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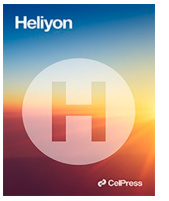

Comparison of changes in adipokine and inflammatory cytokine levels in patients with newly diagnosed type 2 diabetes treated with exenatide, insulin, or pioglitazone: A post-hoc study of the CONFIDENCE trial

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A R T I C L E I N F O

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A B S T R A C T

Background: Adipokines and inflammatory cytokines (ADICs) play important roles in type 2 diabetes mellitus (T2DM). This study aimed to compare the changes of ADIC levels (ΔADICs) in patients with newly diagnosed T2DM treated with different antihyperglycemic agents, and further investigate the impact of these changes on metabolic indices, β-cell function and insulin resistance (IR).

Methods: Four hundred and sixteen patients with newly diagnosed T2DM from 25 centers in China randomly received 48-week intervention with exenatide, insulin or pioglitazone. Anthropometric and laboratory data, indices of β-cell function and IR, and levels of AIDCs, including interleukin-1 beta (IL-1β), interferon-gamma (IFN-γ), leptin, and fibroblast growth factor 21 (FGF21) were detected at baseline and the end of the study.

Results: In total, 281 participants (68 % male, age: 50.3 ± 9.4 years) completed the study. After 48- week treatment, IL-1β and IFN-γ were significantly decreased with exenatide treatment ($P < 0.001$ and $P = 0.001$, respectively), but increased with insulin ($P = 0.009$ and $P = 0.026$, respectively). However, pioglitazone treatment had no impact on ADICs. No significant change in leptin or FGF21 was detected with any of the treatments. After adjustment for baseline values and changes of body weight, waist and HbA1c, the between-group differences were found in ΔIL-1β (exenatide vs. insulin: $P = 0.048$; and exenatide vs. pioglitazone: $P = 0.003$, respectively) and ΔIFN-γ (exenatide vs. insulin: $P = 0.049$; and exenatide vs. pioglitazone: $P < 0.001$, respectively). Multiple linear regression analysis indicated that Δweight was associated with ΔIL-1β ($\beta = 0.753$;

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95 % CI, 0.137–1.369; $P = 0.017$). After adjusting for treatment effects, Δweight was also be correlated with ΔFGF21 ($\beta = 1.097$; 95%CI, 0.250–1.944; $P = 0.012$); furthermore, ΔHOMA-IR was correlated with Δleptin ($\beta = 0.078$; 95%CI, 0.008–0.147; $P = 0.029$) as well. However, ΔHOMA-IR was not significantly associated with ΔIL-1β after adjusting for treatment effects ($P = 0.513$).

Conclusion: Exenatide treatment led to significant changes of inflammatory cytokines levels (IL-1β and IFN-γ), but not adipokines (leptin and FGF21), in newly diagnosed T2DM patients. The exenatide-mediated improvement in weight and IR may be associated with a decrease in inflammatory cytokine levels.

1. Introduction

Type 2 diabetes mellitus (T2DM), as a metabolic disease, is mainly characterized by decreased β-cell function accompanied with increased insulin resistance (IR) [1]. Improving β-cell function and IR may relieve the metabolic disorders associated with the disease and significantly slow its progression [2]. In our previous CONFIDENCE clinical trial, we found that after intervention with exenatide, insulin, or pioglitazone, glycemic and metabolic indices and β-cell function improved in patients with newly diagnosed T2DM. Among the three treatments, exenatide induced the greatest metabolic effects and the best β-cell function improvement [3]. However, the underlying mechanism was not clarified.

Adipose tissue has been proved to play a crucial role in the development of T2DM [4,5] through hormone-like compounds secretion, including adipokines and inflammatory cytokines (ADICs). Adipokines are mainly derived from adipocytes in adipose tissue and function as important modulators in energy expenditure and activity, insulin sensitivity, lipid and glucose metabolism, adipocyte and β-cell function [6]. As the first identified adipokine, leptin was considered a milestone in adipokine research and was found to be the hub of the biomarker correlation network among many adipocytokines in T2DM [7–9]. Fibroblast growth factor 21 (FGF21) is another recently discovered adipokine which is also produced by adipose tissue and liver [10]. Leptin and FGF21 have close relationship and both have important roles as adipokines in the regulation of glucose metabolism and β-cell function [6]. The two adipokines were also proved to be great potential for future clinical application as therapy targets [11,12]. Inflammatory cytokine, another important cytokine, is primarily produced by macrophages and immune cells and mainly have pro-inflammatory function and impair β-cell function, which eventually contribute to the development of T2DM [13–15]. Adipokines have been proposed to link changes of inflammatory markers in T2DM [16]. Adipokines and inflammatory cytokines had an interactional effect in the condition of hyperglycemia [16]. Leptin and FGF21 have been considered to be associated with the regulation of the key pro-diabetic inflammatory cytokines interleukin-1 beta (IL-1β) and interferon-gamma (IFN-γ) [17–19]. Levels of both IL-1β and IFN-γ are increased in animal models of T2DM, and both inflammatory cytokines can impair insulin signaling, induce β-cell apoptosis and dysfunction, and promote insulin resistance [20,21]. Recent studies have also ssessed the potential of IL-1β and IFN-γ as novel therapeutic targets in the treatment of T2DM [22,23]. As a result, adipokines including Leptin and FGF21 and inflammatory cytokines including IL-1β and IFN-γ were investigated together in the present study. Recent studies have suggested that modulating ADIC levels may contribute to the amelioration of T2DM-mediated metabolic disorders and offer new opportunities for pharmacotherapy targeting this condition [24]. Accordingly, research focus has increasingly concentrated on altering ADIC activity in the treatment of T2DM [25].

Many hypoglycemic agents have been demonstrated to alter ADIC levels concomitant with observed improvements in hemoglobin A1c (HbA1c) levels, β-cell function and IR in T2DM patients, leading eventually to the changes in the course of T2DM [24]. Treatment with pioglitazone is indicated to lower leptin levels and increase levels of adiponectin, while also improving IR and HbA1c levels in T2DM [26,27]. In addition, pioglitazone could also suppress the key inflammatory response transcription factors including activator protein-1 (AP-1) and nuclear factor-kappa B (NF-κB) [28]. Insulin treatment is a vital element in the pharmacotherapy of diabetes [2]. The application of exogenous insulin can significantly affect adipokine and inflammatory cytokine expression [29]. The glucagon-like peptide 1 (GLP-1) receptor agonist exenatide exerts multiple actions to regulate glucose by enhancing glucose-dependent insulin secretion, reducing secretion of postprandial glucagon, decreasing appetite and delaying gastric emptying [30]. It has also been observed to significantly alter ADIC expression, accompanied by an improvement in HbA1c levels [31]. The available evidence suggests that pioglitazone, insulin, and exenatide exert more significant effects on adipose tissue, including influencing ADIC expression, compared with other hypoglycemic agents [24]. Nevertheless, few studies directly compared the changes and impact of changes in ADIC expression on not only the metabolic factors, but also β-cell function and IR, in T2DM among these three hypoglycemic agents.

Therefore, a post-hoc study of our previously published CONFIDENCE trial to compare the changes in ADIC expression associated with exenatide, insulin, or pioglitazone was conducted. As illustrated above, we focused on ADICs currently used (leptin) or have a potential to be used as therapeutic tools (fibroblast growth factor 21 [FGF21]) or targets (interferon-gamma [IFN-γ] and interleukin-1β [IL-1β]) [6]. We further investigated the association of ADIC levels with β-cell function and metabolic indices in newly diagnosed, drug-naive T2DM patients following treatment with these three treatments.

2. Material and methods

2.1. Study design

This was a post-hoc study of the CONFIDENCE clinical trial (registered at Clinical Trials.gov under the number NCT01147627). Details of the CONFIDENCE study were published elsewhere [3].

Briefly, this was a multicenter, parallel-group study conducted in 25 centers from 13 provinces of China between August 2010 and August 2012. Four hundred and sixteen patients with newly diagnosed T2DM were 1: 1: 1 randomized to receive 48-week intervention with exenatide, insulin, or pioglitazone.

2.2. Participants

Newly diagnosed T2DM patients aged 30–70 years old who were drug naïve, had HbA1c 7 %–10 % (53–86 mmol/mol) and body mass index (BMI) 20–35 kg/m^2 with stable body weight for more than 3 months were recruited. And participants who had the following situations were excluded: acute or severe chronic micro- and macrovascular complications or comorbidities including nephropathy, retinopathy, neuropathy, hepatic dysfunction, hyperosmotic state, ketoacidosis, and lactic acidosis; positive anti-glutamic acid decarboxylase antibodies; medication influencing gastrointestinal motility, glycemia, bone metabolism and weight; pancreatitis history; triglyceride (TG) levels more than 5 mmol/L; osteoporosis or pathologic fracture history.

Written informed consents were provided by all participants before screening. The study protocol was approved by the Ethics Committee at each site and was undertaken according to Declaration of Helsinki.

2.3. Treatments

Exenatide (Amylin Pharmaceuticals, Inc., San Diego, CA, USA) was subcutaneously injected with 5 μg twice daily initially, and then increased to 10 μg twice daily after 4 weeks. Participants who could not tolerate the adverse events or those who frequently experienced hypoglycemia were instructed to change back to 5 μg twice daily. Premixed insulin (75 % insulin lispro protamine suspension and 25 % insulin lispro injection; Eli Lilly and Company, Indianapolis, IN, USA) was injected 15 min before breakfast and dinner at a dose of 0.4 IU/kg initially, and the dose was divided to 50 %: 50 %. Thus, the doses were titrated according to participants' self-monitored blood glucose (Table Supplementary 1). And pioglitazone (Deyuan Pharmacy, Jiangsu, China) was administered at 30 mg daily initially, and the daily dose was increased to 45 mg after 4 weeks.

The baseline measurements were conducted in two days. On Day 1, the anthropometric data, laboratory indices (fasting plasma glucose, HbA1c, lipid profile, insulin, proinsulin, amylase, and lipase), and ADIC data (IL-1β, IFN-γ, FGF21, and leptin) were collected. Then patients' venous blood samples were collected 0.5 h and 2 h after ingestion to measure glucose and insulin levels during a 162 kcal mixed-meal test (MMT). On Day 2, an intravenous glucose tolerance test (IVGTT) with an injection of 25 g glucose was performed to collect venous blood samples taken at 0, 1, 2, 4, 6, and 10 min for the insulin measurement after an overnight fast. All 2-day baseline assessments were repeated at the end of the study (Week 48).

For the first 12 weeks, the participants were followed up for every 4 weeks. From week 13 to week 48 they were followed up for every 12 weeks. Anthropomorphic data, hypoglycemic episodes and adverse events were recorded each follow-up visit. Fasting plasma glucose (FPG) and HbA1c were measured, and 2-h postprandial glucose (2-h PPG) was obtained after MMT. In addition, participants' information was collected and guidance was provided by telephone calls at week 16, 20, 28, 32, 40, and 44. All participants received diabetes-related education throughout the study.

Homeostasis model assessment of β-cell function was used to evaluate basal β-cell function [HOMA-B = 20 × fasting insulin (FINS)/(FPG–3.5)]. Homeostasis model assessment of IR was used to evaluate insulin resistance [HOMA-IR=FINS × FPG/22.5]. The insulinogenic index [ratio of the MMT 0–30 min increments in insulin to glucose concentrations (mg/dL)] × the Matsuda index was calculated to evaluate the disposition index (DI). The fasting proinsulin-to-insulin ratio (PI/I) and the acute insulin response (AIR) during IVGTT were calculated (the incremental area under the curve using trapezoidal estimation). Serum ADIC concentrations of IL-1β, IFN-γ, and leptin were assessed using luminex (Bio-Rad). The intra-assay and inter-assay coefficients of variation (CV) for IL-1β, IFN-γ and leptin were 3.6 % and 3.2 %, 3.1 % and 3.6 %, 4 % and 4 %, respectively. The FGF21 was tested by ELISA kits (R&D), with the intra-assay and inter-assay CV as 3.4 % and 7.5 %, respectively.

2.4. Statistical analyses

Means ± SD was used to present continuous variables with normal distribution. Non-normally distributed variables (ADIC concentrations, PI/I, HOMA-B, and HOMA-IR and their changes from baseline to endpoint) were expressed as median (interquartile range) and were logarithmically transformed to achieve normal distribution before analysis. The participants' baseline characteristics in different groups were compared using analysis of variance (ANOVA) with Holm-Bonferroni correction for multiple comparisons. Baseline *versus* after-treatment values were compared with paired *t*-test. Changes of ADIC values from baseline to endpoint across groups were compared by analysis of covariance (ANCOVA) with baseline values and changes of body weight, waist and HbA1c adjustment. ANCOVA was also used to compare changes of glycemic and metabolic parameters from baseline among the groups with baseline values adjustment. Chi-squared test was used to compare categorical variables. Pearson's correlation analysis was conducted to evaluate putative associations between changes in ADIC concentrations and other indices. Multiple linear regression analysis was

Table 1
Baseline characteristics and changes from baseline at week 48 in three groups, Variables are shown as means ± SD, medians (interquartile range) or absolute numbers and percentages(n,%).*$P < 0.05$ **$P < 0.01$, compared with baseline; NA, not applicable,
BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HbA1c* hemoglobin A1c, *FPG* fasting plasma glucose, *2-h PPG* 2-h postprandial plasma glucose, *TG* triglycerides, *LDL-C* low density lipoprotein cholesterol, *HOMA-IR* homoeostasis model assessment of insulin resistance, *HOMA-B* homoeostasis model assessment of β-cell function, *AIR* 10-min acute insulin response, *PI/I* fasting proinsulin-to-insulin ratio, *DI* disposition index.

Characteristics	Exenatide	Insulin	Pioglitazone	Overall	P-value Exenatide vs. insulin	Exenatide vs. pioglitazone	Insulin vs. pioglitazone
n	88	84	109				
Men (%)	59 (67.0)	52(61.9)	80(73.4)	0.23	NA	NA	NA
Weight (kg)							
Baseline	71.1 ± 1.5	70.8±1.4	70.9±1.2	0.144	NA	NA	NA
After 48-week intervention	67.3±1.5**	71.6±1.4	70.8±1.2	0.053	NA	NA	NA
Change from baseline	-3.8±0.4	-0.1±0.8	0.1±0.4	<0.001	<0.001	<0.001	0.148
Waist (cm)							
Baseline	91.4 ± 1.2	90.1±1.1	89.6 ± 0.8	0.168	NA	NA	NA
After 48-week intervention	87.2 ± 1.2**	90.0±1.1	89.0±0.9	0.246	NA	NA	NA
Change from baseline	-4.2±0.7	-0.5±0.9	-0.2±0.5	<0.001	<0.001	<0.001	0.779
BMI (kg/m2)							
Baseline	25.9 ± 0.4	25.6±0.4	25.9 ± 0.3	0.089	NA	NA	NA
After 48-week intervention	24.5±0.4**	25.9±0.4	25.9±0.3	0.053	NA	NA	NA
Change from baseline	-1.4±0.2	-0.1±0.3	0.05±0.2	<0.001	<0.001	<0.001	0.216
HbA1c (%)							
Baseline	8.2 ± 0.1	8.1 ± 0.1	8.0 ± 0.1	0.659	NA	NA	NA
After 48-week intervention	6.2±0.1**	6.5±0.1**	6.6±0.1**	0.048	0.114	0.016	0.392
Change from baseline	- 1.9±0.2	-1.6 ±0.3	-1.6±0.2	0.047	0.126	0.015	0.363
FPG (mmol/L)							
Baseline	9.0 ± 0.3	8.8 ± 0.3	9.1 ± 0.2	0.288	NA	NA	NA
After 48-week intervention	6.9±0.2**	7.4±0.3**	7.2±0.2**	0.249	NA	NA	NA
Change from baseline	-2.1±0.3	-1.5± 0.4	-2.2±0.3	0.163	NA	NA	NA
2-h PPG(mmol/L)							
Baseline	13.9 ± 0.4	14.5± 0.4	14.4 ± 0.3	0.300	0.30	0.342	0.928
After 48-week intervention	10.6 ± 0.4**	11.8±0.4**	10.8±0.3**	0.040	0.075	0.486	0.014
Change from baseline	-3.3 ±0.5	-2.5± 0.8	-4.5 ±0.5	0.014	0.096	0.212	0.004
SBP (mmHg)							
Baseline	128 ± 2	125 ± 2	126 ± 1	0.359	NA	NA	NA
After 48-week intervention	125±2	124±2	124±1	0.659	NA	NA	NA
Change from baseline	-2.4±2.3	-1.6 ± 2.3	- 1± 1	0.894	NA	NA	NA
DBP (mmHg)							
Baseline	80 ± 1	79 ± 1	80 ± 1	0.423	NA	NA	NA
After 48-week intervention	78± 1	78±1	77±1**	0.943	NA	NA	NA
Change from baseline	-2.2 ±1.3	- 2.7±1.9	-4 ± 1	0.567	NA	NA	NA
TG (mmol /L)							
Baseline	2.1 ± 0.2	1.7 ± 0.1	2.1 ± 0.2	0.128	NA	NA	NA
After 48-week intervention	1.8±0.2	2.4±0.4	1.9±0.2	0.154	NA	NA	NA
Change from baseline	-0.3±0.2	0.6 ± 0.5	-0.1±0.1	0.021	0.010	0.659	0.029
LDL-C (mmol/ L)							
Baseline	3.2 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	0.866	NA	NA	NA
After 48-week intervention	3.0±0.1*	3.1±0.1	3.2±0.1	0.361	NA	NA	NA
Change from baseline	-0.2 ±0.1	- 0.4±0.2	-0.1 ±0.1	0.582	NA	NA	NA
Amylase (U /L)							
Baseline	49.4 ± 2.1	50.0 ± 2.6	53.6 ± 2.3	0.174	NA	NA	NA

After 48-week intervention	59.1±2.5**	55.4±2.9*	58.7±2.0**	0.157	NA	NA	NA
Change from baseline	11.9±2.4	6.9±2.3	7.4±4.5	0.400	NA	NA	NA
Lipase (U/ L)							
Baseline	81.3±13.1	60.1 ± 8.1	69.3 ± 9.9	0.764	NA	NA	NA
After 48-week intervention	108.6±15.4*	67.5±12.5	78.6±9.7	0.159	NA	NA	NA
Change from baseline	27.4±12.0	7.4±12.3	11.5 ± 14.9	0.248	NA	NA	NA
HOMA-B							
Baseline	46.6(29.8,72.2)	49.1(30.4,69.1)	43.8(26.2,72.9)	0.430	NA	NA	NA
After 48-week intervention	43.6(30.5,69.5)	68.7(45.4,185.0)**	49.2(32.5,71.2)	<0.001	<0.001	0.885	<0.001
Change from baseline	-4.8(-22.9,16.2)	29.0(-3.7,142.2)	4.1(-13.8,25.3)	<0.001	<0.001	0.494	<0.001
HOMA-IR							
Baseline	4.8(3.6,6.3)	4.0(3.3,5.6)	4.5(2.7,6.0)	0.324	NA	NA	NA
After 48-week intervention	2.1(1.4,3.1)**	3.9(2.5,8.6)	2.1(1.7,3.1)**	<0.001	<0.001	0.557	<0.001
Change from baseline	-2.5(-3.9,-1.1)	-0.7(-1.9,4.4)	-2.2(-3.0,-0.6)	<0.001	<0.001	0.362	<0.001
AIR (Uiu/ Ml * min)							
Baseline	12.4±7.0	10.7 ± 6.3	13.0±5.8	0.264	NA	NA	NA
After 48-week intervention	47.4±9.5**	30.5 ± 10.5*	43.9±6.2**	0.357	NA	NA	NA
Change from baseline	35.1±12.3	22.4±16.3	44.9±8.7	0.279	NA	NA	NA
PI/I							
Baseline	2.8(1.7,4.8)	2.7(2.2,3.7)	2.7(1.9,3.7)	0.430	NA	NA	NA
After 48-week intervention	1.7(0.3,5.6)**	0.2(0.1,4.1)**	0.9(0.2,2.5)**	0.013	0.003	0.166	0.112
Change from baseline	-1.2(-3.2,2.3)	-1.7(-2.6,0.5)	-1.4(-2.7,-0.1)	0.038	0.012	0.399	0.095
DI							
Baseline	-0.4±0.2	-0.5±0.1	-0.4±0.07	0.913	NA	NA	NA
After 48-week intervention	0.2±0.1**	0.05±0.1*	-0.04±0.07**	0.013	0.012	0.009	0.902
Change from baseline	0.6±0.2	0.5±0.2	0.2±0.1	0.128	NA	NA	NA

conducted using changes in metabolic indices and parameters of β-cell function as dependent variables and changes of ADIC as independent variables in pooled data.

SPSS 23.0 (IBM corporation, New York, NY, USA) was used to analyze data in this study. Significant difference was defined as *P*-values <0.05.

3. Results

A total of 281 participants with 68 % male and mean age of 50.3 ± 9.4 years completed the study (Figure Supplementary 1). The participants' baseline characteristics were comparable among groups (Table 1).

3.1. Changes of anthropometric, glycemic, and metabolic parameters after intervention in each group

After intervention, HbA1c, FPG, PPG, AIR, DI, and PI/I were significantly improved in all the treatment groups ($P < 0.01$). Weight, BMI, and mean waist circumference were significantly decreased at the endpoint compared with those at baseline in the exenatide group, but not in the other two groups. HOMA-IR showed a significant improvement in both the pioglitazone and exenatide groups; however, the HOMA-β value was significantly altered only in the insulin group.

Diastolic blood pressure significantly decreased in the pioglitazone group; meanwhile, systolic blood pressure did not change from baseline in any of the groups. For the lipid profile, there was an improvement in the LDL-C content in the exenatide group at week 48; however, other lipid parameters did not significantly change in any of the intervention groups (Table 1).

3.2. Changes in ADIC levels after intervention in each group

The detailed data for ADIC levels at baseline and after 48-weeks intervention were shown in Fig. 1. After 48 weeks of treatment, IL-1β ($P < 0.001$) and IFN-γ ($P = 0.001$) were markedly decreased in the exenatide group (Fig. 1A) and significantly increased in the insulin group (Fig. 1B) ($P = 0.009$ and $P = 0.026$ for L-1β and IFN-γ, respectively). However, no changes in ADIC concentrations were detected in the pioglitazone group after intervention (Fig. 1C). Leptin or FGF21 levels did not significantly change in any of the treatment groups.

In the sub-groups analysis of non-obese group and obese group, no significant changes of leptin levels were observed after treatments (all $P > 0.05$) (Table Supplementary 2).

3.3. Correlation between changes in ADIC content and alterations in glycemic and metabolic indices

Results of pearson's correlation analysis was displayed in Table 2. In the exenatide group, change in waist size (Δ waist) was correlated with change in leptin (Δleptin) ($r = 0.301$, $P = 0.047$); change in diastolic blood pressure (ΔDBP) was correlated with change in IFN-γ (ΔIFN-γ) ($r = 0.350$, $P = 0.020$); changes in triglyceride level (ΔTG) and DI(ΔDI) were correlated with change in FGF21 (ΔFGF21) ($r = 0.454$, $P = 0.004$ and $r = 0.558$, $P = 0.011$, respectively); and change in IL-1β (ΔIL-1β) was correlated with ΔFGF21 ($r = 0.340$, $P = 0.030$) and ΔIFN-γ) ($r = 0.857$, $P < 0.001$). ΔIFN-γ was correlated with ΔFGF21($r = 0.356$, $P = 0.022$).

In the insulin group, change in 2-h PPG (Δ2-h PPG) showed correlations with ΔIL-1β ($r = -0.364$, $P = 0.019$) and ΔIFN-γ ($r = -0.363$, $P = 0.020$); change in DI (ΔDI) was correlated with ΔFGF21 ($r = -0.493$, $P = 0.032$); and ΔIFN-γ was significantly correlated with ΔFGF21 ($r = -0.363$, $P = 0.023$) and ΔIL-1β ($r = 0.852$, $P < 0.001$). However, no correlations were observed between Δleptin and other indices in the insulin group.

In the pioglitazone group, Δwaist was correlated with Δleptin ($r = -0.361$, $P = 0.024$); change in LDL-C content (ΔLDL-C) was correlated with ΔFGF21 ($r = -0.356$, $P = 0.036$); and ΔPI/I was correlated with ΔIL-1β ($r = 0.425$, $P = 0.022$). For correlations among ADICs, Δleptin was correlated with ΔFGF21 ($r = -0.339$, $P = 0.047$) and ΔIL-1β was correlated with ΔIFN-γ ($r = 0.723$, $P < 0.001$).

No significant correlation was observed between changes in ADIC levels and change in HbA1c (ΔHbA1c).

3.4. Comparison of changes in glycemic and metabolic parameters from baseline among the groups

A comparison of the changes in glycemic and metabolic parameters from baseline among the groups is displayed in Table 1.

The ΔHbA1c in the exenatide group was significantly greater than that in the pioglitazone group (-1.9 ± 0.2 % *vs* -1.6 ± 0.2 %, $P = 0.015$). ΔHbA1c showed no differences between exenatide group and insulin group or between insulin group and pioglitazone group. The Δ2-h PPG in the pioglitazone group decreased more significantly than that in the insulin group (-4.5 ± 0.5 mmol/L *vs* -2.5 ± 0.8 mmol/L, $P = 0.004$). However, changes in FPG (ΔFPG) were similar among treatment groups.

Δweight, Δwaist, and ΔBMI were significantly greater in the exenatide group than those in the insulin and pioglitazone groups (all $P < 0.001$).

After intervention, ΔTG differed significantly between the exenatide group and the insulin group ($P = 0.010$) and between the insulin group and pioglitazone group ($P = 0.029$). No significant difference was observed among the groups in changes of metabolic indices including SBP (ΔSBP), ΔDBP, and ΔLDL-C.

The change in HOMA-B (ΔHOMA-B) in the insulin group was significantly greater than that in the exenatide and pioglitazone groups (29.0(−3.7142.2) vs −4.8(−22.9,16.2) and 29.0(−3.7142.2) vs 4.1(−13.8,25.3), respectively; all $P < 0.001$). The change in HOMA-IR (ΔHOMA-IR) in the insulin group was also significantly greater than that in the exenatide and pioglitazone groups (−0.7 (−1.9,4.4) vs −2.5(−3.9,-1.1) and −0.7(−1.9,4.4) vs −2.2(−3.0,-0.6), respectively; all $P < 0.001$).

3.5. Comparison of changes in ADIC levels from baseline among the groups

After adjusting for baseline values and changes of body weight, waist and HbA1c, significant differences were seen between the exenatide group and the insulin group and between the exenatide group and the pioglitazone group for both ΔIL-1β ($P = 0.048$ and $P = 0.003$, respectively) (Fig. 2A)and ΔIFN-γ ($P = 0.049$ and $P < 0.001$, respectively) (Fig. 2B).

3.6. Multiple linear regression analysis

Multiple linear regression analysis was conducted in the pooled data using metabolic or β-cell function indices as dependent variables and ADICs as independent variables (Table 3).

Δweight was associated with ΔIL-1β ($\beta = 0.753$; 95 % confidence interval [CI], 0.137–1.369; $P = 0.017$); however, after adjusting for the effects of the antidiabetic agents, this association was lost ($P = 0.647$). Meanwhile, Δweight was not associated with ΔFGF21 before adjustment for the effects of treatments; however, after adjustment, Δweight showed an association with ΔFGF21 ($\beta = 1.097$; 95 % CI, 0.250–1.944; $P = 0.012$).

ΔBMI exhibited the same association as Δweight with ΔIL-1β (before adjusting for the effects of the antidiabetic agents: $\beta = 0.286$; 95 % CI, 0.061–0.511; $P = 0.013$) and ΔFGF21 (after adjusting for the effects of the antidiabetic agents: $\beta = 0.430$; 95 % CI, 0.125–0.735; $P = 0.006$).

Δwaist was associated with ΔFGF21 ($\beta = 1.185$; 95 % CI, 0.0820–2.287; $P = 0.035$) after adjusting for the effects of the antidiabetic

agents while no association was observed before adjustment.

No association was observed between glycemic indices (ΔHbA1c, ΔFPG, and Δ2-h PPG) and ADIC levels.

Multiple linear regression analysis among IR, β-cell function indices, and ADICs revealed that ΔHOMA-IR was associated with ΔLeptin both before ($\beta = 0.085$; 95 % CI, 0.008–0.162; $P = 0.030$) and after ($\beta = 0.078$; 95 % CI, 0.008–0.147; $P = 0.029$) adjusting for the effects of the antidiabetic agents. Additionally, ΔHOMA-IR was associated with ΔIL-1β before ($\beta = 0.067$; 95 % CI, 0.006–0.127; $P = 0.030$), but not after, adjusting for the effects of the antidiabetic agents ($P = 0.513$).

No association was detected between ΔHOMA-B, ΔAIR, ΔDI, ΔPI/I, and ΔADIC.

4. Discussion

ADIC levels can undergo alterations *via* a variety of mechanisms in T2DM, which may also differ markedly from those in healthy individuals [6]. Conversely, alterations in ADIC secretion can change the pathophysiology and progression of T2DM [32]. Increasing evidence has indicated that alterations in ADIC levels may be the major biochemical mediators contributing to the pathophysiology of inflammatory and metabolic diseases, including T2DM [33]. Accordingly, ADICs represent an increasingly promising therapeutic target for T2DM treatment. In this post-hoc study of the CONFIDENCE clinical trial, we found that the levels of IL-1β and IFN-γ, the pro-inflammatory cytokines, were notably decreased after treatment with exenatide, whereas the opposite trend was seen in the insulin treatment group. However, no significant changes were detected in the levels of the adipokines leptin and FGF21 in any of the intervention groups. Significant differences in ΔIL-1β and ΔIFN-γ were seen between treatment groups. Additionally, we found that changes in ADIC contents might contribute to the differences in changes in metabolic factors, IR, and β-cell function among the three groups. To the best of our knowledge, relatively few studies have compared changes in both adipokine and inflammatory cytokine levels while also investigating the associations of metabolic factors, glycemic indices, β-cell function, and ADIC levels with different hypoglycemic agents.

There is abundant evidence supporting that chronic inflammation, marked by increased levels of inflammatory cytokines, plays a crucial role in the pathophysiology of T2DM. However, the mechanisms are still not fully understood [34]. Increased concentrations of circulating inflammatory cytokines may augment the risk of metabolic disorders as well as increase pancreatic islet macrophage infiltration and apoptosis of β cell. And these effects may eventually contribute to the development of T2DM [13–15]. The inflammatory cytokines IL-1β and IFN-γ are key pro-diabetic inflammatory risk factors [19]. The levels of both IL-1β and IFN-γ are increased in animal models of T2DM, and both factors can impair insulin signaling, induce β-cell apoptosis and dysfunction, and promote IR [20,21]. Results in previous studies showing a significant correlation between IL-1β and IFN-γ suggested their close interaction [20,21]. Theoretically, the inhibition of inflammation, accompanied by a reduction in inflammatory cytokine levels, may lead to an improvement in metabolic parameters and β-cell function in T2DM and the amelioration of the associated complications. Accordingly, recent studies have assessed the potential of IL-1β and IFN-γ as novel therapeutic targets in the treatment of T2DM [22,23]. Some hypoglycemic agents have been reported to possess anti-inflammatory characteristics. Studies have consistently shown that intervention with GLP-1 agonists leads to significant reduction of circulating IL-1β and IFN-γ [35], and these anti-inflammatory effects were recapitulated with exenatide intervention in our study. Similar reductions in IL-1β and IFN-γ contents were not observed in the insulin and pioglitazone treatment groups. However, reports on the effects of insulin treatment on inflammatory cytokine levels have been inconsistent. For instance, Aas et al. found that the serum levels of high-sensitivity *C*-reactive protein (hs-CRP) and tumor necrosis factor alpha (TNF-α) were significantly increased after neutral protamine Hagedorn (NPH) insulin intervention [36], whereas the opposite result was reported in the most recent study [37]. Different characteristics of the participants, different effects on body weight or different insulin regimens may explain these discrepant findings. In the present study, we found that IL-1β and IFN-γ levels increased in the group receiving premixed insulin treatment, indicating that insulin, even though it has the strongest hypoglycemic effects of the three agents tested, may play a pro-inflammatory role with long-term administration. This pro-inflammatory effect may offset the benefit of lowering blood glucose. It could partly explain the non-reduction in the number of major adverse cardiovascular events after insulin treatment, even though glucose levels within the target range were achieved [38]. Studies investigating the effects of pioglitazone on IL-1β and IFN-γ are scarce. Here, we found that the levels of circulating IL-1β and IFN-γ decreased in the exenatide group, but not in the pioglitazone group. The accompanying reduction in weight and waist circumference observed in the exenatide group, but not the other two groups, may explain this difference. In addition, in the ANCOVA analysis, after controlling for the effect of baseline, body weight, waist and HbA1c, changes in IL-1β and IFN-γ contents in the exenatide group were significantly greater compared with those in both the insulin and pioglitazone groups. These results further confirmed the anti-inflammatory characteristics

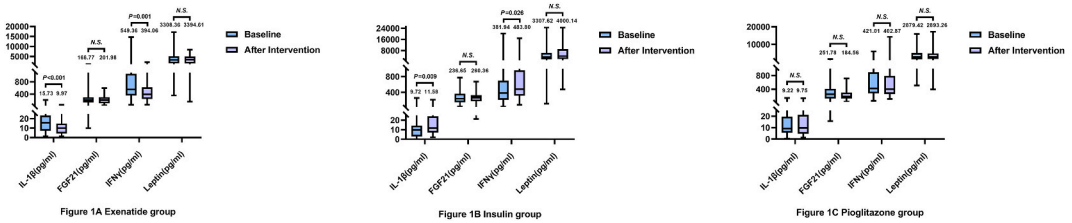

Fig. 1. Adipokine and inflammatory cytokine levels at baseline and after 48 weeks of intervention in the exenatide (A), insulin (B), and pioglitazone (C) groups. FGF21, fibroblast growth factor 21; IL-1β, interleukin-1 beta; IFN-γ, interferon-gamma.

Table 2
Correlation between changes in ADICs and alterations in glycemic and metabolic indices in different groups.

Variables	ΔIL-1β			ΔIFN-γ			ΔFGF21			ΔLeptin		
	Exenatide	Insulin	Pioglitazone	Exenatide	Insulin	Pioglitazone	Exenatide	Insulin	Pioglitazone	Exenatide	Insulin	Pioglitazone
Δ weight	r = 0.141	r = −0.034	r = 0.041	r = 0.019	r = −0.127	r = −0.102	r = 0.202	r = 0.291	r = 0.277	r = 0.258	r = 0.198	r = −0.064
ΔBMI	r = 0.170	r = −0.043	r = 0.050	r = 0.034	r = −0.146	r = −0.083	r = 0.241	r = 0.289	r = 0.287	r = 0.279	r = 0.212	r = −0.054
Δ waist	r = 0.025	r = −0.055	r = 0.039	r = −0.036	r = −0.006	r = −0.058	r = 0.264	r = 0.165	r = 0.179	r = 0.301*	r = 0.062	r = −0.361*
ΔSBP	r = 0.044	r = −0.151	r = −0.198	r = 0.191	r = −0.074	r = −0.204	r = 0.037	r = −0.059	r = 0.011	r = −0.003	r = 0.017	r = 0.038
ΔDBP	r = 0.211	r = −0.066	r = −0.150	r = 0.350*	r = −0.077	r = −0.148	r = −0.002	r = 0.090	r = 0.029	r = 0.132	r = −0.079	r = 0.081
ΔHbA1c	r = 0.147	r = −0.103	r = 0.053	r = 0.134	r = −0.126	r = 0.096	r = −0.104	r = 0.180	r = −0.103	r = 0.090	r = −0.095	r = −0.108
ΔFPG	r = 0.164	r = 0.041	r = 0.236	r = 0.243	r = 0.083	r = 0.161	r = 0.286	r = 0.265	r = −0.057	r = 0.068	r = −0.085	r = 0.001
Δ2-h PPG	r = 0.174	r = −0.364*	r = 0.086	r = 0.243	r = −0.363*	r = 0.058	r = 0.102	r = 0.248	r = −0.081	r = 0.044	r = 0.063	r = 0.130
ΔTG	r = 0.087	r = 0.231	r = −0.109	r = 0.135	r = 0.246	r = −0.144	r = 0.454**	r = −0.212	r = −0.063	r = 0.067	r = 0.032	r = −0.067
Δ LDL-C	r = 0.028	r = −0.010	r = 0.081	r = −0.124	r = 0.090	r = −0.017	r = 0.064	r = 0.057	r = 0.365*	r = 0.138	r = 0.101	r = −0.173
ΔHOMA-IR	r = 0.023	r = 0.108	r = 0.106	r = 0.061	r = 0.006	r = 0.023	r = −0.177	r = 0.217	r = −0.120	r = 0.244	r = 0.208	r = 0.294
ΔHOMA-B	r = −0.077	r = 0.075	r = −0.049	r = −0.106	r = 0.016	r = −0.117	r = −0.409	r = −0.055	r = −0.053	r = 0.045	r = 0.248	r = 0.160
ΔAIR	r = −0.286	r = 0.111	r = −0.034	r = −0.298	r = 0.148	r = 0.023	r = −0.204	r = −0.195	r = 0.062	r = 0.124	r = −0.255	r = −0.260
ΔDI	r = 0.021	r = 0.048	r = −0.222	r = 0.102	r = 0.247	r = 0.009	r = 0.558*	r = −0.493*	r = 0.220	r = −0.068	r = 0.216	r = 0.094
ΔPI/I	r = −0.204	r = −0.287	r = 0.425*	r = −0.168	r = −0.197	r = 0.315	r = 0.076	r = 0.042	r = −0.276	r = −0.294	r = −0.329	r = 0.038
ΔIL-1β	–	–	–	r = 0.857**	r = 0.852**	r = 0.723**	r = 0.340*	r = −0.229	r = −0.116	r = 0.216	r = 0.080	r = 0.257
ΔIFN-γ	r = 0.857**	r = 0.852**	r = 0.723**	–	–	–	r = 0.356*	r = −0.363*	r = −0.326	r = 0.153	r = 0.054	r = 0.316
ΔFGF21	r = 0.340*	r = −0.229	r = −0.116	r = 0.356*	r = −0.363*	r = −0.326	–	–	–	r = −0.027	r = −0.211	r = −0.339*
ΔLeptin	r = 0.216	r = 0.080	r = 0.257	r = 0.153	r = 0.054	r = 0.316	r = −0.027	r = −0.211	r = −0.339*	–	–	–

*$P < 0.05$ **$P < 0.01$.

Δ changes from baseline to the endpoint, *CI* confidence interval, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HbA1c* hemoglobin A1c, *FPG* fasting plasma glucose, *2-h PPG* 2-h postprandial plasma glucose, *TG* triglycerides, *LDL-C* low density lipoprotein cholesterol, *HOMA-IR* homoeostasis model assessment of insulin resistance, *HOMA-B* homoeostasis model assessment of β-cell function, *AIR* 10-min acute insulin response, *PI/I* fasting proinsulin-to-insulin ratio, *DI* disposition index.

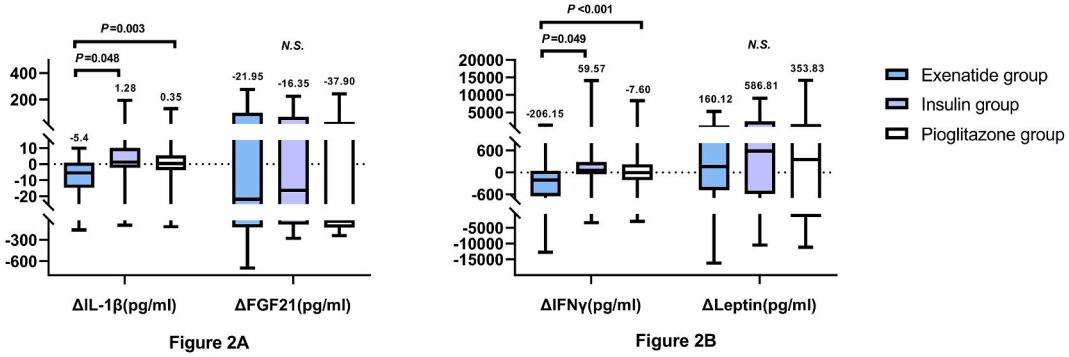

Fig. 2. Comparison of changes in ADIC contents from baseline among the three groups. FGF21, fibroblast growth factor 21; IL-1β, interleukin-1 beta; IFN-γ, interferon-gamma.

of exenatide. Results in the present study demonstrated that exenatide could exert both anti-hyperglycemic and anti-inflammatory effects on diabetes, and its anti-inflammatory effects were superior to those of insulin and pioglitazone, regardless of the changes in body weight and HbA1c. Inflammation has been well recognized as a contributor to atherosclerosis and ultimately cardiovascular disease (CVD) [39]. Decreased IL-1β and IFN-γ contents in the exenatide group in the present study may lead to suppress oxidative stress and foam cell accumulation, reduce smooth muscle cell proliferation and migration into the arterial intima, and stabilize plaque, which may eventually reduce CVD [22,39,40].

We further found that ΔIL-1β was independently associated with Δweight, ΔBMI, and ΔHOMA-IR in the multiple linear regression analysis. These findings suggested that the changes in weight, BMI, and IR were associated with changes in IL-1β levels. However, this association was lost when the effects of the antidiabetic agents were controlled for, implying that IL-1β might play an intermediate role between changes in weight, BMI, HOMA-IR, and antidiabetic agents (mainly exenatide). Exenatide treatment may contribute to a decrease in IL-1β levels along with a reduction in body weight, BMI, and HOMA-IR in this group. However, no associations were found among glycemic indices, β-cell function, and ADIC contents. Our findings suggested that in consideration of weight and IR in T2DM, the anti-inflammatory characteristic of agents should be considered when choosing hypoglycemic agents.

The discovery of leptin, the first adipokine to be identified, was considered a milestone in adipokine research [7,8]. Leptin has an important role in the regulation of satiety, energy expenditure [41], β-cell mass, insulin sensitization, and atherogenesis [42]. A recent study reported that, among the assessed adipocytokines, leptin was found to be the hub of the biomarker correlation network in T2DM [9]. Elevated leptin levels in T2DM have been reported to be associated with the progression of diabetic complications through its stimulatory effects on oxidative stress and inflammation [43,44]. Numerous studies to date have investigated the effects of leptin replacement therapy on IR and hyperglycemic management in animal models of diabetes; however, relatively few studies involved in the investigation on alteration of leptin after medical treatment. Metformin and DDP-IV have been reported to decrease leptin levels in T2DM patients [24]. Nevertheless, data regarding the effects of GLP-1 on leptin levels in T2DM remain limited. Frøssing et al. found that liraglutide treatment in women with PCOS resulted in a significant reduction in the leptin level [45]. Moreover, a different study recently reported that treatment with a combination of metformin and exenatide promoted a reduction in leptin levels in patients with both obesity and T2DM [45]. The mean BMI of participants in that study, however, was greater than 31 kg/m^2, which was significantly higher than the BMI (25 kg/m^2) observed in our study. This might explain the discrepancy between the two studies regarding leptin levels. Reports of the effect of pioglitazone on the leptin level in T2DM have also been contradictory [46], while studies assessing the effect of insulin on leptin levels have been rare. In the present study, the leptin level was not altered in any of the intervention groups, and no differences in changes in leptin levels were observed among the three treatments. Our findings suggested that regular hypoglycemic agents did not affect the leptin level in non-obese diabetic population. Given the limited effect of exogenous leptin treatment on diabetes [47], leptin may not be a good marker or target for the treatment of hyperglycemia regarding this lean group of patients.

FGF21, an adipokine predominantly expressed in the liver, stimulates glucose uptake into adipocytes, promotes an increase in energy expenditure, and improves glucose and lipid metabolism [48]. Patients with T2DM have increased FGF21 levels and decreased expression of FGF receptors resulting from a so-called 'FGF21-resistant state' [48]. Studies have shown that the administration of FGF21 can exert anti-metabolic and anti-diabetic effects in T2DM [10]. Accordingly, regulating the circulating level of FGF21 might enhance its anti-diabetic effects in T2DM and eventually reduce diabetic complications. However, conflicting results have been reported for the effects of hypoglycemic agents on FGF21. Li et al. demonstrated that fasting plasma FGF21 concentrations were decreased after treatment with rosiglitazone in poorly managed T2DM, in contrast to that reported for db/db mice by Muise et al. [49,50]. In patients with T2DM, treatment with a GLP-1 receptor agonist led to a decrease in FGF21 levels [51]. However, no investigation to date has evaluated the effect of insulin on FGF21 expression in diabetes. In the present study, FGF21 levels did not significantly change in newly diagnosed patients with any of the treatments assessed. As FGF21 plasma concentrations exhibited high inter-individual variability (0.05–5.5 ng/mL) in healthy individuals [52], we speculated that variability in FGF21 plasma concentrations might also influence the detection of FGF21 in patients with diabetes, which might partially explain the discrepancies between ours and previous studies. In addition, compared with previous studies, the patients in our study were all newly diagnosed, relatively lean, and had lower HbA1c, which might also contribute to the different results obtained. Given its high inter-individual variability and

Table 3
Multiple linear regression analysis using metabolic or β-cell function indices as dependent variables and ADICs as independent variables.

Variables	ΔIL-1β			ΔIFN-γ		ΔFGF21		ΔLeptin	
		β (95 % CI)	P	*β* (95 % CI)	P	*β* (95 % CI)	P	*β* (95 % CI)	P
Δweight									
	Model 1	0.753 (0.137–1.369)	0.017*	0.502(-0.277 – 1.282)	0.204	0.880(-0.122 – 1.883)	0.085	0.700(-0.078 – 1.478)	0.077
	Model 2	0.136(-0.450 – 0.722)	0.647	−0.278(-1.002 – 0.446)	0.449	1.097(0.250–1.944)	0.012*	0.515(-0.165 – 1.195)	0.136
ΔBMI									
	Model 1	0.286 (0.061–0.511)	0.013*	0.193(-0.092 – 0.478)	0.182	0.350(-0.015 – 0.716)	0.060	0.274(-0.010 – 0.558)	0.059
	Model 2	0.060(-0.153 – 0.273)	0.577	−0.092(-0.356 – 0.171)	0.488	0.430(0.125–0.735)	0.006**	0.206(-0.040 – 0.453)	0.100
Δwaist									
	Model 1	0.672(-0.112 – 1.455)	0.092	0.622(-0.360 – 1.605)	0.212	0.958(-0.298 – 2.214)	0.134	0.359(-0.631 – 1.348)	0.475
	Model 2	0.000(-0.765 – 0.764)	0.999	−0.172 (-1.120–−0.776)	0.720	1.185(0.082–2.287)	0.035*	0.170(-0.724 – 1.064)	0.707
ΔHbA1c									
	Model 1	0.073(-0.135 – 0.281)	0.490	0.105(-0.153 – 0.364)	0.422	−0.097(-0.431 – 0.237)	0.565	−0.022(-0.282– 0.239)	0.870
	Model 2	0.038(-0.187 – 0.264)	0.736	0.070(-0.208 – 0.347)	0.620	−0.087(-0.424 – 0.250)	0.609	−0.033(-0.297– 0.230)	0.801
ΔFPG									
	Model 1	0.325(-0.070 – 0.719)	0.106	0.467(-0.020 – 0.954)	0.060	0.600(-0.056 – 1.255)	0.072	0.017(-0.476 – 0.510)	0.946
	Model 2	0.316(-0.018 – 0.740)	0.143	0.450(-0.071 – 0.972)	0.090	0.615(-0.047 – 1.276)	0.068	−0.004(-0.500 – 0.491)	0.986
Δ2-h PPG									
	Model 1	−0.166(-0.731 – 0.400)	0.562	−0.040(-0.739 – 0.660)	0.911	0.354(-0.524 – 1.230)	0.426	0.308(-0.391 – 1.007)	0.385
	Model 2	−0.172(-0.767 – 0.424)	0.569	−0.072(-0.804 – 0.659)	0.845	0.343(-0.525 – 1.210)	0.435	0.291(-0.399 – 0.980)	0.406
ΔHOMA-IR									
	Model 1	0.067 (0.006–0.127)	0.030*	0.069(0.004–0.141)	0.063	−0.042(-0.132 – 0.048)	0.358	0.085(0.008–0.162)	0.030*
	Model 2	0.023(-0.067 – 0.083)	0.513	0.011(-0.062 – 0.083)	0.772	−0.028(-0.113 – 0.057)	0.513	0.078(0.008–0.147)	0.029*
ΔHOMA-B									
	Model 1	0.044 (0.028–0.116)	0.231	0.035(-0.052 – 0.121)	0.429	−0.104(-0.209 – 0.001)	0.051	0.060(-0.032 – 0.151)	0.199
	Model 2	0.000(-0.073 – 0.074)	0.995	−0.024(-0.112 – 0.065)	0.601	−0.091(-0.192 – 0.010)	0.077	0.054(-0.033 – 0.141)	0.221
ΔAIR									
	Model 1	−6.576(-20.028 – 6.876)	0.334	−8.674(-24.960 – 7.612)	0.293	−11.727 (-33.457–10.002)	0.288	−6.005 (-23.551–11.541)	0.499
	Model 2	−5.633(-20.220 – 8.953)	0.445	−7.455 (-25.294–10.384)	0.409	−13.521(-35.378 – 8.336)	0.222	−4.926 (-22.538–12.686)	0.580
ΔDI									
	Model 1	0.011(-0.180 – 0.202)	0.911	0.162(-0.061 – 0.386)	0.152	0.141(-0.165 – 0.447)	0.361	0.095(-0.171 – 0.362)	0.476
	Model 2	−0.016(-0.221 – 0.189)	0.877	0.134(-0.111 – 0.380)	0.278	0.185(-0.121 – 0.490)	0.231	0.057(-0.211 – 0.325)	0.672
ΔPI/I									
	Model 1	−0.100(-0.230 – 0.030)	0.129	−0.107(-0.266 – 0.051)	0.183	−0.006(-0.189 – 0.177)	0.950	−0.164(-0.332 – 0.004)	0.056
	Model 2	−0.852(-0.190 – 0.086)	0.459	−0.050(-0.219 – 0.119)	0.558	−0.021(-0.204 – 0.163)	0.822	−0.153(-0.319 – 0.013)	0.070

*$P < 0.05$ **$P < 0.01$.
Model 1 were before adjustment.
Model 2 were adjusted for effects of the antidiabetic agents.
Δ changes from baseline to the endpoint, *CI* confidence interval, *HbA1c* hemoglobin A1c, *HOMA-IR* homoeostasis model assessment of insulin resistance, *HOMA-B* homoeostasis model assessment of β-cell function, *AIR* 10-min acute insulin response, *PI/I* fasting proinsulin-to-insulin ratio, *DI* disposition index.

the results in our study, FGF21 may have limited value for clinical use. However, the effects of other hypoglycemic agents on this adipokine need to be investigated to better determine its diagnostic and therapeutic value in diabetes.

Interestingly, during the analysis, we also found a correlation between a reduction in adipokine levels and that in the

concentrations of inflammatory cytokines. A strong positive correlation was observed between plasma and adipose tissue levels of hs-CRP, leptin, and TNF-α. Here, we concluded that there was a strong relationship between adipocytokines and inflammatory markers, suggesting that cytokines secreted by adipose tissue play a role in the increased secretion of inflammatory proteins by the liver [16]. The findings of the present study indicated that adipokines and reduced levels of inflammatory cytokines had an interactional effect in the condition of hyperglycemia. Accordingly, we assessed adipokine and inflammatory cytokine content together in the present study.

One limitation of this study was that metformin was not included in the analysis as this was a post-hoc study of the CONFIDENCE trial. However, we believe that patients without previous treatment or the confounding effects of other hypoglycemic agents would allow for a better comparison of treatment with the three agents on changes in ADIC levels. Secondly, no normal control group was included in the present study. Thirdly, this study was a correlation design and cannot imply causality. Finally, this is a post-hoc analysis of our main study (the CONFIDENCE study) and the sample size calculation was based on the primary outcome of the CONFIDECNE study instead of this post-hoc study. As a result, the possibility that the negative findings in our study could be due to the sample size cannot be ruled out. We intend to address these limitations in a future study.

5. Conclusion

In conclusion, we found significant changes in the levels of inflammatory cytokines (IL-1β and IFN-γ), but not adipokines of leptin or FGF21, after treatment with exenatide in patients newly diagnosed with T2DM. The observed reduction in weight, BMI, and IR may be associated with the decrease in inflammatory cytokine levels induced by the use of the hypoglycemic agent exenatide. Exenatide exerted more favorable hypoglycemic effects compared with insulin and pioglitazone in patients newly diagnosed with T2DM.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee at each site and was undertaken according to Declaration of Helsinki. Written informed consents were obtained from all participants.

Consent for publication

All the authors listed have reviewed the final version of the manuscript and approve it for publication.

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Xubin Yang: Conceptualization, Data curation, Funding acquisition, Project administration, Writing – original draft. **Hongrong Deng:** Conceptualization, Data curation, Project administration, Writing – original draft. **Jing Lv:** Data curation, Investigation, Project administration. **Xueyan Chen:** Data curation, Project administration. **Longyi Zeng:** Conceptualization, Investigation, Supervision. **Jianping Weng:** Conceptualization, Supervision. **Hua Liang:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Wen Xu:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] S.P. Borse, A.S. Chhipa, V. Sharma, D.P. Singh, M. Nivsarkar, Management of type 2 diabetes: current strategies, unfocussed aspects, challenges, and alternatives, Med. Princ. Pract. 30 (2) (2021) 109–121.

[2] American Diabetes Association Professional Practice Committee, 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2022, Diabetes Care 45 (Suppl 1) (2022) S17–S38.
[3] W. Xu, Y. Bi, Z. Sun, et al., Comparison of the effects on glycaemic control and β-cell function in newly diagnosed type 2 diabetes patients of treatment with exenatide, insulin or pioglitazone: a multicentre randomized parallel-group trial (the CONFIDENCE study), J. Intern. Med. 277 (1) (2015) 137–150.
[4] L. Aguirre, N. Napoli, D. Waters, et al., Increasing adiposity is associated with higher adipokine levels and lower bone mineral density in obese older adults, J. Clin. Endocrinol. Metab. 99 (9) (2014) 3290–3297.
[5] J.X. Cheng, K. Yu, New discovered adipokines associated with the pathogenesis of obesity and type 2 diabetes, Diabetes Metab Syndr Obes 15 (2022) 2381–2389.
[6] M. Fasshauer, M. Blüher, Adipokines in health and disease, Trends Pharmacol. Sci. 36 (7) (2015) 461–470.
[7] Y. Zhang, R. Proenca, M. Maffei, et al., Positional cloning of the mouse obese gene and its human homologue, Nature 372 (6505) (1994) 425–432.
[8] M. Blüher, C.S. Mantzoros, From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century, Metabolism 64 (1) (2015) 131–145.
[9] T. Huang, K. Glass, O.A. Zeleznik, et al., A network analysis of biomarkers for type 2 diabetes, Diabetes 68 (2) (2019) 281–290.
[10] N. Itoh, FGF21 as a hepatokine, adipokine, and myokine in metabolism and diseases, Front. Endocrinol. 5 (2014) 107.
[11] A. Kharitonenkov, A.C. Adams, Inventing new medicines: the FGF21 story, Mol. Metabol. 3 (3) (2013) 221–229.
[12] M. Dalamaga, S.H. Chou, K. Shields, et al., Leptin at the intersection of neuroendocrinology and metabolism: current evidence and therapeutic perspectives, Cell Metab 18 (1) (2013) 29–42.
[13] J. Rojas, V. Bermudez, J. Palmar, et al., Pancreatic beta cell death: novel potential mechanisms in diabetes therapy, J. Diabetes Res. 2018 (2018), 9601801.
[14] W. Ying, W. Fu, Y.S. Lee, et al., The role of macrophages in obesity-associated islet inflammation and β-cell abnormalities, Nat. Rev. Endocrinol. 16 (2) (2020) 81–90.
[15] L.A. Berchtold, M. Prause, J. Størling, et al., Cytokines and pancreatic β-cell apoptosis, Adv. Clin. Chem. 75 (2016) 99–158.
[16] X. Unamuno, J. Gómez-Ambrosi, A. Rodríguez, et al., Adipokine dysregulation and adipose tissue inflammation in human obesity, Eur. J. Clin. Invest. 48 (9) (2018), e12997.
[17] T. Reinehr, C.L. Roth, Inflammation markers in type 2 diabetes and the metabolic syndrome in the pediatric population, Curr Diab Rep 18 (12) (2018) 131.
[18] T. Reinehr, B. Karges, T. Meissner, et al., Inflammatory markers in obese adolescents with type 2 diabetes and their relationship to hepatokines and adipokines, J. Pediatr. 173 (2016) 131–135.
[19] V. Wieser, A.R. Moschen, H. Tilg, Inflammation, cytokines and insulin resistance: a clinical perspective, Arch. Immunol. Ther. Exp. 61 (2) (2013) 119–125.
[20] A. Ibarra Urizar, J. Friberg, D.P. Christensen, et al., Inflammatory cytokines stimulate bone morphogenetic protein-2 expression and release from pancreatic beta cells, J. Interferon Cytokine Res. 36 (1) (2016) 20–29.
[21] M. Cieślak, A. Wojtczak, M. Cieślak, Role of pro-inflammatory cytokines of pancreatic islets and prospects of elaboration of new methods for the diabetes treatment, Acta Biochim. Pol. 62 (1) (2015) 15–21.
[22] M.Y. Donath, É. Dalmas, N.S. Sauter, et al., Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity, Cell Metab 17 (6) (2013) 860–872.
[23] N. Abu-Shahba, M. Mahmoud, A.M. El-Erian, et al., Impact of type 2 diabetes mellitus on the immunoregulatory characteristics of adipose tissue-derived mesenchymal stem cells, Int. J. Biochem. Cell Biol. 140 (2021), 106072.
[24] H. Yaribeygi, L.E. Simental-Mendía, G.E. Barreto, et al., Metabolic effects of antidiabetic drugs on adipocytes and adipokine expression, J. Cell. Physiol. 234 (10) (2019) 16987–16997.
[25] J.J. Zhu, J.S. Huang, T. Wang, et al., Sanggenol F exerts anti-diabetic effects via promoting adipocyte differentiation and modifying adipokines expression, Endocrine 56 (1) (2017) 73–81.
[26] L. Negrotto, M.F. Farez, J. Correale, Immunologic effects of metformin and pioglitazone treatment on metabolic syndrome and multiple sclerosis, JAMA Neurol. 73 (5) (2016) 520–528.
[27] L. Yang, M.Q. Song, Q.L. Zhang, et al., Effect of piglitazone and metformin on retinol-binding protein-4 and adiponectin in patients with type 2 diabetes mellitus complicated with non-alcohol fatty acid liver diseases, Zhongguo Yi Xue Ke Xue Yuan Xue Bao 36 (3) (2014) 309–312.
[28] G. Zhou, X. Su, J. Ma, et al., Pioglitazone inhibits high glucose-induced synthesis of extracellular matrix by NF-κB and AP-1 pathways in rat peritoneal mesothelial cells, Mol. Med. Rep. 7 (4) (2013) 1336–1342.
[29] H. Makino, A. Tanaka, H. Hosoda, et al., Effect of basal insulin therapy on vascular endothelial function and adipokine profiles in people with Type 2 diabetes, Diabet. Med. 33 (12) (2016) 1737–1743.
[30] M.A. Nauck, D.R. Quast, J. Wefers, et al., GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art, Mol. Metabol. 46 (2021), 101102.
[31] S.R. Cechin, I. Pérez-Álvarez, E. Fenjves, et al., Anti-inflammatory properties of exenatide in human pancreatic islets, Cell Transplant. 21 (4) (2012) 633–648.
[32] M.Y. Donath, Targeting inflammation in the treatment of type 2 diabetes: time to start, Nat. Rev. Drug Discov. 13 (6) (2014) 465–476.
[33] C.R. Kahn, G. Wang, K.Y. Lee, Altered adipose tissue and adipocyte function in the pathogenesis of metabolic syndrome, J. Clin. Invest. 129 (10) (2019) 3990–4000.
[34] X. Wang, W. Bao, J. Liu, et al., Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis, Diabetes Care 36 (1) (2013) 166–175.
[35] M. Møller, S. Fredholm, M.E. Jensen, et al., Proinflammatory biomarkers are associated with prediabetes in patients with schizophrenia, CNS Spectr. 27 (3) (2022) 347–354.
[36] A.M. Aas, I. Seljeflot, P.A. Torjesen, et al., Blood glucose lowering by means of lifestyle intervention has different effects on adipokines as compared with insulin treatment in subjects with type 2 diabetes, Diabetologia 49 (5) (2006) 872–880.
[37] H. Quan, H. Zhang, W. Wei, et al., A crossover study of the combination therapy of metformin and exenatide or biphasic insulin aspart 30 in overweight or obese patients newly diagnosed with type 2 diabetes mellitus, Exp. Ther. Med. 14 (4) (2017) 3279–3287.
[38] G.G. Schwartz, S.J. Nicholls, P.P. Toth, et al., Relation of insulin treatment for type 2 diabetes to the risk of major adverse cardiovascular events after acute coronary syndrome: an analysis of the BETonMACE randomized clinical trial, Cardiovasc. Diabetol. 20 (1) (2021) 125.
[39] P. Libby, Inflammation and cardiovascular disease mechanisms, Am. J. Clin. Nutr. 83 (2) (2006) 456S–460S.
[40] A. Elyasi, I. Voloshyna, S. Ahmed, et al., The role of interferon-γ in cardiovascular disease: an update, Inflamm. Res. 69 (10) (2020) 975–988.
[41] M. Fasshauer, M. Blüher, M. Stumvoll, Adipokines in gestational diabetes, Lancet Diabetes Endocrinol. 2 (6) (2014) 488–499.
[42] C.S. Mantzoros, F. Magkos, M. Brinkoetter, et al., Leptin in human physiology and pathophysiology, Am. J. Physiol. Endocrinol. Metab. 301 (4) (2011) E567–E584.
[43] M. Kurajoh, H. Koyama, M. Kadoya, et al., Plasma leptin level is associated with cardiac autonomic dysfunction in patients with type 2 diabetes: HSCAA study, Cardiovasc. Diabetol. 14 (2015) 117.
[44] C. Vavruch, T. Länne, M. Fredrikson, et al., Serum leptin levels are independently related to the incidence of ischemic heart disease in a prospective study of patients with type 2 diabetes, Cardiovasc. Diabetol. 14 (2015) 62.
[45] L. Shi, J. Zhu, P. Yang, et al., Comparison of exenatide and acarbose on intra-abdominal fat content in patients with obesity and type-2 diabetes: a randomized controlled trial, Obes. Res. Clin. Pract. 11 (5) (2017) 607–615.
[46] X. Prieur, L. Dollet, M. Takahashi, et al., Thiazolidinediones partially reverse the metabolic disturbances observed in Bscl2/seipin-deficient mice, Diabetologia 56 (8) (2013) 1813–1825.
[47] B. Mittendorfer, J.F. Horowitz, A.M. DePaoli, et al., Recombinant human leptin treatment does not improve insulin action in obese subjects with type 2 diabetes, Diabetes 60 (5) (2011) 1474–1477.
[48] H. Li, J. Zhang, W. Jia, Fibroblast growth factor 21: a novel metabolic regulator from pharmacology to physiology, Front. Med. 7 (1) (2013) 25–30.
[49] K. Li, L. Li, M. Yang, et al., Effects of rosiglitazone on fasting plasma fibroblast growth factor-21 levels in patients with type 2 diabetes mellitus, Eur. J. Endocrinol. 161 (3) (2009) 391–395.

[50] E.S. Muise, B. Azzolina, D.W. Kuo, et al., Adipose fibroblast growth factor 21 is up-regulated by peroxisome proliferator-activated receptor gamma and altered metabolic states, Mol. Pharmacol. 74 (2) (2008) 403–412.
[51] Y. Hu, J. Liu, H. Zhang, et al., Exenatide treatment decreases fasting fibroblast growth factor 21 levels in patients with newly diagnosed type 2 diabetes mellitus, Diabetes Metab. 42 (5) (2016) 358–363.
[52] O.V. Sirotkina, S.G. Khaspekova, A.M. Zabotina, et al., Effects of platelet glycoprotein IIb-IIIa number and glycoprotein IIIa Leu33Pro polymorphism on platelet aggregation and sensitivity to glycoprotein IIb-IIIa antagonists, Platelets 18 (7) (2007) 506–514.