IRS-1 mRNA and protein levels are reported to correlate with insulin resistance [7]. As shown in Fig. 3A and 3B, in subcutaneous adipose tissue, CTRP1 levels in the diabetic group were significantly higher than those in the non-diabetic group (mRNA: 0.882 ± 0.115 fold vs. 0.492 ± 0.083 fold, $p = 0.021$; protein: 0.952 ± 0.324 fold vs. 0.537 ± 0.262

fold, $p = 0.017$), and IRS-1 levels in diabetic subjects were significantly lower than those in the non-diabetic group (mRNA: 0.318 ± 0.142 fold vs. 0.891 ± 0.217 fold, $p = 0.014$; protein: 0.416 ± 0.213 fold vs. 0.643 ± 0.312 fold, $p = 0.024$). Furthermore, we evaluated the correlation of CTRP1 and IRS-1 by Pearson's correlation analysis. In the diabetic group, CTRP1 was positively correlated with IRS-1 (mRNA: $r = 0.226$, $p = 0.031$; protein: $r = 0.207$, $p = 0.038$). However, there was no correlation of CTRP1 and IRS-1 in the non-diabetic group (mRNA: $r = 0.062$, $p = 0.589$; protein: $r = -0.104$, $p = 0.318$). These data indicated that CTRP1 positively regulates IRS-1 only in the state of insulin resistance.

Fig. 2 Changes of glucose utilization rate (GUR) in MADAT and MANDAT after CTRP1 intervention

Glucose utilization rate (GUR) was measured after CTRP1 intervention in the presence of insulin or no insulin. In no insulin group, GUR showed no significant difference in MADAT ($n=5$, GUR with no CTRP1 = 100 ± 3.8 , GUR with CTRP1 = 106.2 ± 2.9 , $p = 0.121$) and MANDAT ($n=5$, GUR with no CTRP1 = 103 ± 3.1 , GUR with CTRP1 = 104.9 ± 2.6 , $p = 0.175$). And in insulin group, GUR was increased after intervention with CTRP1 in MADAT and MANDAT (MADAT, GUR with no CTRP1 = 354 ± 12.9 , GUR with CTRP1 = 419 ± 16.8 , $p = 0.041$; MANDAT, GUR with no CTRP1 = 418 ± 13.1 , GUR with CTRP1 = 450 ± 11.4 , $p = 0.045$). GUR was relative value compared with supposed GUR in non-diabetic group as 100% in the absence of insulin and CTRP1.

Fig. 3 Expression levels of CTRP1 and IRS-1

Relative expression levels of CTRP1 mRNA, IRS-1 mRNA (A) CTRP1 protein and IRS-1 protein (B) in adipose tissue (open bars, non-diabetic group, $n=35$; black bars, diabetic group, $n=35$). Relative expression levels of IRS-1 mRNA (C) and IRS-1 protein (D) in MADAT and MANDAT (open bars, control group, $n=5$; black bars, CTRP1 intervention group, $n=5$). Relative levels were values compared with internal standard (GAPDH). * $p < 0.05$.

To further examine the correlation of CTRP1 and IRS-1, we used CTRP1 to intervene mature adipocytes. After incubation with 40 ng/mL CTRP1 for 12 hours, IRS-1 was higher in MADAT and MANDAT than that in the control group (MADAT: mRNA, 0.560 ± 0.083 fold and 0.388 ± 0.040 fold, $p = 0.027$; protein, 0.607 ± 0.087 fold and 0.415 ± 0.027 fold, $p = 0.031$; MANDAT: mRNA, 1.047 ± 0.160 fold and 0.838 ± 0.075 fold, $p = 0.035$; protein, 0.996 ± 0.060 fold and 0.791 ± 0.105 fold, $p = 0.041$) (Fig. 3C, 3D).

Relationship between CTRP1 and phosphorylation of multi IRS-1 serine sites

IRS serine phosphorylation is a physiological feedback mechanism in insulin signaling to promote insulin resistance [8]. Phosphorylation levels of multi IRS-1 Serine sites were detected to further analyse

the relationship between CTRP1 and IRS-1, including Ser302, Ser307, Ser612, Ser636/639, Ser789, and Ser1101. Results showed that phosphorylation levels of Ser302 and Ser307 in adipose tissue of diabetic subjects were both higher than those in the non-diabetic group (p-Ser302: 0.819 ± 0.164 fold vs. 0.528 ± 0.112 fold, $p = 0.028$; p-Ser307: 0.921 ± 0.194 fold vs. 0.684 ± 0.261 fold, $p = 0.038$). And Ser1101 phosphorylation levels were lower in the diabetic group than those in the non-diabetic group (0.372 ± 0.072 fold vs. 0.664 ± 0.167 fold, $p = 0.018$). There were not significant differences in phosphorylation levels of Ser612, Ser636/639, and Ser789 between groups (p-Ser612: 0.618 ± 0.136 fold vs. 0.528 ± 0.112 fold, $p = 0.063$; p-Ser636/639: 0.576 ± 0.124 fold vs. 0.616 ± 0.211 fold, $p = 0.079$; p-Ser789: 0.568 ± 0.137 fold vs. 0.498 ± 0.135 fold, $p = 0.068$) (Fig. 4A).

Fig. 4 Phosphorylation levels of IRS-1 Serine

Phosphorylation levels of multi IRS-1 Serine sites were measured in adipose tissue (A, open bars, non-diabetic group, $n=35$; black bars, diabetic group, $n=35$) and mature adipocyte *in vitro* (B, open bars, control group, $n=5$; black bars, CTRP1 intervention group, $n=5$), including Ser302, Ser307, Ser612, Ser636/639, Ser789, and Ser1101. Relative levels were values compared with internal standard (GAPDH). * $p < 0.05$.

CTRP1 protein was negatively correlated with Ser1101 phosphorylation in the diabetic group ($r = -0.398$, $p = 0.031$) (Fig. 5). There was no significant correlation between CTRP1 protein and phosphorylation of Ser302, Ser307, Ser612, Ser636/639, and Ser789 (Table 2). CTRP1 protein was not correlated with phosphorylation of all IRS-1 serine sites in the non-diabetic group.

In MADAT and MANDAT, the phosphorylation levels of Ser1101 after incubation with CTRP1 were significantly lower than those in the control group (MADAT, control group: 0.608 ± 0.060 fold, CTRP1 intervention group: 0.419 ± 0.077 fold, $p = 0.012$; MANDAT, control group: 0.657 ± 0.068 fold, CTRP1 intervention group: 0.508 ± 0.055 fold, $p = 0.017$) (Fig. 4B).

Discussion

CTRP1 was a cytokine secreted by adipose tissue. It had been demonstrated to have the biological functions of reducing blood glucose and improving glucose metabolism in animal models [9-11]. Our previous study also showed that plasma CTRP1 was closely related to insulin resistance in type 2 diabetes [3]. However, the correlation between CTRP1 and glucose metabolism in adipose tissue was still not established. Therefore, in this study, we explored the role of CTRP1 in the process of glucose utilization of adipose tissue in type 2 diabetes.

We observed that CTRP1 was significantly increased in type 2 diabetes. This was considered that CTRP1

Fig. 5 The correlation between CTRP1 protein and phosphorylation of IRS-1 Serine1101

The correlation between CTRP1 protein and p-Ser1101 was examined by Pearson correlation analysis. (A) CTRP1 protein was negative correlated with p-Ser1101 in the diabetic group ($r = -0.398$, $p = 0.031$), $n=35$; (B) CTRP1 protein was not correlated with p-Ser1101 in the non-diabetic group ($r = 0.059$, $p = 0.621$), $n=35$.

Table 2 Correlations between of CTRP1 protein and phosphorylation of IRS-1 Serine in adipose tissue

Phosphorylation sites	Diabetic group (n=35)		Non-diabetic group (n=35)	
	Correlation coeff.	<i>p</i>	Correlation coeff.	<i>p</i>
p-Ser302	0.152	0.309	0.178	0.291
p-Ser307	-0.214	0.121	0.096	0.387
p-Ser612	-0.318	0.094	-0.143	0.359
p-Ser636/639	0.263	0.072	0.198	0.124
p-Ser789	-0.193	0.135	0.218	0.095
p-Ser1101	-0.398	0.031	0.059	0.621

participated in the process of regulating glucose metabolism. But interestingly, CTRP1 levels were negatively correlated with the degree of insulin resistance. Thus we hypothesized that CTRP1 was a secreted feedback adipokine under the situation of insulin resistance, which physiological function might improve insulin sensitivity to try to keep steady-state of glucose metabolism. Consistent with our hypotheses, in CTRP1 transgenic mice, Peterson *et al.* reported that CTRP1 overexpression increased insulin sensitivity of peripheral tissues, and reduced blood glucose levels of transgenic mice after glucose load in the state of metabolic emergency caused by high fat feeding [9]. Glucose utilisation test also was used to further identify the role of CTRP1 in improving insulin resistance. We found that glucose utilisation rate of mature adipocytes was improved by CTRP1 in the presence of insulin. However, there was no significant difference in glucose utilisation of mature adipocytes after incubation with CTRP1 in the absence of insulin. Therefore, we speculated that CTRP1 might lower blood sugar by improving insulin resistance in human body. However, the way to regulate the insulin sensitivity of adipocytes by CTRP1 was still unclear. Therefore, we further explored the action mechanism of CTRP1 based on the experimental study of adipose tissues and vitro cell model.

Our data also showed that CTRP1 was closely related to IRS-1 in type 2 diabetes. IRS-1 was activated to improve insulin sensitivity as one of insulin receptor substrates [12, 13]. Moreover, decreased IRS-1 could lead to insulin resistance by blockage of insulin signal transduction in muscle cells and adipocytes [14, 15]. In this study, we found that IRS-1 was increased after incubation with CTRP1 in mature adipocytes *in vitro*. These data suggested that CTRP1 improved the insulin sensitivity of adipose tissue by upregulating IRS-1. However, IRS-1 was low in type 2 diabetic subjects. There were many factors involved in insulin resistance of adipose tissues by regulating IRS-1 levels. For example, inflammatory factors activated intracellular signaling pathway – such as inhibitor of nuclear factor kappa-B kinase β (IKK β) / nuclear factor kappa B (NF- κ B) pathway, c-Jun N-terminal kinase (JNK) / extracellular signalregulated kinases (ERK) pathway, and mammalian target of rapamycin (mTOR) pathway, *etc.* – in the chronic inflammation state in type 2 diabetes [12, 16, 17]. These all led to reduced IRS-1 protein, blockage in signal transduction of insulin effector cells

(*e.g.*, adipocytes, liver cells, and muscle cells) and, eventually, insulin resistance. Therefore, we needed to explore action mechanism of CTRP1 and IRS-1 to further clarify CTRP1 function in type 2 diabetes.

In addition, we found that CTRP1 was not only related to IRS-1 protein, but also closely related to the phosphorylation of IRS-1 serine. IRS-1 contained more than 30 serine phosphorylation sites. Phosphorylation of IRS-1 serine could lead to insulin resistance by affecting the activity of tyrosine kinase [18]. Thereinto, phosphorylation of Ser302 was induced by insulin stimulation, Ser307 was phosphorylated by JNK and I-kappa-B kinase (IKK), phosphorylation of Ser612 and Ser636/639 was regulated by protein kinase C (PKC), phosphorylation of Ser789 was activated by Salt Inducible Kinase 2 (SIK2), and phosphorylation of Ser1101 was mediated by PKC, *etc* [19-22]. Our data showed that phosphorylation levels of Ser1101 were significantly lower in type 2 diabetic group than those in the non-diabetic group. However, phosphorylation levels of Ser302 and Ser307 were higher than those in the control group. Moreover, we found that CTRP1 in adipose tissues were negatively correlated with Ser1101 phosphorylation. Accordingly, we supposed that the expression of IRS-1 protein after incubation with CTRP1 was increased by reducing phosphorylation of IRS-1 Ser1101 in MADAT and MANDAT. Jin UH *et al.* also reported that the phosphorylation of IRS-1 Ser1101 was upregulated by JNK and PKC activation to cause degradation of IRS-1 protein [23]. However, IRS-1 protein degradation was not determined by its mRNA expression level. This was different with our findings. It was suggested that there existed other relation between CTRP1 and IRS-1. So we need to further explore the mechanism for them. In addition, Yu Li *et al.* previously certified that IRS-1 Ser1101 was phosphorylated by PKC θ to block IRS-1 tyrosine phosphorylation and downstream activation of the Akt pathway, which was associated to insulin resistance [24]. Based on the above data, we speculated that CTRP1 improved insulin sensitivity and corrected insulin resistance by reduced phosphorylation of Ser1101.

In conclusion, CTRP1 was over expressed and showed negative relation with insulin resistance in type 2 diabetes. Furthermore, CTRP1 not only improved insulin resistance to increase the glucose utilisation rate of mature adipocytes, but also closely related to the phosphorylation of IRS-1 Ser1101. Therefore, we

proposed that CTRP1 might have a feedback activation to up-regulate its expression under the situation of insulin resistance, and it improved insulin resistance by reducing phosphorylation of IRS-1 Ser1101.

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Disclosure Statement

All authors of this manuscript have no conflict of interest to declare.

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