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EVIDENCE REVIEW

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

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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

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

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 "glycemi*" 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 CESSATION 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 abstenance 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 CESSATION 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^3 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

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

Ref.	Country/region	Study population	Study design	Effect
Dinardo *et al* [24], 2019	United States	T2D, *n* = 282	Cross-sectional, comparison of current smokers *vs* former smokers and never-smokers	Positive
Braffett *et al*[14], 2019	Multicenter (United States and Canada)	T1D, *n* = 1441	Retrospective analysis of the prospective cohort study, comparison of current smokers *vs* former smokers and never-smokers	Positive
Lycett *et al*[17], 2011	United Kingdom	T2D, *n* = 10692	Retrospective cohort study, observation of HbA1c in three groups: Continual smokers, long-term quitters, and relapsers	Negative
Ohkuma *et al* [23], 2015	Japan	T2D, *n* = 2490	Cross-sectional study, comparison of current smokers and former smokers *vs* never-smokers	Positive
Su *et al*[26], 2017	China	T2D, *n* = 7763	Cross-sectional study, comparison of current smokers, former smokers and never-smokers	Positive
Li *et al*[28], 2017	Hong Kong of China	T2D, *n* = 557	A randomized controlled trial, comparison of level of HbA1c and changes from baseline to 12-mo between quitters and non-quitters	Neutral
Kar *et al*[29], 2016	United States, Japan	T1D + T2D, *n* = 13719	Metanalysis, comparison of current smokers *vs* quitters	Neutral
Voulgari *et al* [57], 2011	Greece	T2D, *n* = 193	Prospective study, comparison of smokers *vs* former-smokers	Positive
Feodoroff *et al* [69], 2016	Finland	T1D, *n* = 3613	Prospective study, comparison of smokers *vs* non-smokers and former-smokers *vs* non-smokers	Positive
Reynolds *et al* [68], 2011	United States	T1D, *n* = 2124	Cross-sectional analysis of population-based study, comparison of current smokers *vs* non-smokers and former-smokers *vs* non-smokers	Neutral
Reynolds *et al* [68], 2011	United States	T2D, *n* = 348	Cross-sectional study, comparison of current smokers *vs* non-smokers and former-smokers *vs* non-smokers	Neutral

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

visceral adipose tissue[50]. 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*[58] 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.

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

Ref.	Country	Study population	Study design	Effect
Ohkuma *et al*[23], 2015	Japan	T2D, *n* = 2490	Cross-sectional study, comparison of current smokers and former smokers *vs* never-smokers	Positive
Voulgari *et al*[57], 2011	Greece	T2D, *n* = 193	Prospective study, comparison of smokers *vs* former-smokers	Positive

T1D: Type 1 diabetes; T2D: Type 2 diabetes.

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[63]. 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 3 The results of studies evaluating the effect of smoking cessation on lipid parameters in diabetic patients

Ref.	Country	Study population	Study design	Effect
Reynolds *et al*[68], 2011	United States	T1D, *n* = 2124	Cross-sectional analysis of population-based study, comparison of current smokers *vs* non-smokers and former-smokers *vs* non-smokers	LDL–negative; HDL–neutral; TG–positive
Reynolds *et al*[68], 2011	United States	T2D, *n* = 348	Cross-sectional study, comparison of current smokers *vs* non-smokers and former-smokers *vs* non-smokers	Neutral
Luque-Ramírez *et al*[12], 2018	Spain	T2D, *n* = 890	Cross-sectional, observational study, comparison of smokers *vs* former-smokers	LDL–neutral; HDL–positive; TG–positive
Voulgari *et al*[57], 2011	Greece	T2D, *n* = 193	Prospective study, comparison of smokers *vs* former-smokers	LDL–positive; HDL–positive; TG–positive
Feodoroff *et al*[69], 2016	Finland	T1D, *n* = 3613	Prospective study, comparison of smokers *vs* non-smokers and former-smokers *vs* non-smokers	LDL–neutral; HDL–positive; TG–neutral

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

Table 4 The results of studies evaluating the effect of smoking cessation on biochemical parameters of nephropathy in diabetic patients

Ref.	Country	Study population	Study design	Effect
Ohkuma *et al* [77], 2016	Japan	T2D, *n* = 2770	Cross-sectional study, comparison of smokers, former-smokers and never-smokers	UACR–positive with increasing years after quitting
Voulgari *et al* [57], 2011	Greece	T2D, *n* = 193	Prospective study, comparison of smokers *vs* former-smokers	Microalbuminuria–positive
Feodoroff *et al* [69], 2016	Finland	T1D, *n* = 3613	Prospective study, comparison of smokers *vs* non-smokersand former-smokers *vs* non-smokers	Micro- and macroalbuminuria–positive

T1D: Type 1 diabetes; T2D: Type 2 diabetes; UACR: Albumin-creatinine ratio.

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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REFERENCES

1 **World Health Organization**. Newsroom. Tobacco. [cited 26 July 2021]. Available from: https://www.who.int/news-room/fact-sheets/detail/tobacco
2 **GBD 2015 Tobacco Collaborators**. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990-2015: a systematic analysis from the Global Burden of Disease Study 2015. *Lancet* 2017; **389**: 1885-1906 [PMID: 28390697 DOI: 10.1016/S0140-6736(17)30819-X]
3 **Institute for Health Metrics and Evaluation**. Findings from the Global Burden of Disease Study 2017. Seattle, WA: IHME, 2018. [cited 26 July 2021]. Available from: https://www.healthdata.org/sites/default/files/files/policy_report/2019/GBD_2017_Booklet.pdf
4 **IHME**. Institute for Health Metrics and Evaluation FHM GBD Compare-Viz Hub. 2020. [cited 26 July 2021]. Available from: https://vizhub.healthdata.org/gbd-compare/
5 **Guarino F**, Cantarella G, Caruso M, Russo C, Mancuso S, Arcidiacono G, Cacciola RR, Bernardini R, Polosa R. Endothelial activation and injury by cigarette smoke exposure. *J Biol Regul Homeost Agents* 2011; **25**: 259-268 [PMID: 21880215]
6 **Caponnetto P**, Russo C, Di Maria A, Morjaria JB, Barton S, Guarino F, Basile E, Proiti M, Bertino G, Cacciola RR, Polosa R. Circulating endothelial-coagulative activation markers after smoking cessation: a 12-month observational study. *Eur J Clin Invest* 2011; **41**: 616-626 [PMID: 21198559 DOI: 10.1111/j.1365-2362.2010.02449.x]
7 **Kondo T**, Nakano Y, Adachi S, Murohara T. Effects of Tobacco Smoking on Cardiovascular Disease. *Circ J* 2019; **83**: 1980-1985 [PMID: 31462607 DOI: 10.1253/circj.CJ-19-0323]
8 **Knapp M**, Tu X, Wu R. Vascular endothelial dysfunction, a major mediator in diabetic cardiomyopathy. *Acta Pharmacol Sin* 2019; **40**: 1-8 [PMID: 29867137 DOI: 10.1038/s41401-018-0042-6]
9 **Molla GJ**, Ismail-Beigi F, Larijani B, Khaloo P, Moosaie F, Alemi H, Mansournia MA, Ghadimi T, Ghaemi F, Nakhjavani M, Esteghamati A. Smoking and Diabetes Control in Adults With Type 1 and Type 2 Diabetes: A Nationwide Study From the 2018 National Program for Prevention and Control of Diabetes of Iran. *Can J Diabetes* 2020; **44**: 246-252 [PMID: 31494031 DOI: 10.1016/j.jcjd.2019.07.002]
10 **Pan A**, Wang Y, Talaei M, Hu FB. Relation of Smoking With Total Mortality and Cardiovascular Events Among Patients With Diabetes Mellitus: A Meta-Analysis and Systematic Review. *Circulation* 2015; **132**: 1795-1804 [PMID: 26311724 DOI: 10.1161/CIRCULATIONAHA.115.017926]
11 **Blomster JI**, Woodward M, Zoungas S, Hillis GS, Harrap S, Neal B, Poulter N, Mancia G, Chalmers J, Huxley R. The harms of smoking and benefits of smoking cessation in women compared with men with type 2 diabetes: an observational analysis of the ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron modified release Controlled Evaluation) trial. *BMJ Open* 2016; **6**: e009668 [PMID: 26747037 DOI: 10.1136/bmjopen-2015-009668]
12 **Luque-Ramírez M**, Sanz de Burgoa V; en nombre de los participantes del estudio DIABETES. Impact of smoking cessation on estimated cardiovascular risk in Spanish type 2 diabetes mellitus patients: The DIABETES study. *Rev Clin Esp (Barc)* 2018; **218**: 391-398 [PMID: 29891175 DOI: 10.1016/j.rce.2018.04.014]
13 **Cai X**, Chen Y, Yang W, Gao X, Han X, Ji L. The association of smoking and risk of diabetic retinopathy in patients with type 1 and type 2 diabetes: a meta-analysis. *Endocrine* 2018; **62**: 299-306 [PMID: 30128962 DOI: 10.1007/s12020-018-1697-y]
14 **Braffett BH**, Rice MM, Young HA, Lachin JM. Mediation of the association of smoking and microvascular complications by glycemic control in type 1 diabetes. *PLoS One* 2019; **14**: e0210367 [PMID: 30615671 DOI: 10.1371/journal.pone.0210367]
15 **Pan A**, Wang Y, Talaei M, Hu FB, Wu T. Relation of active, passive, and quitting smoking with incident type 2 diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2015; **3**: 958-967 [PMID: 26388413 DOI: 10.1016/S2213-8587(15)00316-2]
16 **Eisenberg D**, Quinn BC. Estimating the effect of smoking cessation on weight gain: an instrumental variable approach. *Health Serv Res* 2006; **41**: 2255-2266 [PMID: 17116119 DOI: 10.1111/j.1475-6773.2006.00594.x]

17 **Lycett D**, Munafò M, Johnstone E, Murphy M, Aveyard P. Associations between weight change over 8 years and baseline body mass index in a cohort of continuing and quitting smokers. *Addiction* 2011; **106**: 188-196 [PMID: 20925685 DOI: 10.1111/j.1360-0443.2010.03136.x]

18 **Aubin HJ**, Farley A, Lycett D, Lahmek P, Aveyard P. Weight gain in smokers after quitting cigarettes: meta-analysis. *BMJ* 2012; **345**: e4439 [PMID: 22782848 DOI: 10.1136/bmj.e4439]

19 **Zoli M**, Picciotto MR. Nicotinic regulation of energy homeostasis. *Nicotine Tob Res* 2012; **14**: 1270-1290 [PMID: 22990212 DOI: 10.1093/ntr/nts159]

20 **Killi AE**, Baspinar MM, Basat O. Association between post-cessation weight gain and eating behavior changes. *North Clin Istanb* 2020; **7**: 153-160 [PMID: 32259037 DOI: 10.14744/nci.2019.58712]

21 **Hu Y**, Zong G, Liu G, Wang M, Rosner B, Pan A, Willett WC, Manson JE, Hu FB, Sun Q. Smoking Cessation, Weight Change, Type 2 Diabetes, and Mortality. *N Engl J Med* 2018; **379**: 623-632 [PMID: 30110591 DOI: 10.1056/NEJMoa1803626]

22 **Pani LN**, Nathan DM, Grant RW. Clinical predictors of disease progression and medication initiation in untreated patients with type 2 diabetes and A1C less than 7%. *Diabetes Care* 2008; **31**: 386-390 [PMID: 18083790 DOI: 10.2337/dc07-1934]

23 **Ohkuma T**, Iwase M, Fujii H, Kaizu S, Ide H, Jodai T, Kikuchi Y, Idewaki Y, Hirakawa Y, Nakamura U, Kitazono T. Dose- and time-dependent association of smoking and its cessation with glycemic control and insulin resistance in male patients with type 2 diabetes mellitus: the Fukuoka Diabetes Registry. *PLoS One* 2015; **10**: e0122023 [PMID: 25822499 DOI: 10.1371/journal.pone.0122023]

24 **Dinardo MM**, Sereika SM, Korytkowski M, Baniak LM, Weinzierl VA, Hoenstine AL, Chasens ER. Current Smoking: An Independent Predictor of Elevated A1C in Persons With Type 2 Diabetes. *Diabetes Educ* 2019; **45**: 146-154 [PMID: 30755104 DOI: 10.1177/0145721719829068]

25 **Lycett D**, Nichols L, Ryan R, Farley A, Roalfe A, Mohammed MA, Szatkowski L, Coleman T, Morris R, Farmer A, Aveyard P. The association between smoking cessation and glycaemic control in patients with type 2 diabetes: a THIN database cohort study. *Lancet Diabetes Endocrinol* 2015; **3**: 423-430 [PMID: 25935880 DOI: 10.1016/S2213-8587(15)00082-0]

26 **Su J**, Qin Y, Shen C, Gao Y, Pan EC, Pan XQ, Tao R, Zhang YQ, Wu M. [Association between smoking/smoking cessation and glycemic control in male patients with type 2 diabetes]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2017; **38**: 1454-1459 [PMID: 29141328 DOI: 10.3760/cma.j.issn.0254-6450.2017.11.003]

27 **Chen HJ**, Huang WH, Chan HL, Hwang LC. Improvement in Cardiometabolic Risk Factors During Smoking Cessation Treatment in Patients with Type 2 Diabetes: A Retrospective Cohort Study. *Diabetes Metab Syndr Obes* 2021; **14**: 1695-1702 [PMID: 33889004 DOI: 10.2147/DMSO.S303446]

28 **Li WH**, Wang MP, Lam TH, Cheung YT, Cheung DY, Suen YN, Ho KY, Tan KC, Chan SS. Brief intervention to promote smoking cessation and improve glycemic control in smokers with type 2 diabetes: a randomized controlled trial. *Sci Rep* 2017; **7**: 45902 [PMID: 28378764 DOI: 10.1038/srep45902]

29 **Kar D**, Gillies C, Zaccardi F, Webb D, Seidu S, Tesfaye S, Davies M, Khunti K. Relationship of cardiometabolic parameters in non-smokers, current smokers, and quitters in diabetes: a systematic review and meta-analysis. *Cardiovasc Diabetol* 2016; **15**: 158 [PMID: 27881170 DOI: 10.1186/s12933-016-0475-5]

30 **Tong X**, Chaudhry Z, Lee CC, Bone RN, Kanojia S, Maddatu J, Sohn P, Weaver SA, Robertson MA, Petrache I, Evans-Molina C, Kono T. Cigarette smoke exposure impairs β-cell function through activation of oxidative stress and ceramide accumulation. *Mol Metab* 2020; **37**: 100975 [PMID: 32283079 DOI: 10.1016/j.molmet.2020.100975]

31 **Eliasson B**, Taskinen MR, Smith U. Long-term use of nicotine gum is associated with hyperinsulinemia and insulin resistance. *Circulation* 1996; **94**: 878-881 [PMID: 8790020 DOI: 10.1161/01.cir.94.5.878]

32 **Epifano L**, Di Vincenzo A, Fanelli C, Porcellati F, Perriello G, De Feo P, Motolese M, Brunetti P, Bolli GB. Effect of cigarette smoking and of a transdermal nicotine delivery system on glucoregulation in type 2 diabetes mellitus. *Eur J Clin Pharmacol* 1992; **43**: 257-263 [PMID: 1425888 DOI: 10.1007/BF02333019]

33 **Axelsson T**, Jansson PA, Smith U, Eliasson B. Nicotine infusion acutely impairs insulin sensitivity in type 2 diabetic patients but not in healthy subjects. *J Intern Med* 2001; **249**: 539-544 [PMID: 11422660 DOI: 10.1046/j.1365-2796.2001.00840.x]

34 **Lunell E**, Fagerström K, Hughes J, Pendrill R. Pharmacokinetic Comparison of a Novel Non-tobacco-Based Nicotine Pouch (ZYN) With Conventional, Tobacco-Based Swedish Snus and American Moist Snuff. *Nicotine Tob Res* 2020; **22**: 1757-1763 [PMID: 32319528 DOI: 10.1093/ntr/ntaa068]

35 **The European Commission**. Special Eurobarometer 458. Attitudes of Europeans towards tobacco and electronic cigarettes. 2017. [cited 26 July 2021]. Available from: http://ec.europa.eu/commfrontoffice/publicopinion/index.cfm/Survey/getSurveyDetail/instruments/SPECIAL/surveyKy/

36 **Ramström L**, Borland R, Wikmans T. Patterns of Smoking and Snus Use in Sweden: Implications for Public Health. *Int J Environ Res Public Health* 2016; **13** [PMID: 27834883 DOI: 10.3390/ijerph13111110]

37 **Persson PG**, Carlsson S, Svanström L, Ostenson CG, Efendic S, Grill V. Cigarette smoking, oral moist snuff use and glucose intolerance. *J Intern Med* 2000; **248**: 103-110 [PMID: 10947888 DOI: 10.1046/j.1365-2796.2000.00708.x]

38 **Eliasson M**, Asplund K, Nasic S, Rodu B. Influence of smoking and snus on the prevalence and incidence of type 2 diabetes amongst men: the northern Sweden MONICA study. *J Intern Med* 2004; **256**: 101-110 [PMID: 15257722 DOI: 10.1111/j.1365-2796.2004.01344.x]

39 **Wändell PE**, Bolinder G, de Faire U, Hellénius ML. Association between metabolic effects and tobacco use in 60-year-old Swedish men. *Eur J Epidemiol* 2008; **23**: 431-434 [PMID: 18470624 DOI: 10.1007/s10654-008-9260-4]

40 **Neumann A**, Norberg M, Schoffer O, Norström F, Johansson I, Klug SJ, Lindholm L. Risk equations for the development of worsened glucose status and type 2 diabetes mellitus in a Swedish intervention program. *BMC Public Health* 2013; **13**: 1014 [PMID: 24502249 DOI: 10.1186/1471-2458-13-1014]

41 **Rasouli B**, Andersson T, Carlsson PO, Grill V, Groop L, Martinell M, Midthjell K, Storm P, Tuomi T, Carlsson S. Use of Swedish smokeless tobacco (snus) and the risk of Type 2 diabetes and latent autoimmune diabetes of adulthood (LADA). *Diabet Med* 2017; **34**: 514-521 [PMID: 27353226 DOI: 10.1111/dme.13179]

42 **Eliasson M**, Asplund K, Evrin PE, Lundblad D. Relationship of cigarette smoking and snuff dipping to plasma fibrinogen, fibrinolytic variables and serum insulin. The Northern Sweden MONICA Study. *Atherosclerosis* 1995; **113**: 41-53 [PMID: 7755654 DOI: 10.1016/0021-9150(94)05425-i]

43 **Lee PN**, Thornton AJ. The relationship of snus use to diabetes and allied conditions. *Regul Toxicol Pharmacol* 2017; **91**: 86-92 [PMID: 29061372 DOI: 10.1016/j.yrtph.2017.10.017]

44 **Canoy D**, Wareham N, Luben R, Welch A, Bingham S, Day N, Khaw KT. Cigarette smoking and fat distribution in 21,828 British men and women: a population-based study. *Obes Res* 2005; **13**: 1466-1475 [PMID: 16129730 DOI: 10.1038/oby.2005.177]

45 **Troisi RJ**, Heinold JW, Vokonas PS, Weiss ST. Cigarette smoking, dietary intake, and physical activity: effects on body fat distribution--the Normative Aging Study. *Am J Clin Nutr* 1991; **53**: 1104-1111 [PMID: 1850574 DOI: 10.1093/ajcn/53.5.1104]

46 **Karpe F**, Pinnick KE. Biology of upper-body and lower-body adipose tissue--link to whole-body phenotypes. *Nat Rev Endocrinol* 2015; **11**: 90-100 [PMID: 25365922 DOI: 10.1038/nrendo.2014.185]

47 **Westphal SA**. Obesity, abdominal obesity, and insulin resistance. *Clin Cornerstone* 2008; **9**: 23-29; discussion 30 [PMID: 19046737 DOI: 10.1016/s1098-3597(08)60025-3]

48 **Facchini FS**, Hollenbeck CB, Jeppesen J, Chen YD, Reaven GM. Insulin resistance and cigarette smoking. *Lancet* 1992; **339**: 1128-1130 [PMID: 1349365 DOI: 10.1016/0140-6736(92)90730-q]

49 **Pisinger C**, Jorgensen T. Waist circumference and weight following smoking cessation in a general population: the Inter99 study. *Prev Med* 2007; **44**: 290-295 [PMID: 17222450 DOI: 10.1016/j.ypmed.2006.11.015]

50 **Lee K**, Lee CM, Kwon HT, Oh SW, Choi H, Park JH, Cho B. Associations of smoking and smoking cessation with CT-measured visceral obesity in 4656 Korean men. *Prev Med* 2012; **55**: 183-187 [PMID: 22728048 DOI: 10.1016/j.ypmed.2012.06.009]

51 **Graff-Iversen S**, Hewitt S, Forsén L, Grøtvedt L, Ariansen I. Associations of tobacco smoking with body mass distribution; a population-based study of 65,875 men and women in midlife. *BMC Public Health* 2019; **19**: 1439 [PMID: 31675936 DOI: 10.1186/s12889-019-7807-9]

52 **Kim KW**, Kang SG, Song SW, Kim NR, Rho JS, Lee YA. Association between the Time of Length since Smoking Cessation and Insulin Resistance in Asymptomatic Korean Male Ex-Smokers. *J Diabetes Res* 2017; **2017**: 6074760 [PMID: 28706954 DOI: 10.1155/2017/6074760]

53 **Ding R**, Huang T, Han J. Diet/Lifestyle and risk of diabetes and glycemic traits: a Mendelian randomization study. *Lipids Health Dis* 2018; **17**: 18 [PMID: 29375034 DOI: 10.1186/s12944-018-0666-z]

54 **Stadler M**, Tomann L, Storka A, Wolzt M, Peric S, Bieglmayer C, Pacini G, Dickson SL, Brath H, Bech P, Prager R, Korbonits M. Effects of smoking cessation on β-cell function, insulin sensitivity, body weight, and appetite. *Eur J Endocrinol* 2014; **170**: 219-217 [PMID: 24179100 DOI: 10.1530/EJE-13-0590]

55 **Inoue K**, Takeshima F, Kadota K, Yoda A, Tatsuta Y, Nagaura Y, Yoshioka S, Nakamichi S, Nakao K, Ozono Y. Early effects of smoking cessation and weight gain on plasma adiponectin levels and insulin resistance. *Intern Med* 2011; **50**: 707-712 [PMID: 21467702 DOI: 10.2169/internalmedicine.50.4600]

56 **Heggen E**, Svendsen M, Tonstad S. Smoking cessation improves cardiometabolic risk in overweight and obese subjects treated with varenicline and dietary counseling. *Nutr Metab Cardiovasc Dis* 2017; **27**: 335-341 [PMID: 28216282 DOI: 10.1016/j.numecd.2016.12.011]

57 **Voulgari C**, Katsilambros N, Tentolouris N. Smoking cessation predicts amelioration of microalbuminuria in newly diagnosed type 2 diabetes mellitus: a 1-year prospective study. *Metabolism* 2011; **60**: 1456-1464 [PMID: 21489578 DOI: 10.1016/j.metabol.2011.02.014]

58 **Morimoto A**, Tatsumi Y, Deura K, Mizuno S, Ohno Y, Watanabe S. Impact of cigarette smoking on impaired insulin secretion and insulin resistance in Japanese men: The Saku Study. *J Diabetes Investig* 2013; **4**: 274-280 [PMID: 24843666 DOI: 10.1111/jdi.12019]

59 **Bahiru E**, Hsiao R, Phillipson D, Watson KE. Mechanisms and Treatment of Dyslipidemia in Diabetes. *Curr Cardiol Rep* 2021; **23**: 26 [PMID: 33655372 DOI: 10.1007/s11886-021-01455-w]

60 **Gossett LK**, Johnson HM, Piper ME, Fiore MC, Baker TB, Stein JH. Smoking intensity and lipoprotein abnormalities in active smokers. *J Clin Lipidol* 2009; **3**: 372-378 [PMID: 20161531 DOI: 10.1016/j.jacl.2009.10.008]

61 **Chelland Campbell S**, Moffatt RJ, Stamford BA. Smoking and smoking cessation -- the relationship between cardiovascular disease and lipoprotein metabolism: a review. *Atherosclerosis* 2008; **201**: 225-235 [PMID: 18565528 DOI: 10.1016/j.atherosclerosis.2008.04.046]

62 **Kim SW**, Kim HJ, Min K, Lee H, Lee SH, Kim S, Kim JS, Oh B. The relationship between smoking cigarettes and metabolic syndrome: A cross-sectional study with non-single residents of Seoul under 40 years old. *PLoS One* 2021; **16**: e0256257 [PMID: 34411160 DOI: 10.1371/journal.pone.0256257]

63 **Gepner AD**, Piper ME, Johnson HM, Fiore MC, Baker TB, Stein JH. Effects of smoking and smoking cessation on lipids and lipoproteins: outcomes from a randomized clinical trial. *Am Heart J* 2011; **161**: 145-151 [PMID: 21167347 DOI: 10.1016/j.ahj.2010.09.023]

64 **King CC**, Piper ME, Gepner AD, Fiore MC, Baker TB, Stein JH. Longitudinal Impact of Smoking and Smoking Cessation on Inflammatory Markers of Cardiovascular Disease Risk. *Arterioscler Thromb Vasc Biol* 2017; **37**: 374-379 [PMID: 27932354 DOI: 10.1161/ATVBAHA.116.308728]

65 **Takata K**, Imaizumi S, Kawachi E, Suematsu Y, Shimizu T, Abe S, Matsuo Y, Tsukahara H, Noda K, Yahiro E, Zhang B, Uehara Y, Miura S, Saku K. Impact of cigarette smoking cessation on high-density lipoprotein functionality. *Circ J* 2014; **78**: 2955-2962 [PMID: 25319317 DOI: 10.1253/circj.cj-14-0638]

66 **Attard R**, Dingli P, Doggen CJM, Cassar K, Farrugia R, Wettinger SB. The impact of passive and active smoking on inflammation, lipid profile and the risk of myocardial infarction. *Open Heart* 2017; **4**: e000620 [PMID: 28878948 DOI: 10.1136/openhrt-2017-000620]

67 **Nakamura M**, Yamamoto Y, Imaoka W, Kuroshima T, Toragai R, Ito Y, Kanda E, J Schaefer E, Ai M. Relationships between Smoking Status, Cardiovascular Risk Factors, and Lipoproteins in a Large Japanese Population. *J Atheroscler*

Thromb 2021; **28**: 942-953 [PMID: 33116031 DOI: 10.5551/jat.56838]

68 **Reynolds K**, Liese AD, Anderson AM, Dabelea D, Standiford D, Daniels SR, Waitzfelder B, Case D, Loots B, Imperatore G, Lawrence JM. Prevalence of tobacco use and association between cardiometabolic risk factors and cigarette smoking in youth with type 1 or type 2 diabetes mellitus. *J Pediatr* 2011; **158**: 594-601.e1 [PMID: 21129757 DOI: 10.1016/j.jpeds.2010.10.011]

69 **Feodoroff M**, Harjutsalo V, Forsblom C, Thorn L, Wadén J, Tolonen N, Lithovius R, Groop PH. Smoking and progression of diabetic nephropathy in patients with type 1 diabetes. *Acta Diabetol* 2016; **53**: 525-533 [PMID: 26668013 DOI: 10.1007/s00592-015-0822-0]

70 **Afkarian M**, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS, de Boer IH. Clinical Manifestations of Kidney Disease Among US Adults With Diabetes, 1988-2014. *JAMA* 2016; **316**: 602-610 [PMID: 27532915 DOI: 10.1001/jama.2016.10924]

71 **Tziomalos K**, Athyros VG. Diabetic Nephropathy: New Risk Factors and Improvements in Diagnosis. *Rev Diabet Stud* 2015; **12**: 110-118 [PMID: 26676664 DOI: 10.1900/RDS.2015.12.110]

72 **Gambaro G**, Verlato F, Budakovic A, Casara D, Saladini G, Del Prete D, Bertaglia G, Masiero M, Checchetto S, Baggio B. Renal impairment in chronic cigarette smokers. *J Am Soc Nephrol* 1998; **9**: 562-567 [PMID: 9555657 DOI: 10.1681/ASN.V94562]

73 **Ritz E**, Benck U, Franek E, Keller C, Seyfarth M, Clorius J. Effects of smoking on renal hemodynamics in healthy volunteers and in patients with glomerular disease. *J Am Soc Nephrol* 1998; **9**: 1798-1804 [PMID: 9773780 DOI: 10.1681/ASN.V9101798]

74 **Orth SR**. Effects of smoking on systemic and intrarenal hemodynamics: influence on renal function. *J Am Soc Nephrol* 2004; **15** Suppl 1: S58-S63 [PMID: 14684675 DOI: 10.1097/01.asn.0000093461.36097.d5]

75 **Elihimas Júnior UF**, Elihimas HC, Lemos VM, Leão Mde A, Sá MP, França EE, Lemos A, Valente LM, Markman Filho B. Smoking as risk factor for chronic kidney disease: systematic review. *J Bras Nefrol* 2014; **36**: 519-528 [PMID: 25517282 DOI: 10.5935/0101-2800.20140074]

76 **Xia J**, Wang L, Ma Z, Zhong L, Wang Y, Gao Y, He L, Su X. Cigarette smoking and chronic kidney disease in the general population: a systematic review and meta-analysis of prospective cohort studies. *Nephrol Dial Transplant* 2017; **32**: 475-487 [PMID: 28339863 DOI: 10.1093/ndt/gfw452]

77 **Ohkuma T**, Nakamura U, Iwase M, Ide H, Fujii H, Jodai T, Kaizu S, Kikuchi Y, Idewaki Y, Sumi A, Hirakawa Y, Kitazono T. Effects of smoking and its cessation on creatinine- and cystatin C-based estimated glomerular filtration rates and albuminuria in male patients with type 2 diabetes mellitus: the Fukuoka Diabetes Registry. *Hypertens Res* 2016; **39**: 744-751 [PMID: 27250568 DOI: 10.1038/hr.2016.51]

78 **Hammer Y**, Cohen E, Levi A, Krause I. The Relationship between Cigarette Smoking and Renal Function: A Large Cohort Study. *Isr Med Assoc J* 2016; **18**: 553-556 [PMID: 28471605]

79 **Liu G**, Hu Y, Zong G, Pan A, Manson JE, Rexrode KM, Rimm EB, Hu FB, Sun Q. Smoking cessation and weight change in relation to cardiovascular disease incidence and mortality in people with type 2 diabetes: a population-based cohort study. *Lancet Diabetes Endocrinol* 2020; **8**: 125-133 [PMID: 31924561 DOI: 10.1016/S2213-8587(19)30413-9]

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ORIGINAL ARTICLE

Basic Study

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

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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

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 increased levels of endogenous ET-1.

Diabetes is a serious metabolic complication that affects about more than 7% of the world population [14]. An increase in different extracellular matrix (ECM) proteins has been found to be a key pathological factor of diabetes[15,16]. The study by Jain *et al*[12] found an increase in collagen 4α1 and fibronectin both at the mRNA and protein levels. This increase was found in heart and kidney tissues and was found to be ET-1 dependent[12]. In addition, the increase in ECM proteins due to high glucose levels was found to be mediated *via* ET-1[17]**.** ET-1 Levels are in fact increased in patients with diabetes 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 5×10^{10} 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 H_2SO_4 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 2.5×10^{10} 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 A_{450} 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 synthesised. 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

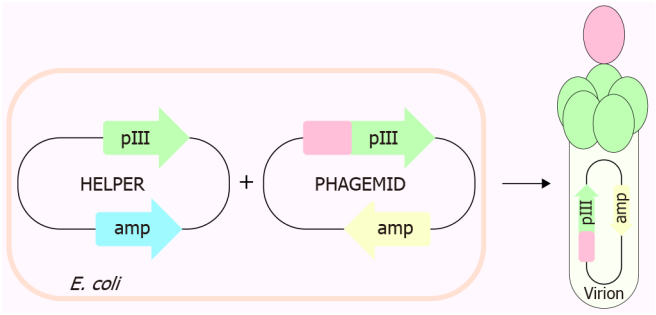

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Figure 1 Depiction of phage display 3 + 3 system.

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 Pharmaceuticals) 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.

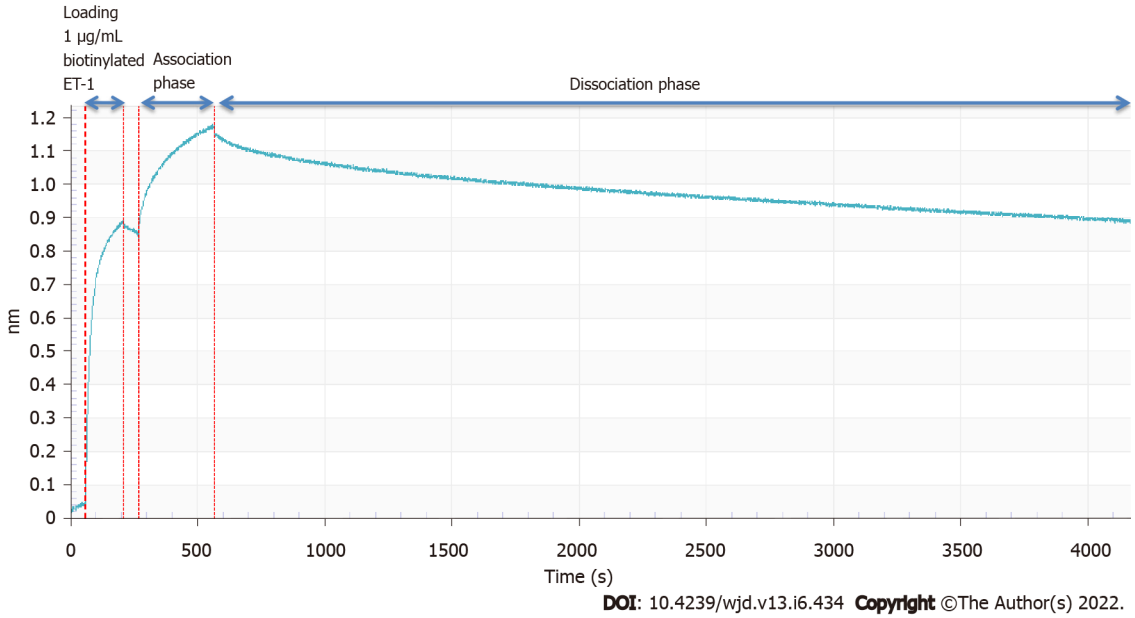

Figure 2 Sensorgram of Fc-β binding to biotinylated endothelin-1. Representative plot shows the binding assay revealed that our selected endothelin (ET)-traps bind strongly to ET-1, displaying double-digit picomolar binding affinity (an average of 73.8 picoMolar, *n* = 6).

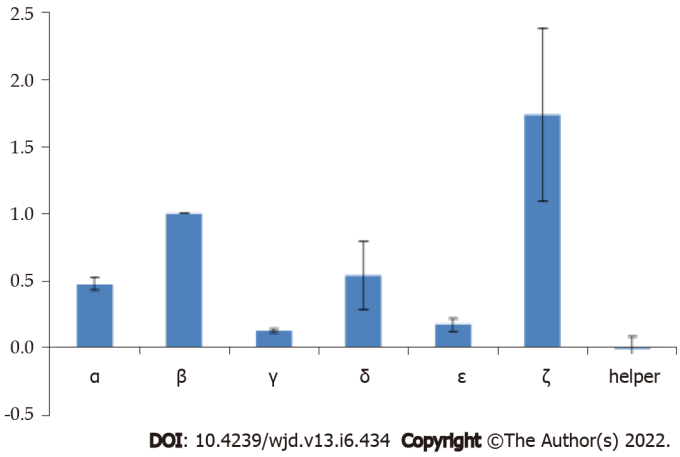

Figure 3 Average relative endothelin-1 binding of endothelin-traps constructs expressing clones using phage display. The final signals are blank-subtracted and normalized to individual expression levels.

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 rM, 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.

Institutional review board statement: This study was approved by ET-traps Limited.

Conflict-of-interest statement: The authors have no conflict of interest.

Data sharing statement: Data available from corresponding author, Dr. Arjun Jain at arjun@et-traps.co.uk.

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REFERENCES

1 **Nelson J**, Bagnato A, Battistini B, Nisen P. The endothelin axis: emerging role in cancer. *Nat Rev Cancer* 2003; **3**: 110-116 [PMID: 12563310 DOI: 10.1038/nrc990]
2 **Böhm F**, Pernow J. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. *Cardiovasc Res* 2007; **76**: 8-18 [PMID: 17617392 DOI: 10.1016/j.cardiores.2007.06.004]
3 **Wernly B**, Jung C. Trapping endothelin-1 to hunt down cardiovascular disease? *Drug Discov Today* 2019; **24**: 2108-2110 [PMID: 31654816 DOI: 10.1016/j.drudis.2019.10.007]
4 **Ergul A**. Endothelin-1 and endothelin receptor antagonists as potential cardiovascular therapeutic agents. *Pharmacotherapy* 2002; **22**: 54-65 [PMID: 11794430 DOI: 10.1592/phco.22.1.54.33505]
5 **Ranno E**, D'Antoni S, Spatuzza M, Berretta A, Laureanti F, Bonaccorso CM, Pellitteri R, Longone P, Spalloni A, Iyer AM, Aronica E, Catania MV. Endothelin-1 is over-expressed in amyotrophic lateral sclerosis and induces motor neuron cell death. *Neurobiol Dis* 2014; **65**: 160-171 [PMID: 24423643 DOI: 10.1016/j.nbd.2014.01.002]
6 **Ferrari CC**, Tarelli R. Parkinson's disease and systemic inflammation. *Parkinsons Dis* 2011; **2011**: 436813 [PMID: 21403862 DOI: 10.4061/2011/436813]
7 **Jain A**. Endothelin-1-induced endoplasmic reticulum stress in disease. *J Pharmacol Exp Ther* 2013; **346**: 163-172 [PMID: 23740603 DOI: 10.1124/jpet.113.205567]
8 **Jain A**, Coffey C, Mehrotra V, Flammer J. Endothelin-1 traps as a potential therapeutic tool: from diabetes to beyond? *Drug Discov Today* 2019; **24**: 1937-1942 [PMID: 31394173 DOI: 10.1016/j.drudis.2019.07.008]
9 **Nelson JB**, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, Simons JW. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. *Nat Med* 1995; **1**: 944-949 [PMID: 7585222 DOI: 10.1038/nm0995-944]
10 **Chiao JW**, Moonga BS, Yang YM, Kancherla R, Mittelman A, Wu-Wong JR, Ahmed T. Endothelin-1 from prostate cancer cells is enhanced by bone contact which blocks osteoclastic bone resorption. *Br J Cancer* 2000; **83**: 360-365 [PMID: 10917552 DOI: 10.1054/bjoc.2000.1261]
11 **Jain A**. Endothelin-1: a key pathological factor in pre-eclampsia? *Reprod Biomed Online* 2012; **25**: 443-449 [PMID: 22995748 DOI: 10.1016/j.rbmo.2012.07.014]
12 **Jain A**, Chen S, Yong H, Chakrabarti S. Endothelin-1 traps potently reduce pathologic markers back to basal levels in an in vitro model of diabetes. *J Diabetes Metab Disord* 2018; **17**: 189-195 [PMID: 30918854 DOI: 10.1007/s40200-018-0360-8]
13 **Jain A**, Mehrotra V, Jha I, Jain A. In vivo studies demonstrate that endothelin-1 traps are a potential therapy for type I diabetes. *J Diabetes Metab Disord* 2019; **18**: 133-143 [PMID: 31275884 DOI: 10.1007/s40200-019-00400-7]
14 **Emerging Risk Factors Collaboration**, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK, Danesh J. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; 375: 2215-2222 [PMID: 20609967 DOI: 10.1016/S0140-6736(10)60484-9]
15 **Chen S**, Mukherjee S, Chakraborty C, Chakrabarti S. High glucose-induced, endothelin-dependent fibronectin synthesis is mediated *via* NF-kappa B and AP-1. *Am J Physiol Cell Physiol* 2003; **284**: C263-C272 [PMID: 12388107 DOI: 10.1152/ajpcell.00192.2002]
16 **Chen S**, Feng B, Thomas AA, Chakrabarti S. miR-146a regulates glucose induced upregulation of inflammatory cytokines extracellular matrix proteins in the retina and kidney in diabetes. *PLoS One* 2017; **12**: e0173918 [PMID: 28301595 DOI: 10.1371/journal.pone.0173918]
17 **Law B**, Fowlkes V, Goldsmith JG, Carver W, Goldsmith EC. Diabetes-induced alterations in the extracellular matrix and their impact on myocardial function. *Microsc Microanal* 2012; **18**: 22-34 [PMID: 22221857 DOI: 10.1017/S1431927611012256]
18 **Seligman BG**, Biolo A, Polanczyk CA, Gross JL, Clausell N. Increased plasma levels of endothelin 1 and von Willebrand factor in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 2000; **23**: 1395-1400 [PMID: 10977040 DOI: 10.2337/diacare.23.9.1395]
19 **Schneider JG**, Tilly N, Hierl T, Sommer U, Hamann A, Dugi K, Leidig-Bruckner G, Kasperk C. Elevated plasma endothelin-1 levels in diabetes mellitus. *Am J Hypertens* 2002; **15**: 967-972 [PMID: 12441216 DOI: 10.1016/s0895-7061(02)03060-1]

20 **Karet FE**, Davenport AP. Endothelin and the human kidney: a potential target for new drugs. *Nephrol Dial Transplant* 1994; **9**: 465-468 [PMID: 8090323 DOI: 10.1093/ndt/9.5.465]

21 **Orry AJ**, Wallace BA. Modeling and docking the endothelin G-protein-coupled receptor. *Biophys J* 2000; **79**: 3083-3094 [PMID: 11106614 DOI: 10.1016/S0006-3495(00)76543-X]

22 **Adachi M**, Hashido K, Trzeciak A, Watanabe T, Furuichi Y, Miyamoto C. Functional domains of human endothelin receptor. *J Cardiovasc Pharmacol* 1993; **22** Suppl 8: S121-S124 [PMID: 7509921 DOI: 10.1097/00005344-199322008-00033]

23 **Davenport AP**, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, Pollock DM, Webb DJ, Maguire JJ. Endothelin. *Pharmacol Rev* 2016; **68**: 357-418 [PMID: 26956245 DOI: 10.1124/pr.115.011833]

24 **Yanagisawa M**, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; **332**: 411-415 [PMID: 2451132 DOI: 10.1038/332411a0]

25 **Inoue A**, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci U S A* 1989; **86**: 2863-2867 [PMID: 2649896 DOI: 10.1073/pnas.86.8.2863]

26 **Ling L**, Maguire JJ, Davenport AP. Endothelin-2, the forgotten isoform: emerging role in the cardiovascular system, ovarian development, immunology and cancer. *Br J Pharmacol* 2013; **168**: 283-295 [PMID: 22118774 DOI: 10.1111/j.1476-5381.2011.01786.x]

27 **Hofstra RM**, Osinga J, Tan-Sindhunata G, Wu Y, Kamsteeg EJ, Stulp RP, van Ravenswaaij-Arts C, Majoor-Krakauer D, Angrist M, Chakravarti A, Meijers C, Buys CH. A homozygous mutation in the endothelin-3 gene associated with a combined Waardenburg type 2 and Hirschsprung phenotype (Shah-Waardenburg syndrome). *Nat Genet* 1996; **12**: 445-447 [PMID: 8630503 DOI: 10.1038/ng0496-445]

28 **Baynash AG**, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE, Yanagisawa M. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell* 1994; **79**: 1277-1285 [PMID: 8001160 DOI: 10.1016/0092-8674(94)90018-3]

29 **Wu CH**, Liu IJ, Lu RM, Wu HC. Advancement and applications of peptide phage display technology in biomedical science. *J Biomed Sci* 2016; **23**: 8 [PMID: 26786672 DOI: 10.1186/s12929-016-0223-x]

30 **Day LA**, Wiseman L. A Comparison of DNA Packaging in the Virions of fd, Xf, and Pf1. Cold Spring Harbor: New York, 1978: 605-625 [DOI: 10.1016/0022-2836(77)90088-2]

31 **Jain A**, Mehrotra V, Yong H, Hiremath K, Jain A, Johnson M, Jha I. Creating a Soluble Binder to Endothelin-1 based on the natural ligand binding domains of the endothelin-1 (G-protein-coupled) receptor. *Int J Pept Res Ther* 2019; **25**: 107-114 [DOI: 10.1007/s10989-017-9653-x]

32 **Smith GP**, Petrenko VA. Phage Display. *Chem Rev* 1997; **97**: 391-410 [PMID: 11848876 DOI: 10.1021/cr960065d]

33 **Zeiher AM**, Goebel H, Schächinger V, Ihling C. Tissue endothelin-1 immunoreactivity in the active coronary atherosclerotic plaque. A clue to the mechanism of increased vasoreactivity of the culprit lesion in unstable angina. *Circulation* 1995; **91**: 941-947 [PMID: 7850978 DOI: 10.1161/01.cir.91.4.941]

34 **Haufschild T**, Shaw SG, Kesselring J, Flammer J. Increased endothelin-1 plasma levels in patients with multiple sclerosis. *J Neuroophthalmol* 2001; **21**: 37-38 [PMID: 11315981 DOI: 10.1097/00041327-200103000-00011]

35 **Pache M**, Kaiser HJ, Akhalbedashvili N, Lienert C, Dubler B, Kappos L, Flammer J. Extraocular blood flow and endothelin-1 plasma levels in patients with multiple sclerosis. *Eur Neurol* 2003; **49**: 164-168 [PMID: 12646761 DOI: 10.1159/000069085]

36 **Sethi AS**, Lees DM, Douthwaite JA, Dawnay AB, Corder R. Homocysteine-induced endothelin-1 release is dependent on hyperglycaemia and reactive oxygen species production in bovine aortic endothelial cells. *J Vasc Res* 2006; **43**: 175-183 [PMID: 16410680 DOI: 10.1159/000090947]

37 **Chang I**, Bramall AN, Baynash AG, Rattner A, Rakheja D, Post M, Joza S, McKerlie C, Stewart DJ, McInnes RR, Yanagisawa M. Endothelin-2 deficiency causes growth retardation, hypothermia, and emphysema in mice. *J Clin Invest* 2013; **123**: 2643-2653 [PMID: 23676500 DOI: 10.1172/JCI66735]

38 **Liu Q**, Cheng J, Lu Y, Zhou J, Wang L, Yang C, Yang G, Yang H, Cao J, Zhang Z, Sun Y. The clinical and genetic research of Waardenburg syndrome type I and II in Chinese families. *Int J Pediatr Otorhinolaryngol* 2020; **130**: 109806 [PMID: 31812001 DOI: 10.1016/j.ijporl.2019.109806]

39 **Sánchez-Mejías A**, Fernández RM, López-Alonso M, Antiñolo G, Borrego S. New roles of EDNRB and EDN3 in the pathogenesis of Hirschsprung disease. *Genet Med* 2010; **12**: 39-43 [PMID: 20009762 DOI: 10.1097/GIM.0b013e3181c371b0]

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ORIGINAL ARTICLE

Basic Study

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

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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 × 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 28.58-34.34 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

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. Moveover, 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 that lowers blood sugar 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 aceti* (*A. aceti*) 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. aceti* 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. aceti* on lowering blood sugar, the present study provides a method for preparing and applying chromium- and zinc-rich *A. aceti*. 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 *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: C11974944); chromium chloride (Shanghai MacLean Biochemical Technology Co., Ltd, batch number: C10717130); zinc chloride (Shanghai MacLean Biochemical Technology Co., Ltd, batch number: C10730413); 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); *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 A. aceti

A. aceti were revived and cultured in a liquid enriched medium, with the concentration of the bacterial solution OD_{600} = 0.9 to 1.5, which is about 3×10^8 to 5×10^8 CFU/mL. To enrich the bacteria with chromium and zinc, *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 1×10^4 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 *A. aceti*.

Detection of coenzymes and metals in A. aceti

Collection of *A. aceti*: Cell suspensions (100 mL) of *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 *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 *A. aceti* suspension taken as the difference OD1-OD2. The *A. aceti* suspension was centrifuged at 8000 rpm for 10 min to remove the supernatant; the precipitate (the *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 *A. aceti* at 0.1 mg/mL.

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

Detection of glucose dehydrogenase in *A. aceti*: Solarbio's "glucose dehydrogenase microplate assay kit" was used to detect glucose dehydrogenase concentrations in *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/6J 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^{-7} mg/mL) and zinc chloride (2×10^{-8} 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 1×10^5 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 1×10^7 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 1×10^5 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 1×10^7 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 1×10^4 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, urinate, 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].

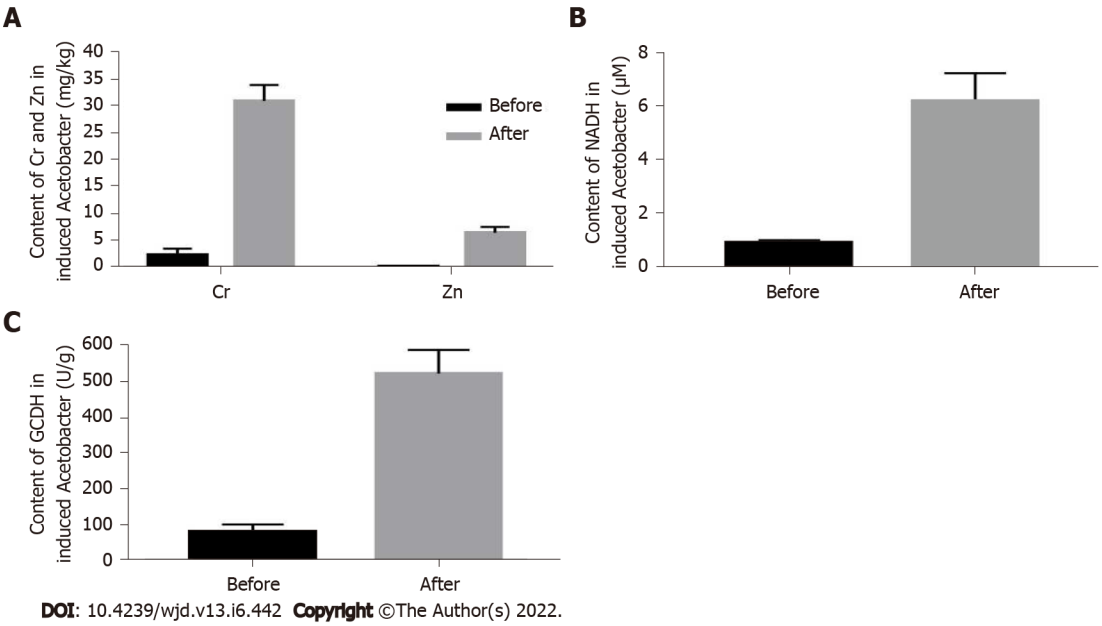


Figure 1 Detection of hypoglycemic components of chromium- and zinc-rich *Acetobacter aceti*. A: Concentration of chromium and zinc; B: Concentration of dihydronicotinamide-adenine dinucleotide; C: Concentration of glucuronic acid dehydrogenase.

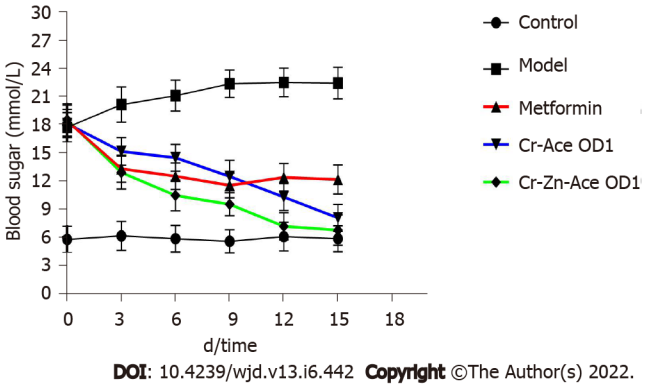
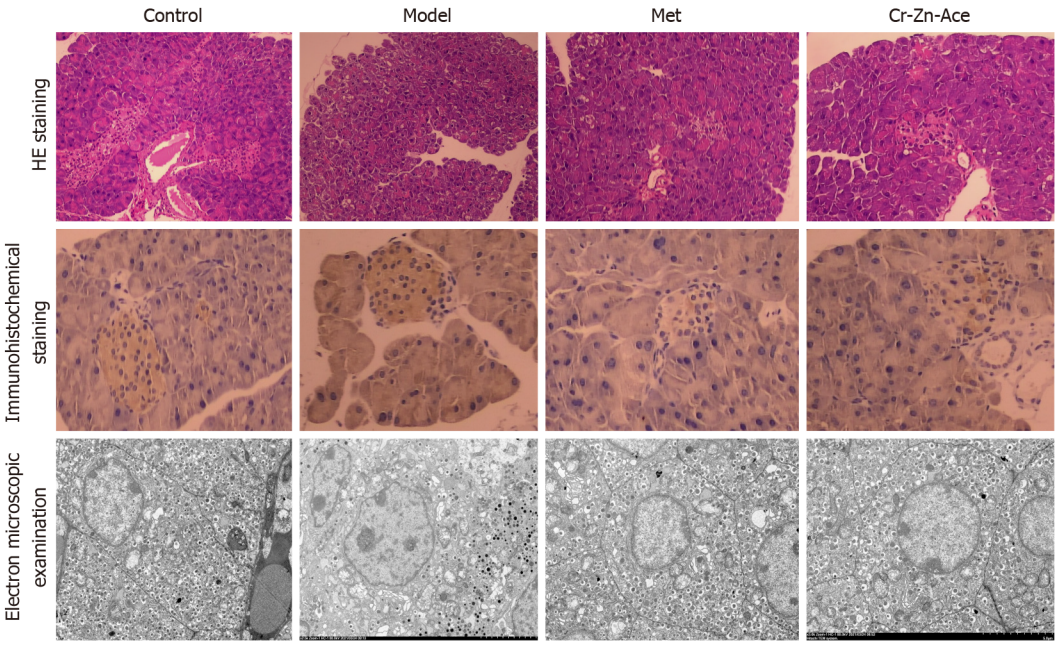


Figure 2 Evaluation of hypoglycemic effects of chromium- and zinc-rich *Acetobacter aceti* on mice with diabetes.

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



DOI: 10.4239/wjd.v13.i6.442

Figure 3 Repair effect of chromium- and zinc-rich *Acetobacter aceti* on tissues and cells of pancreatic islets.

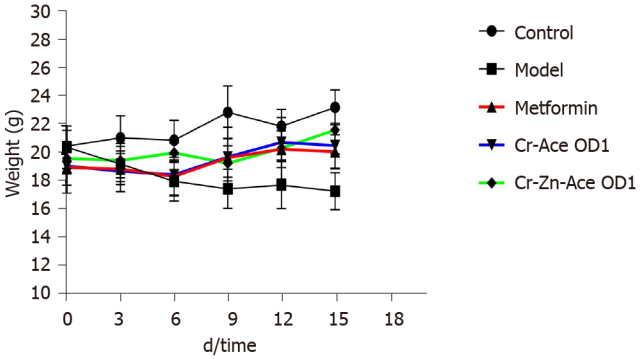


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Figure 4 Effects of chromium- and zinc-rich *Acetobacter aceti* on weight recovery of diabetic mice.

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[31,32]. Studies have found that zinc lowers blood sugar mainly through antioxidant responses, inhibition of inflammatory factors, and anti-apoptosis effects[30,33]. Intracellular zinc has also been found to be regulated by zinc transporters, with the uptake, storage, and distribution regulated by metallothioneins[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[36]. Therefore, increasing the intake of zinc can increase the level of metallothionein, which helps mediate anti-apoptosis, *etc*[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[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 *A. aceti* were co-cultured to induce the production of chromium- and zinc-rich *A. aceti* following simple protocols with high yield. Chromium- and zinc-rich *A. aceti* prepared using this method not only helped *A. aceti* exert the effect of decomposing glucose but also enhanced the hypoglycemic effect seen in untreated *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 *A. aceti* was preliminarily explored, with findings of these bacteria predom-

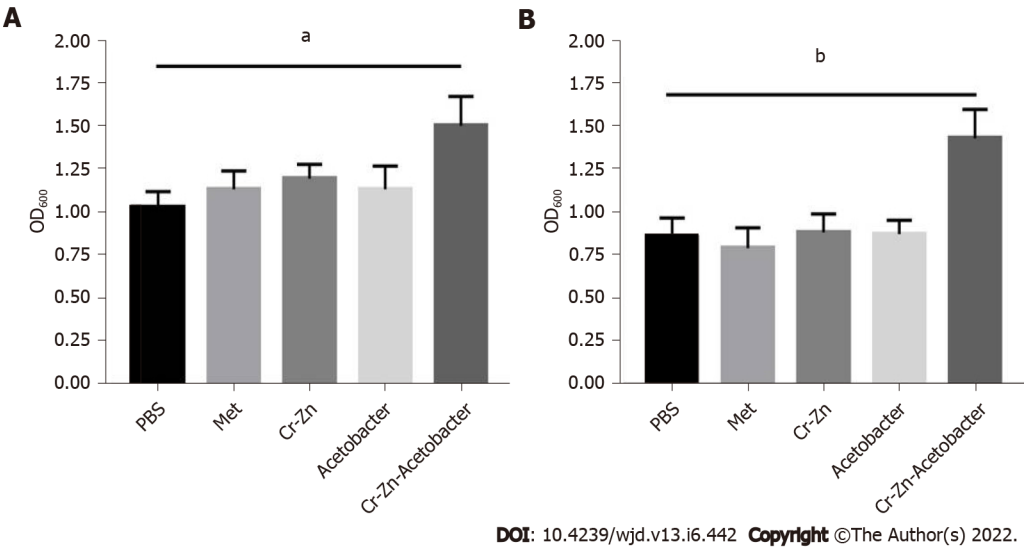

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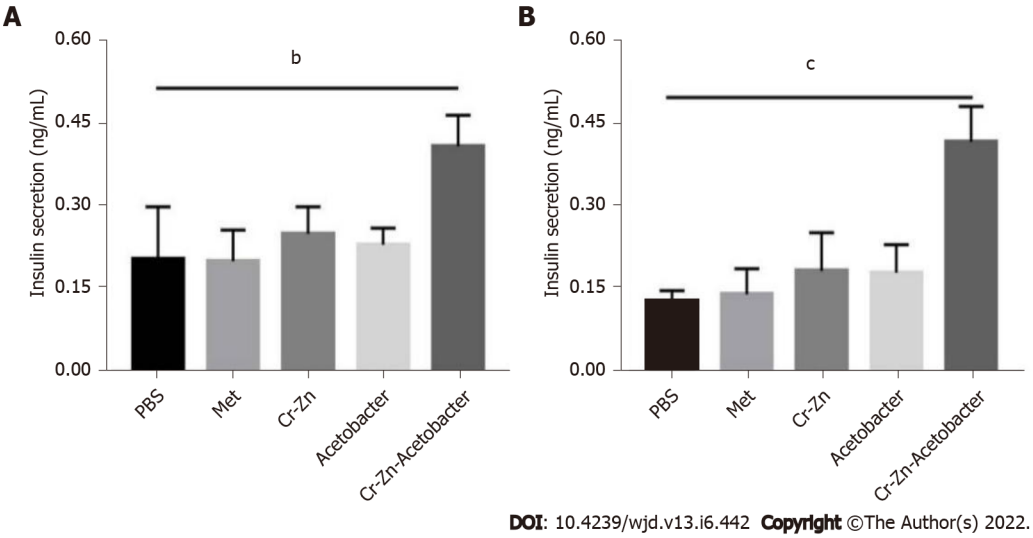
Figure 5 Effects of chromium- and zinc-rich *Acetobacter aceti* on promoting growth of pancreatic islet cells MIN6. A: 5 mmol/L glucose; B: 25 mmol/L glucose. $^{a}P < 0.05$; $^{b}P < 0.01$.



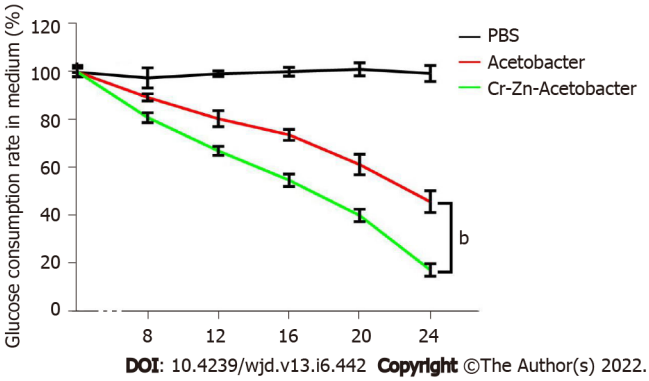
DOI: 10.4239/wjd.v13.i6.442

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.



DOI: 10.4239/wjd.v13.i6.442

Figure 7 Capacity of chromium- and zinc-rich *Acetobacter aceti* for processing glucose. $^{b}P < 0.01$.

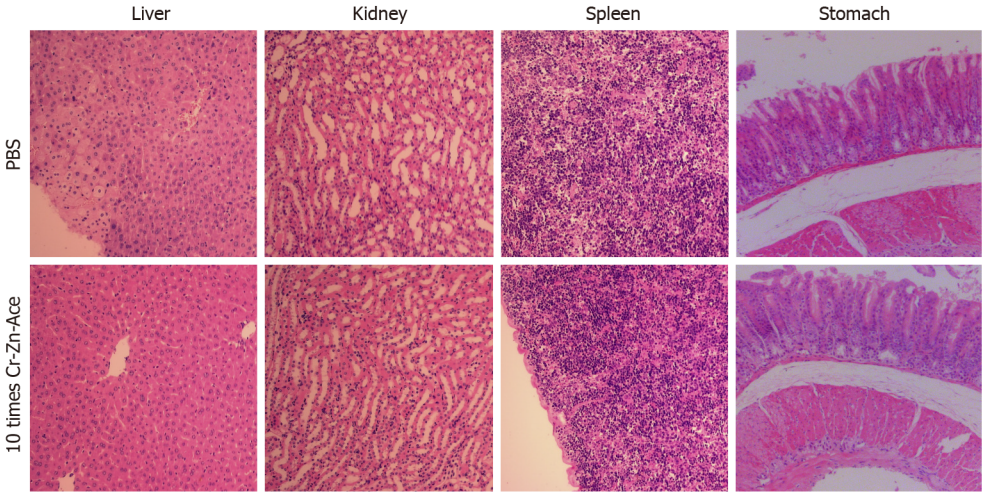


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Figure 8 Safety evaluation of chromium- and zinc-rich *Acetobacter aceti*.

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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Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Data sharing statement: All experimental data are presented in the paper.

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REFERENCES

1 **Kaneko K**, Yatsuya H, Li Y, Uemura M, Chiang C, Hirakawa Y, Ota A, Tamakoshi K, Aoyama A. Risk and population attributable fraction of metabolic syndrome and impaired fasting glucose for the incidence of type 2 diabetes mellitus among middle-aged Japanese individuals: Aichi Worker's Cohort Study. *J Diabetes Investig* 2020; **11**: 1163-1169 [PMID: 32022993 DOI: 10.1111/jdi.13230]

2 **Richter B**, Hemmingsen B, Metzendorf MI, Takwoingi Y. Development of type 2 diabetes mellitus in people with intermediate hyperglycaemia. *Cochrane Database Syst Rev* 2018; **10**: CD012661 [PMID: 30371961 DOI: 10.1002/14651858.CD012661.pub2]

3 National Centre for Statistics & Information of Oman Population Projections 2020-2040, 2017. [cited 1 January 2020]. Available from: https://ncsi.gov.om/Elibrary/LibraryContentDoc/ben_projection-Eng_87f44b27-0bf3-4a80-b2d2-1ead231a0165.pdf

4 **Awad SF**, Al-Mawali A, Al-Lawati JA, Morsi M, Critchley JA, Abu-Raddad LJ. Forecasting the type 2 diabetes mellitus epidemic and the role of key risk factors in Oman up to 2050: Mathematical modeling analyses. *J Diabetes Investig* 2021; **12**: 1162-1174 [PMID: 33112504 DOI: 10.1111/jdi.13452]

5 **Ryder JR**, Northrop E, Rudser KD, Kelly AS, Gao Z, Khoury PR, Kimball TR, Dolan LM, Urbina EM. Accelerated Early Vascular Aging Among Adolescents With Obesity and/or Type 2 Diabetes Mellitus. *J Am Heart Assoc* 2020; **9**: e014891 [PMID: 32370578 DOI: 10.1161/JAHA.119.014891]

6 **Daryabor G**, Atashzar MR, Kabelitz D, Meri S, Kalantar K. The Effects of Type 2 Diabetes Mellitus on Organ Metabolism and the Immune System. *Front Immunol* 2020; **11**: 1582 [PMID: 32793223 DOI: 10.3389/fimmu.2020.01582]

7 **Marín-Peñalver JJ**, Martín-Timón I, Sevillano-Collantes C, Del Cañizo-Gómez FJ. Update on the treatment of type 2 diabetes mellitus. *World J Diabetes* 2016; **7**: 354-395 [PMID: 27660695 DOI: 10.4239/wjd.v7.i17.354]

8 **Chawla R**, Madhu SV, Makkar BM, Ghosh S, Saboo B, Kalra S; RSSDI-ESI Consensus Group. RSSDI-ESI Clinical Practice Recommendations for the Management of Type 2 Diabetes Mellitus 2020. *Indian J Endocrinol Metab* 2020; **24**: 1-

122 [PMID: 32699774 DOI: 10.4103/ijem.IJEM_225_20]
9 **Hemmingsen B**, Sonne DP, Metzendorf MI, Richter B. Dipeptidyl-peptidase (DPP)-4 inhibitors and glucagon-like peptide (GLP)-1 analogues for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk for the development of type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2017; **5**: CD012204 [PMID: 28489279 DOI: 10.1002/14651858.CD012204.pub2]
10 **Blahova J**, Martiniakova M, Babikova M, Kovacova V, Mondockova V, Omelka R. Pharmaceutical Drugs and Natural Therapeutic Products for the Treatment of Type 2 Diabetes Mellitus. *Pharmaceuticals (Basel)* 2021; **14** [PMID: 34451903 DOI: 10.3390/ph14080806]
11 **Nauck MA**. Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. *Drug Des Devel Ther* 2014; **8**: 1335-1380 [PMID: 25246775 DOI: 10.2147/DDDT.S50773]
12 **Artasensi A**, Pedretti A, Vistoli G, Fumagalli L. Type 2 Diabetes Mellitus: A Review of Multi-Target Drugs. *Molecules* 2020; **25** [PMID: 32340373 DOI: 10.3390/molecules25081987]
13 **Zubaidah E**, Afgani CA, Kalsum U, Srianta I, Blanc PJ. Comparison of in vivo antidiabetes activity of snake fruit Kombucha, black tea Kombucha and metformin. *Biocatal Agric Biotechnol* 2018; **17**: 465-469 [DOI: 10.1016/j.bcab.2018.12.026]
14 **Cabral M**, Kuxhaus O, Eichelmann F, Kopp JF, Alker W, Hackler J, Kipp AP, Schwerdtle T, Haase H, Schomburg L, Schulze MB. Trace element profile and incidence of type 2 diabetes, cardiovascular disease and colorectal cancer: results from the EPIC-Potsdam cohort study. *Eur J Nutr* 2021; **60**: 3267-3278 [PMID: 33590281 DOI: 10.1007/s00394-021-02494-3]
15 **Bai J**, Xun P, Morris S, Jacobs DR Jr, Liu K, He K. Chromium exposure and incidence of metabolic syndrome among American young adults over a 23-year follow-up: the CARDIA Trace Element Study. *Sci Rep* 2015; **5**: 15606 [PMID: 26489690 DOI: 10.1038/srep15606]
16 **Nahas R**, Moher M. Complementary and alternative medicine for the treatment of type 2 diabetes. *Can Fam Physician* 2009; **55**: 591-596 [PMID: 19509199]
17 **Chen S**, Jin X, Shan Z, Li S, Yin J, Sun T, Luo C, Yang W, Yao P, Yu K, Zhang Y, Cheng Q, Cheng J, Bao W, Liu L. Inverse Association of Plasma Chromium Levels with Newly Diagnosed Type 2 Diabetes: A Case-Control Study. *Nutrients* 2017; **9** [PMID: 28304331 DOI: 10.3390/nu9030294]
18 **Ngala RA**, Awe MA, Nsiah P. The effects of plasma chromium on lipid profile, glucose metabolism and cardiovascular risk in type 2 diabetes mellitus. A case - control study. *PLoS One* 2018; **13**: e0197977 [PMID: 29975702 DOI: 10.1371/journal.pone.0197977]
19 **Ali A**, Ma Y, Reynolds J, Wise JP Sr, Inzucchi SE, Katz DL. Chromium effects on glucose tolerance and insulin sensitivity in persons at risk for diabetes mellitus. *Endocr Pract* 2011; **17**: 16-25 [PMID: 20634174 DOI: 10.4158/EP10131.OR]
20 **Feng W**, Mao G, Li Q, Wang W, Chen Y, Zhao T, Li F, Zou Y, Wu H, Yang L, Wu X. Effects of chromium malate on glycometabolism, glycometabolism-related enzyme levels and lipid metabolism in type 2 diabetic rats: A dose-response and curative effects study. *J Diabetes Investig* 2015; **6**: 396-407 [PMID: 26221518 DOI: 10.1111/jdi.12350]
21 **Kolahian S**, Sadri H, Shahbazfar AA, Amani M, Mazadeh A, Mirani M. The Effects of Leucine, Zinc, and Chromium Supplements on Inflammatory Events of the Respiratory System in Type 2 Diabetic Rats. *PLoS One* 2015; **10**: e0133374 [PMID: 26185997 DOI: 10.1371/journal.pone.0133374]
22 **Zhang Q**, Sun X, Xiao X, Zheng J, Li M, Yu M, Ping F, Wang Z, Qi C, Wang T, Wang X. Maternal Chromium Restriction Leads to Glucose Metabolism Imbalance in Mice Offspring through Insulin Signaling and Wnt Signaling Pathways. *Int J Mol Sci* 2016; **17** [PMID: 27782077 DOI: 10.3390/ijms17101767]
23 **Hua Y**, Clark S, Ren J, Sreejayan N. Molecular mechanisms of chromium in alleviating insulin resistance. *J Nutr Biochem* 2012; **23**: 313-319 [PMID: 22423897 DOI: 10.1016/j.jnutbio.2011.11.001]
24 **Ruth MR**, Wang Y, Yu HM, Goruk S, Reaney MJ, Proctor SD, Vine DF, Field CJ. Vaccenic and elaidic acid modify plasma and splenocyte membrane phospholipids and mitogen-stimulated cytokine production in obese insulin resistant JCR: LA-cp rats. *Nutrients* 2010; **2**: 181-197 [PMID: 22254015 DOI: 10.3390/nu2020181]
25 **Zhuo J**, Zeng Q, Cai D, Zeng X, Chen Y, Gan H, Huang X, Yao N, Huang D, Zhang C. Evaluation of type 2 diabetic mellitus animal models *via* interactions between insulin and mitogenactivated protein kinase signaling pathways induced by a high fat and sugar diet and streptozotocin. *Mol Med Rep* 2018; **17**: 5132-5142 [PMID: 29393432 DOI: 10.3892/mmr.2018.8504]
26 **Hamedifard Z**, Farrokhian A, Reiner Ž, Bahmani F, Asemi Z, Ghotbi M, Taghizadeh M. The effects of combined magnesium and zinc supplementation on metabolic status in patients with type 2 diabetes mellitus and coronary heart disease. *Lipids Health Dis* 2020; **19**: 112 [PMID: 32466773 DOI: 10.1186/s12944-020-01298-4]
27 **Pompano LM**, Boy E. Effects of Dose and Duration of Zinc Interventions on Risk Factors for Type 2 Diabetes and Cardiovascular Disease: A Systematic Review and Meta-Analysis. *Adv Nutr* 2021; **12**: 141-160 [PMID: 32722790 DOI: 10.1093/advances/nmaa087]
28 **Cheng Y**, Mao N, Fan J. Comment to Sylwia Olechnowicz-Tietz *et al*.: The risk of atherosclerosis in patients with chronic kidney disease. *Int Urol Nephrol* 2013; **45**: 1243-1244 [PMID: 23644777 DOI: 10.1007/s11255-013-0444-9]
29 **Cooper-Capetini V**, de Vasconcelos DAA, Martins AR, Hirabara SM, Donato J Jr, Carpinelli AR, Abdulkader F. Zinc Supplementation Improves Glucose Homeostasis in High Fat-Fed Mice by Enhancing Pancreatic β-Cell Function. *Nutrients* 2017; **9**: 1150 [PMID: 29053582 DOI: 10.3390/nu9101150]
30 **Merriman C**, Huang Q, Rutter GA, Fu D. Lipid-tuned Zinc Transport Activity of Human ZnT8 Protein Correlates with Risk for Type-2 Diabetes. *J Biol Chem* 2016; **291**: 26950-26957 [PMID: 27875315 DOI: 10.1074/jbc.M116.764605]
31 **Fukunaka A**, Fujitani Y. Role of Zinc Homeostasis in the Pathogenesis of Diabetes and Obesity. *Int J Mol Sci* 2018; **19** [PMID: 29415457 DOI: 10.3390/ijms19020476]
32 **Liu Y**, Batchuluun B, Ho L, Zhu D, Prentice KJ, Bhattacharjee A, Zhang M, Pourasgari F, Hardy AB, Taylor KM, Gaisano H, Dai FF, Wheeler MB. Characterization of Zinc Influx Transporters (ZIPs) in Pancreatic β Cells: roles in regulating cytosolic zinc homeostasis and insulin secretion. *J Biol Chem* 2015; **290**: 18757-18769 [PMID: 25969539 DOI: 10.1074/jbc.M115.640524]

33 **Choi S**, Liu X, Pan Z. Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. *Acta Pharmacol Sin* 2018; **39**: 1120-1132 [PMID: 29926844 DOI: 10.1038/aps.2018.25]

34 **Aydemir TB**, Troche C, Kim MH, Cousins RJ. Hepatic ZIP14-mediated Zinc Transport Contributes to Endosomal Insulin Receptor Trafficking and Glucose Metabolism. *J Biol Chem* 2016; **291**: 23939-23951 [PMID: 27703010 DOI: 10.1074/jbc.M116.748632]

35 **Giacconi R**, Cai L, Costarelli L, Cardelli M, Malavolta M, Piacenza F, Provinciali M. Implications of impaired zinc homeostasis in diabetic cardiomyopathy and nephropathy. *Biofactors* 2017; **43**: 770-784 [PMID: 28845600 DOI: 10.1002/biof.1386]

36 **Chang KL**, Hung TC, Hsieh BS, Chen YH, Chen TF, Cheng HL. Zinc at pharmacologic concentrations affects cytokine expression and induces apoptosis of human peripheral blood mononuclear cells. *Nutrition* 2006; **22**: 465-474 [PMID: 16472982 DOI: 10.1016/j.nut.2005.11.009]

37 **Sanna A**, Firinu D, Zavattari P, Valera P. Zinc Status and Autoimmunity: A Systematic Review and Meta-Analysis. *Nutrients* 2018; **10** [PMID: 29324654 DOI: 10.3390/nu10010068]

38 **Fernández-Cao JC**, Warthon-Medina M, H Moran V, Arija V, Doepking C, Serra-Majem L, Lowe NM. Zinc Intake and Status and Risk of Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. *Nutrients* 2019; **11** [PMID: 31071930 DOI: 10.3390/nu11051027]

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ORIGINAL ARTICLE

Retrospective Study

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

Chrong-Reen Wang, Hung-Wen Tsai

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Abstract

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 ± 1.036 *vs* 2.292 ± 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 ± 0.790 *vs* 2.545 ± 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

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.

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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

[21]. This JAKi can act on the JAK/STAT pathway to block the intracellular signaling of multiple cytokines and hormones involved in the pathogeneses 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, naïve 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/m^2 to 26.3 kg/m^2 (22.6 ± 2.0 kg/m^2), following the obesity definition of at least 27 kg/m^2 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 naïve 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 naïve 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 = 0.379, 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 naïve 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 naïve 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 the 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, 89% 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 4.54 to 6.74 (5.265 ± 0.547), 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 ± 1.080, 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 the in low-IR group (0.3589 ± 0.0059 *vs* 0.3648 ± 0.0204, P = 0.813; Figure 2F).

Table 1 Baseline data of 30 active rheumatoid arthritis patients naïve to biologics

Group	All (*n* = 30)	High-IR (*n* = 18)	Low-IR (*n* = 12)	*P* value[1]
Sex (female %)	83.3	77.8	91.7	0.622
Age (yr)	50.3 ± 11.4 (30-74)	49.2 ± 10.5 (30-65)	51.8 ± 12.9 (31-74)	0.445
BMI (kg/m^2)	22.32 ± 1.93 (19.3-26.3)	22.56 ± 2.15 (19.7-26.3)	21.97 ± 1.57 (19.3-24.8)	0.624
Seropositivity (%)	86.7	83.3	91.7	0.632
DAS28	6.291 ± 0.530 (5.16-7.37)	6.499 ± 0.472 (5.56-7.37)	5.980 ± 0.470 (5.16-6.69)	0.008
ESR (mm/h)	51.7 ± 17.2 (26-88)	54.4 ± 18.5 (28-88)	47.6 ± 14.8 (28-70)	0.279
CRP (mg/L)	21.20 ± 6.90 (10.4-36.5)	22.27 ± 7.31 (10.4-36.5)	19.71 ± 6.21 (10.7-29.5)	0.341
Glucose (mg/dL)	88.7 ± 8.5 (66-104)	90.8 ± 9.3 (66-104)	85.6 ± 6.1 (77-98)	0.035
Insulin (μU/mL)	11.870 ± 5.029 (4.64-24.84)	14.710 ± 4.527 (9.07-24.84)	7.605 ± 1.410 (4.64-9.14)	< 0.001
HOMA-IR	2.639 ± 1.185 (1.07-5.89)	3.331 ± 1.036 (2.04-5.89)	1.602 ± 0.294 (1.07-2.00)	< 0.001
QUICKI	0.3353 ± 0.0222 (0.296-0.380)	0.3207 ± 0.0135 (0.296-0.343)	0.3573 ± 0.0117 (0.344-0.380)	< 0.001
Methotrexate (%)	100	100	100	1.0
Dosage (mg/wk)	15	15	15	1.0
Prednisolone (%)	26.7	22.2	33.3	0.678
Daily dosage[2] (mg/d)	5.6 ± 1.8	6.3 ± 2.5	5.0 ± 0.0	1.0
Total dosage[3] (mg)	865.6 ± 258.4	887.5 ± 386.5	843.8 ± 71.8	0.914
Hydroxychloroquine (%)	100	100	100	1.0
Sulfasalazine (%)	20.0	16.7	25.0	0.660
Leflunomide (%)	10.0	11.1	8.3	1.0

[1]High-IR *vs* Low-IR.
[2]Average daily prednisolone dosage in 1-mo period before enrolment into this study.
[3]Total exposure of prednisone dosages in 6-mo period before enrolment into this study.
HOMA-IR: Homeostatic model assessment-insulin resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; BMI: Body mass index; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: Disease Activity Score 28.

DISCUSSION

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 (3.331 ± 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/m^2), 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 *vs* with IR reduction: 24.53 ± 2.07 *vs* 22.49 ± 1.91 kg/m^2, 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

Group	Before	After	*P* value[1]
All (n = 30)			
DAS28	6.291 ± 0.530 (5.16-7.37)	3.101 ± 0.522 (2.08-4.21)	< 0.001
Decrease in DAS28		3.194 ± 0.609 (1.94-4.36)	
HOMA-IR	2.639 ± 1.185 (1.07-5.89)	1.947 ± 0.714 (0.98-4.19)	< 0.001
QUICKI	0.3353 ± 0.0222 (0.296-0.380)	0.3492 ± 0.0183 (0.310-0.385)	< 0.001
High IR (n = 18)			
DAS28	6.499 ± 0.472 (5.56-7.37)	3.006 ± 0.444 (2.52-4.21)	< 0.001
Decrease in DAS28		3.499 ± 0.536 (2.36-4.36)	
HOMA-IR	3.331 ± 1.036 (2.04-5.89)	2.292 ± 0.707 (1.21-4.19)	< 0.001
QUICKI	0.3207 ± 0.0135 (0.296-0.343)	0.3397 ± 0.0154 (0.310-0.372)	< 0.001
Low IR (n = 12)			
DAS28	5.980 ± 0.470 (5.16-6.69)	3.244 ± 0.614 (2.08-3.99)	< 0.001
Decrease in DAS28		2.736 ± 0.389 (1.94-3.23)	
HOMA-IR	1.602 ± 0.294 (1.07-2.00)	1.430 ± 0.293 (0.98-2.02)	0.139
QUICKI	0.3573 ± 0.0117 (0.344-0.380)	0.3634 ± 0.0122 (0.343-0.385)	0.156

[1]Before *vs* after TOF therapy.
HOMA-IR: Homeostatic model assessment-insulin resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; DAS28: Disease Activity Score 28.

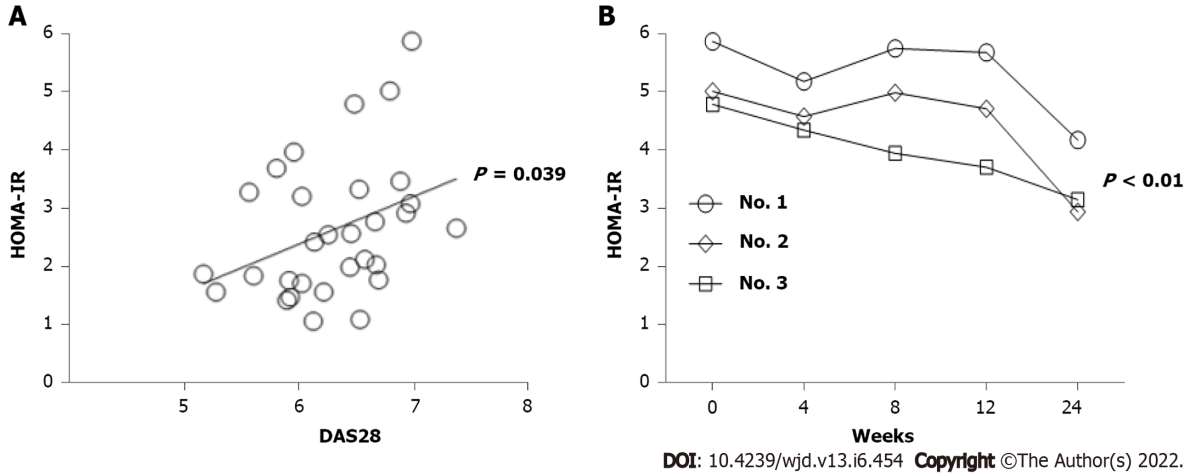

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Figure 1 Characteristics of homeostatic model assessment-insulin resistance levels in active rheumatoid arthritis patients naïve to biologic synthetic disease-modifying anti-rheumatic drugs. A: Positive correlation between 28-joint disease activity 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-TR: 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

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

[1]Before *vs* after TOF therapy.
HOMA-IR: Homeostatic model assessment-insulin resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; TOF: Tofacitinib; DAS28: Disease Activity Score 28.

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.

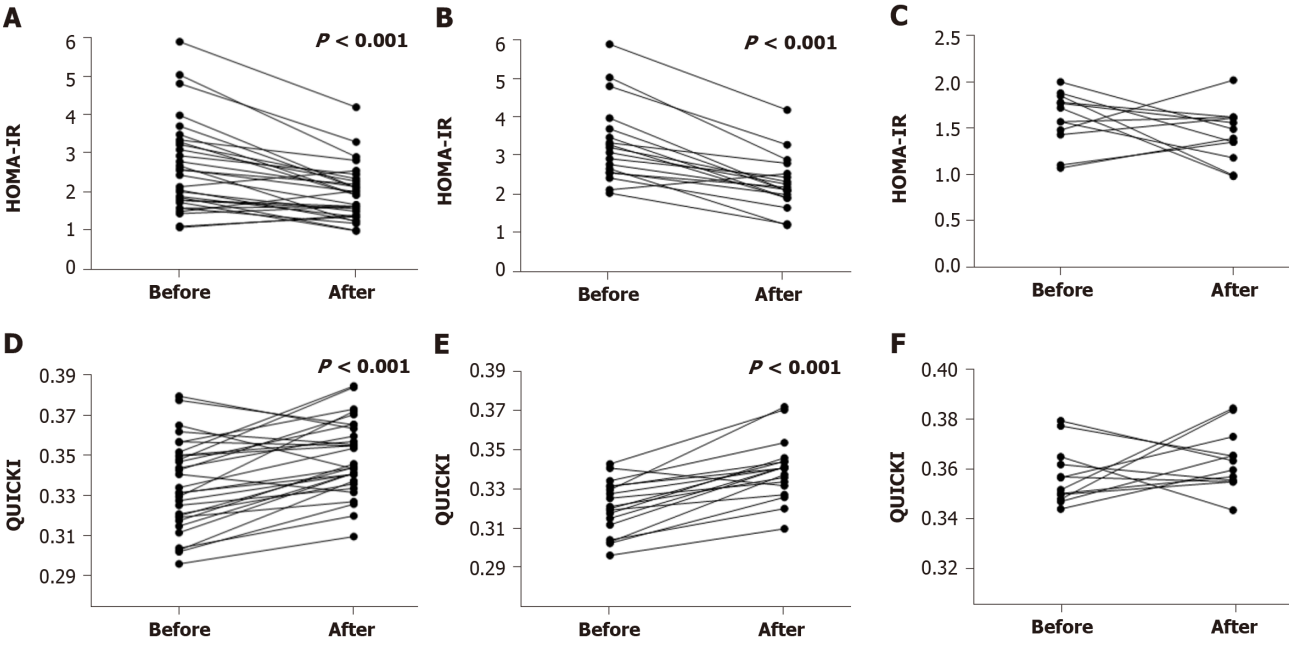

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Figure 2 Homeostatic model assessment-insulin resistance and Quantitative Insulin Sensitivity Check Index levels in 30 active rheumatoid arthritis patients naïve to biologic agents before and 24 wk after tofacitinib therapy. A: Homeostatic model assessment (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.

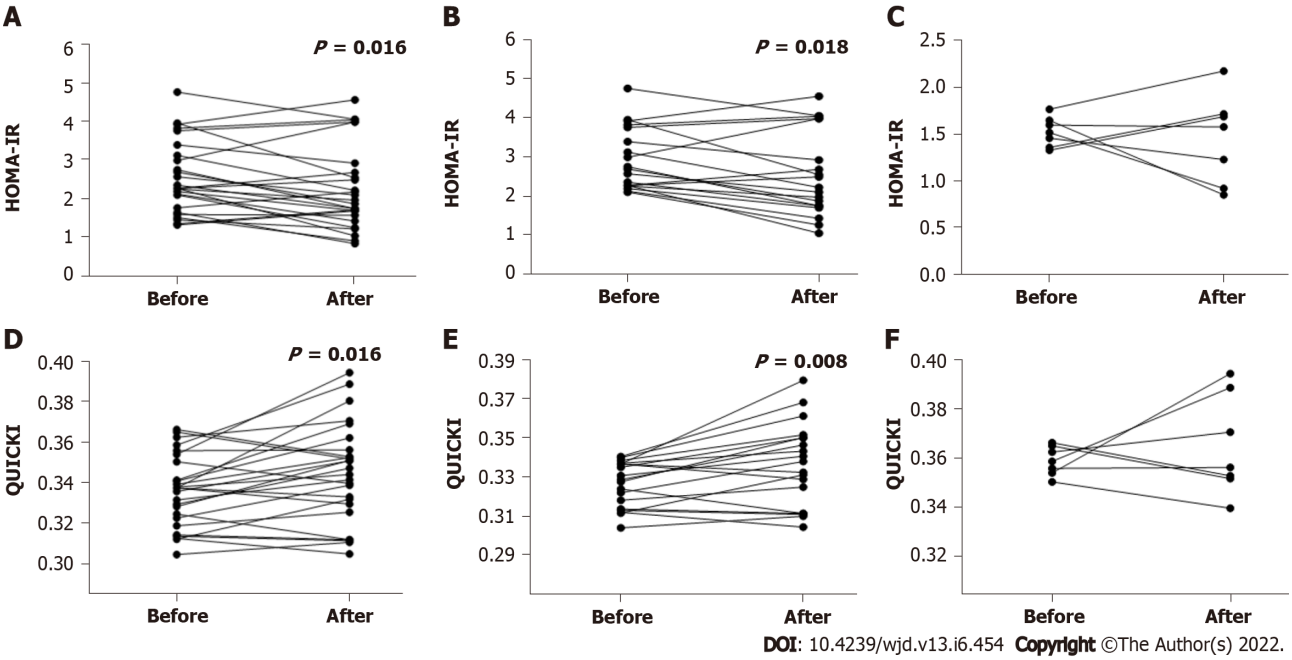

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Figure 3 Homeostatic model assessment-insulin resistance and Quantitative Insulin Sensitivity Check Index levels in 26 active rheumatoid arthritis patients exposed to biologic agents before and 24 wk after tofacitinib therapy. A: Homeostatic model 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-α 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.

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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.

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REFERENCES

1 **Samuel VT**, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest* 2016; **126**: 12-22 [PMID: 26727229 DOI: 10.1172/JCI77812]
2 **Saltiel AR**. Insulin signaling in health and disease. *J Clin Invest* 2021; **131** [PMID: 33393497 DOI: 10.1172/JCI142241]
3 **Khodabandehloo H**, Gorgani-Firuzjaee S, Panahi G, Meshkani R. Molecular and cellular mechanisms linking inflammation to insulin resistance and β-cell dysfunction. *Transl Res* 2016; **167**: 228-256 [PMID: 26408801 DOI: 10.1016/j.trsl.2015.08.011]
4 **Nicolau J**, Lequerré T, Bacquet H, Vittecoq O. Rheumatoid arthritis, insulin resistance, and diabetes. *Joint Bone Spine* 2017; **84**: 411-416 [PMID: 27777170 DOI: 10.1016/j.jbspin.2016.09.001]
5 **Kanety H**, Feinstein R, Papa MZ, Hemi R, Karasik A. Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1. *J Biol Chem* 1995; **270**: 23780-23784 [PMID: 7559552 DOI: 10.1074/jbc.270.40.23780]
6 **Ruan H**, Lodish HF. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. *Cytokine Growth Factor Rev* 2003; **14**: 447-455 [PMID: 12948526 DOI: 10.1016/s1359-6101(03)00052-2]
7 **Wang CR**, Liu MF. Recombinant Soluble TNF-α Receptor Fusion Protein Therapy Reduces Insulin Resistance in Non-Diabetic Active Rheumatoid Arthritis Patients. *ACR Open Rheumatol* 2020; **2**: 401-406 [PMID: 32530139 DOI: 10.1002/acr2.11157]
8 **Fasshauer M**, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, Paschke R. Insulin resistance-inducing cytokines differentially regulate SOCS mRNA expression *via* growth factor- and Jak/Stat-signaling pathways in 3T3-L1 adipocytes. *J Endocrinol* 2004; **181**: 129-138 [PMID: 15072573 DOI: 10.1677/joe.0.1810129]
9 **Rehman K**, Akash MSH, Liaqat A, Kamal S, Qadir MI, Rasul A. Role of Interleukin-6 in Development of Insulin Resistance and Type 2 Diabetes Mellitus. *Crit Rev Eukaryot Gene Expr* 2017; **27**: 229-236 [PMID: 29199608 DOI: 10.1615/CritRevEukaryotGeneExpr.2017019712]
10 **Schultz O**, Oberhauser F, Saech J, Rubbert-Roth A, Hahn M, Krone W, Laudes M. Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases. *PLoS One* 2010; **5**: e14328 [PMID: 21179199 DOI: 10.1371/journal.pone.0014328]
11 **Mirjafari HW**, Wang J, Klearman M, Harari O, Bruce I. FRI0132: Insulin resistance is improved by tocilizumab therapy in rheumatoid arthritis: results from the toward study. *Ann Rheum Dis* 2013; **72**: A12-A15 [DOI: 10.1136/annrheumdis-2013-eular.1259]
12 **Chen DY**, Chen YM, Hsieh TY, Hsieh CW, Lin CC, Lan JL. Significant effects of biologic therapy on lipid profiles and insulin resistance in patients with rheumatoid arthritis. *Arthritis Res Ther* 2015; **17**: 52 [PMID: 25889426 DOI: 10.1186/s13075-015-0559-8]
13 **Castañeda S**, Remuzgo-Martínez S, López-Mejías R, Genre F, Calvo-Alén J, Llorente I, Aurrecoechea E, Ortiz AM, Triguero A, Blanco R, Llorca J, González-Gay MA. Rapid beneficial effect of the IL-6 receptor blockade on insulin resistance and insulin sensitivity in non-diabetic patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2019; **37**: 465-473 [PMID: 30418124]
14 **Gao Z**, Hwang D, Bataille F, Lefevre M, York D, Quon MJ, Ye J. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. *J Biol Chem* 2002; **277**: 48115-48121 [PMID: 12351658 DOI: 10.1074/jbc.M209459200]
15 **Jager J**, Grémeaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF. Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* 2007; **148**: 241-251 [PMID: 17038556 DOI: 10.1210/en.2006-0692]
16 **Ruscitti P**, Masedu F, Alvaro S, Airò P, Battafarano N, Cantarini L, Cantatore FP, Carlino G, D'Abrosca V, Frassi M, Frediani B, Iacono D, Liakouli V, Maggio R, Mulè R, Pantano I, Prevete I, Sinigaglia L, Valenti M, Viapiana O, Cipriani P, Giacomelli R. Anti-interleukin-1 treatment in patients with rheumatoid arthritis and type 2 diabetes (TRACK): A multicentre, open-label, randomised controlled trial. *PLoS Med* 2019; **16**: e1002901 [PMID: 31513665 DOI: 10.1371/journal.pmed.1002901]
17 **Ruscitti P**, Ursini F, Cipriani P, Greco M, Alvaro S, Vasiliki L, Di Benedetto P, Carubbi F, Berardicurti O, Gulletta E, De Sarro G, Giacomelli R. IL-1 inhibition improves insulin resistance and adipokines in rheumatoid arthritis patients with comorbid type 2 diabetes: An observational study. *Medicine (Baltimore)* 2019; **98**: e14587 [PMID: 30762811 DOI: 10.1097/MD.0000000000014587]
18 **Banerjee S**, Biehl A, Gadina M, Hasni S, Schwartz DM. JAK-STAT Signaling as a Target for Inflammatory and Autoimmune Diseases: Current and Future Prospects. *Drugs* 2017; **77**: 521-546 [PMID: 28255960 DOI: 10.1007/s40265-017-0701-9]

19 **Schwartz DM**, Bonelli M, Gadina M, O'Shea JJ. Type I/II cytokines, JAKs, and new strategies for treating autoimmune diseases. *Nat Rev Rheumatol* 2016; **12**: 25-36 [PMID: 26633291 DOI: 10.1038/nrrheum.2015.167]

20 **Schwartz DM**, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat Rev Drug Discov* 2017; **17**: 78 [PMID: 29282366 DOI: 10.1038/nrd.2017.267]

21 **T Virtanen A**, Haikarainen T, Raivola J, Silvennoinen O. Selective JAKinibs: Prospects in Inflammatory and Autoimmune Diseases. *BioDrugs* 2019; **33**: 15-32 [PMID: 30701418 DOI: 10.1007/s40259-019-00333-w]

22 **Wang CR**, Tsai HW. Anti- and non-tumor necrosis factor-α-targeted therapies effects on insulin resistance in rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis. *World J Diabetes* 2021; **12**: 238-260 [PMID: 33758645 DOI: 10.4239/wjd.v12.i3.238]

23 **Migita K**, Izumi Y, Jiuchi Y, Kozuru H, Kawahara C, Izumi M, Sakai T, Nakamura M, Motokawa S, Nakamura T, Kawakami A. Effects of Janus kinase inhibitor tofacitinib on circulating serum amyloid A and interleukin-6 during treatment for rheumatoid arthritis. *Clin Exp Immunol* 2014; **175**: 208-214 [PMID: 24665995 DOI: 10.1111/cei.12234]

24 **Li Y**, Yuan L, Yang J, Lei Y, Zhang H, Xia L, Shen H, Lu J. Changes in Serum Cytokines May Predict Therapeutic Efficacy of Tofacitinib in Rheumatoid Arthritis. *Mediators Inflamm* 2019; **2019**: 5617431 [PMID: 31780862 DOI: 10.1155/2019/5617431]

25 **Chen SK**, Lee H, Jin Y, Liu J, Kim SC. Use of biologic or targeted-synthetic disease-modifying anti-rheumatic drugs and risk of diabetes treatment intensification in patients with rheumatoid arthritis and diabetes mellitus. *Rheumatol Adv Pract* 2020; **4**: rkaa027 [PMID: 32914050 DOI: 10.1093/rap/rkaa027]

26 **Aletaha D**, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, Combe B, Costenbader KH, Dougados M, Emery P, Ferraccioli G, Hazes JM, Hobbs K, Huizinga TW, Kavanaugh A, Kay J, Kvien TK, Laing T, Mease P, Ménard HA, Moreland LW, Naden RL, Pincus T, Smolen JS, Stanislawska-Biernat E, Symmons D, Tak PP, Upchurch KS, Vencovský J, Wolfe F, Hawker G. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010; **62**: 2569-2581 [PMID: 20872595 DOI: 10.1002/art.27584]

27 **Smolen JS**, Breedveld FC, Eberl G, Jones I, Leeming M, Wylie GL, Kirkpatrick J. Validity and reliability of the twenty-eight-joint count for the assessment of rheumatoid arthritis activity. *Arthritis Rheum* 1995; **38**: 38-43 [PMID: 7818569 DOI: 10.1002/art.1780380106]

28 **Singh JA**, Saag KG, Bridges SL Jr, Akl EA, Bannuru RR, Sullivan MC, Vaysbrot E, McNaughton C, Osani M, Shmerling RH, Curtis JR, Furst DE, Parks D, Kavanaugh A, O'Dell J, King C, Leong A, Matteson EL, Schousboe JT, Drevlow B, Ginsberg S, Grober J, St Clair EW, Tindall E, Miller AS, McAlindon T; American College of Rheumatology. 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Care Res (Hoboken)* 2016; **68**: 1-25 [PMID: 26545825 DOI: 10.1002/acr.22783]

29 **Pfützner A**, Kunt T, Hohberg C, Mondok A, Pahler S, Konrad T, Lübben G, Forst T. Fasting intact proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. *Diabetes Care* 2004; **27**: 682-687 [PMID: 14988285 DOI: 10.2337/diacare.27.3.682]

30 **Gallagher L**, Cregan S, Biniecka M, Cunningham C, Veale DJ, Kane DJ, Fearon U, Mullan RH. Insulin-Resistant Pathways Are Associated With Disease Activity in Rheumatoid Arthritis and Are Subject to Disease Modification Through Metabolic Reprogramming: A Potential Novel Therapeutic Approach. *Arthritis Rheumatol* 2020; **72**: 896-902 [PMID: 31840936 DOI: 10.1002/art.41190]

31 **Yun H**, Yang S, Chen L, Xie F, Winthrop K, Baddley JW, Saag KG, Singh J, Curtis JR. Risk of Herpes Zoster in Autoimmune and Inflammatory Diseases: Implications for Vaccination. *Arthritis Rheumatol* 2016; **68**: 2328-2337 [PMID: 26990731 DOI: 10.1002/art.39670]

32 **Liao TL**, Chen YM, Liu HJ, Chen DY. Risk and severity of herpes zoster in patients with rheumatoid arthritis receiving different immunosuppressive medications: a case-control study in Asia. *BMJ Open* 2017; **7**: e014032 [PMID: 28057661 DOI: 10.1136/bmjopen-2016-014032]

33 **Curtis JR**, Xie F, Yun H, Bernatsky S, Winthrop KL. Real-world comparative risks of herpes virus infections in tofacitinib and biologic-treated patients with rheumatoid arthritis. *Ann Rheum Dis* 2016; **75**: 1843-1847 [PMID: 27113415 DOI: 10.1136/annrheumdis-2016-209131]

34 **Johnson AM**, Olefsky JM. The origins and drivers of insulin resistance. *Cell* 2013; **152**: 673-684 [PMID: 23415219 DOI: 10.1016/j.cell.2013.01.041]

35 **Stavropoulos-Kalinoglou A**, Metsios GS, Panoulas VF, Nightingale P, Koutedakis Y, Kitas GD. Anti-tumour necrosis factor alpha therapy improves insulin sensitivity in normal-weight but not in obese patients with rheumatoid arthritis. *Arthritis Res Ther* 2012; **14**: R160 [PMID: 22765047 DOI: 10.1186/ar3900]

36 **van Raalte DH**, Brands M, van der Zijl NJ, Muskiet MH, Pouwels PJ, Ackermans MT, Sauerwein HP, Serlie MJ, Diamant M. Low-dose glucocorticoid treatment affects multiple aspects of intermediary metabolism in healthy humans: a randomised controlled trial. *Diabetologia* 2011; **54**: 2103-2112 [PMID: 21562755 DOI: 10.1007/s00125-011-2174-9]

37 **Peters MJ**, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, McInnes IB, Haentzschel H, Gonzalez-Gay MA, Provan S, Semb A, Sidiropoulos P, Kitas G, Smulders YM, Soubrier M, Szekanecz Z, Sattar N, Nurmohamed MT. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. *Ann Rheum Dis* 2010; **69**: 325-331 [PMID: 19773290 DOI: 10.1136/ard.2009.113696]

38 **Solomon DH**, Massarotti E, Garg R, Liu J, Canning C, Schneeweiss S. Association between disease-modifying antirheumatic drugs and diabetes risk in patients with rheumatoid arthritis and psoriasis. *JAMA* 2011; **305**: 2525-2531 [PMID: 21693740 DOI: 10.1001/jama.2011.878]

39 **Wasko MC**, Hubert HB, Lingala VB, Elliott JR, Luggen ME, Fries JF, Ward MM. Hydroxychloroquine and risk of diabetes in patients with rheumatoid arthritis. *JAMA* 2007; **298**: 187-193 [PMID: 17622600 DOI: 10.1001/jama.298.2.187]

40 **Yazdani-Biuki B**, Stelzl H, Brezinschek HP, Hermann J, Mueller T, Krippl P, Graninger W, Wascher TC. Improvement of insulin sensitivity in insulin resistant subjects during prolonged treatment with the anti-TNF-alpha antibody infliximab. *Eur J Clin Invest* 2004; **34**: 641-642 [PMID: 15379764 DOI: 10.1111/j.1365-2362.2004.01390.x]

41 **Kiortsis DN**, Mavridis AK, Vasakos S, Nikas SN, Drosos AA. Effects of infliximab treatment on insulin resistance in patients with rheumatoid arthritis and ankylosing spondylitis. *Ann Rheum Dis* 2005; **64**: 765-766 [PMID: 15458960 DOI: 10.1136/ard.2004.026534]

42 **Stagakis I**, Bertsias G, Karvounaris S, Kavousanaki M, Virla D, Raptopoulou A, Kardassis D, Boumpas DT, Sidiropoulos PI. Anti-tumor necrosis factor therapy improves insulin resistance, beta cell function and insulin signaling in active rheumatoid arthritis patients with high insulin resistance. *Arthritis Res Ther* 2012; **14**: R141 [PMID: 22691241 DOI: 10.1186/ar3874]

43 **Gurzov EN**, Stanley WJ, Pappas EG, Thomas HE, Gough DJ. The JAK/STAT pathway in obesity and diabetes. *FEBS J* 2016; **283**: 3002-3015 [PMID: 26972840 DOI: 10.1111/febs.13709]

44 **Dodington DW**, Desai HR, Woo M. JAK/STAT - Emerging Players in Metabolism. *Trends Endocrinol Metab* 2018; **29**: 55-65 [PMID: 29191719 DOI: 10.1016/j.tem.2017.11.001]

45 **Shi SY**, Martin RG, Duncan RE, Choi D, Lu SY, Schroer SA, Cai EP, Luk CT, Hopperton KE, Domenichiello AF, Tang C, Naples M, Dekker MJ, Giacca A, Adeli K, Wagner KU, Bazinet RP, Woo M. Hepatocyte-specific deletion of Janus kinase 2 (JAK2) protects against diet-induced steatohepatitis and glucose intolerance. *J Biol Chem* 2012; **287**: 10277-10288 [PMID: 22275361 DOI: 10.1074/jbc.M111.317453]

46 **Corbit KC**, Camporez JPG, Tran JL, Wilson CG, Lowe DA, Nordstrom SM, Ganeshan K, Perry RJ, Shulman GI, Jurczak MJ, Weiss EJ. Adipocyte JAK2 mediates growth hormone-induced hepatic insulin resistance. *JCI Insight* 2017; **2**: e91001 [PMID: 28194444 DOI: 10.1172/jci.insight.91001]

47 **Bako HY**, Ibrahim MA, Isah MS, Ibrahim S. Inhibition of JAK-STAT and NF-κB signalling systems could be a novel therapeutic target against insulin resistance and type 2 diabetes. *Life Sci* 2019; **239**: 117045 [PMID: 31730866 DOI: 10.1016/j.lfs.2019.117045]

48 **Mori T**, Suzuki-Yamazaki N, Takaki S. Lnk/Sh2b3 Regulates Adipose Inflammation and Glucose Tolerance through Group 1 ILCs. *Cell Rep* 2018; **24**: 1830-1841 [PMID: 30110639 DOI: 10.1016/j.celrep.2018.07.036]

49 **Gysemans CA**, Ladrière L, Callewaert H, Rasschaert J, Flamez D, Levy DE, Matthys P, Eizirik DL, Mathieu C. Disruption of the gamma-interferon signaling pathway at the level of signal transducer and activator of transcription-1 prevents immune destruction of beta-cells. *Diabetes* 2005; **54**: 2396-2403 [PMID: 16046307 DOI: 10.2337/diabetes.54.8.2396]

50 **Trivedi PM**, Graham KL, Scott NA, Jenkins MR, Majaw S, Sutherland RM, Fynch S, Lew AM, Burns CJ, Krishnamurthy B, Brodnicki TC, Mannering SI, Kay TW, Thomas HE. Repurposed JAK1/JAK2 Inhibitor Reverses Established Autoimmune Insulitis in NOD Mice. *Diabetes* 2017; **66**: 1650-1660 [PMID: 28292965 DOI: 10.2337/db16-1250]

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LETTER TO THE EDITOR

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

Awadhesh Kumar Singh, Ritu Singh

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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 T2DM having a modest baseline HbA1c (8.0%-8.5%) compared with SGLT-2Is which lowers HbA1c larger in a background of higher baseline HbA1c (> 8.5%-9.0%). These findings suggest that the role of DPP-4Is in the management of type 2 diabetes mellitus cannot be completely ignored even in the era of SGLT-2Is.

Key Words: DPP-4 inhibitors; SGLT-2 inhibitors; GLP-1 receptor agonists; Cardiovascular outcomes; Renal outcomes

Core Tip: Despite the newer anti-diabetic agents such as SGLT-2 inhibitors (SGLT-2Is) and GLP-1 receptor agonists have taken the center stage in the management of type 2 diabetes mellitus due to additional cardiac and renal benefits, the role of DPP-4 inhibitors (DPP-4Is) cannot be undermined. HbA1c lowering potential of DPP-4Is are nearly similar to SGLT-2Is and surprisingly larger in a background of modest baseline HbA1c compared with SGLT-2Is. Moreover, the HbA1c lowering abilities of SGLT-2Is are compromised with declining renal function while DPP-4Is reduce HbA1c favorably in people with chronic kidney disease regardless of impaired kidney functions.

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TO THE EDITOR

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-2I *vs* DPP-4I 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_G), modest baseline HbA1c may not produce further lowering of RT_G.

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 ($P_{interaction}$ = 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 m^2 and in addition, empagliflozin has been granted an additional label of use up to eGFR ≥ 20 mL/min/m^2 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 m^2 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 m^2 unless it is not tolerated or kidney

Table 1 HbA1c reduction with SGLT-2 inhibitors *vs* DPP-4 inhibitors

Ref.	Study duration (wk)	Background therapy	*n* (Active drug)	Baseline HbA1c	SGLT-2I (A) (% HbA1c reduction)	DPP-4I (B) (% HbA1c reduction)	Δ A minus B (95%CI)
HbA1c reduction with SGLT-2Is *vs* DPP-4Is in head-to-head randomized controlled trials							
Rosenstock *et al*[2], 2012	12	Metformin	193	7.6%-7.8%	-0.76 (Cana 100 mg) -0.92 (Cana 300 mg)	-0.74 (Sita 100 mg)	NC, (B) exploratory
Roden *et al*[3], 2013	24	Drug naïve	671	7.9%	-0.66 (Empa 10 mg) -0.78 (Empa 25 mg)	-0.66 (Sita 100 mg)	0.0 (-0.15, 0.14) -0.12 (-0.26, 0.03)
Rosenstock *et al*[4], 2013	12	Metformin	212	7.9%-8.1%	-0.56 (Empa 10 mg) -0.55 (Empa 25 mg)	-0.45 (Sita 100 mg)	NC, (B) exploratory
Ferrannini *et al*[5], 2013	90	Metformin	332	7.9%-8%	-0.34 (Empa 10 mg) -0.63 (Empa 25 mg)	-0.40 (Sita 100 mg)	NC, (B) exploratory
Lavalle-González *et al*[6], 2013	52	Metformin	1079	7.9%	-0.73 (Cana 100 mg) -0.88 (Cana 300 mg[a])	-0.73 (Sita 100 mg)	-0.15[a] (-0.27, -0.03)
Schernthaner *et al*[7], 2013	52	Metformin + SU	755	8.1%	-1.03 (Cana 300 mg[a])	-0.66 (Sita 100 mg)	-0.37[a] (-0.50, -0.25)
Amin *et al*[8], 2015	12	Metformin	328	8.1%	-0.80 (Ertu 5 mg)	-0.87 (Sita 100 mg)	NC, (B) exploratory
Difference in HbA1c reduction with SGLT-2Is *vs* DPP-4Is in meta-analyses							
Pinto *et al*[9], 2015	≥ 12	LSM, Metformin, SU	NR (6 studies)	-	SGLT-2Is	DPP-4Is	-0.15[a] (-0.21, -0.08)
Maruthur *et al*[10], 2016	≤ 52	Metformin	1278 (4 studies)	-	SGLT-2Is	DPP-4Is	(B) minus (A) = +0.17[a] (0.08, 0.26)
Wang *et al*[11], 2018	12-78	Metformin	3454 (7 studies)	-	SGLT-2Is	DPP-4Is	(B) minus (A) = +0.11 (-0.03, 0.25)
Mishriky *et al*[12], 2018	≤ 26	Metformin	2462 (6 studies)	-	SGLT-2Is	DPP-4Is	(B) minus (A) = +0.05 (-0.05, 0.16)
	≥ 52	Metformin	1872 (3 studies)	-	SGLT-2Is	DPP-4Is	(B) minus (A) = +0.11[a] (0.03, 0.20)
HbA1c reduction with SGLT-2Is *vs* DPP-4Is in head-to-head randomized controlled trial stratified on baseline HbA1c							
Rosenstock *et al*[13], 2015	24	Metformin	190	> 9%	-1.87 (Dapa 10 mg)	-1.32 (Saxa 5 mg)	NC
			103	< 8%	-0.45 (Dapa 10 mg)	-0.69 (Saxa 5 mg)	
Lewin *et al*[14], 2015	24	LSM	116	≥ 8.5%	-1.66 (Empa 25 mg) -1.54 (Empa 10 mg	-1.07 (Lina 5 mg)	NC
			473	< 8.5%	-0.66 (Empa 25 mg) -0.56 (Empa 10 mg)	-0.55 (Lina 5 mg)	NC
DeFronzo *et al*[15], 2015	24	Metformin	101	≥ 8.5%	-1.22 (Empa 25 mg) -1.29 (Empa 10 mg)	-0.99 (Lina 5 mg)	NC
			508	< 8.5%	-0.43 (Empa 25 mg) -0.46 (Empa 10 mg)	-0.62 (Lina 5 mg)	NC

[a](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.

replacement therapy is initiated[19]; (5) Although there are no head-to-head randomized controlled trials that compared CV outcomes between DPP-4Is *vs* DPP-4Is, several large real-world, propensity-matched studies showed a significant reduction in HHF with SGLT-2Is compared with DPP-4Is in patients with T2DM, regardless of baseline high CV risk[20]; and (6) Finally, the 2011 European Diabetes Working Party for Older People clinical guideline that recommended DPP-4I as a second-line drug of choice in elderly were made before the US Federal Drug Administration approval of first SGLT-2I

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

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REFERENCES

1 **Florentin M**, Kostapanos MS, Papazafiropoulou AK. Role of dipeptidyl peptidase 4 inhibitors in the new era of antidiabetic treatment. *World J Diabetes* 2022; **13**: 85-96 [PMID: 35211246 DOI: 10.4239/wjd.v13.i2.85]
2 **Rosenstock J**, Aggarwal N, Polidori D, Zhao Y, Arbit D, Usiskin K, Capuano G, Canovatchel W; Canagliflozin DIA 2001 Study Group. Dose-ranging effects of canagliflozin, a sodium-glucose cotransporter 2 inhibitor, as add-on to metformin in subjects with type 2 diabetes. *Diabetes Care* 2012; **35**: 1232-1238 [PMID: 22492586 DOI: 10.2337/dc11-1926]
3 **Roden M**, Weng J, Eilbracht J, Delafont B, Kim G, Woerle HJ, Broedl UC; EMPA-REG MONO trial investigators. Empagliflozin monotherapy with sitagliptin as an active comparator in patients with type 2 diabetes: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Diabetes Endocrinol* 2013; **1**: 208-219 [PMID: 24622369 DOI: 10.1016/S2213-8587(13)70084-6]
4 **Rosenstock J**, Seman LJ, Jelaska A, Hantel S, Pinnetti S, Hach T, Woerle HJ. Efficacy and safety of empagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, as add-on to metformin in type 2 diabetes with mild hyperglycaemia. *Diabetes Obes Metab* 2013; **15**: 1154-1160 [PMID: 23906374 DOI: 10.1111/dom.12185]
5 **Ferrannini E**, Berk A, Hantel S, Pinnetti S, Hach T, Woerle HJ, Broedl UC. Long-term safety and efficacy of empagliflozin, sitagliptin, and metformin: an active-controlled, parallel-group, randomized, 78-week open-label extension study in patients with type 2 diabetes. *Diabetes Care* 2013; **36**: 4015-4021 [PMID: 24186878 DOI: 10.2337/dc13-0663]
6 **Lavalle-González FJ**, Januszewicz A, Davidson J, Tong C, Qiu R, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin compared with placebo and sitagliptin in patients with type 2 diabetes on background metformin monotherapy: a randomised trial. *Diabetologia* 2013; **56**: 2582-2592 [PMID: 24026211 DOI: 10.1007/s00125-013-3039-1]
7 **Schernthaner G**, Gross JL, Rosenstock J, Guarisco M, Fu M, Yee J, Kawaguchi M, Canovatchel W, Meininger G. Canagliflozin compared with sitagliptin for patients with type 2 diabetes who do not have adequate glycemic control with metformin plus sulfonylurea: a 52-week randomized trial. *Diabetes Care* 2013; **36**: 2508-2515 [PMID: 23564919 DOI: 10.2337/dc12-2491]
8 **Amin NB**, Wang X, Jain SM, Lee DS, Nucci G, Rusnak JM. Dose-ranging efficacy and safety study of ertugliflozin, a sodium-glucose co-transporter 2 inhibitor, in patients with type 2 diabetes on a background of metformin. *Diabetes Obes Metab* 2015; **17**: 591-598 [PMID: 25754396 DOI: 10.1111/dom.12460]
9 **Pinto LC**, Rados DV, Remonti LR, Kramer CK, Leitao CB, Gross JL. Efficacy of SGLT2 inhibitors in glycemic control, weight loss and blood pressure reduction: a systematic review and meta-analysis. *Diabetol Metab Syndr* 2015; **7**: A58 [DOI: 10.1186/1758-5996-7-s1-a58]
10 **Maruthur NM**, Tseng E, Hutfless S, Wilson LM, Suarez-Cuervo C, Berger Z, Chu Y, Iyoha E, Segal JB, Bolen S. Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis. *Ann Intern Med* 2016; **164**: 740-751 [PMID: 27088241 DOI: 10.7326/M15-2650]
11 **Wang Z**, Sun J, Han R, Fan D, Dong X, Luan Z, Xiang R, Zhao M, Yang J. Efficacy and safety of sodium-glucose cotransporter-2 inhibitors *vs* dipeptidyl peptidase-4 inhibitors as monotherapy or add-on to metformin in patients with type 2 diabetes mellitus: A systematic review and meta-analysis. *Diabetes Obes Metab* 2018; **20**: 113-120 [PMID: 28656707 DOI: 10.1111/dom.13047]
12 **Mishriky BM**, Tanenberg RJ, Sewell KA, Cummings DM. Comparing SGLT-2 inhibitors to DPP-4 inhibitors as an add-on therapy to metformin in patients with type 2 diabetes: A systematic review and meta-analysis. *Diabetes Metab* 2018; **44**: 112-120 [PMID: 29477373 DOI: 10.1016/j.diabet.2018.01.017]
13 **Rosenstock J**, Hansen L, Zee P, Li Y, Cook W, Hirshberg B, Iqbal N. Dual add-on therapy in type 2 diabetes poorly

controlled with metformin monotherapy: a randomized double-blind trial of saxagliptin plus dapagliflozin addition *vs* single addition of saxagliptin or dapagliflozin to metformin. *Diabetes Care* 2015; **38**: 376-383 [PMID: 25352655 DOI: 10.2337/dc14-1142]

14 **Lewin A**, DeFronzo RA, Patel S, Liu D, Kaste R, Woerle HJ, Broedl UC. Initial combination of empagliflozin and linagliptin in subjects with type 2 diabetes. *Diabetes Care* 2015; **38**: 394-402 [PMID: 25633662 DOI: 10.2337/dc14-2365]

15 **DeFronzo RA**, Lewin A, Patel S, Liu D, Kaste R, Woerle HJ, Broedl UC. Combination of empagliflozin and linagliptin as second-line therapy in subjects with type 2 diabetes inadequately controlled on metformin. *Diabetes Care* 2015; **38**: 384-393 [PMID: 25583754 DOI: 10.2337/dc14-2364]

16 **Martinez FA**, Serenelli M, Nicolau JC, Petrie MC, Chiang CE, Tereshchenko S, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Ponikowski P, Sabatine MS, DeMets DL, Dutkiewicz-Piasecka M, Bengtsson O, Sjöstrand M, Langkilde AM, Jhund PS, McMurray JJV. Efficacy and Safety of Dapagliflozin in Heart Failure With Reduced Ejection Fraction According to Age: Insights From DAPA-HF. *Circulation* 2020; **141**: 100-111 [PMID: 31736328 DOI: 10.1161/CIRCULATIONAHA.119.044133]

17 **Singh AK**, Singh R. Similarities and differences in cardio-renal outcome trials with SGLT-2 inhibitors: call for pharmacogenomic studies? *Pharmacogenomics Res Pers Med* 2022 [DOI: 10.21037/prpm-22-2]

18 **Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group**. . KDIGO 2020 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. *Kidney Int* 2020; **98**: S1-S115 [PMID: 32998798 DOI: 10.1016/j.kint.2020.06.019]

19 KDIGO 2022 Clinical Practice Guidelines for Diabetes Management in Chronic Kidney Disease. [Accessed March 5, 2022]. Available from: https://kdigo.org

20 **Singh AK**, Singh R. Heart failure hospitalization with SGLT-2 inhibitors: a systematic review and meta-analysis of randomized controlled and observational studies. *Expert Rev Clin Pharmacol* 2019; **12**: 299-308 [PMID: 30817235 DOI: 10.1080/17512433.2019.1588110]

21 **Sinclair AJ**, Paolisso G, Castro M, Bourdel-Marchasson I, Gadsby R, Rodriguez Mañas L; European Diabetes Working Party for Older People. European Diabetes Working Party for Older People 2011 clinical guidelines for type 2 diabetes mellitus. Executive summary. *Diabetes Metab* 2011; **37** Suppl 3: S27-S38 [PMID: 22183418 DOI: 10.1016/S1262-3636(11)70962-4]

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