Original Article

A mini-network balance model for evaluating the progression of cardiovascular complications in Goto-Kakizaki rats

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Abstract

Cardiovascular complications represent a leading cause of mortality in patients with type 2 diabetes mellitus (T2DM). During such complicated progression, subtle variations in the cardiovascular risk (CVR)-related biomarkers have been used to identify cardiovascular disease at the incipient stage. In this study we attempt to integrally characterize the progression of cardiovascular complications and to assess the beneficial effects of metformin combined with salvianolic acid A (Sal A), in Goto-Kakizaki (GK) rats with spontaneous T2DM. The rats were treated with metformin ($200 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, ig) alone or in combination with Sal A ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, ip) at ages from 8 to 22 weeks. During the treatment, the levels of asymmetric dimethylarginine, L-arginine, superoxide dismutase, malondialdehyde, glucose, high density lipoprotein and low density lipoprotein were assessed. Based on alterations in these biomarkers, a mini-network balance model was established using matrixes and vectors. Radar charts were created to visually depict the disruption of CVR-related modules (endothelial function, oxidative stress, glycation and lipid profiles). The description for the progression of cardiovascular disorder was quantitatively represented by u , the dynamic parameter of the model. The modeling results suggested that untreated GK rats tended to have more severe cardiovascular complications than the treatment groups. Metformin monotherapy retarded disease deterioration, whereas the combination treatment ameliorated the disease progression via restoring the balance. The current study, which focused on the balance of the mini-network and interactions among CVR-related modules, proposes a novel method for evaluating the progression of cardiovascular complications in T2DM as well as a more beneficial intervention strategy.

Keywords: diabetes; cardiovascular risk; endothelial function; oxidative stress; glycation; lipid profiles; modeling; metformin; salvianolic acid A; biomarkers; network pharmacology; Goto-Kakizaki rats

Acta Pharmacologica Sinica (2017) 38: 362–370; doi: 10.1038/aps.2016.129; published online 2 Jan 2017

Introduction

The worldwide prevalence of diabetes mellitus, particularly type 2 diabetes mellitus (T2DM), has irrepressibly increased in recent years. Despite the tremendous advances in T2DM therapy, cardiovascular disorders constitute the major cause of morbidity and mortality in diabetic patients, leading to a nearly three-fold higher number of the deaths in the diabetic population than in the general population, and the cardiovascular risk (CVR) is far from being eradicated^[1,2].

Recent *in vitro* and *in vivo* studies have revealed that a number of cardiovascular complications are related to endothelial function, lipid profiles, oxidative stress and glycation, which will impair the inherent stability and robustness^[3,4]. The

interactions among these modules in the mini-network are shown in Figure 1. Due to the intrinsic link between different pathophysiological pathways, it is difficult to address such complicated metabolic syndromes and their complications^[5]. Fortunately, recent developments in network pharmacology have provided a new point of view on disease occurrence, proposing that the imbalance of biological networks results in diseases and that therapy for these diseases should aim to restore the balance^[6,7]. It has been observed that the biological network can be subdivided into several small and highly connected functional modules, and this network is considered to be the basis of life^[8]. Moreover, the disease progression model is a credible tool for gaining a quantitative understanding of disease pathogenesis, as it can provide more insight into the therapeutic approaches for diabetes and its complications. Moreover, a multi-marker approach is advocated for CVR prediction in T2DM, including primary prevention^[9], where

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Received 2016-06-27 Accepted 2016-10-12

Figure 1. Scheme of the mini-network balance model for estimating cardiovascular disorders in GK rats. Symbols are defined in the Methods section. Solid lines with arrows indicate conversion to the indicated factors. Dotted lines with arrows indicate the interactions, (+) indicates a stimulatory effect, and (–) indicates a inhibitory effect. Dotted lines outline different cardiovascular risk-related modules in type 2 diabetes mellitus.

subtle variations in plasma precede the clinically significant changes in cardiac function. As a result, based on network pharmacology, the disease progression model and the multi-marker approach, this mini-network balance model was established to assess the progression of diabetes-induced cardiovascular disorders.

As a preferred therapeutic option for T2DM, metformin has a long-standing evidence base for efficacy and safety, and it may reduce the risk of cardiovascular events if it is not contraindicated^[10, 11]. However, several clinical studies have demonstrated that there is some uncertainty regarding the benefit of intensive glycemic control from a cardiovascular standpoint, and whether this strategy could reduce cardiovascular events in diabetic patients is unclear^[12]. In addition, the ACCORD (Action to Control Cardiovascular Risk in Diabetes) trials suggested that an intensive glycemia strategy exerts adverse effects on cardiovascular outcomes during disease progression^[13].

Recently, increasing evidence suggests the necessity of drug combinations for reducing CVR^[14]. Salvianolic acid A (Sal A), one of the main active compounds in an aqueous extract of the herb *Salvia miltiorrhiza*, has been validated to exert a wide-range of cardioprotective effects. Recent studies have demonstrated that Sal A ameliorated diabetes complications (eg, vascular disease and peripheral neuropathy) by increasing peripheral blood perfusion and vascular activities, improving peripheral nerve function and inhibiting AGEs formation^[15]. Moreover, Sal A possessed antidiabetic capacity via AMPK activation and mitochondrial regulation^[16].

Goto-Kakizaki (GK) rats are widely used and recognized

as suitable rodent models for investigating cardiovascular disease in diabetes mellitus progression. GK rats are a lean animal model exhibiting mild type 2 diabetes that is characterized by an early increase in the serum insulin level, mild hyperglycemia, and insulin resistance and exhibits the highest level of similarities to clinical human T2DM syndromes^[17, 18]. In addition, studies have also characterized many abnormalities of various functional modules in this diabetic model, such as endothelial function, lipid profiles, oxidative stress, and glycation^[19–22].

Here, we propose a semi-mechanism-based mini-network balance model with a non-obese rodent animal to estimate the progression of a cardiovascular disorder based on the alterations of relevant serum biomarkers that represent distinct biological modules and to assess the therapeutic benefits of metformin and its combination with Sal A on progression from a different perspective.

Materials and methods

Diet and chemicals

All rats were fed a control diet (consisting of 24.4% protein, 4.9% fat, and 55.0% carbohydrate with a digestible energy content of 2.2 kcal/g), which was purchased from Xietong Biotechnology Co Ltd (Nanjing, China). Asymmetric dimethyl-arginine (ADMA), L-arginine (L-Arg) and H-arginine were obtained from Sigma-Aldrich (Shanghai, China). Metformin and Sal A were kind gifts from Chia Tai Tian Qing Co Ltd (Najing, China) and Qing Feng Co Ltd (Ganzhou, China), respectively. All other chemicals and solvents were of analytical grade. Commercial diagnostic kits were purchased from

the Jiancheng Institute of Biotechnology (Nanjing, China) and the Beijing North Institute of Biological Technology (Beijing, China).

Animals and experimental protocols

GK rats (8 weeks old) and age-matched Wistar rats, which were purchased from Cavens Lab Animal Co Ltd (Changzhou, China), were used. All rats were housed in a standard experimental animal laboratory with free access to food and water, and they were acclimatized for 1 week before the experiments. The use of animals and experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 86-23, revised 1986), and were approved by the Animal Ethics Committee of China Pharmaceutical University (20140828-0217). Based on the pre-experimental data for glucose and body weight, rats were divided into the following four groups: non-diabetic control group (Wistar rats, $n=8$), diabetic control group (GK rats, $n=7$); metformin treatment (200 mg·kg⁻¹·d⁻¹, ig) group of GK rats (MET, $n=7$); combination treatment (metformin at 200 mg·kg⁻¹·d⁻¹, ig and Sal A at 1 mg·kg⁻¹·d⁻¹, ip) group of GK rats (MET+Sal A, $n=7$). During the 14-week study, treatments were performed during the last 10 weeks. Every two weeks during the experiment, an aliquot of 0.5–1 mL of blood was collected in tubes containing EDTA. The blood samples were centrifuged at 4000×g and 4°C for 10 min, and the harvested plasma samples were stored at -70°C before analysis.

Systemic variables

Oral glucose tolerance tests (OGTT), performed as previously described^[22, 23], were conducted at weeks 4 (before treatment) and 14 (after treatment). The concentrations of plasma ADMA and L-Arg were selected to reflect the endothelial function, which were determined using HPLC-MS/MS, as previously established by Tain *et al*^[24]. The plasma levels of glucose were chosen to describe the level of glycation, and lipid profiles were obtained by measuring the high density lipoprotein (HDL) and low density lipoprotein (LDL). The biochemical indexes mentioned above were determined using an automatic biochemistry analyzer (CS-600B, Dirui Medical Technology Co Ltd, Changchun, China). The plasma insulin level was assayed with radioimmunoassay kits (Beijing North Institute of Biological Technology, Beijing, China) according to the instructions provided by the manufacturer. The HbA1c level, plasma malondialdehyde (MDA) level, and superoxide dismutase (SOD) activity were quantified using colorimetric kits (Jiancheng Institute of Biotechnology, Nanjing, China). Among them, SOD and MDA were selected to represent the level of oxidative stress.

Mini-network balance modeling

Based on our previous work^[7], the general scheme for the entire model is shown in Figure 1, where we defined this semi-mechanism-based model as a mini-network balance model. First, the following four CVR-related modules were chosen

to describe the status of CVR burden in diabetes: endothelial function, lipid profiles, anti-oxidative stress capacity and glycemic control. To fit the characteristics of these modules, we used the reciprocal of the concentration values of blood glucose in plasma.

This model was established using matrixes and vectors to describe the CVR status. The blood biochemistry-based modules at different time points were defined as denoting the distribution intensity of the i th module at the j th time point. To avoid the computational error induced by the marker concentrations of different orders of magnitudes, r_i^j , the ratio between c_i^j and c_i^{normal} was used to replace c_i^j :

$$r_i^j = c_i^j / c_i^{\text{normal}} \quad (1)$$

where c_i^{normal} is defined as the normal distribution intensity of the i th module. Here, we used the values obtained from Wistar rats (8 weeks old) as the baseline. A matrix was developed to describe the state of the modules at different time points.

$$R_{n \times m} = \begin{pmatrix} r_1^1 & r_1^2 & \cdots & r_1^m \\ r_2^1 & r_2^2 & \cdots & r_2^m \\ \vdots & \vdots & \ddots & \vdots \\ r_n^1 & r_n^2 & \cdots & r_n^m \end{pmatrix} \quad (2)$$

Each column describes the state of the modules at the same time point, whereas each row represented the state of a module at different time points. The radar charts were created to visually depict the disruption of these modules. The values of the normal group constituted the external contour, which formed a regular square. According to r_i^j , we linked the corresponding value of each module in the sequence and outlined the gray area at the j th time point.

We used vectors to describe the disruption at different time points. The extraction of the j th column vector in $R_{n \times m}$ formed a new vector, describing the state of the model at the j th time point:

$$V_j = (r_1^j, r_2^j, \dots, r_n^j) \quad j=1, 2, 3, \dots, m \quad (3)$$

To systematically describe the variation of these modules during the disease progression of T2DM, three integral dynamic parameters, k , φ and u , were introduced according to the vectors above.

$$k = |V_j| / |V_{\text{normal}}| \quad (4)$$

$$\varphi = \cos^{-1}[V_j \cdot V_{\text{normal}} / (|V_j| \cdot |V_{\text{normal}}|)] \quad (5)$$

$$u = \sqrt{(V_j - V_{\text{normal}})(V_j - V_{\text{normal}})^T} \quad (6)$$

V_j is a vector for the distribution intensity of all the modules in the model during disease progression. V_{normal} is a vector for the distribution intensity of all the modules in the model in the Wistar rat group at the beginning of the study. $|V_j|$ and $|V_{\text{normal}}|$ were their norms. T denotes the transposition of the matrix. Parameters k , φ and u indicate the variations between the V_j and V_{normal} states V_{normal} , and they are related to the alterations of multi-modules. Our previous study illustrated the physiological significance of k , φ and u with the

simulation experimental evidence as follows. The variable k is the response to the multi-marker consistency variation of biomarkers in the mini network. The variable φ responds to the multi-marker inconsistency variation. The variable u is the comprehensive response to both k and φ , representing the quantitative description for the progression of cardiovascular disorder^[25]. Moreover, when the variations of these related modules deviated from the normal state, the k value would stay away from 1, whereas the values of φ and u would increase accordingly.

Statistical analysis

The data are expressed as the mean $\pm$ SEM. Statistical calculations

were performed using SPSS software. The mean values of the data were compared using one-way ANOVA followed by Tukey's *post hoc* test. Significance was defined as $P<0.05$.

Results

Animal characteristics

At the beginning of the experiment, the GK rats exhibited similar fasting plasma glucose levels (Figure 2A). The levels of HbA1c and fasting glycemia and the OGTT were markedly elevated in GK rats compared with non-diabetic rats (Figure 2A–2D). Treatment with metformin alone or the combination of metformin and Sal A for 10 weeks effectively reduced the fasting levels of glucose and the glucose load in diabetic rats at

Figure 2. Animal characteristics. (A) Fasting serum glucose concentrations of each group at weeks 4 and 14. (B) Levels of HbA1c at the end of treatment. (C) Serum glucose concentrations for each group during OGTT before treatment. (D) Serum glucose concentrations for each group during OGTT at week 10 after treatment. (E) The area under the curve for different groups. (F) HOMA-IR levels before treatment and at week 10 after treatment. Values and bars represent the mean $\pm$ SEM ($n=7$) at each time point. # $P<0.05$, ## $P<0.01$ versus the GK control group. * $P<0.05$, ** $P<0.01$ versus the MET group.

the 2 h time point (Figure 2D). The HbA1c levels in the combination treatment group were significantly decreased by 42% ($P<0.01$) compared to the GK rats (Figure 2B). The HOMA-IR levels were significantly higher in GK rats than those of their counterparts, and they were significantly reduced to a normal level in these two treatment groups (Figure 2F). In addition, the combination treatment resulted in a more pronounced reduction of the HOMA-IR level compared with metformin treatment alone. In addition, the AUC of the glucose concentration was markedly higher in the GK group than that in Wistar rats, and it was significantly decreased in diabetic rats that were treated with metformin alone or its combination with Sal A (Figure 2E).

Determination of CVR-related markers

The diabetic rats that received combination treatment had a significantly increased ratio of plasma *L*-Arg/ADMA, except at week 10 ($P<0.01$) (Figure 3A). The ratio tended to be slightly higher in the MET group as compared to the GK control rats after the first 2-week treatment period ($P<0.05$). Both treatment strategies notably returned the blood glucose levels to the non-diabetic level (Figure 3B), but a deterioration of blood glucose was observed in GK rats. The variation of the time curve of the SOD/MDA ratio in the combination treat-

ment group could be divided into two stages (Figure 3C). At the beginning of the therapy, a remarkable increase occurred in this group at weeks 4–8; then, the SOD/MDA ratio value plateaued at approximately 51.8 during the remaining experiment period, which was significantly higher than that of the MET and diabetic groups. Additionally, MET treatment had no significant effect on oxidative stress. Moreover, we also observed time-course changes in plasma HDL and LDL levels over 12 weeks (Figure 3D). The curve of the ratio in the GK group revealed a steeper slope than that in the two treatment groups. Both the MET and combination treatment groups remarkably retarded the declining tendency in the last three weeks.

Analyses of the mini-network balance model

The radar charts of cardiovascular disorder in different groups during the progression are shown in Figure 4, which are used to visually describe the mini-network imbalance. The closer the shape is to square, the less imbalance in the mini network. In the GK group, the radar charts remarkably deviated from the square, and nearly every module shrank, compared to the normal state. The charts of the two treatment groups resembled that of the GK group before treatment. In the MET group, after a 2-week treatment period, the module of the

Figure 3. Variables of CVR-related markers. The plasma *L*-Arg/ADMA ratio (A), blood glucose (B), SOD/MDA ratio (C) and HDL/LDL (D) are presented as the mean $\pm$ SEM ($n=7$). # $P<0.05$, ## $P<0.01$ versus the GK control group. * $P<0.05$, ** $P<0.01$ versus the MET group. The vertical arrows denote the time segmentation of the drug intervention.

Figure 4. Radar charts of cardiovascular disorder in T2DM in the GK, MET and MET+Sal A groups during disease progression. The area circled as a square is the optimal state, whereas the gray area represents the abnormal state at different time points. The endothelial function, glycemic control, anti-oxidative stress capacity and lipid profiles are indicated as a–d, respectively. The vertical arrows denote the time segmentation of the drug intervention.

endothelial function, anti-oxidative stress capacity and glycemic control were notably improved. However, the protective effect of metformin on endothelial function and anti-oxidative stress seemed to be transitory for the entire treatment period. Moreover, in the combination therapy group, the module functions were corrected to nearly normal levels, and the radar charts tended to be regular over time.

The integral dynamic parameters k , ϕ and u were calculated to further evaluate the progression of cardiovascular disorder, and the results are summarized in Figure 5. The k parameter was observed to exhibit a gradual decreasing tendency in GK rats from the 4th week (Figure 5A). Metformin monotherapy partially improved the k value, which was significantly higher than that of the GK group for the last three time points, but this limited effect was insufficient to correct the imbalance. Finally, the combination treatment increased the k value to nearly 1, which signified that metformin plus Sal A could restore the CVR-related network back to a nearly balanced state. The weight of both k and ϕ was considered to define the u level, which represents the quantitative description for the progression of cardiovascular disorder. In the GK group, the u value was persistently elevated during the entire period, which suggested that the untreated diabetic rats tended to have more severe cardiovascular disorders (Figure 5B). However, the metformin treatment retarded the disease deterioration, and the combination treatment continuously and markedly decreased the u value compared with that of the GK rats. These data suggested that the imbalance in the CVR-related network tended to be more severe during T2DM progression, and this imbalance could be improved by metformin treatment. Moreover, the combination treatment seemed to have a higher beneficial effect on restoring the imbalance than monotherapy.

Discussion

Cardiovascular events still constitute the leading cause of mortality in T2DM subjects, despite great progress in prevention strategies or pharmacotherapy^[1]. Multiple studies have focused on obesity-related diabetes, but non-obese diabetes in the specific populations is also important and is also worthy of additional study. In fact, in Asia, where obesity is not prevalent, approximately 60% of the patients with a body mass index (BMI) less than 25 kg/m² have type 2 diabetes^[26]. Clinical trials in different Asian national populations have revealed an increased risk of cardiovascular disease at a lower BMI than in Europeans^[27, 28].

Because hyperglycemic states and insulin resistance involve different pathophysiological pathways, the strategies for reducing CVR in diabetes are controversial. With attempts to understand the mechanism and progression of the disease, the concept of metabolic memory has been proposed, and increasing evidence suggests that intensively decreasing glucose levels may not be conducive to reducing cardiovascular events^[29, 30].

Currently, multiple researchers in the fields of cardiovascular diseases have advocated a multi-marker approach. Although the optimal combination of biomarkers has not been established yet, this approach is considered to be superior to the single biomarker evaluation because of its advantages in diagnosis and prognosis, especially in the risk stratification of patients with complicated diseases^[9, 31]. Nevertheless, the classical multi-marker approaches, including the Cox proportional hazard, multi-marker index and regression trees analyses, have their own limitations in wide applications^[32]. Moreover, these methods ignore the balances in the network and interactions among biomarkers from the dynamic perspective of the system. Model-based disease progression that integrates

Figure 5. The dynamic parameters of the mini-network balance model in GK rats. The integral dynamic parameters k (A), ϕ (B) and u (C) are presented as the mean $\pm$ SEM ($n=7$). * $P<0.05$, ** $P<0.01$ versus the GK control group. * $P<0.05$, ** $P<0.01$ versus the MET group. The vertical arrows denote the time segmentation of the drug intervention.

the biological processes is widely accepted for understanding disease pathogenesis and drug development^[19]. According to the MOSAIC Project, frameworks with multi-level modeling are urgently need for further models of the risk of developing associated complications^[33]. Based on the above, this mini-network balance model was constructed to mine the underlying information of cardiovascular complication progression in T2DM and may provide a novel idea for clinically evaluating cardiovascular disorders.

The present mini-network consisted of the following four CVR-related modules: endothelial function, oxidative stress,

glycemic control and lipid profiles. They interacted with each other, and the balances in this mini-network changed with T2DM progression. Among these modules, the endothelial function module, which is reflected by the L -Arg/ADMA ratio, plays a core role in the occurrence of cardiovascular complications^[34]. Our current study demonstrated that the endothelial function deteriorated in GK rats during T2DM progression, whereas metformin treatment alone had an ameliorative effect, and the combination treatment of metformin and Sal A significantly improved this situation.

Previous studies suggested that increased superoxide production elevates the ADMA level and contributes to vascular derangements^[35]. The oxidative stress module, represented by the ratio between SOD and MDA, is widely regarded as the first and key event in the activation of pathways involved in the pathogenesis of diabetic complications^[36]. In the present investigation, combination treatment was observed to possess potent antioxidant capacity, which was consistent with previous studies^[15, 37]. Simultaneously, hyperglycemia was reported to induce the overproduction of superoxide and inhibit eNOS activity, which accelerated vasculature impairment in diabetes^[38, 39]. In addition, previous studies demonstrated that HDL cholesterol protected endothelial function by decreasing the ADMA level and counteracted the passive effect of ox-LDL on vascular disease^[40]. However, the oxidative modification of LDL was involved in the oxidative stress pathway. Therefore, as one of the major risk factors for cardiovascular disease, the dyslipidemia model was supposed to be considered and may be described by the HDL/LDL ratio^[41]. In our current study, the exacerbation of glycation and dyslipidemia occurred in GK rats, although they were fed a control diet instead of a western diet. By contrast, metformin treatment exerted some hypoglycemic effects and retarded the declining tendency of the HDL/LDL ratio, which is consistent with results in clinical trials. In addition to the comparable hypoglycemic effects, the combination treatment had more powerful ameliorative effects on lipid profiles. Furthermore, several clinical studies indicated that the level of HbA1c was tightly linked to cardiovascular events and exhibited change before the occurrence of cardiac dysfunction^[42, 43]. Of note, each 1% reduction in the updated mean HbA1c was associated with a risk reduction of 21% for any end point related to diabetes in UKPDS^[44]. Thus, we determined the HbA1c level to validate the progressive risk of cardiovascular complications, which preceded the morphological changes. Our results suggested that the combination treatment has a more profound effect on the HbA1c than monotherapy.

Several studies^[11] have demonstrated that diabetic hyperglycemia plays a key role in the pathogenesis of diabetic complications and that individuals with poor blood glucose control are more prone to developing chronic complications. A group-level meta-analysis revealed that the early control of glucose led to a modest, but significant, reduction in major cardiovascular disease outcomes^[45], indicating that glycemic control retarded the progression of disorders in CVR-related modules at the incipient stage. By contrast, the opponents

suggested the restoration of normoglycemia did not affect the occurrence of macrovascular events. The ACCORD trial indicated that the potential risks of intensive glycemic control might outweigh its benefits in patients^[29]. The results of the present study suggested that the single use of metformin retarded the increasing tendency of cardiovascular complications and produced a level of cardiovascular protection during the initial stage. However, with its limited impact on endothelial function, lipid profiles and anti-oxidative stress capacity, the cardiovascular benefit provided by metformin alone seemed feeble in the long-term.

The STENO-2 trial suggested that therapy with multifactorial interventions had sustained beneficial effects with respect to vascular complications in at-risk T2DM patients^[46]. Polyphenols have been reported to regulate glucose and lipid homeostasis, reducing the risk of metabolic syndromes and T2DM complications^[47]. Sal A is a polyphenolic derivative and has recently been validated to exert multifactorial effects and significantly contributes to preventing diabetes and its complications^[15, 16, 37]. In the present study, the radar chart of combination therapy indicated that less homeostatic disruption occurred and that the balances among modules were remarkably restored. The modeling parameters also consistently showed that the combination therapy substantially improved the cardiovascular risk burden and had an advantage over metformin monotherapy, which supports the HbA1c results. These data suggested that mere glycemic control was insufficient for protecting against cardiovascular events and that multifactorial intervention should be reinforced to improve cardiovascular disorders during T2DM progression. Further investigations are imperative to confirm our model and to clarify the pathophysiological mechanisms underlying the observed outcome.

In conclusion, the proposed model, focusing on the balance of the mini-network and interactions among CVR-related modules, satisfactorily described the progression of cardiovascular disorder in T2DM from a different perspective. Our study suggests that the multifactorial intervention should be reinforced to improve the balance of the CVR-related network in diabetes.

Abbreviations

ADMA, asymmetric dimethylarginine; CVR, cardiovascular risk; GK, Goto-Kakizaki; HDL, high density lipoprotein; *L*-Arg, *L*-arginine; LDL, low density lipoprotein; MDA, malondialdehyde; Sal A, salvianolic acid A; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (No 81273588 and 81473274) and Fundamental Research Funds for the Central Universities (ZJ15107). The authors wish to thank Feng ZHANG from the Department of Pharmacology, School of Pharmacy, Nanjing University of Chinese Medicine and Ning LI for constructive advice and help revising the manuscript.

Author contribution

Hao JIANG, Yu-hao WANG, and Xue ZHANG conducted the experiments and participated in the data analysis; Hao JIANG, Chun-xiang WEI, and Hao-chen LIU participated in the modeling data analysis and interpretation; Xiao-quan LIU conceived and designed the study as well as obtained the funding; Hao JIANG drafted the manuscript, and all other authors critically revised it for intellectual content.

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