Control of plasma glucose with alpha-glucosidase inhibitor attenuates oxidative stress and slows the progression of heart failure in mice

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Abstract

Objective: It has been suggested that reduction in glucose levels contributes to the prolongation of life span of rodents in conjunction with restricted food intake, and hyperglycemia has been confirmed as a risk factor for cardiovascular disease (CVD), raising the possibility that better glycemic control could slow the progression of CVD. This study was designed to determine whether impaired glucose tolerance develops during the progression of cardiac hypertrophy and heart failure, and whether tight glycemic control could reduce the severity of heart failure.

Methods: In male C57BL/6 mice, transverse aortic constriction (TAC) was employed to create cardiac hypertrophy and heart failure. The involvement of NADPH in TAC mice and cardiac myocytes in the neonatal rat was investigated.

Results: The random-fed plasma glucose concentration was higher in TAC mice, and it was reduced to about 100 mg/dL by voglibose (an alpha-glucosidase inhibitor). Four weeks after TAC, both the heart weight/body weight ratio and the lung weight/body weight ratio were lower in the voglibose group than in the TAC group. Echocardiographic and invasive hemodynamic examination showed improvement of left ventricular function in voglibose-treated mice. Voglibose treatment decreased the myocardial expression of an NADPH oxidase subunit (p47phox). Glucose dose-dependently increased both neonatal rat myocyte protein synthesis and the expression of p47phox protein, while apocynin (an NADPH oxidase inhibitor) blocked the enhancement of protein synthesis by high glucose.

Conclusion: Improvement of glycemic control through voglibose therapy inhibited cardiac remodeling by decreasing myocardial oxidative stress in mice with cardiac pressure overload.

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Keywords: Glucose metabolism; Myocardial hypertrophy; Oxidative stress; Heart failure

1. Introduction

Hyperglycemia or impaired glucose tolerance (IGT) is a common feature of both acute myocardial infarction [1] and chronic heart failure (CHF) [2,3]. IGT can either be the cause or the result of CHF [4]. Patients with type 2 diabetes have a high propensity to develop CHF [5], and IGT is believed to be an independent risk factor for cardiovascular events [6–8]. Hyperglycemia or IGT can accelerate the progression of CHF [1,9]. On the other hand, increasing evidence supports a reciprocal relationship between CHF and IGT showing CHF patients are susceptible to developing IGT or diabetes [2,3]. These findings suggest the important impact of glycemic levels on the progression of CHF. Because CHF may cause hyperglycemia or IGT via increased sympathetic activity [10] or promotion of the renin–angiotensin system [11], it could be hypothesized that improved glycemic control might ameliorate CHF.
In addition to dietary restrictions, two other approaches are usually employed to control blood glucose levels, namely, increasing glucose utilization and decreasing glucose absorption. Stimulation of carbohydrate oxidation has been shown to have a favorable impact on cardiac function [12,13]. A recent clinical study has also suggested that improvement of glycemic control in patients with IGT by administration of an alpha-glycosidase inhibitor, acarbose, was associated with a reduced risk of cardiovascular disease [14]. In addition, prior retrospective clinical investigations from our laboratory revealed that another alpha-glycosidase inhibitor (voglibose) was also beneficial in the treatment of CHF [15]. It has been established that IGT or hyperglycemia leads to oxidative stress [16,17], which in turn accelerates cardiac remodeling [18,19], further supporting the concept that better glycemic control might slow the progression of cardiac hypertrophy and cardiac failure.

In the present study, we investigated whether IGT develops in mice with left ventricular pressure overload, and explored the beneficial effect of voglibose on cardiac remodeling as well as the possible underlying mechanism.

2. Methods

2.1. Transverse aortic constriction (TAC) model and experimental protocols

All procedures were performed in accordance with our institutional guidelines for animal research conforming to NIH Guidelines. Male C57BL/6 mice (7–8 weeks old, wt 20–25 g) were anesthetized with a mixture of xylazine (5 mg/kg) and ketamine (100 mg/kg) via intraperitoneal injection. TAC was performed to create pressure overload-induced cardiac hypertrophy and heart failure, as described previously [20,21]. Seventy-one mice were included in this study. We treated the mice with water (TAC group, \(n=25\)), Sham group: \(n=21\) or the alpha-glycosidase inhibitor voglibose (supplied gratis by Takeda Pharmaceutical Co. Ltd.) at a daily oral dose of 10 mg/kg (in tap water, TAC+Voglibose group: \(n=19\)); Sham+Voglibose: \(n=6\). The dose of voglibose was set according to the results of a previous study [22]. Mice were fed ad libitum and given free access to water. There were no differences among all the experimental groups with regard to age and body weight before surgery. On the 3rd day following TAC, two mice from the TAC group and the TAC+Voglibose group were used to measure the trans-stenosis pressure gradient to confirm whether the LV pressure overload was similar between the two groups. Mice were euthanized at 4 weeks after TAC for morphometric and molecular analyses. Cell surface area, myocardial and perivascular fibrosis were quantified using 4 hearts from each group, as described previously [21,23].

2.2. Invasive measurement of hemodynamics

To determine the pressure gradient on the third day after TAC, two mice each from the TAC and TAC+Voglibose groups were randomly selected and anesthetized, as mentioned above, and an endotracheal tube was inserted and connected to a volume-cycled rodent ventilator as described elsewhere [20]. Ventilation was necessary to avoid respiratory arrest due to ligation of both carotid arteries. A 1.4 F Millar pressure catheter (Millar Instruments) was inserted into each of the left and right carotid arteries, and the blood pressures were measured simultaneously with a data acquisition and analysis system (PowerLab, AD Instruments). Left ventricular (LV) hemodynamics were evaluated at 4 weeks after TAC. A Millar catheter was inserted via the right carotid artery and carefully introduced into the LV; after which the heart rate, systolic pressure (LVSP), end-diastolic pressure (LVEDP), the maximal slope during the upstroke or downstroke of the pressure wave (max \(dP/dt\) and min \(dP/dt\), max \(dP/dt\) divided by the pressure at the time of max \(dP/dt\) (contractility index) and the exponential time constant of relaxation (Tau) were analyzed using an application program Blood Pressure Module.

2.3. Echocardiography

Transthoracic echocardiography was performed with a Sonos 4500 and a 15–6 L MHz transducer (Philips, the Netherlands). Mice were immobilized without anesthesia. Two-dimensional short-axis views of the LV were obtained for guided M-mode measurement of the LV posterior wall thickness (LVPW), LV end-diastolic diameter (LVEDd), and LV end-systolic diameter (LVESd). LV fractional shortening (FS) was calculated as follows: LVFS=(LVEDd−LVESd)/LVEDd×100. LV volume was calculated using the Teichholz formula: \(V=7D^3/(2.4+D)\), where \(V=vol\)ume and \(D=tech\)ochardiographically measured internal dimension [24]. Accordingly, LV end-diastolic volume (LVEDV)=7(LVEDD)3/(2.4+LVEDD), LV ejection fraction (LVEF %)=SV/LVEDV×100. LV mass was calculated according to cube assumptions and modified with Teichholz formula: LV mass (mg)=1.055[(7(LVEDD)+LVPWd+VSTd)3/(2.4+LVEDD+LVPWd+VSTd)−LVEDV], where 1.055 is the gravity of myocardium, VSTd is diastolic ventricular septal thickness.

2.4. Measurement of glucose and insulin and free fatty acid (FFA)

The plasma concentrations of glucose and insulin were determined under fasting conditions, as described previously [25]. Random plasma glucose levels were also measured in each group. Insulin resistance was determined
by homeostasis assessment: HOMA-IR = (fasting plasma glucose [mg/dL] × fasting serum insulin [ng/mL])/22.5. An intraperitoneal glucose tolerance test (IPGTT) was performed at 4 weeks. IPGTT is extensively used in the study of glucose metabolism in rodent animals [26,27]. After fasting for 14 h, glucose (2 g/kg) was injected intraperitoneally and the plasma glucose level was measured at baseline and at 30, 60, and 120 min. Serum nonesterified (free) fatty acid concentrations were measured by spectrophotometric enzymatic assay (Wako Chemicals).

2.5. RNA preparation and analysis

Total RNA of homogenized mouse whole heart or cultured neonatal rat cardiac myocytes was prepared using RNA-Bee isolation reagent (Tel-Test, Inc.) according to the protocol of the manufacturer. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to generate cDNA templates from extracted RNA. cDNA template (1 µg) was then used for subsequent PCR amplification with primers targeting the genes of atrial natriuretic factor (ANF), collagen IV (procollagen IV alpha) and collagen I. PCR products were loaded onto a 2.0% agarose gel and electrophoresed at 100 V for 45 min. Gels were stained with ethidium bromide and quantified using Scion Image software. β-Actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control.

2.6. Western blot analysis

Membrane proteins were prepared from whole heart tissue homogenate or cultured cardiac myocytes, as described elsewhere [28]. Then immunoblotting was performed to detect the nicotinamide adenine dinucleotide 3-phosphate (NADPH) oxidase subunits p22phox, p47phox, p67phox, and gp91phox (Santa Cruz Biotechnology), and beta-actin was used as a loading control. Immunoreactive bands were visualized by enhanced chemiluminescence (Amersham) and quantified by densitometry with Scion Image software.

2.7. Validation of glucose effects on oxidant stress and cellular hypertrophy in an in vitro model of neonatal rat cardiomyocyte culture

Ventricular myocytes were isolated from neonatal rats (2 to 3 days old) and cultured as described previously [29]. In brief, myocytes were incubated in Dulbecco’s Modified Eagle’s Medium containing 100 mg/dL glucose supplemented with 10% fetal calf serum for 72 h and then grown under serum-free conditions for 48 h. Finally, the myocytes were exposed to 100, 450, or 900 mg/dL glucose with or without the addition of 10^{-4} mol/L apocynin (an inhibitor of superoxide production by NADPH oxidase; Sigma-Aldrich) for 24 h and then harvested for analysis of protein synthesis based on 3H-leucine incorporation [21] and for determination of the expression of NADPH oxidase subunits proteins.

2.8. Statistical analysis

The unpaired Student’s t-test was used for comparisons between two groups, and one-way ANOVA with post hoc analysis by the Tukey–Kramer exact probability test was used for multiple comparisons. Skewed data were log-transformed before parameter testing was performed. Results were expressed as the mean±S.E.M. and P<0.05 was considered statistically significant.

3. Results

3.1. Levels of plasma glucose and serum insulin and FFA

As shown in Fig. 1A, random plasma glucose levels were increased in TAC mice at 3 weeks after surgery, while the administration of voglibose (10 mg/kg/day) returned the plasma glucose level to about 100 mg/dL. Fasting glucose before sacrifice was also higher in the TAC group than in the sham group (Fig. 1B). An increase of serum insulin levels relative to those in sham mice was noted in TAC mice, and this was not changed by voglibose treatment (Fig. 1C). Insulin resistance index HOMA-IR was significantly increased in TAC mice (Fig. 1D). The IPGTT showed significantly lower peak glucose levels in sham and voglibose-treated mice (Fig. 1E), suggesting an improvement of glucose tolerance by voglibose. These findings indicate the development of IGT with postprandial hyperglycemia in TAC mice. On the other hand, the serum FFA level was significantly lower in TAC mice than in the sham group, while no difference was found between TAC and voglibose-treated TAC mice (Fig. 1F).

3.2. Amelioration of cardiac hypertrophy by voglibose

The pressure gradient was about 50 mm Hg on the 3rd day after TAC, indicating that LV pressure overload was similar in the TAC and voglibose groups, which is also supported by the evidence that LVSP was similar between the two groups at 4 weeks after TAC (Table 1). There was no significant difference in body weight at 4 weeks (21.24±0.32 g vs. 21.94±0.46 g in the TAC and TAC+Voglibose groups, respectively). Both the heart weight-to-body weight ratio (HW/BW mg/g) and the cross-sectional surface area of cardiomyocytes were significantly smaller in voglibose-treated TAC mice (Fig. 2A, B, E and F), but there was no significant difference in cardiac fibrosis indexed histologically by Azan staining (myocardial fibrosis: 19.7±4% vs. 17.4±2%; perivascular
fibrosis: 81±12% vs. 73±9%) and expression of the markers of fibrosis, collagen I and IV genes (Fig. 2C and D) between the TAC and TAC+Voglibose groups.

3.3. Improvement of LV hemodynamics by voglibose

It has been reported that high concentrations of glucose impair cardiomyocyte contractility [28]. Since the present study showed that LV pressure overload could induce moderate postprandial hyperglycemia (Fig. 1A), we postulated that inhibition of postprandial hyperglycemia by voglibose might improve cardiac function. As expected, both max dP/dt and contractility index were higher in the voglibose-treated mice compared with the untreated TAC mice (Fig. 3A and Table 1). LVSP was slightly higher, while both LVEDP and Tau were

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>HR (beats/min)</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>Max dP/dt (mm Hg/s)</th>
<th>Min dP/dt (mm Hg/s)</th>
<th>Contractility index</th>
<th>Tau (ms)</th>
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<tr>
<td>Sham</td>
<td>236±25</td>
<td>86±3.0*</td>
<td>8.3±1.6*</td>
<td>3074±344</td>
<td>2695±270</td>
<td>77.3±5.5*</td>
<td>19.2±1.3†</td>
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<tr>
<td>Sham+V</td>
<td>243±37</td>
<td>87±11*</td>
<td>8.9±1.4*</td>
<td>2892±269</td>
<td>2601±195</td>
<td>75.0±9.7*</td>
<td>19.2±1.2†</td>
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<tr>
<td>TAC</td>
<td>188±20</td>
<td>164±5.4</td>
<td>24.5±1.4</td>
<td>3167±106</td>
<td>2995±161</td>
<td>43.6±2.1</td>
<td>24.8±1.3</td>
</tr>
<tr>
<td>TAC+V</td>
<td>170±10</td>
<td>169±13.1</td>
<td>16.3±2.4†</td>
<td>3776±328</td>
<td>3904±369†</td>
<td>54.6±4.7†</td>
<td>18.7±1.6†</td>
</tr>
</tbody>
</table>

TAC, transverse aortic constriction; HR, heart rate; LVSP, maximum left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; Max dP/dt, the steepest slope during the upstroke of the pressure curve; Min dP/dt, the steepest slope during the downstroke of the pressure curve; Contractility index: Max dP/dt divided by the pressure at the time of Max dP/dt; Tau, the exponential time constant of relaxation; Sham+V, sham+ v o g l i b o s e 10 mg/kg/day; TAC+V, TAC+ vogl i b o s e 10 mg/kg/day. The number of mice in groups of Sham, Sham+V, TAC and TAC+V was 10, 6, 11 and 9, respectively. Data are mean±S.E.M.

* P<0.001, compared with TAC.
† P<0.01, compared with TAC.
‡ P<0.05, compared with TAC.

Fig. 1. Glucose and fatty acid metabolism in hypertrophic and failing hearts. (A) Random plasma glucose concentrations at 3 weeks after transverse aortic constriction (TAC) or a sham operation. (B) Fasting plasma glucose concentration at 4 weeks. (C) Fasting serum insulin concentration at 4 weeks. (D) The insulin resistance index HOMA-IR at 4 weeks. A t-test was performed after log-transformation. (E) Intraperitoneal glucose tolerance test. *P<0.05 vs. sham, †P<0.05 vs. voglibose-treated mice, ‡P<0.05 vs. sham. (F) Serum free fatty acid at 4 weeks (FFA).
significantly lower and min dP/dt was significantly higher in voglibose-treated TAC mice (Table 1), and no significant change was found in voglibose-treated sham mice, suggesting that voglibose improves both systolic and diastolic function in TAC mice (Table 1). These results indicate that voglibose treatment delayed the progression of cardiac dysfunction due to pressure overload.

3.4. Pulmonary and echocardiographic findings

Four weeks after TAC, the lung weight-to-body weight ratio (LW/BW) was markedly decreased in voglibose-treated mice (Fig. 3C), suggesting improvement of cardiac function. In agreement with this finding and the data on LV hemodynamics, echocardiography showed a marked increase in LVFS and LVEF in voglibose-treated TAC mice, indicating an improvement in systolic function (Fig. 3B, Table 2). Both LV dimensions and posterior wall thickness were also decreased in TAC mice by voglibose treatment (Table 2), indicating the inhibition of cardiac remodeling. No significant change was found in voglibose-treated sham mice.

3.5. High glucose enhances protein synthesis by cardiac myocytes: role of oxidative stress in vitro

The most commonly used in vitro and in vivo models for myocardial hypertrophy studies are primary culture of neonatal rat cardiac myocytes and the mouse TAC model, respectively [30]. Since mouse cardiac myocyte culture is not an established method for experimental investigation of hypertrophy, we used cultured neonatal rat cardiac myocytes to examine the effect of high glucose levels on protein synthesis to test whether hyperglycemia causes cardiac myocyte hypertrophy. As shown in Fig. 4A, glucose dose-dependently increased 3H-leucine incorporation by cultured cardiac myocytes. Expression of the marker of pathological hypertrophy ANF was upregulated in high glucose-medium cultured cells (Fig. 4B).

To investigate the mechanism underlying glucose-induced myocyte hypertrophy, we examined NADPH
oxidase expression because this enzyme is a potential source of reactive oxygen species (ROS) and makes an important contribution to cardiac hypertrophy and heart failure [19,31,32]. Membrane expression of NADPH subunit proteins (p22phox, p47phox, p67phox) and gp 91 was determined by western blotting. Neonatal rat myocyte expression of p47phox was upregulated by culture in high glucose medium (Fig. 5A) and was

**Table 2**

Echocardiographic findings at 4 weeks after TAC or sham operation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham (n=16)</th>
<th>Sham+Voglubose (n=6)</th>
<th>TAC (n=21)</th>
<th>TAC+Voglubose (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDd (mm)</td>
<td>2.93±0.05*</td>
<td>2.99±0.06††</td>
<td>3.46±0.12</td>
<td>3.09±0.12††</td>
</tr>
<tr>
<td>LVSPd (mmHg)</td>
<td>0.66±0.01*</td>
<td>0.61±0.02*</td>
<td>0.81±0.03</td>
<td>0.76±0.02††</td>
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<tr>
<td>LVESd (mm)</td>
<td>1.27±0.05*</td>
<td>1.35±0.05††</td>
<td>2.23±0.16</td>
<td>1.70±0.17††</td>
</tr>
<tr>
<td>LVFS (%)</td>
<td>56.7±1.3†</td>
<td>55.0±1.4†</td>
<td>37.0±2.8</td>
<td>46.5±3.3††</td>
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<tr>
<td>LVEDV (µL)</td>
<td>33.2±1.3*</td>
<td>34.9±1.7††</td>
<td>51.0±4.4</td>
<td>39.1±4.0††</td>
</tr>
<tr>
<td>LVESV (µL)</td>
<td>4.1±0.4*</td>
<td>4.7±0.4††</td>
<td>20.0±3.4</td>
<td>10.9±2.6††</td>
</tr>
<tr>
<td>SV (µL)</td>
<td>29.1±1.1</td>
<td>30.3±1.4††</td>
<td>31.3±1.3</td>
<td>28.2±1.7</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>88±1*</td>
<td>87±1†</td>
<td>66±3</td>
<td>77±4††</td>
</tr>
<tr>
<td>LV mass (mg)</td>
<td>47.3±1.3*</td>
<td>44.6±1.6*</td>
<td>73.7±2.7</td>
<td>59.7±2.3*</td>
</tr>
</tbody>
</table>

TAC, transverse aortic constriction; LVEDd, left ventricular end-diastolic dimension; LVSPd, left ventricular diastolic posterior wall thickness; LVESd, left ventricular end-diastolic dimension; LVFS: left ventricular fractional shortening; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SV, stroke volume; LVEF, left ventricular ejection fraction; Sham+V, sham+ voglibose 10 mg/kg/day; TAC+V, TAC+ voglibose 10 mg/kg/day. Data are mean±S.E.M.

* P<0.001, compared with TAC.
†† P<0.01, compared with TAC.
†‡ P<0.05, compared with TAC.

Fig. 3. Improvement in heart function by voglibose treatment. (A) Representative graphs of left ventricular pressure and its rate of change (dP/dt) showed a lower diastolic pressure and higher minimum dP/dt. (B) Representative M-mode echocardiographic images. (C) Pulmonary congestion was ameliorated by voglibose treatment at 4 weeks after TAC and the lung weight-to-body weight ratio (LW/BW) was significantly lower in voglibose-treated mice than in the untreated TAC mice. *P<0.01, †P<0.05 vs. TAC.
also upregulated in the hearts of TAC mice at 4 weeks (Fig. 5B). In contrast, the other 3 NADPH subunits were unchanged (data not shown). Treatment with voglibose caused a decrease in p47phox expression ($P < 0.05$ vs. TAC mice, Fig. 5B).

To confirm that the increase in p47phox contributed to the glucose-induced increase in protein synthesis by cardiac myocytes, we used an inhibitor of NADPH oxidase (apocynin) to block protein synthesis. As shown in Fig. 4, co-treatment with $10^{-4}$ mol/L apocynin and high glucose caused a decrease in $^3$H-leucine incorporation by cardiac myocytes.

### 4. Discussion

In this study, we demonstrated that glucose intolerance was induced in mice with cardiac hypertrophy and heart failure due to pressure overload, and that oral treatment with voglibose effectively controlled postprandial hyperglycemia, thereby ameliorating cardiac hypertrophy and slowing progression to heart failure. We further showed that culture with high concentrations of glucose led to enhancement of oxidative stress-mediated protein synthesis by cardiac myocytes, and voglibose inhibited myocardial NADPH oxidase expression via improved control of blood glucose levels.
We speculate that sympathetic activation might have contributed to hyperglycemia associated with CHF. Consistent with previous reports [33–35], we have noted enhancement of sympathetic activity (demonstrated by an increase in plasma catecholamines) in mice with cardiac hypertrophy and cardiac failure [21]. Supportively, alpha1-adrenergic blockade with prazosin [36] has been demonstrated to increase insulin sensitivity and improve glucose metabolism. Moreover, downregulation of glycolytic enzyme encoding genes was observed in pressure-overload-induced hypertrophy in spontaneously hypertensive rats, and a sympathetic inhibitory treatment with carvedilol attenuates the downregulation of glucose metabolic gene expression [37] and increases glucose utilization in hypertension patients [38]. It is also known that the renin–angiotensin system (RAS) plays an important role in the metabolic syndrome, including hyperglycemia and IGT [11], and that inhibition of RAS activity by either angiotensin-converting enzyme inhibitors [39] or angiotensin type 1 receptor blockers [40] is effective both for treating cardiovascular disease and for preventing the onset of diabetes. Evidence from clinical studies also supports our finding that IGT is associated with heart failure. Tenenbaum et al. reported that there was a significantly higher risk of diabetes in patients with advanced heart failure [2]. In 308 non-ischemic heart disease patients with CHF, we have also found a 75% incidence of IGT and diabetes (unpublished data).

The failing heart is postulated to suffer from chronic energy starvation despite an excess of substrates. Therefore, we targeted the excessive supply of glucose by pharmacological intervention in an attempt to improve cardiac function. A very recently published retrospective study by Kosiborod et al. firmly supports the hypothesis that excess glucose is detrimental. They reported that hyperglycemia was common in patients with acute myocardial infarction and the risk of death was higher in hyperglycemic patients without recognized diabetes than in those with diabetes [1]. Furthermore, it was reported that hyperglycemia exacerbates LV remodeling and heart failure in rats after myocardial infarction [9]. Considering these findings, it is plausible that better control of postprandial hyperglycemia by voglibose treatment could ameliorate cardiac hypertrophy and slow the progression to heart failure. We investigated the effect of voglibose on lipid metabolism and did not find any change in serum FFA concentrations, consistent with previous studies showing alpha-glycosidase inhibitors had no effect on FFA metabolism [review [41]]. Except for a reduction in triglycerides, voglibose was reported not to change other lipid profiles such as total cholesterol, low-density lipoprotein cholesterol and FFA levels [42]. In this study, we noted a significantly lower serum FFA level in TAC mice, which could be a result of an increase in fatty acid oxidation. Because fatty acid oxidation requires a greater rate of oxygen consumption than does glucose oxidation for a given rate of ATP synthesis [43], an increase of fatty acid oxidation is likely to increase the consumption of cardiac energy and consequently lead to the depression of contractile performance. This is also supported by the evidence that inhibition of FFA oxidation attenuates the severity of heart failure [33,44,45]. On the other hand, an increased plasma FFA concentration [33] and down regulated fatty acid oxidation were reported to appear in advanced stage CHF [46,47], while moderate heart failure does not decrease fatty acid oxidation [48].

Substantial evidence supports an important role of oxidative stress due to an excess of reactive oxygen species (ROS) in the pathophysiology of cardiac hypertrophy and failure [18,19,49]. NADPH oxidase is a major source of ROS. In this study, we confirmed that high glucose contributed to enhancement of oxidative stress in both cultured cardiac myocytes and mouse hearts. The level of p47phox protein, a subunit of NADPH oxidase, was increased in the hearts of mice with cardiac failure and in cardiac myocytes cultured with high-glucose medium, in agreement with the reported results of clinical and experimental studies [19,28,31,50]. Accordingly, inhibition of p47phox via a decrease in the plasma glucose level could be a mechanism by which voglibose slows the progression of heart failure, as it was shown that high glucose led to a moderate increase in protein synthesis by cardiac myocytes and co-treatment with an NADPH oxidase inhibitor abrogated this effect. Among the NADPH oxidase subunits, p47phox is consistently reported to be upregulated by CHF in both animals and humans [19,28,31]. Pharmacological interventions targeting the inhibition of oxidative stress have been frequently shown to be effective in inhibiting cardiac remodeling [18,49,51]. However, we should note that anti-oxidant stress is not the sole mechanism. The beneficial impact of glycerol control on mechanical efficiency and energy conservation has also been reported to contribute to improved heart function [43,52,53]. In addition, although our data implicate that voglibose improves heart failure independent of the inhibition of cardiac fibrosis, further investigation to obtain firm evidence on the molecular makers of fibrosis from the protein level would be helpful to clarify this issue.

Although the change of glucose concentration in the in vitro model of neonatal rat cardiomyocyte culture was different from that observed in our in vivo model of TAC induced myocardial hypertrophy in mice, the impact on oxidant stress was similar. It has been reported that a long-term decrease in plasma glucose levels by 15 mg/dL in rats contributes to the prolongation of life span in conjunction with caloric restriction via attenuation of oxidant stress [54].

Metabolic modulators, as a novel form of therapy for cardiac hypertrophy and failure, could serve as an effective adjunct to traditional regimens. There is mounting evidence that stimulation of glucose utilization can improve cardiac function in heart failure [12,13]. Our results suggest that a target glucose level of ≤110 mg/dL is beneficial, in agreement with the position statement released by the
American College of Endocrinology [55]. The present study provides the firm evidence that reduction in the postprandial blood glucose level can slow the progression of cardiac hypertrophy and heart failure, which may suggest a novel therapy for addition to the current pharmacological regimens. Alpha-glucosidase inhibitors are devoid of any direct negative hemodynamic or inotropic effects, and no significant side effects such as hypoglycemia were observed in this study, which is another advantage when treating CHF. With this in mind, we are now conducting prospective clinical trials to test the effect of voglibose in patients with CHF or myocardial infarction, and the preliminary results have been encouraging. It seems reasonable that improved glycemic control in combination with approaches to increase energy utilization would be an effective alternative for the treatment of heart failure.

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