ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Technol. Aliment. 24(2) 2025, 189–202
www.food.actapol.net pISSN 1644-0730 eISSN 1898-9594 http://doi.org/10.17306/J.AFS.001322

ORIGINAL PAPER

Received: 18.12.2024
Accepted: 17.03.2025

STEVIOL GLYCOSIDES AFFECT TRACE ELEMENT STATUS IN DIABETIC RATS

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ABSTRACT

Background. Steviol glycosides (stevioside and rebaudioside A) have been reported to have lipid and glucose regulatory potential. The published literature presents conflicting results regarding the impact of hyperglycemia on Fe, Zn, and Cu levels, and almost no data exist on whether supplementary steviol glycosides can affect the status of trace elements in diabetes. This study aimed to evaluate the effect of hyperglycemia and dietary steviol glycoside supplementation on Fe, Zn, and Cu levels and the ratios of these elements in the liver and kidneys of diabetic rats.

Material and methods. The experiment was conducted on 70 male Wistar rats, of which 60 were fed a high-fat diet for 8 weeks, followed by intraperitoneal streptozotocin injection to induce type 2 diabetes, while 10 healthy controls were fed the AIN-93M diet. Thereafter, the diabetic rats were allocated to the following six high-fat diet-fed experimental groups: untreated, supplemented with metformin, or supplemented with stevioside or rebaudioside A (0.5 or 2.5%) for 5 weeks. After the experiment, internal organs were harvested for mineral analyses. The Fe, Zn, and Cu content in tissues were determined using the Atomic Absorption Spectroscopy (AAS) method.

Results. Hyperglycemia was found to significantly elevate the liver Zn/Cu ratio and decrease the kidney Fe level, as well as the kidney Fe/Zn and Zn/Cu ratios, in diabetic non-supplemented rats. In the supplemented groups, steviol glycosides tended to normalize the kidney Zn/Cu ratio, while high doses of steviol glycosides tended to normalize the kidney Fe concentration. Higher doses of steviol normalized the liver Zn/Cu ratio, and higher doses of rebaudioside A normalized the kidney Fe/Zn ratio. The type of glycoside affected the kidney Zn level and the Fe/Zn ratio in diabetic rats.

Conclusion. Hyperglycemia affected Fe, Zn, and Cu balance in diabetic rats, while steviol glycosides showed potential to normalize mineral levels depending on dosage and type. Future research should explore the underlying mechanisms and long-term effects of steviol glycoside supplementation on trace element homeostasis in diabetes.

Keywords: stevia, steviol glycosides, trace elements, diabetes, rats

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is currently a serious global health issue, affecting ~462 million people in 2017 (Khan et al., 2020). The prevalence of the disease is expected to increase due to many factors, such as sedentary lifestyles and population aging (NCD Risk Factor Collaboration, 2016). As the largest proportion of patients is found in developing countries, the highest prevalence of T2DM is commonly observed in

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regions of the Middle East and North Africa (Namazi et al., 2024). While sub-Saharan regions of Africa still show relatively low prevalence, the rate is rapidly rising (Pastakia et al., 2017). Finding new ways to improve the treatment of diabetic patients remains a challenge for healthcare professionals. Nevertheless, this effort is necessary for a few reasons. First, there is still no therapy that combines high efficacy, low risk of side effects, and affordability. Second, T2DM has become a significant economic burden on worldwide healthcare systems (Butt et al., 2024), which is expected to increase further in the future (Bommer et al., 2017). T2DM complications include retinopathy, neuropathy, nephropathy, impaired immunity to infections, and cardiovascular disease (Yu et al., 2023). The latter complication is the leading cause of both morbidity and mortality among patients (Rawshani et al., 2017). Overall lifestyle changes (improved diet and physical activity) and pharmacotherapy are commonly used in T2DM treatment (Davies et al., 2018; Einarson et al., 2018). However, research is ongoing, and in-depth knowledge regarding the molecular basis of T2DM can help physicians develop more effective targeted treatment methods (Zinman et al., 2015). For example, new pharmacological agents, such as GLP-1 receptor agonists and/or SGLT2 inhibitors, are currently being incorporated into disease management strategies (Marso et al., 2016).

The plant *Stevia rebaudiana* Bertoni is a source of compounds that may be useful in diabetes treatment. In particular, its potential lies in steviol glycosides (SG), which are extracted from the leaves of the plant and commonly used as natural sweeteners in the food industry (Huang et al., 2024). Experimental studies have confirmed the safety of SG, and agencies such as the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) permit the use of these compounds as food additives (classified as E960; EFSA Panel on Food Additives and Flavourings (FAF) et al., 2020). In addition to their high sweetness intensity, SGs have been shown to have many health-promoting properties, especially antidiabetic properties that can be used in managing T2DM, as they can regulate blood glucose concentrations, improve insulin sensitivity, and normalize lipid profiles in those diagnosed with the disease (Anton et al., 2010; Chatsudthipong and Muanprasat, 2009; Das et al., 1992; Goyal et al., 2010; Jeppesen et al., 2000; Kurek et al., 2020).

Trace elements such as iron (Fe), zinc (Zn), and copper (Cu) are crucial for various metabolic processes. Abnormalities in their status can impact the development and management of T2DM, and conversely, the disease can affect their status (Kurek et al., 2024; Prasad, 2008). Fe is an essential element for oxygen transport. Both Fe deficiency and Fe excess are risk factors for developing diabetes. The latter effect is most probably due, among other factors, to the role of Fe in oxidative stress and inflammation processes, which impair insulin secretion and increase insulin resistance (Jiang et al., 2004b). Fe deficiency, however, can negatively affect glucose metabolism and amplify diabetic complications in patients (Thomas et al., 2004). Zn is vital for insulin-related processes, including the synthesis, storage, and secretion of insulin (Li, 2014). Experimental studies have shown that Zn deficiency is common in diabetic patients and can amplify glycemic control difficulties and the risk of diabetic complications. Zn supplementation has been shown to improve glycemia in type 1 and 2 diabetic patients (Ranasinghe et al., 2015). Adequate Zn body concentrations are also associated with a reduced risk of developing T2DM, probably because they improve insulin sensitivity (Chausmer, 1998). Cu is involved in enzymatic processes, especially those connected with oxidative stress. Abnormalities in Cu metabolism, including both deficiency and excess, are observed in T2DM. For instance, increased Cu body concentrations can increase oxidative stress and/or inflammation, which can contribute to insulin resistance and pancreas β-cell dysfunction (Eljazzar et al., 2023). However, decreased Cu levels are associated with the impairment of glucose metabolism and more severe diabetic complications (DiNicolantonio et al., 2018). The balance between these trace elements is also crucial for glucose metabolism. High Fe intake can, for instance, impair Zn absorption, while sufficient Zn concentrations protect against the toxicity of Cu. Thus, maintaining homeostasis of these trace elements and their respective ratios is important for the prevention and management of T2DM (Sanjeevi et al., 2018).

In light of these considerations, the goal of this study was to verify the effect of SG supplementation on levels of trace elements, such as Fe, Zn, and Cu, and their ratios in the liver and kidneys, as the main storage organs, in rats with induced T2DM.

MATERIAL AND METHODS

Detailed descriptions of the materials and methods used in the experiment are provided in a previous article (Kurek et al., 2020), and are therefore not be repeated in full here. This article presents information specifically related to the topic of trace elements.

Experimental agents

The following dietary supplements were used in the experiment: metformin (Met; metformin hydrochloride from the antidiabetic drug Metformax; Teva Operations Poland Ltd., Kraków, Poland), stevioside (ST; Anhui Minmetals Development Co., Hefei, China) and rebaudioside A (RA; Anhui Minmetals Development Co., Hefei, China). Streptozotocin was used in the protocol for the laboratory model of T2DM induction (Sigma-Aldrich Ltd., Poznań, Poland).

Metformin

The dose of metformin used in animal studies can vary depending on the species, the purpose of the study, and the experimental design. In studies involving rodents, metformin is often administered at doses ranging from 50 to 500 mg/kg body weight per day (Kim et al., 2020; Sahu et al., 2024). Based on these guidelines, we used metformin at a dose of 0.15 % of the diet in our experiment, which is equivalent to 150 mg/kg b.w./day.

Steviol Glycosides

The test doses of SG (ST, RA) were established on the basis of previous data showing that chronic exposure to ST up to 967 mg/kg b.w./day has no adverse effects in rats (NOAEL) (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010).

The "pharmacologic" dose of SG was set at 2,500 mg/kg b.w./day for short-term exposure (5 weeks). Following these assumptions, we established two experimental dosages of ST and RA at 0.5% and 2.5%, equal to 500 and 2,500 mg/kg b.w./day, respectively, aligning with this range.

Experimental diets

A diet in the form of pellets (∅ 10–12 mm) was manufactured by a local company (Urszula Borgiasz Zoolab, Sędziszów, Poland). The control group (C) received the AIN-93M standard maintenance diet (4% fat, 7.5% energy) (Reeves, 1997) throughout the entire experiment. The diabetic group (Db) received a high-fat (HF) diet (21.4% w.w., 40% energy from fat). The dietary sources of fat were: soybean oil (2.5%) and lard (15.4% w.w.). The diabetic group Db + Met received a HF diet enriched with metformin (0.15%, equal to 150 mg/kg b.w./day). Other diabetic groups (Db + S1, Db + S2, Db + R1, Db + R2) received HF diets enriched with SG (St or RA) in two different doses (0.5% or 2.5%, equal to 500 or 2500 mg/kg b.w./day).

The mineral content (Fe, Zn, Cu) was standardized across all the diets (in mg/kg d.m.): 60.6 ±5.9; 49.9 ±4.7; 6.2 ±0.8 for Fe, Zn, and Cu, respectively, which was confirmed by relevant analyses.

Study protocol

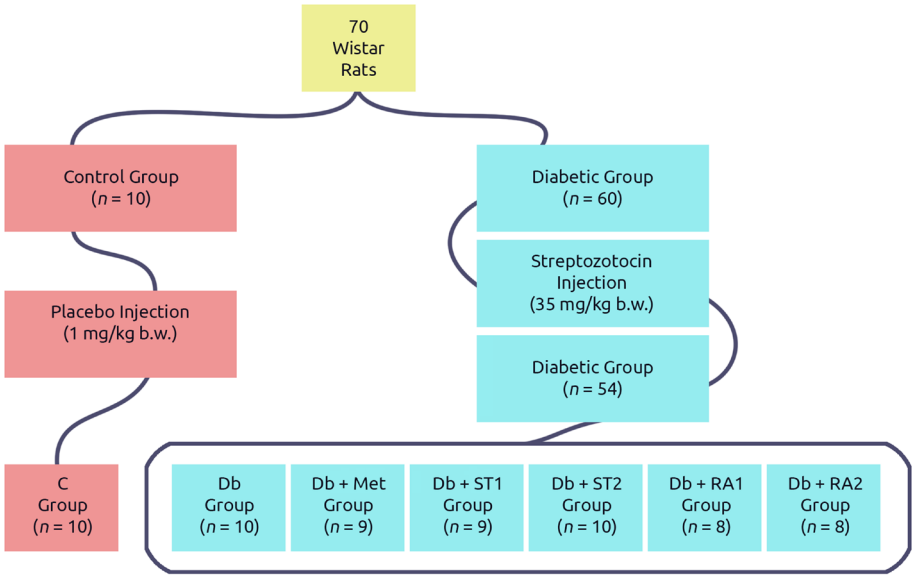
The animals (n = 70, Wistar rats, outbred strain of *Rattus norvegicus* ♂ at an age of 6 weeks and an initial body weight of approximately 223 g) used in the experiment originated from Charles River Laboratories, Inc. (Sulzfeld, Germany) and were provided by Animalab Ltd. (Poznań, Poland). The conditions of the experiment were strictly controlled: 12/12 h light/dark cycle, ~18°C, humidity ~57%. The experimental stage of steviol glycoside supplementation lasted 5 weeks, preceded by 8 weeks of HF diet-feeding to induce insulin resistance in the diabetic rats.

Hyperglycemia was induced with intraperitoneal injections of streptozotocin (35 mg/kg b.w., i.p.) (Srinivasan et al., 2005). Six rats died due to severe diabetic complications. At the end of the experiment, the animals were sacrificed, and blood and organs were collected for further analyses. A detailed scheme of the animal grouping is shown in Figure 1.

The protocol was approved by the Local Ethical Commission in Poznań (No. 31/2019). The experiment was conducted in the Association for Assessment and Accreditation of Laboratory Animal Care International-approved Animal Care Facility in Poznań University of Life Sciences.

Trace element analysis

The organs were pre-digested in 65% HNO_3 (Merck KGaA, Darmstadt, Germany) followed by mineralization in a Speedwave XPERT (Berghof Products, Instruments GmbH, Eningen, Germany) digestion system. The obtained samples were analyzed in terms



C group: control healthy rats, standard AIN-93M diet; Db Group: diabetic rats, HF diet (40% energy from fat); Db + Met Group: diabetic rats, HF diet + 0.15% metformin (150 mg/kg b.w./day); Db + ST1 Group: diabetic rats, HF diet + 0.5% stevioside (500 mg/kg b.w./day); Db + ST2 Group: diabetic rats, HF diet + 2.5% stevioside (2,500 mg/kg b.w./day); Db + RA1 Group: diabetic rats, HF diet + 0.5% rebaudioside A (500 mg/kg b.w./day); Db + RA2 Group: diabetic rats, HF diet + 2.5% rebaudioside A (2,500 mg/kg b.w./day).

Fig. 1. Experimental design – animal grouping scheme

of trace element concentrations using the atomic absorption spectrometry method with a Carl-Zeiss Jena AAS-3 atomic absorption spectrometer (Jena, Germany). To validate the accuracy of the method, certified reference materials (INCT-SBF-4, Institute of Nuclear Chemistry and Technology, Warsaw, Poland; NCS ZC 73030, LGC Standards Ltd., Teddington, UK; BCR 679, Institute for Reference Materials and Measurements, Geel, Belgium; NIST1577C, NIST®, Gaithersburg, MD, USA) were used.

Statistical analysis

All obtained values were first collected and organized in MS Excel 2019 (Microsoft Corporation, Redmond, USA). Data were then analyzed in Statistica 13.3 (TIBCO Software Inc., Palo Alto, USA). The following statistical methods were applied: the Shapiro–Wilk test ($p < 0.05$), a one–way analysis of variance (ANOVA) ($p < 0.05$), a multivariate analysis of variance (MANOVA), and Fisher's LSD post hoc test ($p < 0.05$). The results shown in the article refer to the stage of the experiment after T2DM induction.

RESULTS

Determining trace element status in both animals and humans is a difficult task due to the multitude of functions they serve and their specific compartmentalization and distribution in the body. Trace element status is usually determined by measuring trace element concentrations in available fluids or tissues. In this experiment, trace element status was evaluated by measuring Fe, Cu, and Zn concentrations in liver and kidney samples. Given the key role of these trace elements in the maintenance of the antioxidant system, the Fe/Zn, Fe/Cu, and Zn/Cu ratios in these organs were calculated to evaluate how their balance is affected by diabetes.

Liver trace element status

The effects of the tested diet on liver trace element status are presented in Tables 1 & 2.

The results indicate that induced diabetes did not affect the total content of any of the analyzed trace elements in the liver. However, the liver Zn/Cu ratio was significantly elevated in the Db group, whereas

Table 1. Effects of SG on liver and kidney trace element status in animals

Parameter	C	Db	Db + Met	Db + ST1	Db + ST2	Db + RA1	Db + RA2
Liver							
Fe, µg/g d.m.	487.51 ±68.79	543.82 ±132.39	550.31 ±139.79	559.57 ±130.30	556.75 ±85.21	514.94 ±0.40	554.74 ±70.53
Zn, µg/g d.m.	108.89 ±12.35	123.73 ±17.53	110.36 ±16.27	117.72 ±19.35	111.48 ±8.64	113.09 ±12.40	113.98 ±17.68
Cu, µg/g d.m.	13.68 ±1.78	13.01 ±2.31	16.60 ±5.53	14.43 ±2.87	13.85 ±1.47	13.60 ±2.69	14.06 ±1.36
Fe/Zn ratio	4.61 ±0.93	4.41 ±1.03	5.03 ±1.19	4.74 ±0.66	5.00 ±0.65	4.57 ±0.73	4.94 ±0.87
Fe/Cu ratio	36.60 ±10.11	42.96 ±11.45	36.34 ±15.06	38.95 ±5.68	40.77 ±8.04	39.73 ±13.04	40.00 ±7.56
Zn/Cu ratio	8.04 ±1.15^{ab}	9.36 ±1.07^{c}	7.14 ±2.04^{a}	8.23 ±0.73^{abc}	8.11 ±0.85^{ab}	8.54 ±1.58^{bc}	8.13 ±1.10^{abc}
Kidney							
Fe, µg/g d.m.	314.24 ±21.12^{b}	246.60 ±37.18^{a}	269.50 ±48.69^{a}	260.59 ±30.75^{a}	282.17 ±53.55^{ab}	262.35 ±43.76^{a}	282.51 ±35.30^{ab}
Zn, µg/g d.m.	115.76 ±8.86^{a}	122.20 ±16.96^{a}	131.15 ±8.74^{ab}	126.36 ±19.99^{a}	145.08 ±33.72^{b}	122.24 ±13.50^{a}	114.00 ±17.47^{a}
Cu, µg/g d.m.	37.29 ±7.20^{a}	50.56 ±14.26^{ab}	129.19 ±5.26^{c}	71.42 ±38.66^{b}	49.13 ±14.92^{ab}	74.88 ±33.87^{b}	70.92 ±34.22^{b}
Fe/Zn ratio	2.72 ±0.23^{c}	2.03 ±0.29^{a}	2.08 ±0.46^{a}	2.11 ±0.42^{a}	1.93 ±0.51^{a}	2.15 ±0.28^{ab}	2.52 ±0.47^{bc}
Fe/Cu ratio	8.69 ±1.65^{c}	5.57 ±2.95^{b}	2.82 ±2.52^{a}	4.61 ±2.33^{ab}	6.38 ±2.79^{bc}	4.68 ±3.80^{ab}	4.89 ±2.31^{ab}
Zn/Cu ratio	3.25 ±0.58^{c}	2.65 ±1.04^{bc}	1.37 ±0.88^{a}	2.12 ±0.80^{ab}	3.16 ±1.05^{c}	2.14 ±1.53^{ab}	1.93 ±0.82^{ab}

Data are presented as mean ±standard deviation. Different letters (a–c) indicate statistically significant differences between values (Fisher's LSD post-hoc test; a < b; $p < 0.05$).

Table 2. Main effects of SG (multivariate analysis)

Index	Main Effects			
	Glycoside (ST vs RA)	*p*	Dose (0.5% vs 2.5%)	*p*
1	2	3	4	5
Liver				
Fe, µg/g d.m.	558.16 ±106.81	NS	538.57 ±112.21	NS
	534.84 ±80.98		555.80 ±76.21	
Zn, µg/g d.m.	114.44 ±14.63	NS	115.54 ±16.13	NS
	113.54 ±14.76		112.59 ±13.04	
Cu, µg/g d.m.	14.13 ±2.20	NS	14.04 ±2.73	NS
	13.83 ±2.07		13.94 ±1.39	
Fe/Zn ratio	4.87 ±0.65	NS	4.66 ±0.68	NS
	4.76 ±0.80		4.97 ±0.74	
Fe/Cu ratio	39.86 ±6.82	NS	39.32 ±9.53	NS
	39.86 ±10.30		40.41 ±7.58	

Table 2. – cont.

1	2	3	4	5
Zn/Cu ratio	8.17 ±0.77	NS	8.38 ±1.17	NS
	8.33 ±1.33		8.12 ±0.94	
		Kidney		
Fe, µg/g d.m.	271.38 ±43.79	NS	261.36 ±35.65	NS
	273.10 ±39.39		282.33 ±44.48	
Zn, µg/g d.m.	136.21 ±28.95	0.03905	124.56 ±17.04	NS
	117.85 ±15.77*		131.27 ±31.31	
Cu, µg/g d.m.	59.69 ±30.11	NS	72.94 ±35.49	NS
	72.77 ±32.89		58.81 ±26.91	
Fe/Zn ratio	2.02 ±0.46	0.04612	2.13 ±0.36	NS
	2.35 ±0.42*		2.21 ±0.56	
Fe/Cu ratio	5.49 ±2.66	NS	4.64 ±2.94	NS
	4.79 ±2.98		5.68 ±2.61	
Zn/Cu ratio	2.67 ±1.06	NS	2.13 ±1.13	NS
	2.03 ±1.16		2.61 ±1.12	

Data are presented as mean ± standard deviation ($^*p < 0.05$).

supplement treatment tended to normalize this ratio in diabetic rats. The multivariate analysis did not reveal any additional effects of experimental factors on the liver and kidney trace element content or ratios in diabetic rats.

Kidney trace element status

The effects of the tested diets on kidney trace element status are presented in Tables 1 & 2. The only trace element directly affected by T2DM in the kidney was Fe, as significantly lower Fe levels were observed in untreated diabetic rats (Db group). Furthermore, supplementary SG treatment was generally ineffective in restoring kidney Fe content, even though a slight (insignificant) elevation of this parameter was observed with higher doses of SG (Db + ST2, Db + RA2). Regarding the relevant kidney trace element ratios, some more statistically significant effects were observed. In particular, T2DM was associated with a decrease in both Fe/Zn and Fe/Cu ratios in this organ. In most cases, the administration of a diet enriched with SG was ineffective in mitigating these changes; however, a high dose of RA (Db + RA2) resulted in normalization of the kidney Fe/Zn ratio in diabetic rats.

The multivariate analysis revealed that the type of SG affected Zn content in the kidney and the kidney Fe/Zn ratio in diabetic rats. Interestingly, regardless of dosage, diabetic rats treated with RA had significantly lower kidney Zn levels, while the Fe/Zn ratio was higher compared to those treated with ST.

DISCUSSION

Diabetes (T2DM) can significantly alter the status of trace elements in organs such as the liver and kidneys. The primary aim of the study was to evaluate tissue levels of essential trace elements, namely Fe, Zn, and Cu, in diabetic rats subjected to SG supplementation, as these metals are known to be involved in processes of lipid peroxidation and play an important role in the pathogenesis and exacerbation of diabetic complications (Özenç et al., 2015). Usually, the assessment of

trace element status, particularly in human subjects, is based on a limited number of biomarkers in the blood, mainly by measuring their total or fractional concentration in blood or serum, depending on the element. For example, Fe status can be conveniently evaluated by recording an array of hematologic indices, or the serum ferritin level, and comparing them with reference values. For Zn and Cu, the available biomarkers in blood or serum have certain limitations, as they do not always correspond to their metabolic or storage pools. There are conflicting results in the available literature regarding trace element levels in the blood and serum of diabetic individuals that will not be addressed in detail in this study. The variability of data may result from differences in sample sizes, the age of patients, environmental factors, disease duration, ethnicity, nutritional habits and status, or the glycemic control of the patients enrolled in different studies.

Oxidative stress has been associated with T2DM, as the generation of reactive oxygen species (ROS) is a common phenomenon in this disease (Mohamed et al., 1999). Oxidative damage to DNA, proteins, and lipids has been observed and correlated with diabetic complications (Dinçer et al., 2002; Ziegler et al., 2004). Several studies have shown that hyperglycemia plays a role in inducing oxidative stress in diabetes (Forbes et al., 2008). However, high levels of glucose are not the only factor responsible for the generation of ROS. Some studies imply an important role of transition metals as catalysts of oxidative stress.

The biology of transition metals, such as Cu, Zn, Mn, Mo, Cr, V, and Fe, has been evaluated in the context of T2DM (Viktorínová et al., 2009; Zheng et al., 2008). The generation mechanisms of ROS induced by transition elements have been reported in a variety of publications and therefore will not be discussed in detail in this paper (Fenton, 1894; Haber et al., 1997; Halliwell and Gutteridge, 1990; Henle and Linn, 1997; Koppenol, 1993; Minotti and Aust, 1987; Walling, 1975). In brief, increased ROS production brought about by chronic hyperglycemia (glucotoxicity) leads to oxidative stress that has been implicated as a contributor to both the onset and progression of diabetes and its associated complications (Rains and Jain, 2011). Some of the consequences of an oxidative environment are the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance, and mitochondrial dysfunction, which can ultimately lead to a diabetic disease state. Experimental and clinical data suggest an inverse association between insulin sensitivity and ROS levels.

In biological organisms, essential trace elements (Fe, Zn, Cu) usually take the form of metalloproteins distributed between a few metabolic pools that remain in a dynamic balance through homeostatic mechanisms. It has been reported that aberrant alterations in their homeostasis can lead to the release of free species that in turn undergo redox cycling reactions (e.g., Fenton reaction) to produce ROS (Valko et al., 2016). Trace elements (Fe, Zn, Cu) in the forms of various metalloproteins are among the protective agents preventing oxidative stress and the development of diabetes and diabetic complications (Wei et al., 2009).

The role of Fe in diabetes has been the subject of many studies which have clarified its involvement in the development and progression of diabetes. However, the mechanisms underlying its actions are multiple and not fully understood (Fernández-Real et al., 2005; Howard et al., 1991; Jehn et al., 2004; Jiang et al., 2004a; Lao and Tam, 1997; Nishiya et al., 1996; Swaminathan et al., 2007). The central importance of Fe in the pathophysiology of disease is attributed to the ease with which Fe is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes Fe potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radicals (Swaminathan et al., 2007). In the liver specifically, T2DM may disturb Fe homeostasis. Some patients exhibit elevated hepatic Fe concentrations and thus have an increased risk of liver damage and excessive oxidative stress (Fernández-Real and Manco, 2014). Experimental studies suggest that Fe liver overload is associated with the development of insulin resistance and can amplify both liver inflammation and fibrosis (Simcox and McClain, 2013). T2DM can also significantly change the status of this trace element in the kidneys. Increased Fe deposition can contribute to nephropathy and further kidney damage through oxidative stress-related mechanisms (Swaminathan et al., 2007). Animal studies have provided considerable evidence for the role of Fe and oxidants in diabetic nephropathy. Oxidative stress from hyperglycemia, advanced glycation end products, and hyperlipidemia further contribute to the

availability of intracellular Fe, which can generate and severely worsen oxidative stress and renal damage. Renal Fe content has been shown to be changed (increased or decreased) in an animal model of diabetes (Johnson and Evans, 1984), as urinary Fe excretion is increased early in the course of diabetic renal disease in humans (Howard et al., 1991; Nishiya et al., 1996). Another feature of the role of Fe in this disease is the link between increased dietary Fe intake, particularly red meat consumption, increased body Fe stores, and the development of diabetes. A causative link with Fe overload is suggested by the improvement in insulin sensitivity and insulin secretion with frequent blood donation and decreased Fe stores (Fernández-Real et al., 2005; Jiang et al., 2004a). Furthermore, high body Fe stores have been linked to insulin resistance (Sheu et al., 2003), metabolic syndrome (Jehn et al., 2004), and gestational diabetes (Lao and Tam, 1997). In summary, on the one hand, chronic hyperglycemia leads to a disturbance of Fe metabolism that in turn exacerbates diabetic complications via oxidative stress mechanisms; on the other hand, Fe overload can contribute to the development of diabetes, hence restoring Fe balance is crucial in the management of diabetes.

The protective role of Zn in diabetes has been reported in many studies. In brief, hyperglycemia can significantly impact Zn homeostasis in the body through various interrelated mechanisms. Some of the key effects include: increased Zn excretion through urine (hyperzincuria), leading to lower zinc levels in the body (de Carvalho et al., 2017); reduced Zn absorption, because high blood sugar levels can interfere with the absorption of zinc in the intestines (Fernández-Cao et al., 2019); and altered Zn homeostasis, because hyperglycemia can disrupt the balance of Zn in the body, leading to deficiencies that affect various metabolic processes (de Carvalho et al., 2017). Zn deficiency caused by the above has an impact on insulin function that can in turn exacerbate issues related to insulin resistance and beta-cell dysfunction. As an antioxidant trace element, Zn not only potentiates the action of insulin but is also crucial for insulin production (Chistiakov and Voronova, 2009). Zn deficiency reported in T2DM is believed to result from increased urinary losses of this trace element, and reduced Zn levels are negatively correlated with hyperglycemia and poor glycemic control (Bandeira et al., 2017). In the course of T2DM, Zn concentrations are frequently lowered, which can result in impaired insulin signaling and glucose metabolism (Puri et al., 2013). Decreased Zn concentrations in the liver may be associated with oxidative damage and abnormal liver regeneration processes (Chasapis et al., 2012). Kidney Zn levels are often observed to be lowered in the course of T2DM. This can result in impaired antioxidant defense mechanisms in the organ and further development of diabetic nephropathy (Gembillo et al., 2022b). Therefore, adequate kidney Zn levels are crucial for renal health and the prevention of oxidative damage in T2DM (Takeda and Tamano, 2009).

The role of Cu in diabetes has also been examined in many studies, which have shed light on its possible mechanisms of action (Chang and Li, 2023). Cu has been shown to play a role in various aspects of diabetes. Furthermore, Cu is a controversial element due to its bidirectional functions in the body. Both Cu deficiency and excess can lead to poor glucose tolerance and exacerbate diabetic complications (Gembillo et al., 2022a; Shirur et al., 2024). Copper is a strong prooxidant; thus, elevated levels of Cu are not only implicated in increased oxidative stress in T2DM but also contribute to insulin resistance and hyperglycemia (Bo et al., 2008; Park et al., 2009). On the other hand, Cu, together with Zn, is an integral component of superoxide dismutase (SOD), one of the key endogenous enzymes protecting against ROS. Despite these observations, some data on serum Cu levels in T2DM contradict claims that Cu concentrations remain unchanged in the disease (Car et al., 1992). As regards liver Cu levels in T2DM, research shows that disease complications may lead to either an accumulation or a deficiency of this element in the organ, increasing the risk of disrupted liver function and increased oxidative stress (Gembillo et al., 2022a). Cu imbalance is common in the kidneys of T2DM patients. This state can significantly impair renal function and contribute to the progression of diabetic complications, mostly by amplifying oxidative stress (Arredondo and Núñez, 2005).

In this experiment, the liver and kidney Fe, Zn, and Cu levels were used to evaluate trace element status, as they better reflect the storage pools of these elements, rather than the mobile pools. Furthermore, the liver and kidney Fe/Zn, Fe/Cu, and Zn/Cu ratios were taken

into account, based on the working assumption that any significant deviations in these ratios (compared with a suitable reference – here, the control group of healthy rats) may indicate metabolic distress—particularly that associated with increased oxidative stress in the respective tissues. In the present study, liver and kidney Fe, Zn, and Cu levels, and the ratios of some of these elements, were altered in diabetic rats compared to the healthy control group. There is a scarcity of data in the literature discussing trace element ratios in these tissues, as presented in this article, which poses a challenge for understanding the mechanisms underlying the observed results. These mechanisms might be intricate and involve multiple variables or processes, as discussed *vide supra*. In this experiment, we used a high fat diet followed by STZ to induce hyperglycemia. The effect of a high fat diet on trace element (Fe, Zn, Cu) status and oxidative stress is very complex, as described above. An extensive discussion on this topic would exceed the scope of this article. In brief, a high fat diet leads to insulin resistance, hyperlipidemia, and finally overt diabetes. Hyperglycemia leads to protein glycation, which is well known in the pathogenesis of diabetic vascular complications. Transition metals (Fe, Cu) also play a role in protein glycation induced by hyperglycemia. It has been shown that glycated proteins have a substantial affinity for the transition metals (Fe, Cu), and the bound metal retains redox activity and participates in catalytic oxidation (Qian et al., 1998). Thus, it is possible that glycochelates formed in vivo increase oxidative damage in tissues, which in turn may contribute to the vascular complications of diabetes (Sullivan, 1981).

For the purposes of comparison, we used data reported in some experimental studies performed on diabetic rats (similar models to the present study) in which the liver and kidney Fe/Zn, Fe/Cu, Zn/Cu ratios were calculated, revealing diverse patterns, including both increasing and decreasing trends in trace element ratios in these organs of diabetic rats compared to healthy control rats. For example, in the liver of diabetic rats, increasing trends have been reported for the Fe/Zn ratio (Król et al., 2012; 2016; Salvador et al., 2016), Fe/Cu ratio (Król et al., 2012; 2016; 2020; Salvador et al., 2016) and Zn/Cu ratio (Król et al., 2012, 2016; Salvador et al., 2016), whereas decreasing trends have been reported for the Zn/Cu ratio (Król et al., 2014) relative to healthy controls. Furthermore, in the kidneys of diabetic rats, increasing trends have been reported for the Fe/Zn ratio (Król et al., 2014; 2020) and Fe/Cu ratio (Król et al., 2012; 2016), whereas decreasing trends have been observed for the Fe/Cu and Zn/Cu ratios (Król et al., 2014; 2020; Salvador et al., 2016) relative to healthy controls.

In light of scientific reports evaluating the metabolic consequences of altered trace element ratios in tissues (here: Fe/Zn, Fe/Cu, and Zn/Cu), an attempt has been made to cautiously interpret the experimental data obtained, based on the available knowledge about the role of these elements in maintaining oxidant-antioxidant balance in biological systems. It can be hypothesized that altered (increased or decreased) Fe/Zn, Fe/Cu, and Zn/Cu ratios in liver and kidney tissues may imply some metabolic abnormalities associated with oxidative stress

In this experiment, the type of SG significantly affected only kidney Zn levels, as well as the kidney Fe/Zn ratio. RA in particular, irrespective of dosage, was associated with markedly lower values compared to ST. The reason for these effects remains unclear, but taking into account previous findings (Kurek et al., 2020) showing that RA appears to be less effective in correction of histological damage compared to ST, it can be hypothesized that the efficacy of SG in mitigating metabolic distress correlates with changes in the Fe/Zn ratio.

The main limitation of this study is the lack of information on trace element levels in other tissues (e.g. blood, serum, muscle) and their respective biomarkers (e.g. ferritin, Zn, and Cu-dependent enzymes such as SOD). However, this limitation has been acknowledged in the discussion and conclusions. The primary strength of this study is the assessment of Fe, Zn, and Cu concentrations and ratios in the storage tissues (liver, kidney), which are in dynamic homeostatic with other functional pools of these elements (e.g. blood, muscle).

CONCLUSION

Chronic hyperglycemia significantly elevated the liver Zn/Cu ratio, while decreasing the kidney Fe level and reducing Fe/Zn and Zn/Cu ratios in diabetic rats. SG supplementation (ST, RA) tended to normalize the

kidney Zn/Cu ratio, while high doses of SG tended to normalize the kidney Fe concentration in diabetic rats. Interestingly, irrespective of dosage, the type of SG (ST, RA) appears to affect the kidney Zn concentration and the Fe/Zn ratio in diabetic rats.

The results of this study contribute to the body of knowledge about the functional properties of SG and their potential to mitigate metabolic alterations caused by hyperglycemia in diabetes. Nevertheless, more studies are warranted to clarify the mechanisms and significance of these effects in both animal and human subjects.

FUNDING SOURCE

The presented work is an integral part of the research project National Science Centre, Poland, NCN 2017/27/B/NZ9/00677.

DECLARATIONS

Ethics Approval

The experimental protocol was approved by the Local Ethical Commission in Poznań (approval #31/2019).

Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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