The effect of short-term intensive insulin therapy on inflammatory cytokines in patients with newly diagnosed type 2 diabetes

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Abstract

Background: Diabetes mellitus was a chronic low-grade inflammatory disease and had increased circulating inflammatory cytokines and acute phase proteins. We aimed to identify the changes of inflammatory cytokines in newly diagnosed type 2 diabetic patients after short-term intensive insulin therapy using continuous subcutaneous insulin infusion (CSII).

Methods: Thirty-three newly diagnosed type 2 diabetic patients were enrolled between September 2020 to December 2020. Expression of 40 inflammatory cytokines of the patients were tested with RayBiotech antibody array before and after 1 week of intensive insulin therapy of CSII. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was carried out to explore the signaling pathway involved in the therapy.

Results: Five inflammatory cytokines were downregulated significantly after 1 week of CSII therapy. They were interleukin-6 receptor (IL-6R), regulated upon activation normal T-cell expressed and secreted (RANTES), intercellular adhesion molecule-1 (ICAM-1), tissue inhibitor of metalloproteinase-1 (TIMP-1), and platelet-derived growth factor type BB (PDGF-BB) \((p < 0.05\) and foldchange <0.83). Among patients with baseline glycated hemoglobin (HbA1c) < 10%, three proinflammatory cytokines were decreased significantly after therapy: IL-6R, RANTES, and ICAM-1. As for the patients with baseline HbA1c \(\geq\) 10%, eight inflammatory cytokines were inhibited significantly after the treatment, including ICAM-1, IL-6R, RANTES, TIMP-1, TIMP-2, macrophage inflammatory protein-1 beta (MIP-1β), PDGF-BB, and tumor necrosis factor receptor type II (TNF RII). No matter which subgroup of baseline HbA1c level was considered, the decreased cytokines after CSII therapy were significantly involved in TNF signaling pathway. Nuclear factor-kappa B (NF-κB) signaling pathway was mainly enriched in patients with baseline HbA1c \(\geq\) 10%.

Junyu He and Peiji Dai contributed equally to this study.

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Conclusions: A panel of 40 inflammatory cytokines, measured by protein microarray, were evaluated for 1 week of CSII treatment in newly diagnosed type 2 diabetic patients. After treatment, many proinflammatory cytokines decreased. In the higher baseline HbA1c subgroup, more proinflammatory cytokines improved. No matter which subgroup of HbA1c level was considered, IL-6R, RANTES, and ICAM-1, which were involved in TNF signaling pathway, decreased significantly after CSII therapy. This was the first report showing that the cytokines of IL-6R, TIMP-2, PDGF-BB, and TNF RII decreased after the CSII therapy.

KEYWORDS
antibody array, inflammatory cytokines, short-term intensive insulin therapy, type 2 diabetes

Highlights
- Many proinflammatory cytokines could be decreased after 1 week of intensive insulin therapy using continuous subcutaneous insulin infusion (CSII).
- This was the first report showing that the cytokines of interleukin-6 receptor (IL-6R), tissue inhibitor of metalloproteinase-2 (TIMP-2), platelet-derived growth factor type BB (PDGF-BB), and tumor necrosis factor receptor type II (TNF RII) decreased after the CSII therapy.
- The circulating levels of IL-6R, regulated upon activation normal T-cell expressed and secreted (RANTES), and intercellular adhesion molecule-1 (ICAM-1) were decreased after 1 week of intensive insulin therapy using CSII no matter which subgroup of glycated hemoglobin was considered.

1 INTRODUCTION

Type 2 diabetes is one of the most common chronic metabolic diseases. It is also a chronic low-grade inflammatory disease. Diabetic patients had increased circulating inflammatory cytokines and acute phase proteins, such as tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP), which will lead to systemic metabolic dysfunction.1 It is probably because hyperglycemia-induced oxidative stress and hyperglycemia could activate the inflammatory pathway as well.2 Meanwhile, insulin resistance and dyslipidemia of type 2 diabetes could promote the release of inflammatory cytokines.3 Insulin has an immunomodulatory effect, which can inhibit the production of proinflammatory cytokines and promote the release of anti-inflammatory cytokines.4 Cytokines mediate signals between immune cells. They are divided into proinflammatory and anti-inflammatory cytokines, which promote and inhibit inflammatory responses. For example, TNF-α, interferon-γ (IFN-γ), interleukin-1β (IL-1β) and interleukin-6 (IL-6) belongs to proinflammatory cytokines, while interleukin-4 (IL-4) and interleukin-10 (IL-10) are anti-inflammatory cytokines.5 Besides, insulin could reduce the lipopolysaccharide-induced increases in reactive oxygen species (ROS) generation and some important mediators of oxidative, nitrosative, and inflammatory stress, including thiobarbituric acid-reacting substances, nitrite and nitrate, and plasma-free fatty acids.6

Short-term intensive insulin therapy (STII) has been used as one of the first-line treatment for newly diagnosed type 2 diabetes.7 It could improve glycemic levels, reserve β-cell dysfunction, and restore the acute insulin response by reducing glucotoxicity and subsequent β-cell overload.8 After 2 weeks of STII using continuous subcutaneous insulin infusion (CSII) for newly diagnosed type 2 diabetes, about 50% of patients achieved glucose remission for 1 year without any followed glucose-lowering agents, accompanied with insulin sensitivity and β-cell function remarkably improved.7 Besides, STII had anti-inflammatory effects in type 2 diabetic patients by lowering blood glycemic levels, improving insulin resistance, and inhibiting hyperglycemia-induced oxidative stress.9,10 Long-term intensive insulin therapy could also improve dyslipidemia, therefore reducing the release of
TABLE 1  Clinical data of the newly diagnosed type 2 diabetes patients before and after STII using the continuous subcutaneous insulin infusion treatment

|                        | Total (n = 33) | HbA1c<10% (n = 15) | HbA1c ≥10% (n = 18) |
|------------------------|---------------|------------------|-------------------|
|                        | Before STII   | 1 week after STII| Before STII       | 1 week after STII |
| Age (years)            | 51.9 ± 7.07   | 52.73 ± 7.95     | 51.17 ± 12.13     |
| Sex (male/female)      | 23/10         | 10/5             | 13/5              |
| BMI (Kg/ m²)           | 25.42 ± 2.85  | 24.54 ± 2.51     | 26.16 ± 2.9       |
| FPG (mmol/L)           | 11.16 ± 2.94  | 5.75 ± 1.02***   | 9.26 ± 2.03       |
| FINS (µU/ml)           | 6.89 ± 2.93   | 6.99 ± 3.87      | 6.72 ± 3.02       |
| HbA1c(%)               | 10.65 ± 2.01  | 9.13 ± 1.84***   | 8.75 ± 0.71       |
| TG (mmol/L)            | 1.96 (1.35)   | 1.3 (0.46)**     | 2.34 ± 1.34       |
| Cholesterol (mmol/L)   | 4.7 (1.4)     | 4.6 (1.95)       | 5.02 ± 1.37       |
| HDL-C (mmol/L)         | 1.00 ± 0.29   | 1.10 ± 0.27***   | 1.07 ± 0.38       |
| LDL-C (mmol/L)         | 3.24 ± 0.76   | 3.07 ± 0.80      | 3.26 ± 0.98       |
| Serum creatinine(µmol/L)| 61.33 ± 15.69| 70.79 ± 18.23*** | 64.8 ± 19.2       |
| HOMA-β                 | 17.96(16.97)  | 60.2(36.78)***   | 22.63 (25.61)     |
| Insulin dose (IU/kg/d) | 0.74 ± 0.16a  | 0.5 ± 0.25b      | 0.72 ± 0.18a      |

Note: Continuous parametric variables were expressed as means ± SD. Continuous nonparametric variables were expressed as medians (interquartile ranges). And categorical variables were expressed as proportion. Abbreviations: BMI, body mass, index; FINS, fasting insulin; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of β-cell function; LDL-C, low-density lipoprotein cholesterol; STII, short-term intensive insulin therapy; TG, triglyceride.

*aThe largest insulin dose during the treatment.

*bThe insulin dose at the end of the treatment.

*p < 0.05; **p < 0.01; ***p < 0.005 when compared with baseline.
proinflammatory cytokines. STII therapy using CSII for 2 weeks could decrease the ratio of type 1 T helper/type 2 T helper cells and increase the proportion of regulatory T cells, which would alleviate the inflammatory responses. It was found that intensive insulin therapy could decrease and improve proinflammatory cytokines in patients with severe trauma, such as TNF-α, IL-1β, IL-6, IFN-γ, and CRP, and it could increase anti-inflammatory cytokines, such as IL-2, IL-4, and IL-10. Another research found that insulin infusion inhibited monocyte chemoattractant protein 1 (MCP-1) and soluble intercellular adhesion molecule 1 (sICAM-1) in obese nondiabetic patients. However, the types of inflammatory cytokines analyzed in CSII treatment were limited at present, mainly including IL-1, IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ, TNF-α, MCP-1, regulated upon activation normal T-cell expressed and secreted (RANTES), eotaxin, C-X3-C motif chemokine ligand 1 (CX3CL1), ICAM-1, macrophage migration inhibitory factor (MIF), tissue inhibitor of metalloproteinase-1 (TIMP-1), and tumor necrosis factor receptor type 1 (TNF-RI). The changes of other cytokines after CSII treatment were not clear. The levels of cytokines were analyzed by ELISA in previous studies, which could not detect a large number of proteins simultaneously in a strict special clinical condition.

Recently, protein microarrays have become a useful tool for biological research in tumor, inflammation, and...
autoimmune diseases. It could analyze a large number of proteins simultaneously in various biological samples, such as serum and tissue. The Quantibody array, also known as antibody-based sandwich microarray, is one type of the protein microarrays, which uses cytokine specific antibodies for detection. It combines the advantages of the high detection sensitivity, specificity of ELISA, and the high throughput of arrays with only a small amount of sample volume.

Here, we use Quantibody human inflammation array 3 (QAH-INF-3, RayBiotech, Peachtree Corners, GA, USA) to systematically measure the changes of 40 common inflammatory cytokines in newly diagnosed type 2 diabetic patients after 1 week of intensive insulin therapy using CSII. Bioinformatics analysis was used to study the possible related mechanism.

2 | METHODS

2.1 | Subjects

The study was from an independent randomized controlled trial (granted by two Chinese research programs, 2018YFC1314100 and 2019B020230001) and has been registered in the public trials registry. It was approved by the academic research department of the First Affiliated Hospital of Sun Yat-sen University ([2019]174-1). Data and blood samples were collected from patients with newly diagnosed type 2 diabetes mellitus who came to our hospital for short-term CSII intensive treatment from September 2020 to December 2020. The patients who were enrolled in this study were diagnosed as type 2 diabetes mellitus according to the criteria of the World Health Organization. (A–C) For the patients with baseline glycated hemoglobin (HbA1c) < 10%, histograms of the concentration of ICAM-1 (intercellular adhesion molecule-1), IL-6R (interleukin-6 receptor), and RANTES (regulated upon activation normal T-cell expressed and secreted) before and after CSII therapy; (D) Heatmap of the concentration distribution of ICAM-1, IL-6R, and RANTES before and after CSII therapy. **, p < 0.01 versus before STII. CSII, continuous subcutaneous insulin infusion; STII, short-term intensive insulin therapy
Health Organization (1999) for no more than 1 year and have never received any hypoglycemic therapy (oral hypoglycemic drugs or insulin), aged between 18 and 70 years old, with glycated hemoglobin (HbA1c) ≥ 7.0% and body mass index between 20 and 35 kg/m². The exclusion criteria included (1) patients diagnosed as type 1 diabetes; (2) patients with acute complications of diabetes or severe microvascular and macrovascular complications; (3) patients with systemic infection or cancer; (4) patients with chronic cardiac insufficiency (New York Heart Association class II-IV) or renal insufficiency (estimated glomerular filtration rate [eGFR] < 50 ml/min/1.73m² according to Chronic Kidney Disease Epidemiology Collaboration formula); (5) patients whose blood pressure was higher than 180/110 mm Hg and could not be controlled within 160/110 mm Hg; (6) patients who took any medicine that may affect glycemic levels for more than 1 week (glucocorticoid, growth hormone, estrogen, progestogen, and antipsychotic drugs); and (7) pregnant or breastfeeding women. In all, 33 patients were enrolled in the study and informed consent was obtained from them.

2.2 | Study design and sample collection

All subjects were hospitalized for CSII intensive treatment. After admission, baseline assessments were carried out and blood samples were collected. Then on
the second day, CSII intensive therapy was administered. The initial insulin daily dose was 0.4-0.8 IU/Kg/d, half dose was used as basal insulin for 24 h and the other half was divided equally before three meals. The insulin infusion regimen was adjusted according to capillary blood glucose levels, which were tested eight times daily (fasting in the morning, before and 2 h after three meals, and 3 AM at night). The strict glycemic target was 4.4-6 mmol/L for pre-meals and 4.4-8 mmol/L for 2 h postprandial glucose level. When the glycemic target was reached, the treatment was maintained for another 7 days and usually insulin dosage was down-titrated. Then CSII was discontinued after dinner of the last day and the blood samples were collected on the next morning. The blood samples before and after intensive insulin therapy were centrifuged at 3000 rpm for 10 min and the supernatant was separated and stored at −80°C.

2.3 | Measurement

Quantibody human inflammation array 3 (QAH-INF-3, RayBiotech) was used for quantitative measurement of 40 inflammatory cytokines of serum samples before and after CSII insulin therapy. The 40 inflammatory cytokines included B lymphocyte chemoattractant (BL/CXCL13), eotaxin-1 (CCL11), eotaxin-2 (MPIF2/CCL24), granulocyte colony stimulating factor, granulocyte-macrophage colony-stimulating factor, I-309 (TCA-3/CCL1), ICAM-1, IFN-γ, IL-1α, IL-1β, IL-1 ra, IL-2, IL-4, IL-5, IL-6, IL-6 R, IL-7, IL-8, interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 p40 (IL-12 p40), IL-12 p70, IL-13, IL-15, IL-16, IL-17A, MCP-1, monocytic colony stimulating factor, monokine induced by gamma interferon, macrophage inflammatory protein-1 alpha (MIP-1α/CCL3), macrophage inflammatory protein-1 beta (MIP-1β/CCL4), nuclear factor kappa; TNF, tumor necrosis factor.
macrophage inflammatory protein-1 delta (MIP-1δ/CCL15), platelet-derived growth factor type BB (PDGF-BB), RANTES/CCL5, tTIMP-1, TIMP-2, TNF-α, TNF-β, TNF RI, and TNF RII. The experiment was performed according to the manufacturer's protocol. First of all, 100 μL diluted serum were added into the array and incubated for 2 h at room temperature. After washing the array for 5 times, 80 μL secondary antibody solution was added and incubated for 2 h at room temperature. After washing the array again, 80 μL detection solution was added and incubated for 1 h at room temperature. Finally, the microarray was scanned and tested by InnoScan 310 microarray scanner.

2.4 Statistical analysis

The data analysis was conducted with SPSS 20.0 software. Normally distributed continuity variables were expressed as mean ± SD and compared by Student t-tests or paired sample t-tests. Nonnormally distributed variables were expressed as medians (interquartile ranges) and analyzed by Wilcoxon rank-sum test. The changes of inflammatory cytokines were considered significant according to p value less than 0.05 and foldchange greater than 1.2 or less than 0.83. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was carried out to explore the possible mechanism of inflammation changes before and after CSII therapy by using the open source program R (version 3.6.3).

| Total HbA1c<10% | HbA1c ≥ 10% |
|----------------|-------------|
| Pathway | p.adjust | Pathway | p.adjust | Pathway | p.adjust |
| EGFR tyrosine kinase inhibitor resistance | 0.019 | Rheumatoid arthritis | 0.007 | Viral protein interaction with cytokine and cytokine receptor | 4.12E-05 |
| Rheumatoid arthritis | 0.019 | Viral protein interaction with cytokine and cytokine receptor | 0.007 | Cytokine-cytokine receptor interaction | 0.002 |
| Viral protein interaction with cytokine and cytokine receptor | 0.019 | TNF signaling pathway | 0.007 | TNF signaling pathway | 0.002 |
| HIF-1 signaling pathway | 0.019 | Influenza A | 0.012 | Human cytomegalovirus infection | 0.009 |
| TNF signaling pathway | 0.019 | Lipid and atherosclerosis | 0.014 | Cytosolic DNA-sensing pathway | 0.013 |
| Fluid shear stress and atherosclerosis | 0.024 | Human cytomegalovirus infection | 0.014 | EGFR tyrosine kinase inhibitor resistance | 0.018 |
| JAK–STAT signaling pathway | 0.027 | Cytokine-cytokine receptor interaction | 0.02 | Rheumatoid arthritis | 0.02 |
| Influenza A | 0.027 | NF-kappa B signaling pathway | 0.02 | |
| Kaposi sarcoma-associated herpesvirus infection | 0.031 | Toll-like receptor signaling pathway | 0.02 | |
| Lipid and atherosclerosis | 0.034 | HIF-1 signaling pathway | 0.02 | |
| Human cytomegalovirus infection | 0.034 | Fluid shear stress and atherosclerosis | 0.029 | |
| JAK–STAT signaling pathway | 0.036 | Influenza A | 0.037 | |
| Chemokine signaling pathway | 0.041 | | |
| Kaposi sarcoma-associated herpesvirus infection | 0.041 | |
| Lipid and atherosclerosis | 0.047 | |

Note: p.adjust<0.05 was considered as significant.

Abbreviations: CSII, continuous subcutaneous insulin infusion; EGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HIF-1, hypoxia inducible factor; JAK–STAT, Janus kinase–signal transducer and activator of transcription; KEGG, Kyoto Encyclopedia of Genes and Genomes; NF kappa, nuclear factor kappa; TNF, tumor necrosis factor.
RESULTS

3.1 Baseline characteristics

Thirty-three qualified patients were enrolled and received the CSII therapy. As shown in Table 1, glucose and lipid profiles were improved after CSII therapy. Fasting plasma glucose (FPG) decreased from $11.16 \pm 2.94$ mmol/L to $5.75 \pm 1.02$ mmol/L after treatment ($p < 0.05$). HbA1c dropped from $10.65 \pm 2.01\%$ to $9.13 \pm 1.84\%$ after treatment ($p < 0.05$). The triglyceride levels decreased significantly ($p < 0.05$) and high-density lipoprotein cholesterol levels were increased ($p < 0.05$). Cholesterol and low-density lipoprotein cholesterol (LDL-C) levels were also decreased but without significant difference. When we divided the patients into different baseline HbA1c levels, we found that cholesterol levels and LDL-C levels decreased only in patients with HbA1c < 10%. The homeostasis model assessment of β-cell function value was significantly increased after the therapy, which showed that the functions of islet β cells were greatly improved.

3.2 Cytokines after 1-week CSII therapy

We selected the cytokines whose foldchange was $\geq 1.2$ or $< 0.83$ and $p$ value $< 0.05$. Among the 40 cytokines, 5 were decreased after insulin therapy: IL-6R, RANTES, ICAM-1, TIMP-1, and PDGF-BB (Figure 1).

3.3 Subgroup analysis

The patients were divided into two subgroups: baseline HbA1c < 10% ($n = 15$) and HbA1c $\geq 10\%$ ($n = 18$). In the subgroup of HbA1c < 10%, the levels of ICAM-1, IL-6R, and RANTES decreased significantly after treatment ($p < 0.05$, Figure 2). There were more cytokines decreased significantly after therapy in patients with HbA1c $\geq 10\%$, including ICAM-1, IL-6R, RANTES, TIMP-1, TIMP-2, MIP-1β, PDGF-BB, and TNF RII ($p < 0.05$, Figure 3).

3.4 Pathway analysis

KEGG enrichment analysis showed that the inflammation cytokines that decreased after CSII treatment were mainly involved in the processes of eGFR tyrosine kinase inhibitor resistance, rheumatoid arthritis, viral protein interaction with cytokine and cytokine receptor, hypoxia inducible factor-1, and TNF signaling pathway (Figure 4A). When the patients were divided into different baseline HbA1c levels, we found that the decreased cytokines were significantly enriched in rheumatoid arthritis, viral protein interaction with cytokine and cytokine receptor, and TNF signaling pathway in patients with HbA1c < 10% (Figure 4B). As for patients with HbA1c $\geq 10\%$, the differentially expressed cytokines were associated with the processes of viral protein interaction with cytokine and cytokine receptor, cytokine-cytokine receptor interaction, TNF, and nuclear factor-kappa B (NF-κB) signaling pathway by KEGG enrichment analysis (Figure 4C). The data were shown in Table 2.

DISCUSSION

Type 2 diabetes mellitus is a chronic low-grade inflammatory disease in which several proinflammatory cytokines are increased, including TNF-α, IL-1, and CRP. Intensive insulin therapy had an anti-inflammatory effect and could decrease the levels of proinflammatory cytokines. However, the current analysis about cytokine changes after CSII therapy was limited, mainly including IL-1, IL-6, IL-10, IFN-γ, TNF-α, MCP-1, RANTES, ICAM-1, and so on. The function of many inflammatory cytokines in CSII therapy remains unclear. Here QuantiBody human inflammation array was used to evaluate the levels of inflammatory cytokines in CSII therapy for the first time, which combines the advantages of the high detection sensitivity of ELISA and the high throughput of arrays. The patients were divided into different baseline HbA1c levels and the levels of inflammatory cytokines were analyzed in different groups.

The expression of three cytokines, IL-6R, RANTES, and ICAM-1, decreased after CSII therapy in both subgroups. As we know, IL-6 is a proinflammatory cytokine involved in inflammation, insulin resistance, and β cell function. IL-6R is the receptor of IL-6, which exists as transmembrane IL-6 receptor or soluble forms of IL-6R. It was found that IL-6 level is higher in type 2 diabetic patients and insulin therapy could decrease IL-6 level in diabetic patients. But it has not been reported before that IL-6R levels were decreased significantly after 1 week of intensive insulin therapy in newly diagnosed type 2 diabetic patients. RANTES is a proinflammatory chemokine. As we know, chemokines are important in inflammation by mediating the arrival of inflammatory cells to the sites of acute and chronic inflammation. They can be divided into CC and CXC chemokines according to the structure. CC chemokines include MCP 1–5, RANTES, eotaxins 1–3, and MIP-1α and -β. It was found that RANTES levels of type 2 diabetic patients were higher than normal people. However, they were decreased after 5 weeks of...
insulin therapy compared with before, which is inconsistent with our result. ICAM-1 is one of the surface glycoproteins in the Ig superfamily. ICAM-1 is involved in the progression of inflammation because it is the key molecular for leukocyte adhesion to endothelium and leukocyte migration into tissues, leading to endothelial cell injury.22 Hyglycemia will lead to the increase of ICAM-1, whereas insulin intensive therapy can inhibit the release of ICAM-1.23 In type 2 diabetic patients, 2u/h insulin infusion could reduce sICAM-1 level while controlling blood glycemic level.15 Intensive insulin therapy could also reduce the urinary ICAM-1/creatinine ratio.24 It was found that insulin could inhibit the NF-κB signaling pathway and increase IκB, which binds to cytoplasmic NF-κB and prevents its translocation to the nucleus, thereby reducing the expression of proinflammatory cytokines such as IL-6, ICAM-1, and CRP and leading to ROS generation.25 Insulin could also suppress activator protein-1 and early growth response-1, which are proinflammatory transcription factors as well. The cytokines regulated by these transcription factors are also inhibited by insulin, such as ICAM-1 and tissue factor.26

Patients with higher hyperglycemia, baseline HbA1c ≥ 10%, had more serious inflammation. More proinflammatory cytokines decreased and improved after CSII therapy. One explanation was that glucose intake resulted in an increase in ROS generation by leucocytes and increased oxidative load, which led to inflammatory stress.27 Patients with higher glycemic levels are associated with more serious inflammatory stress. Insulin infusion could inhibit the ROS generation and had an anti-inflammatory effect.27 In addition to the above three cytokines (IL-6R, RANTES, and ICAM-1), we found that the cytokines of TIMP-1, TIMP-2, MIP-1β, PDGF-BB, and TNF RII also decreased after treatment in the subgroup of baseline HbA1c ≥ 10%. As we know, TIMP-1 is one of the TIMPs, which can inhibit the proteolysis mediated by zinc enzymes, also known as matrix metalloproteinases.28 It is also involved in vascular stromal fibrosis.29 The chronic inflammation of type 2 diabetes increased the levels of TIMP-1 and TIMP-2, leading to increased risk of diabetic vasculopathy.30 TIMP-1 levels were decreased in diabetic patients complicated with hypertension after 1 year of intensive insulin therapy, antplatelet, pressure-lowering and lipid-lowering treatment.29 We found that CSII therapy for 1 week can significantly reduce TIMP-1 levels in newly diagnosed type 2 diabetic patients, especially in patients with HbA1c ≥ 10%, indicating that short-term intensive insulin therapy plays an important role in improving chronic inflammation and preventing vascular lesions. Interestingly, TIMP-1 can also reduce the apoptosis of pancreatic islet β cells in type 1 diabetic patients and enhance the proliferation of β cells, which may be a therapeutic target for reversing type 1 diabetes.31 So further experiment is needed on the relationship and mechanism between TIMP-1 and diabetes. TIMP-2 is also one type of TIMPs and is closely associated with acute kidney injury.22 It has been reported that TIMP-2 was increased in patients with metabolic syndrome (MetS) and it was even higher in patients with diabetes compared with nondiabetic MetS.32 The mechanism is not clear. We found that intensive insulin therapy reduced TIMP-2 levels in patients with HbA1c ≥ 10%, which had not been discovered before. More basic research is warranted to study the relationship of TIMP-2 and insulin therapy. MIP-1β is a kind of proinflammatory chemokine, also known as chemokine CC motif ligand 4 (CCL4). It plays an important role in the progression of diabetes mellitus, especially diabetic vasculopathy.34 MIP-1β expression is increased in type 2 diabetic patients.35,36 It was also found that low-dose insulin infusion for 4 h inhibited the expression of MIP-1β in monocytes in patients with type 2 diabetes. But no significant changes were found in circulating MIP-1β levels.39 We found that subcutaneous intensive insulin therapy for 1 week significantly reduced circulating MIP-1β levels, possibly because hyperglycemia could stimulate the monocytes to secrete more MIP-1β.34 In addition, circulating MIP-1β levels would be increased with the dysfunction of islet β cells, and exogenous insulin could suppress MIP-1β by reducing glycemic levels and unloading the β-cells. Circulating MIP-1β levels were negatively correlated with proinsulin levels.37 TNF-RII is one of the receptors of TNF-α, which is closely related to the progression of diabetes, especially diabetic nephropathy.38 The previous studies did not find significant reduction in TNF-RII levels after 14 weeks of insulin therapy in diabetic patients, probably because of low baseline HbA1c levels (7%–10%).39 We found that TNF-RII levels were decreased after CSII therapy only in patients with HbA1c ≥ 10%. The mechanism of it needs to be further studied. Another inflammatory cytokine was PDGF-BB, which was also decreased after therapy. It belongs to PDGF and is closely related to wound healing.40 Circulating PDGF-BB levels were higher in type 1 diabetic patients compared with healthy people.41 Low levels of circulating PDGF-BB are thought to be associated with cardiovascular complications of type 2 diabetes.42 It could also be used to predict diabetic macular edema.43 At present there are few studies of the relationship between PDGF and diabetes. Our research demonstrated that CSII therapy could reduce the PDGF-BB levels in newly diagnosed type 2 diabetic patients and it was not discovered before. We still need more research to confirm it. In a word, more proinflammatory cytokines decreased after CSII therapy, which indicated that intensive insulin therapy had a stronger anti-inflammatory effect in patients with poor glycemic control.
We also analyzed the signaling pathways related to these inflammatory cytokines by KEGG enrichment analysis and provide a direction for subsequent basic research. We found that no matter which subgroup of baseline HbA1c level was considered, the differentially expressed cytokines in CSII therapy were significantly involved in TNF signaling pathway, which played an important role in the development of type 2 diabetes mellitus by inhibiting the insulin signaling pathway through serine phosphorylation. 44 In addition, NF-κB signaling pathway was mainly enriched in patients with higher HbA1c level (HbA1c ≥ 10%). It is a typical proinflammatory signaling pathway because it is involved in the expression of multiple proinflammatory cytokines and chemokines. 45 It was found that insulin could inhibit NF-κB signaling pathway by increasing cellular IκB, which binds to cytoplasmic NF-κB and prevents its translocation to the nucleus. 25 However, we found that different signaling pathways were involved in CSII therapy in different baseline HbA1c populations and the mechanism remains unclear, which still needs more basic research.

However, the proinflammatory cytokines, IL-1β, IL-6, and TNF-α, were decreased after CSII treatment but without significant difference (p>0.05, data not shown). It was found that IL-6 levels were decreased in newly diagnosed type 2 diabetic patients after 4 weeks of intensive insulin therapy with multiple subcutaneous insulin injection. 4 IL-1β and TNF-α levels were also reduced after 2 weeks of intensive insulin therapy by CSII or premixed 30/70 insulin in a previous study. 36,47 It might be for the following reasons. First, the duration of intensive insulin therapy in our study was short, only 1 week. The insulin treatment lasted for more than 2 weeks in the previous study. Second, the hyperglycemic levels of patients (FPG 14.62 ± 1.68 mmol/L, HbA1c 11.9 ± 2%) in the previous study might be higher than the patients here (FPG 11.16 ± 2.94 mmol/L, HbA1c 10.65 ± 2.01%). 46 Besides, the limited number of subjects in our study resulted in relatively large SD of the measured concentrations of inflammatory cytokines, leading to nonsignificant difference in statistical analysis.

There were two limitations in the study. First, the number of subjects was small here. Fortunately, the analysis results were significant, despite the small sample size. In the future, more subjects should be included to validate the differential expression of these cytokines after STII therapy. Second, we used antibody microarray-based technology to test the expression of inflammatory cytokines. Other methods, such as ELISA, should be done to verify the changes of these cytokines, especially for the four newly reported cytokines, IL-6R, TIMP-2, TNF-RII, and PDGF-BB.

In summary, we found that five proinflammatory cytokines were reduced after only 1 week of CSII therapy in newly diagnosed type 2 diabetic patients. They were IL-6R, RANTES, ICAM-1, TIMP-1, and PDGF-BB. In patients with baseline HbA1c ≥ 10%, the levels of TIMP-2, MIP-1β, and TNF RII also reduced beside the five cytokines, which further indicated that intensive insulin therapy has an anti-inflammatory effect. The worse the hyperglycemia levels were, the more benefits of anti-inflammatory effect were obtained. The reduction of IL-6R, TIMP-2, TNF-RII, and PDGF-BB were reported for the first time. No matter which subgroup of baseline HbA1c level was considered, the decreased cytokines after CSII therapy were significantly involved in TNF signaling pathway. NF-κB signaling pathway was mainly enriched in patients with HbA1c ≥ 10%. In the future, more basic research, in vivo or vitro, is required to study the signaling pathways involved in these cytokines and their connections with the micro- or macro-complications for various hypoglycemic treatments.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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