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A SGLT2 inhibitor dapagliflozin comparison with insulin exerts important effects on Zn\textsuperscript{2+}-transporters in cardiomyocytes from insulin-resistant metabolic syndrome rats through inhibition of oxidative stress

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Abstract

Sodium-glucose cotransporter 2 (SGLT2)-inhibitors showed significant effect in patients with diabetes or metabolic syndrome, MetS with high cardiovascular-risk. Although the increased intracellular Zn\(^{2+}\) level ([Zn\(^{2+}\)]\(_i\)), oxidative stress and altered cardiac matrix metalloproteinases (MMPs) in diabetic cardiomyopathy can intersect with different signaling pathways, the exact mechanisms are not known yet. Since either MMPs or SGLT2 have important role in cardiac-fibrosis under hyperglycemia, we aimed to examine the role of SGLT2-inhibitor dapagliflozin (DAP) on cardiac Zn\(^{2+}\)-transporters responsible from [Zn\(^{2+}\)]\(_i\)-regulation, comparison to insulin (INS), together with MMP levels and systemic oxidative-stress-status in MetS-rats. High-carbohydrated diet-induced MetS-rats received DAP or INS for two weeks. DAP but not INS in MetS-rats significantly decreased high blood-glucose level, while both treatments exerted benefits on increased total-oxidative-status and decreased total-antioxidant-status in MetS-rat plasma as well as in heart tissue. Protein levels of Zn\(^{2+}\)-transporters, responsible from Zn\(^{2+}\)-influx into cytosol, ZIP7 and ZIP14 were decreased with no change in ZIP8 of MetS-rat cardiomyoctes, while Zn\(^{2+}\)-transporters, responsible from cytosolic Zn\(^{2+}\)-efflux, ZnT7 was decreased. Both treatments induced significant beneficial effects on altered ZIP14, ZIP8 and ZnT7 levels. Furthermore, both treatments exerted also benefits on depressed gelatin-zymography and protein expression levels of MMP-2 and MMP-9 in MetS-rat ventricular cardiomyocytes. The direct effect of DAP on heart was also confirmed with measurement of left ventricular developed pressure. Overall, we showed that DAP has important antioxidant-like cardio-protective effect in MetS-rats, similar to INS-effect, affecting Zn\(^{2+}\)-regulation via Zn\(^{2+}\)-transporters, MMPs and oxidative-
stress. Therefore one can suggest that SGLT2-inhibitors can be new therapeutic agents for cardio-protection not only in hyperglycemia but also in failing heart.

Key words: diabetes, SGLT2 inhibitors, heart function, oxidative stress, matrix metalloproteins, heart.
Introduction

The global epidemic of the metabolic syndrome (MetS), known also as insulin resistance syndrome, is a pathologic condition characterized by abdominal obesity, hypertension, and hyperlipidemia besides insulin resistance, and is not a single disease but a mixture of risk factors for cardiovascular diseases (Aguilar et al. 2010; Lauer et al. 1992; Saklayen 2018). It is now well accepted that the MetS often is followed with high incidence of obesity and type 2 diabetes (Genser et al. 2016). However, regardless of the underlying genetic and environmental influences that mediate the prevalence of the MetS, a higher prevalence will undoubtedly lead to undesirable outcomes among humans worldwide. Clinical data documented that the risk of cardiovascular diseases between diabetic and obese individuals is approximately two-to four-fold higher than in those without them (Cameron et al. 2012; Moreira et al. 2014).

The pathogenesis of the MetS is multiple and still poorly understood. Experimental evidence well documented the important role of oxidative stress in pathogenesis of MetS, while oxidative stress is a common mediator in pathogenicity of established cardiovascular risk factors (Mahjoub and Masrour-Roudsari 2012). In this regard, we previously have shown that important increases in oxidative stress at cellular and systemic levels in diabetic and MetS rats with marked depression in cardiac contractile activity. Furthermore, experimental data have pointed out that there is important contribution of increased cytosolic free Zn$^{2+}$ level ($[\text{Zn}^{2+}]_i$) to these alterations. Additionally, it was demonstrated that the depressed level of matrix metalloproteinases, MMPs (mainly MMP-2 and MMP-9) via a degradation in their expression levels (Yaras et al. 2008). Moreover, the remodeling of cardiovascular system in MetS individuals has a multifactorial pathogenesis that includes increased
oxidative stress and also an altered MMPs expression, which, in turn, degrade extracellular matrix proteins, such as collagen and fibronectin (Berg et al. 2014; Hopps and Caimi 2012; Kadoglou et al. 2010). In addition, it is known that MMPs are a family belonging to another family being structurally related Zn\(^{2+}\) and Ca\(^{2+}\)-dependent endopeptidases, involved in the cleavage of extracellular matrix proteins (Shapiro 1998).

Identifying the oxidative stress markers in cells/tissues has been the focus of many researchers as they have the potential to act as modulator of many signalling pathways, driving cardiovascular pathobiology (Ilkun and Boudina 2013). Authors pointed out the interrelationship between cardiac dysfunction and oxidative stress in MetS individuals and beneficial effects antioxidant therapies on these relations in via lipid accumulation, increased fibrosis and stiffness, altered Ca\(^{2+}\)-homeostasis, altered substrate utilization and mitochondrial dysfunction (Bonomini et al. 2015; Ilkun and Boudina 2013). Functional significance of the oxidative modifications enhances their validity as a proposed biological marker of cardiovascular disease, and is the strength of the redox cysteine modifications. In this regard, in early studies, the experimental data had implied that [Zn\(^{2+}\)]\(_{i}\) did increase rapidly in cardiomyocytes due to Zn\(^{2+}\) release from intracellular stores, at least, from metalloproteins via protein thiol oxidation under increased oxidative stress as well as hyperglycemia (Tuncay et al. 2013; Tuncay and Turan 2016; Turan et al. 1997).

Recent studies emphasized the important role of sodium-glucose cotransporter 2 (SGLT2)-selective inhibitor dapagliflozin (DAP), for use as an antidiabetic agent in normal and diabetic rats (Han et al. 2008; Hansen et al. 2014; Oku et al. 1999). In the clinical studies, it has been demonstrated that SGLT2 inhibitors, in addition to standard care of diabetics, provided important benefits against cardiovascular
disorders (Ahmadieh et al. 2017; Lahnwong et al. 2018; Sato et al. 2017; Zinman et al. 2015). Although these above data reported the important benefits of SGLT2 inhibitor trials in obesity and diabetes by clinicians for their benefits in prevention of cardiovascular disease, rather than focusing on glycemic control, the mechanisms by which SGLT2 inhibition improves cardiovascular outcomes are not fully understood.

Therefore, in the present study, we aimed to test whether the beneficial effect of a SGLT2 inhibitor on cardiac dysfunction in insulin-resistant overweight MetS rats via a way of affecting Zn\(^{2+}\)-regulation via Zn\(^{2+}\)-transporters, MMPs and oxidative-stress. For this aim, we used isolated left ventricular cardiomyocytes from SGLT2 inhibitor dapagliflozin (DAP) treated high-carbohydrated diet-induced metabolic syndrome (MetS) rats comparison to those of insulin (INS) treated MetS rats. Our data indicate that DAP, comparison with INS, has important an anti-oxidant like beneficial cardiac effect, directly targeting heart, in MetS-rats affecting Zn\(^{2+}\)-regulation via Zn\(^{2+}\)-transporters, MMPs and oxidative-stress, similar to INS-effects.

**Materials and methods**

**Animals**

Metabolic syndrome (MetS) was induced in male Wistar rats as described, previously (Okatan et al. 2015). Shortly, 2-month old male rats were fed with drinking water containing 32% sucrose besides their daily standard rat diet for 24 months. Following confirmation the MetS induction in these animals (by measuring body weight, fasting blood glucose level, insulin level, oral glucose tolerance test and and the development of insulin resistance with HOMO-IR index), they were treated with either dapagliflozin (DAP; 5 mg/kg, Bristo-myers Squibb Manufacturing Company, Humacao, Porto Riko), insulin (INS; 0.15 mg/kg, Humalog Mix25 Kwikpen, Lilly) or
vehicle (MetS) for 2 weeks. Experimental animals are exposed to a 12-h light–dark cycle and had free access to tap water. All animals were fed standard chow ad libitum and are housed in the standard rat cages.

**Langendorff-perfused heart measurement**

The left ventricular developed pressure changes (LVDP) are measured with a water-filled latex balloon inserted into the left ventricle, as described previously (Durak et al. 2017b). Briefly, hearts are electrically stimulated (DCS; Harward) at 300 beats/min with 1.5 ms square waves (at twice the threshold voltage) and all data are recorded online then stored and processed (Model 1050BP; BIOPAC Systems, Goleta, California, USA). The results are given as percentage changes with respect to the controls.

**Isolation of left ventricular cardiomyocytes**

The rats were anesthetized with pentobarbital sodium (30 mg/kg body weight, ip) and the hearts were removed from the body immediately. Blood samples were collected into heparinized tubes during the heart removal procedure.

Cardiomyocytes were isolated freshly from left ventricle of heart by enzymatic method, as described previously (Turan et al. 1997). Briefly, isolated hearts were initially perfused with a Ca\(^{2+}\)-free, HEPES-buffered solution for 5 min at 37°C and then hearts were perfused (6-8 mL/min) with fresh buffer supplemented 1.3 mg/ml collagenase (Type A, w) for 30 min. Following perfusion, we used the left ventricle part to get lived cardiomyocytes into HEPES-buffered solution. Isolated cardiomyocytes put into the HEPES buffer with 1 mM Ca\(^{2+}\) and 0.5 % bovine serum albumin (pH 7.4) and then they were stored -80°C for biochemical experiments.
Total antioxidant status (TAS) and total oxidant status (TOS) measurement in plasma and cardiomyocytes

TOS and TAS levels were measured in plasma as well as in cardiomyocytes by using commercially available kit as described, previously (Okatan et al. 2015). The TAS level in plasma was measured by using commercially available kit (RL0024, Rel Assay Diagnostics, Turkey), as described previously (Erel 2004). Briefly, we used the novel automated method, which is based on the bleaching of characteristic color of a more stable ABTS (2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The results are expressed as mmol Trolox equivalent/L. The TOS level in plasma was measured using commercially available kits (RL0024, Rel Assay Diagnostics, Turkey) as described previously (Erel 2004). Shortly, the oxidation reaction is enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with H₂O₂ and the results are expressed in terms of µM H₂O₂ equivalent/L.

Western-blotting

The stored cardiomyocytes were used through all measurements. The experimental procedure for Western-blot analysis were performed as described elsewhere (Tuncay et al. 2017). Briefly, the cells following harvested in lysis buffer, were homogenated and then centrifuged at 12,000×g for 15-min at 4°C. The protein concentration from supernatants from was determined by using the Bradford assay. Equal amount of protein preparations were run on SDS-polyacrylamide gels, electro-transferred to polyvinylidine difluoride membranes, and blotted with a primary antibody against ZIP7 (Santa Cruz, sc-83858, 1:500), ZIP8 (Protein Tech, 20459-1-AP; 1:500), ZIP14
(Thermo, PA5-21077; 1:500), ZnT7 (Santa Cruz, sc-160948; 1:500), ZnT8 (Santa Cruz, sc-98243, 1:500), MMP-2 (Santa Cruz, sc-6838, 1:500), MMP-9 (Santa Cruz sc-6841, 1/500) and GAPDH (Cell Signalling, D16H11, 1/5000). Immunoreactive bands were detected by a chemiluminescent reaction (ECL kit, Amersham Pharmacia, USA).

**Gelatin zymography**

Gelatin zymography for MMP-2 and MMP-9 activities were performed as described (Sawicki et al. 1998). Briefly, we loaded the non-reduced proteins onto an 8% polyacrylamide gel containing gelatin and then gelatinolytic activities were detected as transparent bands against the background of Coomassie blue-stained gelatin. Gelatinolytic activity was identified using HT1080 cell culture medium as a standard. The intensities of the bands were analysed using SigmaGel (Jandel).

**Chemicals and statistics**

Chemicals are obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. The results are expressed as means ± SEM. Statistical significance is tested with one-way ANOVA followed by Tukey post-test and significance level is considered as p<0.05.

**Results**

**General status of animals**

As presented previously, the mean (±SEM) body weight of MetS rats was significantly high (460±10 g) comparison with the aged-matched controls (400±12 g). Either DAP treatment of INS or treatment of MetS rats did not significantly prevent the extra weight gain of the rats comparison to those of untreated MetS rats. However, DAP treatment but not INS treatment of MetS rats modestly but
significantly decreased blood glucose (95±3 mg/dL vs. 88±3 mg/dL in MetS vs. MetS+DAP). Similarly, both treatment of MetS rats had no significant effect on the OGTT during 60 min monitorization, as comparison with those of vehicle-treated MetS rats.

**DAP or INS treatment have beneficial effects on oxidative stress status in systemic and organ levels of MetS rats**

Similar to our previous study (Durak et al. 2017a), the total oxidative status (TOS) level in plasma of MetS rats was significantly high comparison to those of aged-matched controls (Fig.1A). Furthermore, the total antioxidant status (TAS) level was significantly low in MetS rats comparison to aged-matched controls (Fig.1B). Either DAP or INS treatment of MetS rats for 2 weeks similarly and significantly induced positive effects. Interestingly, although it has been not shown any direct antioxidant actions of either DAP or INS, the systemic recovery in increased oxidative stress seems very important for MetS individuals.

We also measured the TOS and TAS levels in cardiomyocytes in order to detect the direct effect of DAP treatment of MetS rats targeting of the heart. As can be seen in Fig.1 C and D, the TOS level in isolated cardiomyocytes was markedly high while the TAS level was significantly low comparison with those of controls. Either DAP or INS treatment of MetS rats preserved the antioxidant defense of the heart, significantly via targeting it directly.

**Either DAP or INS treatment of Mets rats provided significant benefits on cellular Zn$^{2+}$-transporter protein expression levels**

Taken into consideration our previously published data on the contribution of increased [Zn$^{2+}$], ievil in depressed cardiac function under hyperglycemia (Olgar et al.
2018b; Tuncay and Turan 2016), in here, we determined first the protein expression levels of some Zn\(^{+2}\)-transporters responsible for Zn\(^{+2}\)–influx into cytosol of cardiomyocytes, such as ZIP7, ZIP8 and ZIP14, which have been previously shown in mammalian cardiomyocytes (Olgar et al. 2018a; Tuncay et al. 2018). As can be seen in Fig. 2 (A to C), the protein expression level of ZIP7 significantly increased, similar to in streptozotocin-induced diabetic rat heart (Tuncay et al. 2017). Similar to the our previous data in the heart tissue from human heart failure, the ZIP8 level is found to be markedly decreased with significantly increased ZIP14 level, similar to those of heart homogenates from human heart failure. Either DAP or INS treatment of MetS rats did not change the ZIP7 level, however, altered levels of both ZIP7 and ZIP14 were almost normalized with both types treatments.

The second groups Zn\(^{+2}\)-transporters, ZIP7 and ZnT8 were also examined. As can be seen in Fig. 3, the ZnT7 level (A) decreased with no change in the ZnT8 level (B) in cardiomyocytes isolated from MetS rats. Interestingly, INS but not DAP treatment induced fully recovery in ZnT7 level with no effect on the ZnT8 level.

**Either DAP or INS treatment provided important protection against MMP degradation in cardiomyocytes from MetS rats**

First, in here, we demonstrated the already known relation between oxidative stress and degradation of MMPs in the heart under hyperglycemia via determination of both protein expression levels and activities of MMP-2 and MMP-9 (Yaras et al. 2008). Fig. 4 shows the markedly decreased protein expression levels of both MMP-2 and MMP-9 in cardiomyocytes from MetS rats. Either DAP or INS treatment of MetS rats provided a similar significant and fully protection against MetS associated degradation in these MMPs.
DAP treatment of MetS rats provides benefits directly affecting the depressed cardiac function in hyperglycemic and hyperinsulinemic rats

Previously, we have demonstrated that the left ventricular developed pressure (LVDP) depressed significantly in MetS rats comparison to those of aged-matched controls (about 35-40%) (Okatan et al. 2015). In order to demonstrate the direct beneficial effect of DAP treatment on cardiac function via affecting the mechanical activity of the heart, we used hyperglycemic and hyperinsulinemic condition for Langendorff-perfusion of heart preparations. As can be seen in Fig. 5B, a direct exposure of DAP (0.1 μM; (Han et al. 2008)) or INS (0.1 μM) induced significant increase (about 40% w.r.t. control) in left ventricular developed pressure, LVDP in normal male rat heart preparations. The original pressure changes are given in Fig.5A. When we exposed heart preparations to DAP or INS following the MetS-mimicing via using hyperglycemic and hyperinsulinemic perfussion (25 mM glucose and 0.1 nM insulin, respectively), the contractile activity responses were about 20% compared with those of controls. These data confirmed that DAP application has important cardio-protective effect directly affecting the heart mechanical activity in insulin resistant MetS rats.

Discussion

We previously have demonstrated that cellular free Zn²⁺-changes are primarily coordinated by Zn²⁺-transporters and free Zn²⁺ releases during cardiac cycle (Tuncay et al. 2011) results mostly in a cytosolic free Zn²⁺ increase, further triggering higher production of pro-oxidant species and leading to cellular oxidative damage including proteins and kinases from contractile machinery (Tuncay et al. 2013). Furthermore, we have also been shown that either acute or chronic oxidant exposure induces...
marked increases in cytosolic free $\text{Zn}^{2+}$ in cardiomyocytes (Ayaz and Turan 2006; Turan et al. 1997) while either acute or chronic hyperglycemia causes oxidative stress and increased levels of cytosolic free $\text{Zn}^{2+}$, being responsible from cardiac dysfunction (Ayaz and Turan 2006; Tuncay and Turan 2016). Having all these previous documents in diabetic samples, in here, we examined the effect of a SGLT2 inhibitor dapagliflozin (DAP) on protein expression levels of $\text{Zn}^{2+}$-transporters, which have important roles in cellular $\text{Zn}^{2+}$-regulation in cardiomyocytes from insulin-resistant MetS rats. In order to confirm the DAP effect is associated with insulin resistance, and insulin treatment is used in diabetic and/or insulin resistant individuals, we also compared the DAP effects with those of insulin (INS) effects. In here, we have shown that DAP treatment of MetS rats provided important antioxidant-like benefits, in some aspects similar to INS effects, not only in systemic system of the rats but also directly targeting the heart against increased oxidative stress. Therefore, our present data provided important information related with how the SGLT2 inhibitors exert cardiac benefits when they used in MetS rats as in vivo. We, in here, also demonstrated that INS treatment could exert an anti-oxidant like action in MetS rats via inducing significant increase in TAS levels with marked decrease in TOS levels determined in both plasma and isolated cardiomyocytes from MetS rats. Supporing our present data, recently, it has been demonstrated that treatment of Zucker diabetic fatty (ZDF) rats with another SGLT2 inhibitor empagliflozin, an inhibitor of the renal SGLT2, provided benefical effects on high blood glucose level as well as endothelial dysfunction and increased oxidative stress in isolated aortic preparations of diabetic rats (Steven et al. 2017). Interestingly, since there were persisting hyperlipidemia and hyperinsulinemia in their animals, their data showed that empagliflozin could able to reduce glucotoxicity and thereby might prevent the development of endothelial
dysfunction together with an important reduction in oxidative stress of the experimental animals. Therefore, our present, similar to the above data, can support truly the previous pre-clinical observations with SGLT2 inhibitors. Most of pre-clinical data (EMPA-REG trial and others) provide important insights about the benefits of SGLT2 inhibitors on cardiovascular system in hyperglycemic and hyperinsulinemic individuals via marked reduction in cardiovascular mortality among the patients (Kashiwagi and Maegawa 2017; Kramer and Zinman 2016; Tanaka et al. 2017; Zinman et al. 2015). In this regard, experimental and clinical studies suggested that those risk factors beyond glucose that can potentially be modulated positively with SGLT2 inhibitors include blood pressure, weight, visceral adiposity, hyperinsulinaemia, arterial stiffness, albuminuria, circulating uric acid levels and oxidative stress (Kramer and Zinman 2016; Steven et al. 2017; Zinman et al. 2015). Other study results also provided important data on beneficial effects of SGLT2 inhibitors on hyperglycemia, hyperlipidemia, hepatic steatosis, oxidative stress, inflammation, and obesity in type 2 diabetic mice (Tahara et al. 2013) and prevention of oxidative stress in the kidney of diabetic rats (Osorio et al. 2012). However, they could not provide any data underlying the mechanisms of beneficial effects of SGLT2 inhibition on depressed cardiovascular function, independent of diuretic effect or blood pressure lowering effect. Although SGLT2 inhibition seems to be a promising therapeutic strategy for diabetic, obese or prediabetic MetS, further studies are also needed.

The cardioprotective effect of SGLT2 inhibitors, particularly via effecting vascular system have been demonstrated recently in diabetics as well as in prediabetics with MetS (Hammoudi et al. 2017; Kusaka et al. 2016; Lee et al. 2018). Their data demonstrated that empagliflozin significantly lowered HbA1c and slightly reduced
body weight compared to vehicle treatment with no obvious changes in insulin levels, however, it improved markedly left ventricular maximum pressure and in vivo indices of diastolic function. In this aspect, Baartscheer A et al (2017) treated animals with empagliflozin and measured cytoplasmic Na\(^+\) and Ca\(^{2+}\) concentrations, mitochondrial Ca\(^{2+}\) concentration and Na\(^+\)/H\(^+\) exchanger activity in isolated ventricular myocytes (Baartscheer et al. 2017). Their data have demonstrated that extracellular high glucose related changes in these above parameters could be preserved with SGLT2 inhibitor treatment via implying its direct cardiac effects by lowering myocardial cytosolic ionic mechanisms particularly associated with cardiac contractile activity.

Previous studies pointed out that enhanced oxidative stress or diabetes-induced oxidative stress, under both in vitro and in vivo conditions, is known to increase the basal levels of [Ca\(^{2+}\)]\(_i\), as well as [Zn\(^{2+}\)]\(_i\), which is also known as a strong indicator of the cellular level of oxidative stress in cardiomyocytes (Ayaz and Turan 2006; Tuncay et al. 2013; Turan et al. 1997). In these aspects, we, previously, have shown that exogenously application of oxidants could increase both [Ca\(^{2+}\)]\(_i\) and [Zn\(^{2+}\)]\(_i\) with deleterious effects on proteins and kinases (RyR2, PKA, CaMKII, PKC, PLB) of contractile machinery in cardiomyocytes, which, in turn, induce significant depression effect on heart contractility. In addition, our previous data also demonstrated that either acute or chronic hyperglycemia could induce significant increases in both [Ca\(^{2+}\)]\(_i\) and [Zn\(^{2+}\)]\(_i\) as well as reactive oxygen and nitrogen species (Tuncay et al. 2011; Tuncay et al. 2013; Tuncay and Turan 2016). Taken into consideration all these published data on increased [Ca\(^{2+}\)]\(_i\) and [Zn\(^{2+}\)]\(_i\) and increased oxidative stress under hyperglycemia, the benefical effects of DAP treatment of MetS rats associated with well-controlled systemic oxidative stress and antioxidant defense system together with possible well-controlled [Zn\(^{2+}\)]\(_i\) through normalized protein expression levels of Zn\(^{2+}\)-
transporters will get much more interest for further clinical trials with SGLT2 inhibitors in diabetic and/or obese individuals or individuals with cardiac diseases. Zn\(^{2+}\) is essential for numerous cellular functions although it is toxic for live cells (Vallee and Falchuk 1993). Zn\(^{2+}\)-homeostasis is therefore dynamically maintained by a variety of transporters, stores, and other proteins distributed in distinct cellular compartments.

In the present study, we have also shown that DAP treatment of MetS rats significantly preserved the depressed expression levels of MMP-2 and MMP-9 in isolated left ventricular cardiomyocytes. Similarly, we previously have demonstrated the significantly depressed level of MMP-2 and MMP-9 via degradation in their expression levels (Yaras et al. 2008). Moreover, the remodeling of cardiovascular system in MetS individuals has a multifactorial pathogenesis that includes increased oxidative stress and also an altered MMPs expression, which, in turn, degrade extracellular matrix proteins, such as collagen and fibronectin (Berg et al. 2014; Hopps and Caimi 2012; Kadoglou et al. 2010). In these aspects, it is known that glomerular hypertrophy and extracellular matrix accumulation involve in the pathogenesis of diabetic nephropathy through an increased matrix synthesis and degradative pathway in diabetic kidney due to event series including dynamic process involving synthesis as well as degradation in the regulation of extracellular matrix. The latter is tightly regulated by a number of MMPs. In line with these events, Liu Bi-cheng, Xu Yan, Ma Kun-ling et al. (2005) treated streptozotocin-induced diabetic rats with a SGLT2 inhibitor and demonstrated its beneficial effect on renal MMP-2 level (Liu et al. 2005). Additionally, it is known that MMPs play an important role during physiological tissue remodeling in pathophysiological conditions, including diabetes and obesity while MMP expression and activity are regulated by different factors such
as insulin resistance and obesity. The role of Zn\textsuperscript{2+} level and its binding-groups to MMPs on the activities of MMPs and their roles in several pathological conditions is greatly discussed by Jacobsen JA et al (2010) (Jacobsen et al. 2010). Therefore, in here, we first demonstrated the degradation of MMPs in MetS rat cardiomyocytes and their preservation with DAP treatment. At least, our data imply the possible cross-talk between [Zn\textsuperscript{2+}], via Zn\textsuperscript{2+}-transporters and MMPs (MMP-2 and MMP-9) in MetS cardiac function and the beneficial effect of DAP treatment on this cross-talk. Taken into consideration the relation between this cross-talk and oxidative stress, the importance of DAP treatment of individuals with hyperglycemia raises much more rapidly between clinical trials.

Taken into consideration of the direct effect of DAP on the heart, overall, we showed that DAP has important antioxidant-like cardio-protective effect in MetS-rats, in some aspects similar to INS-effect, affecting Zn\textsuperscript{2+}-regulation via Zn\textsuperscript{2+}-transporters, MMPs and oxidative-stress. Therefore one can suggest that SGLT2-inhibitors can be new therapeutic agents for cardio-protection not only in hyperglycemia but also in failing heart.
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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

This study consists of animal data and is devoid of any human data.

Ethics approval and consent to participate

The experimental protocols with rats were performed in accordance with the the standards of the European Community guidelines on the care and use of laboratory animals" adhere to the to the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and had been approved by the local ethics committee of Ankara University (No:2015-12-137).

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References


Figure legends

Fig. 1. Assessment of in vivo DAP or INS treatment on systemic oxidative status in plasma and cardiomyocytes of MetS rats. The total oxidant status (TOS) (A and C) and the total antioxidant status (TAS) (B and D) measured in plasma and cardiomyocytes of MetS rats comparison with aged-matched rats (Con). Data are presenting as mean (±SEM) as percentage changes with respect to the corresponding controls. The total number of rats in each group; n= 5-6 rats. *p<0.05 vs. Con; #p<0.05 vs. MetS.

Fig. 2. The protein expression levels of Zn$^{2+}$-transporters responsible from Zn$^{2+}$-influx into cytosol in cardiomyocytes from either DAP or INS treated MetS rats. Western-blot analysis of ZIP7, ZIP8 and ZIP 14 (at 51 kDa) protein expression levels (with respect to GAPDH at 37 kDa) (A, B, C, respectively). Data are presenting as mean (±SEM) as percentage changes with respect to the corresponding controls. Number of animals /group; n= 5-7. *p<0.05 vs. Con; #p<0.05 vs. MetS.

Fig. 3. The protein expression levels of Zn$^{2+}$-transporters responsible from cytosolic Zn$^{2+}$-efflux in cardiomyocytes from either DAP or INS treated MetS rats. Western-blot analysis of ZnT7 (A) and ZnT8 (B) (at 41 kDa) protein expression level (with respect to GAPDH at 37 kDa). Data are presenting as mean (±SEM) as percentage changes with respect to the corresponding controls. Number of animals /group; n= 5-7. *p<0.05 vs. Con; #p<0.05 vs. MetS.

Fig. 4. The protein expression levels and activities of matrix metalloproteinases (MMPs) in cardiomyocytes from either DAP or INS treated MetS rats. The protein expression levels and activities of MMP-2 at 72 kDa and MMP-9 at 92 kDa determined by Western-blot (with respect to GAPDH at 37 kDa) and zymography (in
A and C, respectively and in B and D, respectively). Data are presenting as mean (±SEM) as percentage changes with respect to the corresponding controls. Number of animals/group; n=5-7. *p<0.05 vs. Con; #p<0.05 vs. MetS.

Fig. 5. The beneficial effect of DAP on hemodynamic parameters of the heart. The original left ventricular developed (LVDP) traces with different protocols such as either DAP (0.1 μM) or INS (0.1 μM) exposures (A). (B) To mimic MetS (MetS_m) in cardiac preparations (hyperglycemic and hyperinsulinemic), high glucose (25 mM) and high circulating insulin (0.1 nM) were used for perfusion of the heart. Data are presenting as mean (±SEM) as percentage changes with respect to the corresponding controls. Number of animals; n=4-5/group, *p<0.05 vs. Con, #p<0.05 vs. MetS_m.
Assessment of in vivo DAP or INS treatment on systemic oxidative status in plasma and cardiomyocytes of MetS rats

254x190mm (300 x 300 DPI)
The protein expression levels of Zn2+–transporters responsible for Zn2+–influx into cytosol in cardiomyocytes from either DAP or INS treated MetS rats.
The protein expression levels of Zn2+-transporters responsible for cytosolic Zn2+-efflux in cardiomyocytes from either DAP or INS treated MetS rats.

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