Contents

EVIDENCE REVIEW

422 Impact of stopping smoking on metabolic parameters in diabetes mellitus: A scoping review

ORIGINAL ARTICLE

Basic Study

434 Investigating the specificity of endothelin-traps as a potential therapeutic tool for endothelin-1 related disorders
Jain A, Bozovicar K, Mehrotra V, Bratkovic T, Johnson MH, Jha I

442 Preparation and hypoglycemic effects of chromium- and zinc-rich Acetobacter aceti
Huang YY, Qin XK, Dai YY, Huang L, Huang GR, Qin YC, Wei X, Huang YQ

Retrospective Study

454 Immediate-release tofacitinib reduces insulin resistance in non-diabetic active rheumatoid arthritis patients: A single-center retrospective study
Wang CR, Tsai HW

LETTER TO THE EDITOR

466 Relook at DPP-4 inhibitors in the era of SGLT-2 inhibitors
Singh AK, Singh R
ABOUT COVER

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The primary aim of *World Journal of Diabetes* (*WJD, World J Diabetes*) is to provide scholars and readers from various fields of diabetes with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

*WJD* mainly publishes articles reporting research results and findings obtained in the field of diabetes and covering a wide range of topics including risk factors for diabetes, diabetes complications, experimental diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, diabetic angiopathies, diabetic cardiomyopathies, diabetic coma, diabetic ketoacidosis, diabetic nephropathies, diabetic neuropathies, Donohue syndrome, fetal macrosomia, and prediabetic state.

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Impact of stopping smoking on metabolic parameters in diabetes mellitus: A scoping review

Magdalena Walicka, Cristina Russo, Michael Baxter, Isaac John, Grazia Caci, Riccardo Polosa

Abstract

The purpose of this scoping review is to create a single narrative that describes the impact of smoking cessation on metabolic parameters in people with diabetes. It is generally well accepted that smoking enhances the harmful effects of elevated blood glucose levels, accelerating the vascular damage seen in patients with diabetes. Smoking cessation has clear benefits in terms of reducing cardiovascular morbidity and mortality. However, there is less evidence for the impact of smoking cessation on other diabetes-related complications. Studies in people with diabetes have shown improvement as well as temporary deterioration in glycemic control after ceasing smoking. Only a few studies have described the effect of quitting smoking on insulin resistance and lipid parameters, however, their results have been inconclusive. In this situation, healthcare professionals should
not assume that cessation of smoking will improve metabolic parameters in patients with diabetes. It seems they should, first of all, emphasize the prevention of weight gain that may be associated with quitting smoking. The lack of data regarding the metabolic effects of smoking and smoking cessation in diabetes is very disappointing and this area needs to be addressed.

Key Words: Smoking; Smoking cessation; Diabetes; Insulin resistance; Glucose; Lipids

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Core Tip: Results of the studies regarding the impact of smoking cessation on metabolic parameters in patients with diabetes are inconsistent. Healthcare professionals should not assume that metabolic parameters in patients with diabetes who stop smoking will improve. It seems that the top priority after smoking cessation should be the prevention of weight gain. Further studies of the effects of quitting smoking on metabolic parameters among people with diabetes are required to provide an evidence base for healthcare advice to managed patients and to assist healthcare providers to implement the most effective interventions.

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INTRODUCTION

Approximately 1.3 billion people worldwide use tobacco, most commonly in the form of tobacco smoking, and more than 7 million people die every year as a result of smoking related conditions[1,2]. Smoking is the main cause of lung cancer, chronic obstructive pulmonary disease, and cardiovascular disease[3,4].

Exposure to cigarette smoke is associated with vascular damage, endothelial dysfunction, and activation of oxidative stress, inflammatory pathways, coagulation, and fibrinolysis[5,6]. A similar mechanism of endothelial dysfunction is described for people with diabetes. It is therefore not surprising that smoking enhances the combined harmful effects of elevated blood glucose levels, accelerating vascular damage in diabetic patients who smoke[7,8].

Smokers with diabetes [both type 1 diabetes (T1D) and type 2 diabetes (T2D)] may be at a higher risk due to the direct effect of vascular damage as well as the indirect adverse effect that smoking has on glycemic control and lipid levels[9].

The risk of cardiovascular events in diabetic patients is reduced with smoking cessation[10]. In the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation study, smoking cessation in those with diabetes was associated with a 30% reduction in all-cause mortality [11]. A comprehensive evaluation of predicted coronary heart disease (CHD) among current and ex-smokers who had T2D in Spain found that ex-smokers had approximately 20% lower CHD risk at 10 years compared to current smokers[12].

Although there is evidence that patients with diabetes can reduce the risk of macrovascular complications by giving up smoking, there is no conclusive evidence for the impact on the risk of microvascular complications[9,13,14]. The impact of quitting smoking on microvascular complications of diabetes and its metabolic indices is unclear. Furthermore, stopping smoking is known to cause weight gain which in turn may have unpredictable metabolic effect in patients with diabetes.

To the best of our knowledge, there have been no published systematic reviews to quantify the health benefits of smoking cessation in the diabetes population to date. The purpose of this scoping review is to create a single narrative describing the impact of smoking cessation in people with diabetes on glycemic control, insulin resistance and insulin secretion, and lipid abnormalities as well as biochemical parameters of nephropathy.

SEARCH METHODS

The published literature on the impact of stopping smoking on metabolic indices, including glycemic control, insulin resistance, and lipid abnormalities was systematically reviewed in September and October 2021. The studies on biochemical parameters of nephropathy were also included. The literature
Walicka M et al. Stopping smoking and metabolic parameters

search was conducted using the following databases: PubMed, Embase, ScienceDirect library, Database of Abstracts of Reviews of Effects, Scopus, and Google Scholar, using medical subject headings. We also used an artificial intelligence technology-based open multidisciplinary citation analysis database named Reference Citation Analysis. Search queries were developed by a trained librarian experienced in developing search strategies for reviews and were based on diabetes, smoking cessation, fasting plasma glucose (FPG) levels, hemoglobin A1c (HbA1c), insulin resistance, insulin secretion, lipids [total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoproteins (VLDL)], microalbuminuria, creatinine. More specifically, search terms included (“smoking cessation” OR “former smokers” OR “ex-smokers” OR “stop smoking” OR “quitting”) AND (“diabetes”) AND (“glucose” OR “glycemia” OR “HbA1c” OR “insulin resistance” OR “HOMA” OR “insulin secretion” OR “total cholesterol” OR “HDL” OR “LDL” OR “VLDL” OR “microalbuminuria” OR “albuminuria” OR “creatinine” OR “GFR”). Search results were filtered to include only human studies and published from 1980. The titles, abstracts, and full texts of the search results were sequentially and independently screened by MW and GC for inclusion. A few studies were identified, including cross-sectional, case-control, and cohort studies, randomized clinical trials, and observational clinical studies, as well as systematic reviews and meta-analyses. The references of relevant studies were also manually reviewed for additional eligible citations.

SMOKING CESSTIATION AND INCIDENCE OF DIABETES

According to a meta-analysis conducted by Pan et al[15], recent quitters are at higher risk for developing diabetes, although this risk progressively declines with time.[15]. It is often found that giving up smoking leads to a significant increase in weight.[16,17]. According to a large prospective United Kingdom study, smoking abstinence was associated with an average weight gain of 8.79 kg at eight years, while continuing smokers gained only 2.24 kg.[17]. This has been confirmed in a meta-analysis showing that quitting smoking is associated with a bodyweight gain of 4-5 kg after 12 mo of abstinence, with most of the weight gain occurring between the third and the sixth month after quitting.[18]. As nicotine (in tobacco cigarettes) suppresses appetite and increases resting metabolic rate[19], people who stop smoking gain weight because they have diminished resting energy expenditure and increased appetite. Moreover, quitters often substitute smoking with excessive eating/snacking, as shown in several studies of eating behaviors[20]. It is likely that the weight gain associated with stopping smoking is responsible for the initial increase in risk of developing T2D. The increase in the risk of T2D after quitting was directly proportional to weight gain, but not increased among quitters without weight gain[21].

SMOKING CESSTIATION AND GLYCAEMIC CONTROL

Patients with diabetes can become more insulin resistant with worsening glycemic control when they gain weight. Pani et al[22], examining the predictors of diabetes progression (defined as HbA1c ≥ 7% or the initiation of hypoglycemic therapy), found that weight gain was an independent predictor. Each extra pound of weight that is gained increases the risk of developing diabetes by 2%. In patients with diabetes, quitting smoking may cause increased appetite, caloric intake, and weight gain, which would predictably lead to the worsening of glycemic control. In contrast, stopping smoking appears to have a beneficial effect on carbohydrate metabolism in the long run which may potentially mitigate the initial adverse metabolic effects of smoking cessation[23]. Considering the complex interplay among factors that affect glycemic control, some uncertainty in the findings of studies that investigate the impact of stopping smoking on glycemic control might be anticipated. Studies comparing smokers to ex-smokers, both with T1D and T2D, demonstrated that active smoking is associated with worse glycemic control. In the study of Dinardo et al[24] current smokers (with an average smoking history of 30 years, an average daily habit of one pack of cigarettes per day) had higher mean HbA1c in comparison with former smokers. In the multiple linear regression analysis, current smoking was independently and significantly associated with higher HbA1c. Braffett et al[14], using the data of a well-characterized cohort group with T1D from the Diabetes Control and Complications Trial (1983-1993), showed that in comparison to former smokers (subjects who previously smoked but quit > 3 mo prior to baseline), current smokers (subjects who currently smoked or quit < 3 mo prior to baseline) had higher mean HbA1c levels (average difference of 0.31%) over an average of 6.5 years of follow-up. The mean HbA1c levels for former smokers were similar to those of whom have never smoked. In relation to not only the current smoking status but also to its lifetime intensity and duration, the mean HbA1c levels were higher (average difference 0.22%) for current smokers with more than 10 pack-years in comparison to former smokers with less than 10 pack-years.

In an observational study of 10692 adult smokers with T2D, 29% of patients who had quit smoking and remained abstinent for at least 1 year revealed an increase in HbA1c of 0.21% with the need to intensify glucose-lowering treatment.[25]. In further observation, HbA1c level decreased as abstinence...
continued, and became comparable to this in people who continued to smoke after a 3-year follow-up. Patients who stopped smoking gained weight (4.68 kg on average), but the results suggested that the change in weight was not directly related to the increase in HbA1c.

In Asiatic patients quitting smoking is generally associated with an improvement in glycemic control. In a study of 2490 male Japanese patients with T2D, HbA1c decreased linearly with the years after stopping smoking; however, there was no correlation with FPG.[23] Similarly, in a study of 7763 Chinese men with T2D, the HbA1c level decreased progressively with each year that the patients had stopped smoking; in this study, FPG levels decreased.[26] In a smaller retrospective cohort study comprising 241 Taiwanese patients with T2D, the group completing the smoking cessation program showed a significant decrease in FPG and HbA1c levels at 3-mo follow-up compared to baseline. Due to the fact that the analyses of cardiometabolic factors were carried out before and after participation in the smoking cessation program in the whole group (regardless of the outcome of the smoking cessation program), it is difficult to interpret these results.[27]

In contrast, there are a number of studies on Asian patients failing to show improvement in glycemic control after stopping smoking. In a randomized controlled trial conducted in China, results of quitting smoking did not affect HbA1c levels at 1-year follow-up. The study included 557 smokers with T2D.[28]

In the meta-analysis published by Kar et al.[29] there was no statistically significant difference in HbA1c between smokers and quitters. However, when the meta-analysis was reanalyzed including studies comparing nonsmokers and active smokers, a statistically significant difference was demonstrated and this was positively associated with smoking duration; increasing as the years of smoking increased.

A summary of the studies evaluating smoking cessation’s effect on HbA1c is shown in Table 1. The table also includes HbA1c data from studies of smokers with diabetic nephropathy.

SMOKING CESSATION AND INSULIN RESISTANCE AND INSULIN SECRETION

The pathogenic mechanisms underlying T2D are a balance between insulin resistance and beta-cell dysfunction. Smoking has been shown to influence both insulin resistance and insulin secretion. Studies on animals have shown that cigarette smoke can impair insulin production and secretion in addition to reducing beta-cell viability and proliferation.[30]

There has also been speculation that nicotine in tobacco smoke could play a significant role in promoting insulin resistance. Although chronic exposure to nicotine may be necessary to impact insulin sensitivity in nicotine naive subjects, acute exposure to nicotine can cause negative effects on insulin sensitivity in individuals with pre-existing insulin resistance.[31-33]

However, the direct effect of nicotine on insulin resistance is not supported in studies looking at the use of snus. Snus is an oral tobacco product that delivers significant levels of nicotine without producing any toxic combustion byproducts.[34] Since the 1980s, snus consumption has been growing in popularity in Sweden, gradually replacing cigarette smoking.[35,36]

With the exception of one study, which has methodological issues including a flawed cross-sectional design and the lack of adjustment for smoking history in snus users[37], there is clear evidence that snus use does not produce a significant rise in diabetes risk.[38-41]. Moreover, there was no association between snus use and insulin levels or glucose tolerance in a large study involving 1266 subjects and primarily focused on cardiovascular risk factors.[42] Insignificant relative risks for T2D were reported in a meta-analysis for never-smoking current, former and ever-snus users.[43]. In addition, impaired glucose tolerance and related endpoints were not associated in any significant way.

It has been demonstrated that smokers have greater waist-to-hip circumference ratios.[44,45] Waist-to-hip circumference ratio is one of the most pragmatic clinical measures of central obesity. One of the major contributing factors in obesity-related metabolic complications is fat distribution. The visceral abdominal depot (abdominal obesity) is linked to metabolic dysfunction (cardiovascular disease, insulin resistance, T2D). Conversely, lower body adiposity (gluteofemoral obesity) is associated with improved cardiovascular and metabolic profiles.[46]. The abdomen adipose tissue is characterized by the rapid uptake of diet-derived fat and a high lipid turnover that is easily stimulated by stress hormones.[46]. Increased release of free fatty acids and abnormalities in adipokine secretion observed in people with abdominal obesity promote insulin resistance.[47]

Compared with nonsmokers, smokers are characterized by greater insulin resistance and hyperinsulinemia.[48]. However, little research has been conducted on the impact of smoking cessation on insulin resistance and insulin secretion.

Smoking cessation may be associated with worsening fat distribution. In a population-based study (Inter99 Study) performed in Copenhagen, the mean increase in waist circumference after quitting at the one-year follow-up was 3.88 cm (42% of the quitters had increased their waist circumference by ≥ 5 cm). Quitters with high baseline tobacco consumption were more likely to have substantially increased waist circumference. In this study, abstinence from smoking was the most important predictor of substantial weight gain and a substantial increase in waist circumference.[49]. Likewise, a study with the use of computed tomography showed that both current and former smoking is associated with increased
Walicka M et al. Stopping smoking and metabolic parameters

### Table 1 The results of studies evaluating the effect of smoking cessation on hemoglobin A1c in diabetic patients

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Country/region</th>
<th>Study population</th>
<th>Study design</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinardo et al [24], 2019</td>
<td>United States</td>
<td>T2D, n = 282</td>
<td>Cross-sectional, comparison of current smokers vs former smokers and never-smokers</td>
<td>Positive</td>
</tr>
<tr>
<td>Beaufort et al [14], 2019</td>
<td>Multicenter (United States and Canada)</td>
<td>T2D, n = 1441</td>
<td>Retrospective analysis of the prospective cohort study, comparison of current smokers vs former smokers and never-smokers</td>
<td>Positive</td>
</tr>
<tr>
<td>Lycett et al [17], 2011</td>
<td>United Kingdom</td>
<td>T2D, n = 10692</td>
<td>Retrospective cohort study, observation of HbA1c in three groups: Continual smokers, long-term quitters, and relapers</td>
<td>Negative</td>
</tr>
<tr>
<td>Okhuma et al [23], 2015</td>
<td>Japan</td>
<td>T2D, n = 2400</td>
<td>Cross-sectional study, comparison of current smokers and former smokers vs never-smokers</td>
<td>Positive</td>
</tr>
<tr>
<td>Su et al [24], 2017</td>
<td>China</td>
<td>T2D, n = 7763</td>
<td>Cross-sectional study, comparison of current smokers, former smokers and never-smokers</td>
<td>Positive</td>
</tr>
<tr>
<td>Li et al [28], 2017</td>
<td>Hong Kong of China</td>
<td>T2D, n = 557</td>
<td>A randomized controlled trial, comparison of level of HbA1c and changes from baseline to 12-mo between quitters and non-quitters</td>
<td>Neutral</td>
</tr>
<tr>
<td>Kar et al [29], 2016</td>
<td>United States, Japan</td>
<td>T2D + T2D, n = 13719</td>
<td>Metanalysis, comparison of current smokers vs quitters</td>
<td>Neutral</td>
</tr>
<tr>
<td>Voulgaris et al [57], 2011</td>
<td>Greece</td>
<td>T2D, n = 193</td>
<td>Prospective study, comparison of smokers vs former-smokers</td>
<td>Positive</td>
</tr>
<tr>
<td>Fedoroff et al [69], 2016</td>
<td>Finland</td>
<td>T2D, n = 3613</td>
<td>Prospective study, comparison of smokers vs non-smokers and former-smokers vs non-smokers</td>
<td>Positive</td>
</tr>
<tr>
<td>Reynolds et al [68], 2011</td>
<td>United States</td>
<td>T2D, n = 2124</td>
<td>Cross-sectional analysis of population-based study, comparison of current smokers vs non-smokers and former-smokers vs non-smokers</td>
<td>Neutral</td>
</tr>
<tr>
<td>Reynolds et al [68], 2011</td>
<td>United States</td>
<td>T2D, n = 348</td>
<td>Cross-sectional study, comparison of current smokers vs non-smokers and former-smokers vs non-smokers</td>
<td>Neutral</td>
</tr>
</tbody>
</table>

T1D: Type 1 diabetes; T2D: Type 2 diabetes; HbA1c: Hemoglobin A1c;

In a population-based study performed in Norway, former smokers compared with current smokers had a lower waist to hip ratio (additionally among women, waist circumference was lower)[51].

However, studies assessing insulin resistance indicators in quitters do not have consistent results. There was a statistically significant negative correlation between homeostasis model assessment-estimated insulin resistance (HOMA-IR) values among asymptomatic, Korean male ex-smokers without diabetes[52]. In contrast, other studies (also in the groups without diabetes) showed that quitting smoking was associated with greater insulin resistance as measured by Quicki or HOMA-IR[53,54].

The observed discordance amongst the insulin sensitivity findings is likely to be due to a change in body weight. After stopping smoking, insulin sensitivity is likely to change because of weight fluctuations. It was shown that the HOMA-IR index after quitting significantly increases in weight gainers, but not in weight maintainers[55]. In the study by Heggen et al[56], no differences were found in HOMA-IR between quitters and smokers but the findings must be interpreted within the context of similar modest body weight changes in quitters and smokers at 3-mo follow-up.

These studies have most commonly included people without diabetes. The only study investigating the relationship between insulin resistance and smoking cessation among patients with diabetes is that of Ohkuma et al[23]. The authors found that smoking cessation has a time-dependent link with insulin resistance in Japanese patients with T2D; HOMA-IR levels decreased in ex-smokers over time relative to current smokers. HOMA-IR was also assessed in the prospective study, evaluating the effect of smoking on the progression of microalbuminuria in T2D. Smoking cessation was associated with the amelioration of insulin resistance parameters in spite of the small but significant increase in body mass index. This observation may be explained by the fact that many quitters increased their physical activity [57].

A summary of the studies evaluating smoking cessation’s effect on HOMA-IR is shown in Table 2.

The search for publications on quitting and insulin secretion in patients with diabetes was unproductive. In the population without diabetes, Morimoto et al[38] found that the risk of impaired insulin secretion in an ex-smoker is similar to that in never-smokers, where the risk is almost twice as high in current smokers when compared with never smokers, with the magnitude of this increase being dose-dependent (i.e. increasing with a number of pack-years). Stadler et al[54] showed a 31% increase in beta-cell secretion (as measured by insulinogenic index 140) after > 3 mo of not smoking.
SMOKING CESSATION AND LIPIDS ABNORMALITIES

Patients with T2D characteristically have abnormal plasma lipids profiles which are marked by hypertriglyceridemia, reduced HDL cholesterol levels, and increased concentration of small dense LDL. These abnormalities are a result of a multifactorial process, including abdominal obesity, insulin resistance, increased free fatty acid flux, and inflammation\[^{[59]}\]. Cigarette smoke has been shown to increase the atherogenic nature of the lipid profile\[^{[60]}\]. Smoking is associated with increased triglycerides (TG), total cholesterol, and LDL, as well as reduced levels of cardioprotective HDL\[^{[61]}\]. In a prospective study of 808 young Asian adults, smokers were three times more likely to have low HDL cholesterol and were 2.6 times more likely to develop hypertriglyceridemia\[^{[62]}\]. There is a clear assumption in healthcare messaging that stopping smoking may correct dyslipidemia, which is especially relevant in smokers with diabetes. Studies, conducted on patients without diabetes, indicate that quitting smoking increases HDL levels\[^{[63,64]}\]. The increase in HDL has frequently been observed in spite of weight gain experienced after cessation of smoking\[^{[65]}\]. Evidence also indicates that smokers may have improved HDL function (increased cholesterol efflux capacity and decreased HDL inflammatory index) after quitting smoking\[^{[65]}\].

Data on TG levels are conflicting. Some studies performed in the group without diabetes showed that smoking cessation is associated with a reduction of this lipid fraction\[^{[66]}\], however, others studies have failed to confirm this\[^{[64]}\].

Data on LDL is also limited, but evidence seems to suggest that smoking cessation does not affect LDL levels or LDL size\[^{[63,67]}\].

A few studies have tested diabetic patients’ lipid profiles after quitting smoking. Results are inconsistent. In Reynolds et al\[^{[68]}\], 3466 youth who had T1D (n = 2887) or T2D (n = 579) and were smokers were examined for prevalence of tobacco use and the coexistence of cardiovascular risk factors. Compared to patients who were non-smokers, past smokers with T1D had significantly higher odds of having high LDL cholesterol levels, and those who were current smokers had significantly higher chances of having high TG levels. Patients with T2D did not exhibit these relationships, but the smaller numbers of patients included in the study could have influenced the statistical significance of the results.

In the study of Luque-Ramírez et al\[^{[12]}\] patients with T2D who smoke had lower HDL and higher TG levels compared to their nonsmoking counterparts.

Lipid parameters were examined in two studies in patients with diabetic nephropathy. After stopping smoking for at least 1 year, patients had significantly lower total cholesterol, LDL, and HDL levels than those who continued to smoke\[^{[57]}\]. In a similar study of patients with T1D, total cholesterol, TG, and LDL levels of current and former smokers were higher than those of non-smokers, whereas lower HDL levels were observed in current smokers\[^{[69]}\].

A summary of the studies evaluating smoking cessation’s effect on lipid parameters is shown in Table 3.

SMOKING CESSATION AND BIOCHEMICAL PARAMETERS OF NEPHROPATHY

It is well known that chronic kidney disease (CKD) and end-stage renal disease (ESRD) can complicate diabetes mellitus. Diabetic nephropathy is characterized by proteinuria and/or the decline of renal function [e.g. reduced glomerular filtration rate (GFR)][\[^{[70]}\]. Aside from high blood sugar levels, other risk factors that contribute to the development and progression of diabetic kidney disease include high blood pressure, dyslipidemia, and genetic predisposition\[^{[71]}\]. Smoking may also be a factor in the development and progression of kidney failure possibly through a mechanism of progressive arteriolar damage, increased renovascular resistance, and increased intraglomerular capillary pressure\[^{[72-75]}\]. While many studies have examined the relationship between cigarette smoking and kidney disease with conflicting results, a meta-analysis of 15 prospective cohort studies with 65064 incident cases of CKD suggests that smoking is as an independent risk factor in the general population\[^{[76]}\].

### Table 2 The results of studies evaluating the effect of smoking cessation on homeostasis model assessment-estimated insulin resistance in diabetic patients

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Country</th>
<th>Study population</th>
<th>Study design</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ohkuma et al[^{[23]}], 2015</td>
<td>Japan</td>
<td>T2D, n = 2490</td>
<td>Cross-sectional study, comparison of current smokers and former smokers vs never-smokers</td>
<td>Positive</td>
</tr>
<tr>
<td>Voulgari et al[^{[57]}], 2011</td>
<td>Greece</td>
<td>T2D, n = 193</td>
<td>Prospective study, comparison of smokers vs former-smokers</td>
<td>Positive</td>
</tr>
</tbody>
</table>

T1D: Type 1 diabetes; T2D: Type 2 diabetes.
Table 3 The results of studies evaluating the effect of smoking cessation on lipid parameters in diabetic patients

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Country</th>
<th>Study population</th>
<th>Study design</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Reynolds et al[68], 2011</td>
<td>United States</td>
<td>T1D, n = 2124</td>
<td>Cross-sectional analysis of population-based study, comparison of current smokers vs non-smokers and former-smokers vs non-smokers</td>
<td>LDL–negative; HDL–neutral; TG–positive</td>
</tr>
<tr>
<td>Reynolds et al[68], 2011</td>
<td>United States</td>
<td>T2D, n = 348</td>
<td>Cross-sectional study, comparison of current smokers vs non-smokers and former-smokers vs non-smokers</td>
<td>Neutral</td>
</tr>
<tr>
<td>Luque-Ramírez et al[12], 2016</td>
<td>Spain</td>
<td>T2D, n = 890</td>
<td>Cross-sectional, observational study, comparison of smokers vs former-smokers</td>
<td>LDL–neutral; HDL–neutral; TG–positive</td>
</tr>
<tr>
<td>Voulgari et al[57], 2011</td>
<td>Greece</td>
<td>T2D, n = 193</td>
<td>Prospective study, comparison of smokers vs former-smokers</td>
<td>LDL–positive; HDL–positive; TG–positive</td>
</tr>
<tr>
<td>Feodoroff et al[69], 2016</td>
<td>Finland</td>
<td>T1D, n = 3613</td>
<td>Prospective study, comparison of smokers vs non-smokers and former-smokers vs non-smokers</td>
<td>LDL–neutral; HDL–neutral; TG–neutral</td>
</tr>
</tbody>
</table>

T1D: Type 1 diabetes; T2D: Type 2 diabetes; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglycerides.

There is no clear impact of stopping smoking on characteristics associated with diabetic nephropathy. The effect of smoking cessation on microalbuminuria was investigated by Voulgari et al[57] in subjects with newly diagnosed T2D mellitus. Within a year of stopping smoking, the prevalence of those with microalbuminuria markedly declined compared to those who continued smoking. However, eGFR was comparable between the two study groups. Smokers who stopped smoking had a lower microalbuminuria rate irrespective of the effect of drug therapy (antihypertensive, hypolipidemic, and antidiabetic).

In a study of 2770 patients with T2D, Ohkuma et al[77] investigated the association of smoking and its abstinence with parameters of CKD. In comparison to non-smokers, former and current smokers had higher urinary albumin-creatinine ratios. In the former smokers, this ratio decreases linearly with increasing years after quitting smoking. Furthermore, current smokers’ risk is related to how many cigarettes per day they smoke. After quitting smoking, age-adjusted creatinine-based GFR declined compared to the never-smokers but increased in parallel with increasing cigarette consumption. The increased eGFR of smokers may be related to glomerular hyperfiltration which is implicated as a mechanism for the progression of diabetic nephropathy[78].

Progressive kidney damage can result from glomerular hyperfiltration over time. According to Ohkuma’s study, the proportion of smokers with CKD increased with the number of cigarettes they smoked per day (compared with never-smokers). However, as the years passed since quitting, the proportion of patients with CKD decreased. There was a significant increased HbA1c level for current smokers and a greater proportion of hypertension for ex-smokers compared with never smokers and current smokers with respect to the other risk factors for nephropathy in this study[77].

Using data from the Finnish Diabetic Nephropathy Study, which included 3613 T1D patients, the 12-year cumulative risk of microalbuminuria, macroalbuminuria, and ESRD by smoking status was calculated. Current and former smokers were more likely to have micro- and macro-albuminuria (ESRD for current smokers only) than non-smokers. There were no statistically significant differences in the 12-year cumulative risk of microalbuminuria and macroalbuminuria between former smokers and never smokers. There were significantly poorer glycemic control and lipid parameters for smokers compared to nonsmokers. Adjusting for HbA1c and lipid variables, the increased risk of diabetic nephropathy progression among current and former smokers was attenuated. Smoking-related changes in lipids and glucose control may account for the majority of nephropathic changes due to diabetes[69]. This observation suggests that poor glucose control and lipid alterations in smokers are the main drivers of nephropathic changes in diabetes.

A summary of the studies evaluating smoking cessation’s effect on characteristics associated with diabetic nephropathy is shown in Table 4.

### CONCLUSION

In addition to reducing overall and cardiovascular mortality, stopping smoking may provide significant additional health benefits to people with diabetes. It is important to note, however, that weight gain experienced after stopping smoking may attenuate some of these health benefits[79].

When considering the potential impact of stopping smoking on metabolic parameters in patients with diabetes, the benefits of cessation of smoking are less clear because the expected outcomes have not been consistently demonstrated. Studies have shown both improvements and temporary deterioration in glycemic control after quitting smoking. Only a few available studies have investigated the effect of
quitting smoking on insulin resistance and lipid parameters in diabetic patients. These studies also report inconsistent results. Smoking cessation appears to have a clear beneficial effect on markers of nephropathy, particularly after longer periods of smoking abstinence.

The review of the published literature found only a few studies, many of which had design and methodological shortcomings, and as such need to be interpreted with caution. A major issue for this area of study is the lack of randomized controlled trials that have been carried out to date.

In an era of evidence-based medicine, the lack of data regarding the metabolic effects of smoking and smoking cessation in diabetes is very disappointing and needs to be addressed. Diabetes is one of the major population health issues, the consequence of which appears to be amplified by smoking. The lack of good quality research on the impact of smoking cessation on metabolic parameters in this population hampers clinicians’ ability to give informed advice on the effectiveness and management of stopping smoking. This is a complex medical and sociological issue that demands a greater research focus to better inform people with diabetes and assist healthcare providers to implement the most effective interventions.

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**FOOTNOTES**

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Walicka M et al. Stopping smoking and metabolic parameters

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Walicka M et al. Stopping smoking and metabolic parameters


Walicka M et al. Stopping smoking and metabolic parameters


Basic Study

Investigating the specificity of endothelin-traps as a potential therapeutic tool for endothelin-1 related disorders

Arjun Jain, Kristof Bozovicar, Vidhi Mehrotra, Tomaz Bratkovic, Martin H Johnson, Ira Jha

Abstract

BACKGROUND
Endothelin (ET)-traps are Fc-fusion proteins with a design based on the physiological receptors of ET-1. Previous work has shown that use of the selected ET-traps potently and significantly reduces different markers of diabetes pathology back to normal, non-disease levels.

AIM
To demonstrate the selected ET-traps potently and significantly bind to ET-1.

METHODS
We performed phage display experiments to test different constructs of ET-traps, and conducted bio-layer interferometry binding assays to verify that the selected ET-traps bind specifically to ET-1 and display binding affinity in the double-digit picomolar range (an average of 73.8 rM, n = 6).

RESULTS
These experiments have confirmed our choice of the final ET-traps and provided proof-of-concept for the potential use of constructs as effective biologics for diseases associated with pathologically elevated ET-1.

CONCLUSION
There is increased need for such therapeutics as they could help save millions of lives around the world.
Key Words: Endothelin-1; Endothelin-traps; Diabetes; Heart failure; Chronic kidney disease; Novel therapeutic

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Core Tip: This study verified the specificity of endothelin (ET)-traps, which are an Fc-based fusion protein that acts as a potential therapeutic for various cET-1 related disorders, including diabetes and chronic kidney disease. ET-traps, unlike ET receptor antagonists, do not completely block the ET system and hence have minimal side effects. ET-traps would help save millions of lives around the world.

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INTRODUCTION

Endothelin-1 (ET-1) is a vasoactive peptide synthesized and secreted by a diverse range of cells, and thus implicated in signaling events in a wide variety of target tissues[1]. ET-1 plays a key role in physiological functions. However, supraphysiological levels of ET-1 induce pathology and are implicated in a host of different diseases, including cardiovascular disease[2-4], neurodegenerative disorders[5-8], chronic kidney disease, different cancers, such as prostate cancer[1,9,10], pregnancy disorders like preeclampsia[7,11], as well as diabetes[8,12,13]. Given that a key feature of these diseases is elevated ET-1 Levels, one proposed strategy of therapeutic intervention is to target the increased levels of ET-1. To this end, we have created ET-traps, molecular constructs that bind and sequester ET-1 to have an efficacious effect on cells treated with a pathological dose of ET-1, as well as those treated compared with control subjects[18,19]. Accordingly, our previous in vitro work confirmed the ET-traps to have an efficacious effect on cells treated with a pathological dose of ET-1, as well as those treated with pathologically high glucose (25 mmol/L)[12]. We also established the proof-of-concept (PoC) for ET-traps as a therapeutic in the diabetes disease space at the in vivo level. The use of ET-traps gave a significant reduction in different markers of diabetes disease pathology, which suggested the ET-traps could be considered a therapeutic for diabetes with a novel mechanism of action. Importantly, the ET-traps were found to be non-toxic at the proposed therapeutic concentration both in vitro and in vivo[12,13].

ET-1

ET-1 exerts its effects by binding to the endothelin A and B receptors, two highly homologous cell-surface proteins that belong to the G-protein-coupled receptor superfamily[20]. The two receptors share about 60% similarity at the level of primary structure[1], i.e. both receptors exhibit a high polypeptide sequence identity with each other. Nevertheless, the two receptors show a clear distinction in ligand binding selectivity based on their ligand-binding domains.

Orry et al[21] constructed a model of interaction of the ET-1 peptide with the endothelin A receptor, where ET-1 makes contacts with both the N-terminal receptor domain and two different extracellular loops (ECL).

Further, amino acids of the C-terminal and residues in the third intracellular loop are important for ET-1 binding[22]. In this study, we performed binding affinity experiments to ascertain that our selected ET-traps bind specifically just to ET-1.

Homologs of ET-1

The ETs are a family of potent vasoactive peptides. ET-1 has two paralogs in the ET family; ET-2 and ET-3[23]. ET-1 was identified by Yanagisawa et al[24] in 1988. A year later, 2 homologs of ET-1 were discovered; ET-2 and ET-3[25].
ET-2 is a peptide encoded by the EDN2 gene located on chromosome 1 in humans[26]. ET-2 has a key role in ovarian physiology[25]. Previous research findings have also revealed that ET-2 is critical for the growth and survival of postnatal mice and plays important roles in energy homeostasis, thermoregulation, and the maintenance of lung function[26].

ET-3 is a peptide that in humans is encoded by the EDN3 gene[27]. The active peptide is a ligand for ET receptor type B (EDNRB). The interaction of this ET with EDNRB is essential for development of neural crest-derived cell lineages, such as melanocytes and enteric neurons[28].

Therefore, both the ET-1 paralogs (ET-2 and ET-3) are essential for different physiological processes and so it is important that any ET-1 sequestering agent selectively targets ET-1 and hence the problems associated with increased expression of ET-1 to avoid disrupting the remaining processes of the ET system.

In this study, we first performed phage display experiments to ascertain the binding of the ET-traps to ET-1. Phage display is one of the most powerful and widely used laboratory techniques for the study of protein–protein, protein–peptide and protein–DNA interactions[29]. This technology is based on expressing the protein or peptide of interest on bacterial virus protein coat, allowing the study of molecular interaction between the virion-displayed ligand (in this case ET-traps) and an immobilized target (i.e. ET-1).

MATERIALS AND METHODS

Sub-cloning ET-traps into pIT2 phagemid vector
ET-traps constructs were cloned and displayed in a monovalent phage display system using the 3 + 3 display approach (Figure 1). Each codon-optimized construct (Genscript) was amplified by polymerase chain reaction from its parent plasmid pUC18 using forward and reverse primers with overhangs harboring NcoI and NotI restriction sites. Reaction mixtures were subjected to agarose gel electrophoresis, amplicons were purified with QIAEX II gel extraction kit (Qiagen), and subsequently digested with NcoI/NotI restriction enzymes alongside pIT2 phagemid vector.

Digested inserts were ligated into pIT2 phagemid vector and chemically competent Escherichia coli TG1 were transformed with the resulting recombinant phagemids with the heat-shock method. Phages were amplified and rescued by superinfection with KM13 helper phage. PEG/NaCl was used to precipitate and isolate phage clones which were spectrophotometrically quantified with NanoDrop 1000. Phage titers were calculated using equation 1 (derived by Day and Wiseman)[30] and subjected to phage enzyme-linked immunosorbent assay (ELISA).

Verifying ET-traps construct display
MaxiSorp microtiter plate (Nunc) wells were coated with anti-cMyc antibodies (1 µg/mL in PBS) overnight at 4 °C. Wells were blocked with 5% skimmed milk and 100 µL of 5 × 10⁶ phage clone virions in 0.5% milk/0.1% PBST were added and incubated for 1 h with gentle agitation. After extensive washing, bound phages were detected with anti-M13 monoclonal antibodies conjugated with horseradish peroxidase (GE Healthcare) and chromogenic substrate (3,3’,5,5’- tetramethylbenzidine). Reaction was terminated with 2 M H₂SO₄ and absorbance was measured at 450 nm. The signals generated were later used for normalization of ET-1 binding activity.

Analysis of ET- traps: ET-1 binding
To increase the adsorption surface area, and thus the detection signal, N-biotin-ET-1 (Phoenix pharma) was coupled to paramagnetic streptavidin beads (MyOne Streptavidin T1, Thermo Fisher Scientific; 10 mg/mL beads) as an alternative to the conventional phage ELISA on 96-well plates. 5 µL of beads were incubated with 2.5 × 10⁹ ET-traps-displaying phages for 1 h in 500 µL of 0.5% milk/0.1% PBST. After washing and detection reaction, paramagnetic beads were captured on a magnet, and the supernatants were transferred to a 96-well plate for absorbance measurements. In parallel, binding of ET-trap constructs to streptavidin beads in absence of ET-1 was analyzed. Absorbance signals were blank-subtracted and normalized according to the relative display levels as determined in anti-cMyc phage ELISA assay.

Creating an Fc-fusion construct and measuring binding affinity to ET-1
The gene for the ET-traps was designed and optimized for expression in mammalian cells (HEK293) prior to being synthetised. The sequence was then sub-cloned into a cloning and expression vector for human Fc fusion proteins.

In brief, HEK293 cells were passaged to the optimum stage for transient transfection. Cells were transiently transfected with the appropriate expression vector and cultured for a further 6-14 d. An appropriate volume of cells was transfected with the aim of obtaining 1-5 mg of purified Fc fusion protein. Cultures were harvested and one-step purification performed using affinity chromatography. For this, culture supernatant containing Fc fusion protein was loaded onto a MabSelect SuRe Protein A
column at 4 mL/min and washed with PBS pH 7.2. A step elution was performed with sodium citrate buffer (pH 3.0). Eluted protein was neutralised with 10% (v/v) Tris buffer (pH 9.0). Upon purification, the Fc fusion protein was buffer exchanged into PBS pH 7.4. The protein was analysed for purity by SDS-PAGE and concentration determined by UV spectroscopy (at 280 nm).

For the binding affinity measurement, we employed the use of the Octet Red96 system (Patel et al 2013). In brief, the kinetics of the selected ET-traps binding to biotinylated ET-1 (Phoenix Pharmacueticals) was determined using the Octet Red96 system (ForteBio, Menlo Park, CA). The buffer for the assays was PBS with 0.01% (w/v) bovine serum albumin and 0.002% Tween20. The measurements were carried out at 30 °C. 1 µg/mL bio-ET-1 was captured on dip-and-read streptavidin sensors, followed by binding of the selected ET-traps at 500 nM concentration. The ForteBio Octet analysis software (ForteBio, Menlo Park, CA) was used to generate the sensorgram.

RESULTS

Previous work identified a strong binder to ET-1[31]. In this study, we tested different sequence combinations of ET-traps that could also bind ET-1. We performed phage display experiments to ascertain this.

Phage display experiments

Phage display is a powerful technique commonly used today to identify different protein–protein interactions. We displayed individual ET-traps in a monovalent setting (i.e. 3 + 3 display type[32]) to prevent avidity effects on binding to ET-1. The cMyc-tag peptide present in the linker region that tethers ET-traps to the anchoring phage coat protein p3 allows for assessment of constructs’ display levels by phage ELISA against anti-cMyc antibody. These were, in turn, used to normalize signals from phage ELISA where binding of ET-traps to biotinylated ET-1 was analyzed (Figure 2).

The construct ζ gave strong binding to ET-1 in phage display experiments, but the Fc-fusion molecule was not stable and the results could not be replicated with the soluble fusion protein. The phage experiments confirmed that construct β indeed gave consistent, high binding. It was further observed that in the form of an Fc-fusion construct β showed high binding affinity to ET-1 consistently (Figure 3).

DISCUSSION

This paper discusses the characterization of ET-traps that might be useful in the treatment of ET-1 related diseases or disorders, such as preeclampsia, cardiovascular diseases, chronic kidney disease, diabetes or neurodegenerative disorders[11,18,19,33-36]. Previous work has shown that the use of the selected ET-traps gave a therapeutic effect on reducing different markers of diabetes-induced disease pathology[12,13]. The ET-traps helped reduce different markers of diabetes disease pathology, such as over-expression of ECM proteins, proteinuria and tissue damage to kidneys and heart. This effect was found to be statistically significant both in vitro and in vivo. The ET-traps were designed based on a previous study[22]. The purpose of this study was to ascertain our selection of the ET-traps. Both ECL2 and ECL3, including the flanking transmembrane regions, were found to play an important role in ligand selection[22]. Further, residues in the intracellular loop and of the C-terminus are important for ET-1 binding[22]. These domains were used to create the final ET-traps that gave an efficacious, therapeutic effect in our proof-of-concept studies done both at the in vitro and in vivo levels in the diabetes disease space[12,13]. The final design of our ET-traps ensured that the selected ET-traps do not bind the ET-1 paralogs, which is important for selective activity and thus fewer potential adverse effects.
Figure 2 Sensorgram of Fc-β binding to biotinylated endothelin-1. Representative plot shows the binding assay revealed that our selected endothelin (ET)-traps bind strongly to ET-1, displaying double-digit picomolar binding affinity (an average of 73.8 picoMolar, n = 6).

Figure 3 Average relative endothelin-1 binding of endothelin-traps constructs expressing clones using phage display. The final signals are blank-subtracted and normalized to individual expression levels.

We began with phage display experiments to test different combinations of potential ET-traps, including the previously selected ET-traps that were used to perform the proof of concept (PoC) work [12,13]. These experiments confirmed the selection of the most efficient ET-traps. Phage experiments were performed on different combinations of sequences (Figure 3). These experiments allowed us to confirm our final ET-traps selection and we then proceeded to test the binding of its cognate Fc fusion to ET-1.

As ET-1 is abundant in the body while ET-2 is almost undetectable, ET-1 was more convenient to research; this assumption has meant ET-2 is relatively under-researched [26]. However, recent research evidence suggests distinct roles and features of ET-2. In mice with the ET-2 gene knocked-out, the animals displayed growth retardation, and were hypothermic and hypoglycemic, which resulted in early mortality [37].

ET receptor antagonists (ERAs) can have a deleterious effect on physiological ET-2 functions by completely blocking the receptors and thereby inhibiting the physiological actions of ET-2. With our ET-traps, we would overcome this; the ET-traps specifically bind to ET-1 and do not block the receptors to effect ET-2 actions like an inhibitor to the ET system might do. The ET-traps have been designed to specifically bind ET-1.

Aberrations in the EDN3 gene that is responsible for producing ET-3 have been associated with congenital disorders involving neural crest-derived cells, like Hirschsprung disease and Waardenburg syndrome [38,39]. This shows that ET-3 is one of the important peptides of the ET family, which is
involved in various developmental processes. Further, use of ERAs would essentially block the function of this molecule thereby potentially causing serious birth defects. This again precludes completely blocking the physiological functions of ET-3, as it is one of the important factors for essential developmental processes. Again, with the ET-traps, we would not completely be blocking the ET system, rather just targeting elevated ET-1 Levels upstream. As found in this study, the selected ET-traps bind ET-1 with a high binding affinity in the double-digit picomolar range (an average of 73.8 nM, n = 6). This was also previously found and reported by Jain et al.[12] in their diabetes PoC study. This work showed that the selected ET-traps have an efficacious, therapeutic effect in ameliorating diabetes disease pathology [12,13]. This was not associated with any toxic effects as evinced by the toxicology data. This corroborates that the selected ET-traps are efficacious at the working concentration and specific to just ET-1.

CONCLUSION
The ET-traps were designed to specifically bind ET-1. The results of this study confirm that our selected ET-traps specifically bind to ET-1. This is in agreement with previous PoC studies that detected no toxic effects of the selected ET-traps at the working concentration. This is an important factor for the potential use of ET-traps as a therapeutic.

ARTICLE HIGHLIGHTS

Research background
Endothelin (ET)-1 is a very potent vasoactive peptide that is significantly elevated in different diseases.

Research motivation
We wanted to develop a cure that would target this peptide and would help save millions of lives around the world.

Research objectives
To develop a tool that specifically targets ET-1.

Research methods
We employed phage display and binding assays.

Research results
A very high binding affinity was observed for our selected tool.

Research conclusions
Developed a potent tool targeting ET-1.

Research perspectives
A new target in drug discovery and development.

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FOOTNOTES

Author contributions: Jain A and Bozovičar K contributed equally to this work; Jain A, Bozovičar K, Mehrotra V, Bratkovič T performed the experimental analyses and contributed towards writing the article and Johnson M and Jha I revised it critically for important intellectual content.

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Data sharing statement: Data available from corresponding author, Dr. Arjun Jain at arjun@et-traps.co.uk.
Jain A et al. Specificity of ET-traps for ET-1

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Basic Study

Preparation and hypoglycemic effects of chromium- and zinc-rich *Acetobacter aceti*

Yong-Yi Huang, Xiang-Kun Qin, Yuan-Yuan Dai, Liang Huang, Gan-Rong Huang, Yan-Chun Qin, Xian Wei, Yan-Qiang Huang

**Abstract**

**BACKGROUND**

At present, there is no ideal method to cure diabetes, and there are few reports on the treatment of diabetes with probiotics.

**AIM**

To propose a method for preparing a new type of chromium- and zinc-rich *Acetobacter aceti* (*A. aceti*) and explore its ability to enhance the hypoglycemic effects of probiotics in the treatment of diabetes.

**METHODS**

*A. aceti* was cultured in a liquid medium that contained chromium trichloride and zinc chloride, both at a concentration of 64 mg/mL, with the initial concentration of the bacterial solution $1 \times 10^4$ CFU/mL. After the bacterial solution had been inducted for 48 h, the culture media was changed and the induction was repeated once. The levels of chromium and zinc in the bacteria were detected by inductively coupled plasma mass spectrometry, and the contents of NADH and glucose dehydrogenase were determined using an NAD/NADH kit and glucose dehydrogenase kit, respectively. Streptozotocin was used to establish a mouse model to evaluate the hypoglycemic effects of the proposed chromium- and zinc-rich *A. aceti*. Ten-times the therapeutic dose was administered to evaluate its biological safety. The effect on MIN6 islet cells was also assessed in vitro.

**RESULTS**

The levels of chromium metal, metallic zinc, NADH coenzyme, and glucose dehydrogenase in *A. aceti* prepared by this method were 28.38-34.34 mg/kg, 5.35-
7.52 mg/kg, 5.13-7.26 μM, and 446.812-567.138 U/g, respectively. The use of these bacteria resulted in a better hypoglycemic effect than metformin, promoting the repair of tissues and cells of pancreatic islets in vivo and facilitating the growth of MIN6 pancreatic islet cells and increasing insulin secretion in vitro. Ten-times the therapeutic dose of treatment was non-toxic to mice.

**CONCLUSION**

Chromium trichloride and zinc chloride can be employed to induce the preparation of chromium- and zinc-rich *A. aceti*, which can then promote the hypoglycemic effect found in normal *A. aceti*. The bacteria biotransforms the chromium and zinc in a way that could increase their safety as a treatment for diabetes.

**Key Words:** *Acetobacter aceti*; Chromium; Zinc; Enrichment; Blood sugar decrease

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**Core Tip:** At present, there are no ideal drugs to treat diabetes. Acetobacter and other probiotics can be used in the treatment of diabetes, but their effect is not significant. The focus of this study is to determine if enriching chromium and zinc in *Acetobacter aceti* could enhance the hypoglycemic effect of this probiotic. In this study, metal compounds were used to induce *A. aceti* to enrich chromium and zinc concentrations, and the effects of these metal-enriched bacteria on the hypoglycemic effect were assessed. These chromium- and zinc-rich bacteria were able to increase the hypoglycemic effect and, due to low toxicity, have good prospects as a treatment for diabetes.

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**INTRODUCTION**

Diabetes mellitus is a disease of chronic glucose metabolism disorders [1,2]. In 2020, the world’s population with diabetes was about 495 million, and this is expected to increase to 700 million by 2045 [3,4]. The pain and burden of diabetes and its complications are major health and socio-economic concerns for people all over the world. Diabetic patients are often challenged by serious complications, such as diabetic nephropathy, diabetic retinopathy, cardiovascular system disease, etc, which pose a serious threat to human life and health [5-7]. Patients with type 2 diabetes mellitus (T2DM) account for 90% to 95% of all diabetic patients, making T2DM an important focus of much research into the epidemiology of diabetes. Scholars have heavily researched the causes of diabetes and methods for its prevention, for many years; however, there remains no effective cure, due to the disease’s unclear causes and complicated pathogenesis. Moreover, its underlying mechanism has yet to be fully elucidated [8,9]. The current comprehensive prevention and treatment measures for diabetes mainly include diet control, exercise, and drug therapy [10-12].

The drugs used for the treatment of diabetes are mainly chemical agents, with few biological drugs available. Health drinks, including kombucha (which contains yeast, lactic acid bacteria, and acetic acid bacteria and their metabolites) and black tea, can lower blood sugar, blood pressure, blood lipids, etc, and exert certain auxiliary effects on patients with hypertension, hyperlipidemia, and hyperglycemia; the main active ingredients in these drinks are D-glucaric acid-1, 4-lactone and tea polyphenols, respectively [13]. However, the effect of bacterial metabolites from these drinks on reducing blood sugar is not fully elucidated. For example, *Acetobacter acetii* (*A. acetii*) contains dihydronicotinamide-adenine dinucleotide and glucuronic acid dehydrogenase, among other metabolites, which decompose glucose through glycolysis; however, kombucha fails to improve the effect of reducing blood sugar by fully utilizing the effects of these metabolic enzymes. Therefore, *A. acetii* can only be used as a healthy drink for adjuvant therapy. In order to capitalize on the ability of these bacteria to metabolize glucose, increase the contents of dihydronicotinamide-adenine dinucleotide, glucuronic acid dehydrogenase, and other metabolic enzymes, and exert their hypoglycemic effects, their full effectiveness must be realized in vivo before these bacteria can be used as a hypoglycemic drug.

To improve the effect of *A. acetii* on lowering blood sugar, the present study provides a method for preparing and applying chromium- and zinc-rich *A. acetii*. This method demonstrated a significant increase in the amount of dihydronicotinamide-adenine dinucleotide, glucuronic acid dehydrogenase, chromium, and zinc inside the cells and other microelements in the specimens we prepared. These
enriched bacteria had a notable repair effect on pancreatic islet cells, promoted insulin secretion, and demonstrated a good hypoglycemic effect. Therefore, chromium- and zinc-rich \textit{A. aceti} can be used as a candidate drug for the treatment of T2DM.

**MATERIALS AND METHODS**

**Materials**

Glucose (Guangdong Guanghua Technology Co., Ltd, batch number: 20200403); yeast extract (Beijing Aoboxing Bio-tech Co., Ltd, batch number: 20200422); calcium carbonate (Shanghai Titan Scientific Co., Ltd, batch number: P1260108); agar (Beijing Solarbio Technology Co., Ltd, batch number: 310C022); anhydrous alcohol (Shanghai MacLean Biochemical Technology Co., Ltd, batch number: C1974944); chromium chloride (Shanghai MacLean Biochemical Technology Co., Ltd, batch number: C10717130); zinc chloride (Shanghai MacLean Biochemical Technology Co., Ltd, batch number: C10790413); 50-mL centrifuge tubes and EP tubes (Jiangsu Lexinkang Medical Equipment Co., Ltd); streptozotocin (STZ) (Shanghai McLean Biochemical Technology Co., Ltd, batch number: C20PA038100B); citric acid (Shanghai McLean Biochemical Technology Co., Ltd, batch number: C10723907); sodium citrate (Shanghai McLean Biochemical Technology Co., Ltd, batch number: C10712912); universal pH indicator paper (Hangzhou Test Three Technology Co., Ltd); metformin hydrochloride tablets (Beijing Jingfeng Pharmaceutical Group Co., Ltd, batch number 2004032); glucose test strips (ACCU-CHEK; Roche Diabetes Care GmbH, batch number: 26020933, etc); specific pathogen-free (SPF) C57BL/6 mice, aged 6-8 wk (Changsha Tianqin Biological Co., Ltd); \textit{A. aceti}, number: GIM1.67 (Guangdong Microorganism Conservation Centre); and MIN6 cells (China Centre for Type Culture Collection) were used. The Animal Experiment Ethics Approval Number was 20200620.

**Preparation of chromium- and zinc-rich \textit{A. aceti}**

\textit{A. aceti} were revived and cultured in a liquid enriched medium, with the concentration of the bacterial solution $\text{OD}_{600} = 0.9$ to 1.5, which is about $3 \times 10^6$ to $5 \times 10^8$ CFU/mL. To enrich the bacteria with chromium and zinc, \textit{A. aceti} were cultured in a liquid medium (the concentrations of chromium trichloride and zinc chloride were both 64 mg/mL) with the initial concentration of the bacterial solution set to $1 \times 10^8$ CFU/mL and shaken for 48 h at 250 rpm; this cultivation was repeated once. Following the enrichment cultivations, the bacterial solution was collected, centrifuged to remove the supernatant, and washed by phosphate buffer saline (PBS); the precipitate was the chromium- and zinc-rich \textit{A. aceti}.

**Detection of coenzymes and metals in \textit{A. aceti}**

**Collection of \textit{A. aceti}:** Cell suspensions (100 mL) of \textit{A. aceti} were removed and distributed across a sterile 96-microwell plate, with the culture solution used as a blank control. The absorption of the suspension and the \textit{A. aceti} culture solution were measured at 600 nm using the microplate reader and calculated as OD1 and OD2, respectively, with the final OD value of the \textit{A. aceti} suspension taken as the difference OD1-OD2. The \textit{A. aceti} suspension was centrifuged at 8000 rpm for 10 min to remove the supernatant; the precipitate (the \textit{A. aceti}) was then washed once with 1 mL sterile PBS and centrifuged at 13000 rpm for 1 min to remove the supernatant. Thereafter, the precipitate was weighed and resuspended with sterile water for a final concentration of \textit{A. aceti} at 0.1 mg/mL.

**Detection of chromium and zinc in \textit{A. aceti}:** Collected samples were sent to Shanghai WEIPU Chemical Technology Service Co., Ltd, and the contents of chromium and zinc were detected by inductively coupled plasma mass spectrometry.

**Detection of NAD+/NADH in \textit{A. aceti}:** The concentrations of NAD+/NADH in the \textit{A. aceti} was determined using the NAD+/NADH assay kit with WST-8 (Beyotime Biotechnology).

**Detection of glucose dehydrogenase in \textit{A. aceti}:** Solarbio’s “glucose dehydrogenase microplate assay kit” was used to detect glucose dehydrogenase concentrations in \textit{A. aceti}, according to the instruction manual.

**Construction of a diabetes mouse model:** For the preparation of citrate buffer, 2.10 g of citric acid was treated with 100 mL of double distilled water to make a citric acid mother liquor (solution A), after which 2.94 g of trisodium citrate was treated with 100 mL of double-distilled water to make a sodium citrate mother liquor (solution B). Solution A and solution B were mixed in a ratio of 1:1.32 (or 1:1), and the pH value was measured with a pH meter and adjusted from 4.2 to 4.5. This represented the 0.1 mol/L sodium citrate-hydrochloric acid buffer solution required to prepare streptozotocin (STZ).

Seventy SPF grade C57BL/6 female mice, aged 6 wk and weighing 20 ± 2 g, were allowed to eat and drink without restrictions during 5 d of adjustable feeding. Six mice were randomly selected as the normal control group, and the rest were used for model construction.
To prepare the STZ required to inject the mice to cause diabetes, 24 mL of 0.1 mol/L sodium citrate buffer was treated with 120 mg of STZ away from the light (equivalent to a concentration of 5 mg/mL) and placed in an ice environment. The mice were made to fast for 10 h and then treated with STZ at a dose of 0.15 mL/10 g of mouse weight (equivalent to 75 mg/kg). STZ was administered by intraperitoneal injection for 3 consecutive days. Before each intraperitoneal injection, the STZ liquid was carefully pipetted with a 1 mL syringe to mix the precipitate before it was extracted to maintain the STZ concentration. After each intraperitoneal injection, mice were deprived of food and water for 90 min. On the 7th d after the last administration (the mice were made to fast for 10 h before blood collection), blood was collected from their caudal veins, and the fasting blood glucose (FBG) levels of the mice were measured with a Roche glucometer. Pathological models of mice with diabetes were confirmed as having been successfully established when FBG ≥ 16.7 mmol/L.

The diabetic mice were designated from high to low, according to their blood glucose levels, and the model mice were divided into a model group (PBS), positive control group (metformin), metal chromium plus zinc group (concentrations of chromium and zinc calculated as 1 × 10⁻⁷ mg/mL and 2 × 10⁻⁸ mg/mL, respectively, according to the highest content of chromium- and zinc-rich A. aceti, A. aceti group (OD = 1), and chromium- and zinc-rich A. aceti group (OD = 1). There were six mice in each group, and each was given 0.5 mL of treatment by gavage.

**Evaluation of hypoglycemic activity in vivo:** After the diabetic models were successfully established, the mice were given intragastric administration of treatment on the second day, once a day, for 15 consecutive days. The normal control group and the model group were given the same amount of PBS-water. The positive drug control group was given diformin tablets (ground into powder, prepared into a suspension with reverse osmosis water, and administered intragastrically at 0.320 g/kg/d). The metal chromium plus zinc group was given chromium trichloride (1 × 10⁻² mg/mL) and zinc chloride (2 × 10⁻⁴ mg/mL) intragastrically. Through general observation, the activity and spirit of the mice, their eating and drinking, urine and feces, and the dryness and wetness of the bedding were observed every day during the 15 d of administration. Starting at the beginning of treatment, fasting blood glucose was measured every 3 d. After 15 d of administration, the mice were fasted for 10 h and blood was collected from the eyeballs. Thereafter, the mice were sacrificed, with the tissue taken from their pancreas islets, fixed with formaldehyde, and processed for hematoxylin and eosin (HE) staining and immunohistochemistry with the apoptosis-related Bax proteins. Tissue from pancreatic islets was also prepared for electron microscopy to analyze the ultrastructural damage and possible repair on tissues and cells of pancreatic islets. The weights of mice were recorded every 3 d.

**Evaluation of MIN6 cell growth:** A. aceti was collected (OD = 10), sonicated, and stored at -20 °C for later use. MIN6 cells, a pancreatic islet cell line, were revived, the cell concentration adjusted to 1 × 10⁶ CFU/mL, and then cultured in 5 mmol/L and 25 mmol/L high-glucose 1640 medium on a 96-well plate with 90 mL/well. After 12 h, the cells were diluted 10-fold and dosed with 10 mL of collected A. aceti (with or without metal enrichment; OD = 10), the dose of which was equivalent to 1 × 10⁵ CFU/mL of bacteria. A positive drug control group (diformin tablets) and negative drug control group (PBS) were included. Approximately 24 h after dosing, cell growth was detected with the CCK-8 kit (Beyotime Biotechnology).

**Determination of insulin secretion in MIN6 cells:** A. aceti were collected (OD = 10), sonicated, and stored at -20 °C for later use. MIN6 pancreatic islet cells were revived, the cell concentration adjusted to 1 × 10⁵ CFU/mL, and cultured in 5 mmol/L and 25 mmol/L high-glucose 1640 medium on a 6-well plate with 1.98 mL/well. After 12 h, the cells were dosed with 20 mL A. aceti (with or without metal enrichment; OD = 10), the dose of which was equivalent to 1 × 10⁶ CFU/mL of bacteria. A positive drug control group (diformin tablets) and negative drug control group (PBS) were included. Some 24 h after dosing, the cell supernatant was collected, with the insulin content detected by a commercially-available enzyme-immunized mouse insulin ELISA kit.

**Detection of glucose processing by A. aceti**
Chromium- and zinc-rich A. aceti and control A. aceti were collected, with the initial concentration adjusted to 1 × 10⁶ CFU/mL. The glucose concentration of the liquid medium was detected with a blood glucometer in A. aceti cultured at 30 °C for 12 h, 24 h, 36 h, and 48 h.

**Safety evaluation of chromium- and zinc-rich A. aceti**
Chromium- and zinc-rich A. aceti were collected and adjusted to 10 times the therapeutic dose (OD = 10). Aliquots of 1-mL were administered, respectively, to the mice three times a day for 7 consecutive days, with the body weights and the pathological changes of the organs detected.
RESULTS

**Determination of coenzymes and metals in chromium- and zinc-rich *A. aceti***

The concentration of chromium metal in the *A. aceti* prepared as described in the methods section above was 28.58-34.34 mg/kg, and the zinc metal concentration was 5.35-7.52 mg/kg, both of which were significantly higher than those in the untreated *A. aceti* (chromium: 1.05-2.29 mg/kg; zinc: 0.18-0.26 mg/kg) (**Figure 1A**). The concentration of NADH was 5.13 to 7.26 mM, which was significantly higher than that in the non-cultured *A. aceti* (0.86 to 1.02 mM) (**Figure 1B**). The concentration of glucose dehydrogenase was 446.812-567.138 U/g, which was significantly higher than that of non-cultivated *A. aceti* (54.126-93.651 U/g) (**Figure 1C**).

**Evaluation of the therapeutic effects of chromium- and zinc-rich *A. aceti* on mice with diabetes**

After 7 d of treatment, the diabetic mice (mice with FBG ≥ 16.7 mmol/L) treated with chromium- and zinc-rich *A. aceti* were found to be in a good mental state, with bright eyes and normal activity, normal intake of food and water, urination, and normal defecation, with their beddings dry. The fasting blood glucose was detected every 3 d after the initial administration of treatment, and the diabetic mice treated with chromium- and zinc-rich *A. aceti* (OD = 1) had significantly lower blood glucose than mice in both the positive drug control group (diformin tablets) and the metal chromium plus zinc group (**Figure 2**).

After 15 d of treatment, HE staining of the pancreas tissue indicated fewer cells undergoing apoptosis, less structural atrophy, and hardly any vacuoles detected in the chromium- and zinc-rich *A. aceti* group (OD = 1) (**Figure 3**). Immunohistochemistry analysis of apoptosis-related Bax proteins confirmed that the apoptosis of islet cells was significantly reduced (**Figure 3**). Electron microscopy of pancreas islet tissue confirmed that the ultrastructural damage was alleviated after treatment with the chromium- and zinc-rich *A. aceti*, with a small expansion range of endoplasmic reticulum, slightly swollen mitochondria, and the amount of autophagic vacuolization significantly reduced (**Figure 3**).

The body weights of the mice after treatment with chromium- and zinc-rich *A. aceti* were recorded every 3 d. The weights of diabetic mice in the chromium- and zinc-rich *A. aceti* (OD = 1) were found to recover well, with no significant difference from those of the positive drug control group (metformin), as displayed in **Figure 4**.

**Evaluation of chromium- and zinc-rich *A. aceti* on pancreatic islet cells MIN6**

Chromium- and zinc-rich *A. aceti* promoted the growth of MIN6 cells significantly better than both the positive and negative control groups in the high glucose 1640 medium, as indicated by cytotoxicity measured with the CCK-8 kit (**Figure 5**). This result implied that chromium- and zinc-rich *A. aceti* could promote the growth of MIN6 pancreatic islet cells.

The insulin content of the supernatant in MIN6 cells incubated in the 25 mmol/L high-glucose 1640 medium treated with chromium- and zinc-rich *A. aceti* (OD = 10) group was significantly higher than that in the positive and negative control groups (**Figure 6**). This result implies that chromium- and zinc-rich *A. aceti* could promote insulin secretion of MIN6 islet cells.

**Detection of the capacity of chromium- and zinc-rich *A. aceti* for processing glucose**

The glucose content in medium containing chromium- and zinc-rich *A. aceti* and control *A. aceti* both decreased significantly as incubation times increased; however, this decrease was significantly more pronounced for the chromium- and zinc-rich *A. aceti* compared to the *A. aceti* group (**Figure 7**). This result implies that chromium- and zinc-rich *A. aceti* are better at decomposing glucose than the untreated bacteria.

**Safety evaluation of chromium- and zinc-rich *A. aceti***

Ten times the dose of chromium- and zinc-rich *A. aceti* was administered to mice by gavage with no change detected in the weights and no pathological damage found in the liver, spleen, kidneys, and stomach (**Figure 8**). This result implies that *A. aceti* is biosafe.

DISCUSSION

At present, the incidence of diabetes is high, with no known cure. The conventional therapy is long-term use of hypoglycemic drugs and symptomatic treatment. However, in the long-term treatment process, drugs are prone to resistance among non-compliant patients who fail to adhere to treatment regimens. Therefore, in addition to helping patients control blood sugar long-term, it is vital that we clearly identify the risk factors for diabetes to prevent patients from developing this disease. An important area that may be significant in both the long-term treatment and prevention of diabetes is the use of supplemental micronutrients, as diabetic patients are often deficient in B vitamins and micronutrients, such as chromium, zinc, and selenium[14-16].
Chromium was designated as an essential microelement for the human body in 1989. The body absorbs Cr\(^{3+}\) mainly from food, including meat, whole grain, millet, pepper, etc. The daily intake for adults recommended by the United States Food and Drug Administration’s Center for Food Safety and Applied Nutrition Food Safety Recommendations Committee is 25-35 μg (as of 2001). Studies have found that plasma chromium levels are negatively correlated with the risk of type 2 diabetes and prediabetes. Chromium deficiency may lead to impaired glucose tolerance, insulin resistance, and elevated blood sugar. Chromium levels in the human body will decrease with age, with chromium deficiency becoming severe in old people\(^{17-19}\).

According to evidence-based medicine meta-analysis studies, chromium increased insulin sensitivity mainly by activating insulin receptor kinase activity, inhibiting phosphatase activity, and increasing phosphorylation of insulin receptors\(^{20,21}\). Low molecular weight chromium binding substance was found to be combined with insulin receptors and activate tyrosine kinase activity on insulin P receptors, thus enhancing insulin signal transduction. However, at an insulin concentration of 100 nmol/L, the tyrosine kinase activity in insulin receptors was significantly enhanced if insulin was dosed with chromium\(^{22,23}\). In addition, it was found that chromium could also promote the translocation of GLUT4 to the cell membrane by activating protein kinase B and adenosine monophosphate to activate protein kinase signaling pathways and by activating the activity of p38 mitogen-activated protein kinase\(^{24,25}\). Despite much related research, the specific mechanism of lowering blood sugar through chromium supplementation remains unclear.

Zinc ions are an important part of the insulin molecule, maintaining the stability and biological effects of insulin\(^{26-28}\). Zinc plays a key role in the synthesis, storage, and secretion of insulin in pancreatic β cells and can increase the activity of the insulin signaling pathway. However, zinc deficiency can lead to a decrease in insulin secretion\(^{29,30}\). In addition, the formation of hexamers that contain zinc is
required in the synthesis of insulin; therefore, zinc deficiency will lead to restricted insulin synthesis and reduced insulin sensitivity, thus increasing the risk of diabetes\cite{31,32}. Studies have found that zinc lowers blood sugar mainly through antioxidant responses, inhibition of inflammatory factors, and anti-apoptosis effects\cite{30,33}. Intracellular zinc has also been found to be regulated by zinc transporters, with the uptake, storage, and distribution regulated by metallothioneins\cite{34,35}. Studies have found that zinc can inhibit the inflammatory response and has an anti-apoptosis effect at a concentration higher than 100 μmol/L\cite{36}. Therefore, increasing the intake of zinc can increase the level of metallothionein, which helps mediate anti-apoptosis, etc\cite{31,37}. Some scholars have suggested that the effects of a high-zinc intake on lowering blood sugar may be associated with its ability to reduce variation in zinc transporters\cite{38}. Therefore, given the important role of zinc in glycemic control, a deficiency of zinc can lead to glucose metabolism disorders, while high intake may reduce the risk of glucose metabolism disorders and diabetes.

Above all, chromium and zinc supplementation can stabilize blood sugar in an indisputable manner. The key is how to supplement chromium and zinc through a proper, safe, and scientific approach. In the present study, chromium trichloride, zinc chloride, and \textit{A. aceti} were co-cultured to induce the production of chromium- and zinc-rich \textit{A. aceti} following simple protocols with high yield. Chromium- and zinc-rich \textit{A. aceti} prepared using this method not only helped \textit{A. aceti} exert the effect of decomposing glucose but also enhanced the hypoglycemic effect seen in untreated \textit{A. aceti}. Because the chromium and zinc was transformed by the bacteria, biological safety was ensured, which means that it is possible that this can be used as a new hypoglycemic biological drug. The hypoglycemic mechanism of chromium- and zinc-rich \textit{A. aceti} was preliminarily explored, with findings of these bacteria predom-
Huang YY et al. A new *Acetobacter aceti* hypoglycemic method

**Figure 5** Effects of chromium- and zinc-rich *Acetobacter aceti* on promoting growth of pancreatic islet cells MIN6. A: 5 mmol/L glucose; B: 25 mmol/L glucose. *a* P < 0.05; *b* P < 0.01.

**Figure 6** Effects of chromium- and zinc-rich *Acetobacter aceti* on facilitating growth and insulin secretion of islet MIN6 cells. A: 5 mmol/L glucose; B: 25 mmol/L glucose. *b* P < 0.01; *c* P < 0.001.

Inantly leading to increased dihydronicotinamide-adenine dinucleotide and glucuronide dehydrogenase levels in *A. aceti*, enhancing the ability to degrade glucose. In addition, its hypoglycemic mechanism was found to be not much different from those of chromium and zinc, which are metal microelements. However, because the source of nutrition for the growth of *A. aceti* is ethanol, these bacteria do not survive for long in the body, and, therefore, they cannot exert long-term hypoglycemic effects, which will be further addressed in future research.

**CONCLUSION**

Chromium trichloride and zinc chloride can be employed to induce the preparation of chromium- and zinc-rich *A. aceti*, which has a significantly enhanced hypoglycemic effect relative to normal *A. aceti* and can biotransform chromium and zinc in a way that improves the safety of administering these metals as a treatment for diabetes.
Figure 7 Capacity of chromium- and zinc-rich *Acetobacter aceti* for processing glucose. *P < 0.01.*

Figure 8 Safety evaluation of chromium- and zinc-rich *Acetobacter aceti*.

**ARTICLE HIGHLIGHTS**

**Research background**
At present, there are no ideal drugs to treat diabetes. *Acetobacter* and other probiotics can play a role in the treatment of diabetes; however, their effect is not significant. In this study, metal compounds were used to enrich *Acetobacter* with chromium and zinc in an effort to enhance the hypoglycemic effect of these bacteria.

**Research motivation**
This research provides a theoretical basis for the application of new chromium- and zinc-rich *Acetobacter aceti* (*A. aceti*) to treat diabetes.

**Research objectives**
To prepare a new type of chromium- and zinc-rich *A. aceti* and explore its hypoglycemic effects on enhancing the application of probiotics in the treatment of diabetes.

**Research methods**
*A. aceti* was cultured in a liquid medium that contained chromium trichloride and zinc chloride.

**Research results**
A new type of chromium and zinc rich *A. aceti* was successfully prepared.

**Research conclusions**
Chromium- and zinc-rich *A. aceti* has a significantly enhanced hypoglycemic effect and can biotransform chromium and zinc to improve safety for administering these metals as a treatment.
Research perspectives
The new method described has very good application prospects.

FOOTNOTES

Author contributions: Huang YY was responsible for the experimental research; Qin XK, Dai YY, Huang L, and Huang GR consulted the literature and wrote the first draft, then corrected and improved the manuscript; Wei X and Huang YQ designed, checked, modified, and finalized the manuscript, contributed equally to this work, and agreed to serve as co-corresponding authors; All authors proofread the revised manuscript.

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Acetobacter aceti cytosolic zinc homeostasis and insulin secretion.


Immediate-release tofacitinib reduces insulin resistance in non-diabetic active rheumatoid arthritis patients: A single-center retrospective study

Chrong-Reen Wang, Hung-Wen Tsai

BACKGROUND
An increased risk of insulin resistance (IR) has been identified in rheumatoid arthritis (RA), a chronic inflammatory disorder with elevated levels of pathogenic cytokines. Biologics targeting proinflammatory cytokines can control the disease and improve insulin sensitivity in RA. Although Janus kinase (JAK) signaling can regulate cytokine receptors and participate in RA pathogenesis, it remains to be elucidated whether there is a reduction of IR in such patients under JAK inhibitor (JAKi) therapy.

AIM
To study the effect of JAKi treatment on the reduction of IR in RA patients with active disease.

METHODS
A retrospective study was carried out from April 1, 2017 to March 31, 2021 in a population of non-diabetic patients with active RA who were undergoing tofacitinib (TOF) therapy with 5 mg twice-daily immediate-release formulation.

RESULTS
Fifty-six RA patients, aged 30 years to 75 years (mean ± SD: 52.3 ± 11.1) with disease activity score 28 values ranging from 4.54 to 7.37 (5.82 ± 0.74), were classified into high-IR (> 2.0) and low-IR (≤ 2.0) groups based on their baseline homeostatic model assessment (HOMA)-IR levels. They had no previous exposure to JAKi, and received TOF therapy for no less than 6 mo. In 30 patients who were naïve to biologics, after a 24-week therapeutic period, the high-IR...
group showed reduced HOMA-IR levels ($3.331 \pm 1.036$ vs $2.292 \pm 0.707$, $P < 0.001$). In another 26 patients who were exposed to tumor necrosis factor-α or interleukin-6 blockers, the high-IR group, despite having achieved a decrease but with lower magnitude than in naïve patients, showed reduced HOMA-IR levels ($2.924 \pm 0.790$ vs $2.545 \pm 1.080$, $P = 0.018$).

**CONCLUSION**

In this retrospective study, reduced IR was achieved in non-diabetic active RA patients following 24 wk of TOF therapy.

**Key Words:** Insulin resistance; Rheumatoid arthritis; Diabetes mellitus; Tofacitinib; Janus kinase inhibitor

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**Core Tip:** An increased risk of insulin resistance (IR) has been identified in rheumatoid arthritis (RA), a chronic inflammatory disorder with elevated levels of pathogenic cytokines. In addition to controlling RA activity, biologics targeting proinflammatory cytokines have been shown to reduce IR, while it remains to be elucidated whether Janus kinase inhibitor therapy can cause IR reduction in such patients. This retrospective study carried out in non-diabetic active RA patients classified into high-IR and low-IR groups before tofacitinib (TOF) therapy demonstrated reduced IR by 24 wk of TOF treatment in the active RA patients with high baseline IR status.

**INTRODUCTION**

A critical mechanism causing diabetes development is the resistance of target cells to the action of insulin, with ineffective strength of signaling from the receptor to the final action substrates and requiring beyond-normal insulin concentrations to maintain euglycemic status[1,2]. Insulin resistance (IR) manifests from a blockade of tissues to the insulin action upon the uptake, metabolism or storage of glucose, a common feature of human disorders such as diabetes, hyperlipidemia, metabolic syndrome, fatty liver, and obesity[1]. Furthermore, an increased risk of IR has been identified in various inflammatory disorders with increased levels of proinflammatory cytokines like interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α[3].

Rheumatoid arthritis (RA), a chronic inflammatory disorder with elevated levels of proinflammatory cytokines, has been demonstrated to be associated with IR during its activity[4]. TNF-α is involved in IR pathogenesis through the phosphorylation of inhibitory serine residue of insulin receptor substrate-1 (IRS-1) and reduction of tyrosine phosphorylation of IRS-1 and the β-subunit of the insulin receptor[5,6]. Inactivation of TNF-α by use of recombinant soluble receptor fusion proteins or monoclonal antibodies for IR reduction has been successfully demonstrated in active RA[7]. IL-6 can exert a negative influence on insulin signaling by decreasing tyrosine phosphorylation of IRS-1, inducing recruitment of IRS-1 to its receptor complex for serine phosphorylation, and reducing autophosphorylation of tyrosine residues in the insulin receptor[8,9]. Under treatment with tocilizumab (TCZ; an IL-6 receptor antibody) to inhibit IL-6 signaling in RA, decreased IR was identified in an investigation of 221 active patients as well as in other studies with smaller sample sizes[10-13]. IL-1β is able to impair insulin signaling through activation of the IKKβ/NF-κB pathway to target IRS-1 through serine phosphorylation[14,15]. Anakinra, an IL-1 receptor antagonist, has been shown to reduce IR in active RA with comorbid type 2 diabetes[16,17]. Altogether, these observations indicate that, biologic therapy targeting pathogenic cytokines can not only control disease activity but also improve insulin sensitivity in active RA patients.

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, including JAKs 1 to 3, STATs 1 to 6, and tyrosine kinase 2, regulates many cytokine and hormone receptors with pathogenic roles in a variety of inflammatory disorders[18]. Notably, different cytokine receptors can recruit their own combinations of JAKs and STATs to activate distinct processes in individual targeted cells, while antagonizing a JAK can suppress more than one cytokine pathway, expanding the efficacy in using such an antagonist in cytokine-targeted therapy[19]. Notably, tofacitinib (TOF) is the first small-molecule pan-JAK inhibitor (JAKi) targeting JAKs 1 to 3[20]. It has been approved by the United States’ Federal Food and Drug Administration in 2012 and by European Medicines Agency in 2017 for the treatment of RA patients with moderate to high activity and an inadequate response to methotrexate...
Wang CR et al. Tofacitinib reduces insulin resistance in RA

[21]. This JAKi can act on the JAK/STAT pathway to block the intracellular signaling of multiple cytokines and hormones involved in the pathogenesis of RA and IR [20,22]. In RA patients, significantly reduced circulating levels of pro-inflammatory cytokines IL-6 and TNF-α, two crucial mediators of IR, were observed since week 4 after initiation of TOF therapy [23,24]. Furthermore, in a recent large-scale survey of 10019 RA patients with type 1 or 2 diabetic co-morbidity, the diabetic treatment intensification, i.e. addition of a new anti-diabetic medication, was found to be lower for those using TOF than for those using other TNF-α inhibitors or non-TNF-α-targeted biologics [25]. Based on the above findings, there is a therapeutic potential to reduce the IR in active RA patients by TOF therapy.

In this retrospective investigation, the effect of TOF treatment (specifically, 5 mg twice-daily immediate-release formulation) on IR reduction was investigated in 56 non-diabetic patients with active RA, naive or exposed to biologic therapy and classified into high- and low-IR groups according to the baseline levels of the homeostatic model assessment (HOMA)-IR score.

MATERIALS AND METHODS

Study design and patients
This study was carried out to analyze the effect of TOF on IR in active RA patients who met the 2010 American College of Rheumatology/European League Against Rheumatism (EULAR) classification criteria [26]. Each patient received regular monthly follow-up at an outpatient rheumatology clinic of National Cheng Kung University Hospital from April 1, 2017 to March 31, 2021. This study was approved by the Institutional Review Board and conducted according to the guidelines of Declaration of Helsinki. Before receiving the 5 mg twice-daily immediate-release TOF formulation, all patients had manifested inadequate therapeutic responses to methotrexate for at least 6 mo, having received a weekly dosage of up to 15 mg and at least one conventional synthetic disease-modifying anti-rheumatic drug (DMARD) at an adequate daily dosage. In addition, low-dose prednisolone was selectively prescribed (daily dosage of no more than 10 mg). Furthermore, patients were excluded from this study if they had previous exposure to targeted synthetic DMARDs treatment or were known to have diabetes, endocrine abnormalities, or critical medical disorders involving heart, lung, liver, and kidney.

Data collection and measurements
A detailed review was performed to collect data on the patients’ demographic, clinical, laboratory and medication profiles. In addition to body mass index (BMI), clinical data included the 28-joint Disease Activity Score (DAS28) for RA activity [27], classifying as high (> 5.1), moderate (3.2-5.1) or low activity (2.6-3.2) and remission (< 2.6) [28]. Laboratory parameters included rheumatoid factor (RF)/anti-citrullinated peptide antibody (ACPA), C-reactive protein/erythrocyte sedimentation rate, and fasting blood levels of glucose and insulin. Seropositive RA was defined by the presence of either ACPA or RF. In addition to TOF, medication profiles were reviewed for use of prednisolone, conventional synthetic DMARDs with cyclosporin, hydroxychloroquine, leflunomide and sulfasalazine, and biologic synthetic DMARDs with abatacept, adalimumab (ADA), etanercept (ETA), golimumab (GOL), rituximab, and TCZ. For the calculation of IR, HOMA-IR, insulin (μU/mL) × glucose (mg/dL)/405, and Quantitative Insulin Sensitivity Check Index (QUICKI), 1/(log insulin (μU/mL) + glucose (mg/dL)) were used in this study. The baseline HOMA-IR levels before TOF therapy were used to classify patients into high-IR (> 2.0) and low-IR (≤ 2.0) groups [7,29]. HOMA-IR and QUICKI measurements were obtained from all participants before and after a 24-wk therapeutic period. Furthermore, in the high-IR group, serial calculation data were available in selected patients before and after the TOF treatment.

Statistical analyses
Results were expressed as the mean ± SD. Serial HOMA-IR levels before and after starting TOF therapy were compared with the two-way analysis of variance with a post-hoc test. DAS28, HOMA-IR and QUICKI levels before and after a 24-wk therapeutic period were compared by using the Wilcoxon signed rank test. Different values and frequencies between high-IR and low-IR groups were compared using the Mann-Whitney and the chi-square/Fisher’s exact tests, respectively. Spearman correlation coefficient test was used to correlate DAS28 values and HOMA-IR levels. A P value less than 0.05 was considered as significant in this study.

RESULTS

Baseline characteristics of active RA patients before TOF therapy
Fifty-six patients with 84% females and 88% seropositivity, aged 30 years to 75 years (52.3 ± 11.1 years), received TOF therapy for no less than 6 mo. They had BMI ranging from 19.2 kg/m² to 26.3 kg/m² (22.6 ± 2.0 kg/m²), following the obesity definition of at least 27 kg/m² by the Ministry of Health and Welfare, Taiwan. Their DAS28 values varied from 4.54 to 7.37 (5.82 ± 0.74), all with moderate to high activity.
None had exposure to JAKi or succumbed to diabetes, endocrine or critical medical disorders involving major organs, fulfilling the selection criteria in this study.

Before the TOF treatment, 30 patients were naive to biologic synthetic DMARDs therapy, and their DAS28 values varied from 5.16 to 7.37 (6.291 ± 0.530), all with high disease activity. Table 1 shows the demographic, clinical, laboratory and medication data for 30 naive patients, classified into high- (n = 18) and low-IR (n = 12) groups according to their baseline HOMA-IR levels. There were no differences between high- and low-IR groups regarding age, sex, BMI, seropositivity and medication profile with prescription frequencies of various conventional synthetic DMARDs and low-dose prednisolone, as well as weekly methotrexate or daily/total prednisolone dosages. Before TOF therapy, there was a positive correlation between DAS28 values and HOMA-IR levels (r = 0.357, P = 0.039; Figure 1A), whereas a negative correlation was found between DAS28 values and QUICKI levels (r = -0.423, P = 0.020).

Furthermore, higher DAS28 values were found in the high-IR group compared to the low-IR group (6.499 ± 0.472 vs 5.980 ± 0.470, P = 0.008), indicating that IR is driven by disease activity in RA patients[7, 30]. Notably, there were no changes in the patients’ medication profiles during the 24-wk therapeutic period, with the exception of additional use of TOF.

In addition, 2 patients had an episode of single-dermatome herpes zoster (HZ) infection, both of which responded to valacyclovir therapy, with an incidence rate of 3.03 per 100 person-years. There is a general increased risk of HZ infection in RA patients[31], but especially in those receiving specific immunosuppressive therapy, including prednisolone (no less than 10 mg/d), methotrexate and anti-TNF-α biologics[32]. Interestingly, by analyzing health plan data from the United States, TOF-treated RA patients show an incidence rate of 3.87 per 100 person-years in HZ infection[33].

Effects of TOF therapy on IR in 30 active RA patients naive to biologics

For 3 patients in the high-IR group, there were serial HOMA-IR calculations available for baseline at week 0 and after starting TOF therapy at weeks 4, 8, 12 and 24 (Figure 1B). In comparison with baseline levels, these patients who were naive to biologics showed significantly lower levels only at week 24 but not at weeks 4, 8 or 12 (Figure 1B, week 0 vs weeks 24, 5.243 ± 0.571 vs 3.433 ± 0.664, P < 0.01). Further comparison with baseline HOMA-IR levels was carried out at week 24.

The levels of HOMA-IR and QUICKI before and after TOF therapy in the high-IR and low-IR groups are shown in Table 2 and Figure 2. There were significantly reduced DAS28 values in both the high-IR and low-IR groups after the 24-wk TOF treatment (high-IR: 6.499 ± 0.472 vs 3.006 ± 0.445, P < 0.001; low-IR: 5.980 ± 0.470 vs 3.244 ± 0.614, P < 0.001). Significantly decreased HOMA-IR levels were found in the high-IR group (3.331 ± 1.036 vs 2.292 ± 0.707, P < 0.001; Figure 2B) but not in the low-IR group (1.602 ± 0.294 vs 1.430 ± 0.293, P = 0.139; Figure 2C), while significantly increased QUICKI levels were observed in the high-IR group (0.3207 ± 0.0135 vs 0.3397 ± 0.0154, P < 0.001; Figure 2E) but not in the low-IR group (0.3573 ± 0.0117 vs 0.3634 ± 0.0122, P = 0.156; Figure 2F). Furthermore, reduced HOMA-IR levels were observed in 17 patients in the high-IR group, while 7 patients in the low-IR group had a reduction in IR (high-IR vs low-IR: 94.4% vs 58.3%, P = 0.026). Despite observing no reduced IR after the TOF treatment in the low-IR group, a greater decrease in the values of DAS28 was found in 7 patients with decreased HOMA-IR levels, compared to 5 patients who showed no decrease (2.977 ± 0.237 vs 2.529 ± 0.362, P = 0.018), implicating reduced IR involvement in the responses to TOF therapy in active RA patients.

Effects of TOF therapy on IR in 26 active RA patients exposed to biologics

Before TOF therapy, 26 patients had been exposed to biologic synthetic DMARDs for at least 6 mo; the DMARDs included ADA, ETA, GOL and TCZ. This group of patients was consisted of 85% females, 69% with seropositivity, ages 40 years to 75 years (54.7 ± 10.6) and BMI 19.2 to 26.2 (22.96 ± 2.02). Their DAS28 values varied from 5.16 to 7.37 (6.291 ± 0.530), lower than that in those naïve to biologics (5.16 to 7.37, 6.291 ± 0.530, P < 0.001). The patients were divided into high- (n = 19) and low-IR (n = 7) groups according to the baseline levels of HOMA-IR. All patients received methotrexate, while 5 patients in the high-IR group and 1 patient in the low-IR group received low-dose prednisolone therapy. No differences were found in the prescription frequencies of conventional synthetic DMARDs and low-dose prednisolone between two groups of patients.

The levels of HOMA-IR and QUICKI before and after TOF therapy in the high-IR and low-IR groups are shown in Table 3 and Figure 3. There were significantly reduced DAS28 values in both the high-IR and low-IR groups after the 24-wk TOF treatment (high-IR: 5.316 ± 0.807 vs 3.070 ± 0.466, P = 0.001; low-IR: 5.124 ± 0.470 vs 3.000 ± 0.672, P = 0.016). Significantly decreased HOMA-IR levels were found in the high-IR group (2.924 ± 0.790 vs 2.545 ± 1.808, P = 0.018; Figure 3B) but not in the low-IR group (1.527 ± 0.159 vs 1.453 ± 0.478, P = 0.781; Figure 3C), while significantly increased QUICKI levels were observed in the high-IR group (0.3273 ± 0.0117 vs 0.3372 ± 0.0214, P = 0.008; Figure 3E) but not in the low-IR group (0.3589 ± 0.0059 vs 0.3648 ± 0.0204, P = 0.813; Figure 2F).
In this retrospective study, active RA patients receiving a 24-wk TOF treatment had significantly reduced IR among those with high baseline HOMA-IR levels. Furthermore, the clinical use of biologic synthetic DMARDs, including IL-6 and TNF-α blockers, has been demonstrated to reduce IR in non-diabetic active RA patients[22]. For patients with high IR before TOF therapy, baseline HOMA-IR levels were greater in those naïve to biologic agents than in those with an exposure history to anti-IL-6/TNF-α blocker (3.33 ± 1.036 vs 2.924 ± 0.790), while after therapy, there was a decrease in HOMA-IR levels with higher magnitude in naïve than exposed patients (31% vs 13% reduction, respectively). These results demonstrated, in this study, the effect of prescribed biologics on IR in active RA patients before TOF therapy. In addition to type 2 diabetes, IR is a crucial pathophysiological feature of obesity, with both conditions being characterized by persistent low-grade inflammation with increased levels of proinflammatory cytokines[34]. A reduction in IR has been identified in RA patients with a normal weight but not in those with obese status under anti-TNF-α therapy[35]. Despite no identified obesity in the present investigation (all patients had BMI < 27 kg/m²), there were higher BMI levels for patients without IR reduction (n = 7) when compared to those with reduced IR (n = 30) in the high-IR group of patients naïve or exposed to biologic therapy (without IR reduction: 24.53 ± 2.07 vs 22.49 ± 1.91 kg/m², P = 0.019), reflecting an influence of increased BMI on IR.

Recent investigations have indicated that when prescribed chronically, glucocorticoid (GC) can impair glucose tolerance and induce IR through stimulation of hepatic gluconeogenesis, alteration of insulin release from pancreatic β cells, and decrease in the sensitivity of the liver and muscle to insulin[36]. Since GC therapy is associated with a risk of developing type 2 diabetes, the EULAR recommends to wean RA patients off prednisolone use as early as possible[37]. Although methotrexate may enhance the actions of insulin on glucose transport and metabolism by increasing the extracellular concentration of adenosine, a retrospective study with 21340 RA patients under a 12-year follow-up demonstrated that the risk of type 2 diabetes was not lower with the use of methotrexate[38]. Hydroxychloroquine has...
### Table 2: Insulin resistance change in 30 active rheumatoid arthritis patients naïve to biologics by tofacitinib therapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>P value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All (n = 30)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS28</td>
<td>6.291 ± 0.530 (5.16-7.37)</td>
<td>3.101 ± 0.522 (2.08-4.21)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Decrease in DAS28</td>
<td>3.194 ± 0.609 (1.94-4.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.639 ± 1.185 (1.07-5.89)</td>
<td>1.947 ± 0.714 (0.98-4.19)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.3353 ± 0.0222 (0.296-0.380)</td>
<td>0.3492 ± 0.0183 (0.310-0.385)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>High IR (n = 18)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>DAS28</td>
<td>6.499 ± 0.472 (5.56-7.37)</td>
<td>3.006 ± 0.444 (2.52-4.21)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Decrease in DAS28</td>
<td>3.493 ± 0.536 (2.36-4.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.331 ± 1.036 (2.04-5.89)</td>
<td>2.292 ± 0.707 (1.21-4.19)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.3207 ± 0.0135 (0.296-0.343)</td>
<td>0.3397 ± 0.0154 (0.310-0.372)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Low IR (n = 12)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS28</td>
<td>5.980 ± 0.470 (5.16-6.69)</td>
<td>3.244 ± 0.614 (2.08-3.99)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Decrease in DAS28</td>
<td>2.736 ± 0.389 (1.94-3.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.602 ± 0.294 (1.07-2.00)</td>
<td>1.430 ± 0.293 (0.98-2.02)</td>
<td>0.139</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.3573 ± 0.0117 (0.344-0.380)</td>
<td>0.3634 ± 0.0122 (0.343-0.385)</td>
<td>0.156</td>
</tr>
</tbody>
</table>

\(^1\)Before vs after TOF therapy.


---

**Figure 1** Characteristics of homeostatic model assessment-insulin resistance levels in active rheumatoid arthritis patients naïve to biologic synthetic disease-modifying anti-rheumatic drugs.

- **A:** Positive correlation between 28-joint disease activity score 28 values and homeostatic model assessment (HOMA)-insulin resistance (IR) levels (P = 0.039) before tofacitinib (TOF) therapy; **B:** Serial calculations of HOMA-IR levels in 3 patients with high baseline IR at weeks 0, 4, 8, 12 and 24 after TOF therapy. There were significantly lower levels at week 24 as compared with those at week 0 (P < 0.01). HOMA-IR: Homeostatic model assessment-insulin resistance.

Beneficial effects on the release and sensitivity of insulin, and a multicenter prospective study with 4950 RA patients showed a lower risk of developing type 2 diabetes in those receiving hydroxychloroquine treatment\[^{39}\]. In this study, only 14 patients (25%) received low-dose prednisolone prescription before TOF therapy, and most of them (86%) had reduced HOMA-IR levels after therapy. Furthermore, there were no differences in the prescription frequencies and the dosages of various conventional synthetic DMARDs between the two patient groups with different baseline IRs, and their medication profiles were stable throughout the therapeutic period. In the present investigation, the effects of 24-wk TOF therapy on IR reduction could be identified in RA patients with high baseline DAS28 values and HOMA-IR levels. Notably, reduced IR in active RA only with high baseline IR has been demonstrated by studies with IR classification occurring before anti-IL-6 or anti-TNF-α therapy\[^{7,11,35,40-42}\].
### Table 3 Insulin resistance change in 26 active rheumatoid arthritis patients exposed to biologics by tofacitinib therapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>P value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 26)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DAS28</td>
<td>5.265 ± 0.547 (4.54-6.74)</td>
<td>3.051 ± 0.516 (2.11-3.99)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Decrease in DAS28</td>
<td>2.214 ± 0.688 (1.08-3.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.548 ± 0.925 (1.33-4.75)</td>
<td>2.251 ± 1.067 (0.85-4.55)</td>
<td>0.016</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.3358 ± 0.0177 (0.305-0.366)</td>
<td>0.3446 ± 0.0242 (0.305-0.394)</td>
<td>0.016</td>
</tr>
<tr>
<td>High IR (n = 19)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS28</td>
<td>5.316 ± 0.807 (4.63-6.74)</td>
<td>3.070 ± 0.466 (2.42-3.90)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Decrease in DAS28</td>
<td>2.246 ± 0.672 (1.08-3.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.924 ± 0.790 (2.10-4.75)</td>
<td>2.545 ± 1.080 (1.05-4.55)</td>
<td>0.018</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.3273 ± 0.0117 (0.305-0.341)</td>
<td>0.3372 ± 0.0214 (0.305-0.380)</td>
<td>0.008</td>
</tr>
<tr>
<td>Low IR (n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS28</td>
<td>5.124 ± 0.332 (4.54-5.48)</td>
<td>3.000 ± 0.672 (2.11-3.99)</td>
<td>0.016</td>
</tr>
<tr>
<td>Decrease in DAS28</td>
<td>2.124 ± 0.778 (1.25-3.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.527 ± 0.159 (1.33-1.77)</td>
<td>1.453 ± 0.478 (0.85-2.18)</td>
<td>0.781</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.3589 ± 0.0059 (0.350-0.366)</td>
<td>0.3648 ± 0.0204 (0.340-0.394)</td>
<td>0.813</td>
</tr>
</tbody>
</table>

\(^1\)Before vs after TOF therapy.


Accumulated evidence has indicated that the JAK-STAT pathway is required for normal homeostasis of metabolic processes, and when it is dysregulated it contributes to the development of obesity and diabetes type 2 associated with chronic low-grade inflammatory response\(^{[43]}\). Numerous investigations have found the involvement of JAK-STAT signaling in peripheral metabolic organs with adipose, liver, muscle and pancreas, and in diabetes types 1 and 2\(^{[44]}\). A crucial role of JAK signaling, involving JAK2 in particular, has been recognized in regulating metabolic processes with glucose tolerance, insulin sensitivity and adiposity through studies using conditional genetic ablation mouse models. Mice with hepatocyte-specific deletion of JAK2 had reduced adiposity, increased pancreatic β-cell mass and complete protection against high-fat diet (HFD)-induced IR and glucose intolerance\(^{[45]}\). Mice with adipocyte-specific loss of JAK2 showed increased insulin sensitivity and resistance to HFD-induced metabolic inflammation\(^{[46]}\). Furthermore, besides an involvement in the activation of cytokine signaling pathways, the JAK-STAT pathway has been shown to regulate the function and survival of the β cells\(^{[43,44]}\). In the non-obese diabetic mouse model, disruption of STAT1 could inhibit interferon-γ-induced β cell apoptosis\(^{[47]}\), while treating mice with a JAK1/JAK2 inhibitor reversed diabetes through blockade of the MHC class I upregulation on β cells\(^{[48]}\). Notably, experiments with diabetic animal models have demonstrated that systemic administration of TOF, a pan-JAKi, could normalize impaired glucose tolerance and insulin response in Lnk deficient mice, and reduce IR and improve β-cell function in fructose/streptozotocin-induced rats\(^{[49,50]}\). In this clinical study, oral TOF therapy showed a beneficent effect on IR reduction in active RA patients. In sum, these findings implicate JAK-STAT signaling as a pharmacological target in diabetes and the potential for JAKi use in treating diabetic patients.

**CONCLUSION**

In this retrospective study, we observed a reduction of IR following 24-wk TOF therapy with 5 mg twice-daily immediate-release formulation in non-diabetic RA patients with active disease. Further prospective studies can be performed in both non-diabetic patients and those with comorbid diabetes to clearly elucidate the effect of TOF on IR in active RA.
Figure 2 Homeostatic model assessment-insulin resistance and Quantitative Insulin Sensitivity Check Index levels in 30 active rheumatoid arthritis patients naïve to biologic agents before and 24 wk after tofacitinib therapy. A: Homeostatic model assessment (HOMA)-insulin resistance (IR) levels in all 30 patients at weeks 0 and 24 after tofacitinib (TOF) therapy ($P < 0.001$); B: HOMA-IR levels in the high-IR group with 18 patients at weeks 0 and 24 after TOF therapy ($P < 0.001$); C: HOMA-IR levels in the low-IR group with 12 patients at weeks 0 and 24 after TOF therapy; D: Quantitative Insulin Sensitivity Check Index (QUICKI) levels in all 30 patients at weeks 0 and 24 after TOF therapy ($P < 0.001$); E: QUICKI levels in the high-IR group with 18 patients at weeks 0 and 24 after TOF therapy ($P < 0.001$); F: QUICKI levels in the low-IR group with 12 patients at weeks 0 and 24 after TOF therapy. QUICKI: Quantitative Insulin Sensitivity Check Index; HOMA-TR: Homeostatic model assessment-insulin resistance.

Figure 3 Homeostatic model assessment-insulin resistance and Quantitative Insulin Sensitivity Check Index levels in 26 active rheumatoid arthritis patients exposed to biologic agents before and 24 wk after tofacitinib therapy. A: Homeostatic model assessment (HOMA)-insulin resistance (IR) levels in all 26 patients at weeks 0 and 24 after tofacitinib (TOF) therapy ($P = 0.016$); B: HOMA-IR levels in the high-IR group with 19 patients at weeks 0 and 24 after TOF therapy ($P = 0.018$); C: HOMA-IR levels in the low-IR group with 7 patients at weeks 0 and 24 after TOF therapy; D: Quantitative Insulin Sensitivity Check Index (QUICKI) levels in all 26 patients at weeks 0 and 24 after TOF therapy ($P = 0.016$); E: QUICKI levels in the high-IR group with 19 patients at weeks 0 and 24 after TOF therapy ($P = 0.008$); F: QUICKI levels in the low-IR group with 7 patients at weeks 0 and 24 after TOF therapy. QUICKI: Quantitative Insulin Sensitivity Check Index; HOMA-TR: Homeostatic model assessment-insulin resistance.
ARTICLE HIGHLIGHTS

Research background
An increased risk of insulin resistance (IR) has been identified in rheumatoid arthritis (RA), a chronic inflammatory disorder with elevated levels of pathogenic cytokines. Biologics targeting proinflammatory cytokines can control the disease and improve insulin sensitivity in RA.

Research motivation
Although Janus kinase (JAK) signaling can regulate cytokine receptors and participate in RA pathogenesis, it remains to be elucidated whether there is a reduction of IR in such patients under JAK inhibitor (JAKi) therapy.

Research objectives
This study examined the effect of JAKi treatment on the reduction of IR in RA with active disease.

Research methods
A retrospective study was carried out in non-diabetic active RA patients under tofacitinib (TOF) therapy with 5 mg twice-daily immediate-release formulation from 2017 to 2021.

Research results
Fifty-six RA patients aged 30 years to 75 years (52.3 ± 11.1) with DAS 28 values 4.54 to 7.37 (5.82 ± 0.74), were classified into high- and low-IR groups based on the baseline homeostatic model assessment (HOMA)-IR levels. For the 30 patients naive to biologics, after a 24-wk therapeutic period, reduced levels of HOMA-IR were observed in the high-IR group (3.331 ± 1.036 vs 2.292 ± 0.707, \( P < 0.001 \)). In another 26 patients exposed to tumor necrosis factor-\( \alpha \) or interleukin-6 blockers, despite showing a decrease with lower magnitude than that observed in the naïve patients, reduced HOMA-IR levels were also identified in the high-IR group (2.924 ± 0.790 vs 2.545 ± 1.080, \( P = 0.018 \)).

Research conclusions
In this retrospective study, our results demonstrated reduced IR following 24-wk TOF therapy in non-diabetic active RA patients.

Research perspectives
Further prospective studies can be performed in both non-diabetic patients and those with comorbid diabetes to clearly elucidate the effect of TOF on IR in active RA.

ACKNOWLEDGEMENTS
The authors are indebted to all of the doctors/nurses involved in the management of RA patients at the National Cheng Kung University Hospital (NCKUH). The statistical analyses of this study were performed with help from the Biostatistics Consulting Center at the NCKUH.

FOOTNOTES

Author contributions: Wang CR designed the report, collected the clinical data, and wrote the paper; Wang CR and Tsai HW analyzed the clinical data.

Institutional review board statement: This study was approved by the Ethics Committee of National Cheng Kung University Hospital, No. B-ER-105-108.

Informed consent statement: The Institutional Review Board waived the requirement of informed consent from each patient due to the study being classified as a retrospective review of medical records.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The data of this study can be provided to researchers by the corresponding author upon reasonable request.

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P-Editor: Fan JR

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Wang CR et al. Tofacitinib reduces insulin resistance in RA


Wang CR et al. Tofacitinib reduces insulin resistance in RA


Relook at DPP-4 inhibitors in the era of SGLT-2 inhibitors

Awadhesh Kumar Singh, Ritu Singh

Abstract

SGLT-2 inhibitors (SGLT-2Is) have significantly improved cardio-renal outcomes and are preferred agents in people with cardiovascular diseases, heart failure, and diabetic kidney disease. Similarly, GLP-1 receptor agonists (GLP-1RAs) have significantly improved atherosclerotic cardiovascular outcomes. To this end, DPP-4 inhibitors (DPP-4Is) are cardiac-neutral drugs. While long-acting GLP-1RAs have shown a favorable HbA1c lowering compared to DPP-4Is, there is no clinically meaningful HbA1c lowering difference between SGLT-2Is vs DPP-4Is. Moreover, the glucose-lowering potential of SGLT-2Is gets compromised with a progressive decline in renal functions, unlike DPP-4Is. Furthermore, the HbA1c lowering potential of DPP-4Is is favorable in people with chronic kidney disease regardless of impaired kidney functions.

Key Words: DPP-4 inhibitors; SGLT-2 inhibitors; GLP-1 receptor agonists; Cardiovascular outcomes; Renal outcomes
We read with interest a minireview by Florentin et al [1] putting their arguments in favor of DPP-4 inhibitors (DPP-4Is) as a second-line drug after metformin in people with type 2 diabetes mellitus (T2DM) in particular who are elderly and have chronic kidney disease (CKD) stage 3A or lower. This wonderfully written minireview discusses the role of DPP-4Is in the era of two other novel anti-diabetic agents such as SGLT-2 inhibitors (SGLT-2Is) and GLP-1 receptor agonists (GLP-1RAs) that have shown a remarkably beneficial effect on cardiovascular (CV) and renal endpoints making them an ideal second or arguably even first-line drug in people with T2DM having established CV disease (CVD), heart failure (HF) and CKD. While authors have discussed the pharmacological differences amongst different DPP-4Is and put a perspective on the CV outcome trials in the era of SGLT-2Is and GLP-1RAs, few vital details seem to be missing and some of the statements appear rather ambiguous that need clarification.

The most important area that is surprisingly missing in this review is the efficacy comparison between DPP-4Is vs SGLT-2Is or GLP-1RAs. Expectedly, the HbA1c lowering effect of DPP-4Is would be inferior to GLP-1RAs owing to their mechanism of action that causes a physiological vs pharmacological rise of GLP-1 respectively and indeed, several head-to-head studies of long-acting GLP-1RAs have shown a superior HbA1c lowering beside a significant reduction in weight and systolic blood pressure (SBP) when compared with DPP-4Is. However, the HbA1c lowering effect of DPP-4Is is not clinically meaningful different from SGLT-2Is. To this end, several studies have evaluated the HbA1c lowering effect of SGLT-2Is vs DPP4Is in the past decade [2-8]. Although in most of these SGLT-2Is head-to-head studies with DPP-4Is, HbA1c reduction was similar between the two drug classes; DPP-4Is were used as an open-label active comparator arm only for exploratory analysis. One study that compared empagliflozin 10 and 25 mg with 100 mg sitagliptin as an active comparator in a double-blind randomized fashion found no difference in HbA1c lowering [3]. However, two studies that compared canagliflozin 100 and 300 mg with sitagliptin 100 mg as an active comparator in a double-blind randomized fashion, found 300 mg canagliflozin to be superior to 100 mg sitagliptin in HbA1c lowering, though no difference was noted with 100 mg canagliflozin (Table 1) [6,7]. Meta-analyses that compared HbA1c lowering with DPP-4Is vs SGLT-2Is yielded discordant results [9-12]. While some found no difference in HbA1c lowering, others showed a small but significant HbA1c lowering with SGLT-2Is compared to DPP-4Is (Table 1). Notably, weight and SBP reduction were consistently superior with SGLT-2Is vs DPP-4Is in all these head-to-head studies including meta-analyses. Another interesting piece of missing information that needs discussion is the differential HbA1c lowering effect of DPP-4Is vs. SGLT-2Is stratified on baseline HbA1c. While the SGLT-2Is appear to lower the HbA1c more favorably compared with DPP-4Is in the background of higher baseline value (HbA1c 8.5%-9.0%), DPP-4Is lowered HbA1c more favorably compared with SGLT-2I in people having a modest baseline HbA1c value (< 8%-8.5%) (Table 1) [13-15]. This finding suggests DPP-4Is may have a favorable effect on HbA1c lowering compared to SGLT-2Is in people with T2DM having a modest baseline HbA1c, in absence of high CV risk. Although a reduction in HbA1c is always larger when baseline HbA1c is high, we do not know exactly why DPP-4Is reduce HbA1c larger compared to the SGLT-2Is when the baseline value is modest. Since SGLT-2Is HbA1c lowering ability is dependent on the renal threshold of glucose excretion (RT), modest baseline HbA1c may not produce further lowering of RT.

Nevertheless, we humbly disagree with the author’s conclusion about “the lack of evidence with SGLT-2Is and GLP-1RAs in elderly patients with diabetes as well as the contraindication of SGLT-2Is in patients with CKD, grade 3A and lower, make DPP-4Is a safe choice in such populations.” Let us recall that: (1) About one-fourth patients population (24.2%) in HF trial of SGLT-2I dapagliflozin were elderly [≥ 75 years, median age 79 years (76-82 years)] and they benefitted equally [Hazard ratio (HR), 0.68; 95% Confidence interval (CI), 0.53-0.88] when compared to the overall population (HR, 0.74; 95% CI, 0.65-0.85) in terms of reduction of the primary composite endpoint of CV death or HF hospitalization (HHF) or urgent HF visits (Pinteraction = 0.76) [16]; (2) Mean age of the population in CV-, HF- and renal-outcome trials of SGLT-2Is varied from as low as 62 years in renal outcome trial of dapagliflozin (DAPA-CKD) to as high as 72 years in HF trial of empagliflozin (EMPEROR-Preserved) that found a significantly beneficial renal and CV effect respectively [17]; (3) Current guidelines recommend using SGLT-2Is in patients with CKD if eGFR is ≥ 30 mL/min/1.73 m2 and in addition, empagliflozin has been granted an additional label of use up to eGFR ≥ 20 mL/min/m2 in patients with HF with reduced ejection fraction and CKD [18]; (4) The latest Kidney Disease: Improving Global Outcomes 2022 guideline which is currently under public review recommend using SGLT-2Is in patients with CKD if eGFR ≥ 20 mL/min/1.73 m2 regardless of background HF. Moreover, once SGLT-2Is are initiated it is reasonable to continue even if the eGFR falls below 20 mL/min per 1.73 m2 unless it is not tolerated or kidney
Table 1 HbA1c reduction with SGLT-2 inhibitors vs DPP-4 inhibitors

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Study duration (wk)</th>
<th>Background therapy</th>
<th>n (Active drug)</th>
<th>Baseline HbA1c (%)</th>
<th>SGLT-2I (A) (% HbA1c reduction)</th>
<th>DPP-4I (B) (% HbA1c reduction)</th>
<th>Δ A minus B (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh AK et al.[1], 2012</td>
<td>12</td>
<td>Metformin</td>
<td>193</td>
<td>7.6%-7.8%</td>
<td>-0.76 (Cana 100 mg)</td>
<td>-0.74 (Sita 100 mg)</td>
<td>NC, (B) exploratory</td>
</tr>
<tr>
<td>Rosenstock et al.[2], 2013</td>
<td>24</td>
<td>Drug naive</td>
<td>671</td>
<td>7.9%</td>
<td>-0.66 (Empa 10 mg)</td>
<td>-0.66 (Sita 100 mg)</td>
<td>0.0 (-0.15, 0.14)</td>
</tr>
<tr>
<td>Rosenstock et al.[3], 2013</td>
<td>12</td>
<td>Metformin</td>
<td>212</td>
<td>7.9%-8.1%</td>
<td>-0.56 (Empa 10 mg)</td>
<td>-0.45 (Sita 100 mg)</td>
<td>NC, (B) exploratory</td>
</tr>
<tr>
<td>Ferrannini et al.[4], 2013</td>
<td>90</td>
<td>Metformin</td>
<td>332</td>
<td>7.9%-8%</td>
<td>-0.78 (Empa 25 mg)</td>
<td>-0.66 (Sita 100 mg)</td>
<td>NC, (B) exploratory</td>
</tr>
<tr>
<td>Lavalle-González et al.[5], 2013</td>
<td>52</td>
<td>Metformin</td>
<td>1079</td>
<td>7.9%</td>
<td>-0.88 (Cana 300 mg)</td>
<td>-0.73 (Sita 100 mg)</td>
<td>-0.15 (-0.27, -0.03)</td>
</tr>
<tr>
<td>Schernthaner et al.[6], 2013</td>
<td>328</td>
<td>Metformin + SU</td>
<td>755</td>
<td>8.1%</td>
<td>-1.03 (Cana 300 mg)</td>
<td>-0.66 (Sita 100 mg)</td>
<td>-0.37 (-0.50, -0.25)</td>
</tr>
<tr>
<td>Pinto et al.[9], 2015</td>
<td>≥ 12</td>
<td>LSM, Metformin, SU</td>
<td>NR (6 studies)</td>
<td>-</td>
<td>SGLT-2Is</td>
<td>DPP-4Is</td>
<td>-0.15 (-0.21, -0.08)</td>
</tr>
<tr>
<td>Maruthur et al.[10], 2016</td>
<td>≤ 52</td>
<td>Metformin</td>
<td>1278 (4 studies)</td>
<td>-</td>
<td>SGLT-2Is</td>
<td>DPP-4Is</td>
<td>(B) minus (A) = +0.17 (0.08, 0.26)</td>
</tr>
<tr>
<td>Wang et al.[11], 2018</td>
<td>12-78</td>
<td>Metformin</td>
<td>3454 (7 studies)</td>
<td>-</td>
<td>SGLT-2Is</td>
<td>DPP-4Is</td>
<td>(B) minus (A) = +0.11 (-0.03, 0.25)</td>
</tr>
<tr>
<td>Mishriky et al.[12], 2018</td>
<td>≤ 26</td>
<td>Metformin</td>
<td>2462 (6 studies)</td>
<td>-</td>
<td>SGLT-2Is</td>
<td>DPP-4Is</td>
<td>(B) minus (A) = +0.05 (-0.05, 0.16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Study duration (wk)</th>
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<th>Δ A minus B (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosenstock et al.[13], 2015</td>
<td>24</td>
<td>Metformin</td>
<td>103</td>
<td>&gt; 9%</td>
<td>-1.87 (Dapa 10 mg)</td>
<td>-1.32 (Saxa 5 mg)</td>
<td>NC</td>
</tr>
<tr>
<td>Lewin et al.[14], 2015</td>
<td>24</td>
<td>LSM</td>
<td>166</td>
<td>&lt; 8%</td>
<td>-1.66 (Empa 25 mg)</td>
<td>-1.07 (Lina 5 mg)</td>
<td>NC</td>
</tr>
<tr>
<td>DeFronzo et al.[15], 2015</td>
<td>24</td>
<td>Metformin</td>
<td>473</td>
<td>≥ 8.5%</td>
<td>-1.54 (Empa 10 mg)</td>
<td>-0.56 (Empa 10 mg)</td>
<td>NC</td>
</tr>
<tr>
<td>DeFronzo et al.[15], 2015</td>
<td>508</td>
<td>Metformin</td>
<td>101</td>
<td>≥ 8.5%</td>
<td>-1.22 (Empa 25 mg)</td>
<td>-0.29 (Empa 10 mg)</td>
<td>NC</td>
</tr>
</tbody>
</table>

* (A) superior over (B).
SGLT-2Is: SGLT-2 inhibitors; DPP-4Is: DPP4 inhibitors; Cana: Canagliflozin; Empa: Empagliflozin; Dapa: Dapagliflozin; Ertu: Ertugliflozin; Sita: Sitagliptin; Saxa: Saxagliptin; Lina: Linagliptin; SU: Sulfonylureas; LSM: Life style modification; NC: Not compared.

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SGLT-2Is: SGLT-2 inhibitors; DPP-4Is: DPP4 inhibitors; Cana: Canagliflozin; Empa: Empagliflozin; Dapa: Dapagliflozin; Ertu: Ertugliflozin; Sita: Sitagliptin; Saxa: Saxagliptin; Lina: Linagliptin; SU: Sulfonylureas; LSM: Life style modification; NC: Not compared.
canagliflozin in 2013 and first positive CV outcome with empagliflozin in 2015. These findings suggest author’s conclusion is discordant with the available evidence[21].

FOOTNOTES

Author contributions: Singh AK designed the research; Singh R performed the research, Singh AK and Singh R analyzed the data; Singh AK wrote the letter, and Singh R revised the manuscript.

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