厚生労働科学研究費補助金

臨床研究基盤整備推進研究事業

食後血糖上昇の抑制による心筋梗塞二次予防に関する大規模薬剤介入臨床研究

（若手医師・協力者活用に要する研究）

平成18年度　総括研究報告書

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（ 国立循環器病センター ）

平成19（2007）年 3月

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研究要旨

現在わが国における心臓死は全国民死亡原因の第2位を占めている。なかでも慢性心不全患者は増加し続けており、その原因として心筋梗塞後心ポンプ機能低下が重要である。慢性心不全による繰り返す入院、多種類の治療薬の使用は医療費増加につながり、厚生行政上の重要課題となっている。また臨床的見地からも、梗塞後慢性心不全患者のQOLは低く、健康寿命の延長のためにも梗塞後心不全の発症を抑制することは極めて重要な案件である。 今後わが国が迎える超高齢化社会の到来を考慮すると、心筋梗塞の二次予防というアプローチが特に重要となる。心筋梗塞のリスクファクターである生活習慣病のうち、発症およびその予後に最も影響を与える糖尿病は、食後高血糖のみを有する糖尿病予備軍とともに非常な勢いで増加している。血糖値の上昇は酸化ストレスを引き起こすことが知られており、食後の高血糖のみがすでに大血管障害のリスクとなり、心筋梗塞の発症リスクを高めることが報告されている(Donahue RP, et al. Diabetes 36: 689-692,1987)。 そこで心筋梗塞後患者の食後の血糖上昇を抑えることが、心筋梗塞二次予防につながると考え、我々は本研究を立案した。本研究は急性心筋梗塞で入院した症例に対してαグルコシダーゼ阻害薬を投与し、その心血管イベントの抑制効果の有無を全国100施設と共同した多施設大規模臨床試験にて4000症例をエントリーして検討する。かかる大規模多施設臨床研究の成否にはサンプルサイズおよび質の高いデータの確保が重要な要素となる。当該年度は詳細な実施手順案の作成を完了しており参加予定施設によるキックオフミーティングを開催し試験計画の詳細を決定した。国立循環器病センターでは倫理委員会の承認を得て、エントリーが開始されている。

A.採択された研究事業での研究概要

[本研究の目的、必要性]

現在わが国において、心臓死は全国民死亡原因の第2位を占めている。なかでも慢性心不全患者は増加し続けており、その原因としては心筋梗塞後の心機能低下が重要である。くり返す入院、多種類の治療薬の使用は医療費増加につながり、厚生行政上の重要課題である。また臨床的見地からも、梗塞後心不全患者のQOLは低く、健康寿命の延長のためにも梗塞後の心不全発症を抑制することは極めて重要な案件である。

くり返す心筋梗塞後の心機能低下に対して、二次予防および心筋リモデリング抑制というアプローチがあげられる。加齢そのものが発症のリスクファクターであるため、今後わが国が迎える超高齢化社会の到来にあたり、心筋梗塞二次予防の重要性が増している。一方で心筋梗塞のリスクファクターである生活習慣病のうち、その発症・予後に最も影響を与えるとされる糖尿病およびその予備軍は、生活習慣の変化、高齢化をうけて非常な勢いで増加している。したがって今後わが国の心筋梗塞二次予防において、耐糖能異常への新たな対処の必要性が高まっている。

血糖の上昇は酸化ストレスを引き起こすことが知られており、食後高血糖のみがすでに大血管障害のリスクとなり、心筋梗塞の発症リスクを高めることがわかっている(Donahue RP, et al. Diabetes 36: 689-692,1987)。そこで心筋梗塞後の症例に対して、αグルコシダーゼ阻害薬により食後の血糖上昇を抑えることが、心筋梗塞二次予防につながると考え我々は本研究を立案した。当該患者数が相当数にのぼる治療法は、当然のことながら検討段階から安全な薬剤の使用が必須である。われわれが考慮しているαグルコシダーゼ阻害薬は、血中には原則取り込まれず、消化管にとどまり糖分の吸収を抑える薬剤であるため、幅広い患者層への適用が可能であると考えられる。

［期待される結果］

1. 心筋梗塞の二次予防により慢性心不全患者の増加を抑制できれば、厚生行政面においては大幅な医療費抑制効果が期待され、また医療面においては患者のQOLの著明な改善、健康寿命の延長が期待できる。
2. 包括医療制度の導入により急性心筋梗塞を含めた心血管イベントの発症数の減少は、そのまま医療費の抑制につながる。

心筋梗塞後患者への食後高血糖からの早期治療が普及することにより、糖尿病の発症抑制が期待できる。

B. 採択された研究事業での研究実績

1年目　研究体制の整備、症例エントリーの開始、動物実験による検討

H16年度は、参加予定施設を含めたキックオフミーティングを開催しプロトコルの確定を行った。また、インターネットを用いた登録のためホームページを立ち上げ、予定通りエントリーを開始した。また、実験動物を使った評価系において耐糖能異常と心不全の関連について検討した。

2年目　症例のエントリー、エントリーされた症例の観察、動物実験による検討

H17年度は、参加施設にて倫理委員会の通過を得て、目標症例数を目指しエントリーを開始した。これまでに予備登録症例を含め約1100症例の登録が終了している。また、マウスTACモデルおよび慢性非虚血性心不全症例においてボグリボースが心不全を改善することが示唆された。このことから一過性高血糖は心不全の増悪因子であり、αグルコシダーゼ阻害剤には心筋保護効果があると考えられた。

3年目　エントリーされた症例の観察、データ解析

平成18年度は、目標症例数を目指しエントリーを推進した。今後も目標症例数の4000症例までエントリーを継続する予定である。現在までの登録症例の観察では心臓死・急性心筋梗塞発症症例は全体として12症例であり急性心筋梗塞を扱ったJ-WIND1試験より少ないイベント発生率となっていた。割付群別ではαGI群4例、対照群8例(p=0.25)でありαGI群でイベント発生が少ない傾向にあった。

本採択研究は4000例の登録・フォローアップを予定しており、試験体制立ち上げ時もさることながらエントリー開始後の努力によりデータの質が大きく左右される。また、各施設への試験実施に関する問い合わせ業務も非常に重要であり、定期的なニュースレターの発行・電子メールや電話による問い合わせなど幅広く行っているが、実際に参加予定施設に招かれ説明会を行うことなどもあり、人的資源が必要となる。さらには、有害事象などは計画書の文面と各施設からの報告内容の摺り合わせが必要となり、医学的な知識を持ちながら臨床研究の流れを十分に理解した、かかる臨床研究実施チームの人材は貴重であり研究遂行上必要不可欠であった。

（倫理面への配慮）

以下の点を明記し、倫理委員会の承認手続きを経て研究を開始した。各参加施設でも同様の手続きを開始している。

（1）医学研究及び医療行為の対象となる個人の人権の擁護

本研究は遺伝子情報を扱わず臨床情報のみの解析であるが、臨床データの収集は「連結匿名化」を行った上で中央解析施設（国立循環器病センター）に集積し、解析時には個人特定に繋がるデータとは切り離した状態での解析を行う。

（2）医学研究及び医療行為の対象となる個人への利益と不利益

本研究で使用される薬剤は臨床の現場で日常使われている薬剤であり、開発途中のいわゆる治験薬とは根本的に異なる。本研究ではコントロール目標を立て厳密にコントロールするので、より細やかな治療を受けられる可能性がある。また検査項目に関して一般の診療に必要な物に限っており新たな採血等の必要がなく対象となる個人への負担は少ない。個人情報の保護に努めれば個人への不利益は少ないものと考えられる。

（3）医学的貢献度

我が国における心不全による死亡は全死因の第二位を占めており、その抑止は社会的急務となっている。特に心筋梗塞後の心機能低下に起因する慢性心不全については、5年生存率が50%以下と低いことから、その改善が急がれている。心筋梗塞後の心機能低下は再梗塞によりその危険性が増大する。心筋梗塞再発予防における大規模薬剤介入を念頭に置いた本研究は、医学的貢献度も非

常に高いと考える。

（4）医学研究及び医療行為の対象となる個人に理解を求め同意を得る方法
患者さん用説明文書を用いて、研究遂行者の担当者が説明し、別紙の同意文書により同意を得る。

（5）動物を用いた実験について
動物実験は施設の倫理規定に基づき審査に通過した実験のみを行い、マウス等動物の生命を最大限尊重し、効率的に実験を進める。

C.考察
本研究遂行のため臨床研究チームが人的資源の不足を補うだけでなく、中心的な活動を行った。しかしながら、4000例の登録・フォローアップに当たっては、現チームの人数では十分とは言えず、目標症例数まで達しなかったのは残念である。

D.健康危険情報
特記なし

E.その他実施した臨床研究・治験の概要及び実績

（臨床研究）
申請者らは厚生労働省科研　効果的医療技術の確立推進臨床研究事業　虚血・再潅流における心筋保護に関する大規模無作為薬剤効果比較試験（課題番号H14—心筋—006、JWIND試験）にて、心筋梗塞における急性期の薬剤介入という困難な性格であるにもかかわらず、全国68施設との共同で1200例の登録が完了した実績を有する。心筋梗塞の多施設臨床研究チームとしてネットワークがすでに確立されている我々の研究グループは、そのインフラストラクチャーを活用することにより、本研究の遂行能力を十分有するものと考えられる。さらに本研究では全国100施設と共同で4000例を目指して順調に登録を継続している。本研究の登録を継続するに当たり、マンパワー（人的資源）が研究成功の鍵を握っていることを痛感した。本採用研究においては、全国100施設と共同で4000症例の登録を目指しておりさらに多くのマンパワーが必要であると予想された。各エントリーに関して欠損データなどを最小限にとどめより質の高い研究を目指すため、各施設との連携を今まで以上に推進していく必要がある。現チームの人数では十分とは言えず、目標症例数まで達しなかった。目標達成の為には、マンパワーの補充が必要不可欠であり臨床研究実施チームの整備が必要となる。

（治験）
国立循環器病センターでは、受託研究取扱規程に基づき平成13年度は34課題、14年度は41課題、15年度は47課題、16年度は33課題（12月末現在）の治験契約を締結した。申請者が所属する心臓内科の課題に関する詳細（対象疾患、使用薬剤、実施症例数、契約症例数、プロトコル概略）は以下の通り。

申請者が所属する心臓内科の課題は以下の通り。
1）原発性肺高血圧症に対するボセンタンの一般臨床試験

2）AMDivの致死性心室性不整脈に対する第Ⅱ相非盲検下・非対照臨床試験
3）SMP-536のﾌｧﾌﾞﾘｰ病患者に対する臨床試験（再継続投与）

4）症候姓虚血姓心疾患患者におけるJJ-CRD02ステントの薬物動態試験　第2期

5）新鮮心房細動・心房粗動における洞調律維持に関する有効性及び安全性を実薬対照（Calibrator）として塩酸アミオダロンを用いて評価するプラセボ対照二重盲検用量検討試験

6）ネイティブ冠動脈の新規病変に対する薬剤溶出型冠動脈ｽﾃﾝﾄ治療の　臨床評価″

7）アルガトロバンのヘパリン起因性血小板減少症に対する臨床治験（自ら実施する治験）主要評価項目は致死性不整脈の発現頻度。

研究成果の刊行に関する一覧表

（業績一覧）

課題名 ： 食後血糖上昇の抑制による心筋梗塞二次予防に関する大規模薬剤介入臨床研究
（若手医師・協力者活用に要する研究）

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	頁	出版年
Komamura. K(2 人略 4 番目), Kim. J(11 人略 16 番目),**Kitakaze M.**	Heart-type fatty acid binding protein is a novel prognostic marker in patients with non-ischemic dilated cardiomyopathy.	Heart	92	615-618	2006
Maruo. T, Nakatani S (3 人略 6 番目) **Kitakaze M**, (以下 2 人略)	Circadian variation of endothelial function in idiopathic dilated cardiomyopathy.	Am J Cardiol	97	699-702	2006
Saeki.M (10 人略 12 番目) **Kitakaze M**,Tomoike,H (以下 13 人略)	Haplotype Structures of the *UGT1A* Gene Complex in a Japanese Population.	Pharmacogenomics J	6(1)	63-75	2006
Hirata.A (13 人略 15 番目) **Kitakaze M**, Hori.M	Erythropoietin enhances neovascularization of ischemic myocardium and improves left ventricular dysfunction after myocardial infarction in dogs.	J Am Coll Cardiol	48(1)	176-184	2006
Tsukamoto.O (13 人略 15 番目) Hori.M, **Kitakaze M.**	Depression of proteasome activities during the progression of cardiac dysfunction in pressure-overloaded heart of mice.	Biochem Biophys Res Commun	340	1125-1133	2006

Tsukamoto.O (10 人略 12 番目) Tomoike.H, Hori.M, **Kitakaze M.**	The antagonism of aldosterone receptor prevents the development of hypertensive heart failure induced by chronic inhibition of nitric oxide synthesis in rats.	Cardiovasc Drugs Ther	20(2)	93-102	2006
Okazaki.H (2 人略 4 番目)Kim.J,(5 人略 8 番目) **Kitakaze M.**	Angiotensin II type 1 receptor blocker prevents atrial structural remodeling in rats with hypertension by chronic nitric oxide inhibition.	Hypertension Res	29	277-284	2006
Liao.Y (5 人略 7 番目)Kim.J, Fujita.M, Hori.M, **Kitakaze M.**	Control of plasma glucose with alpha-glucosidase inhibitor attenuates oxidative stress and slows the progression of heart failure in mice.	Cardiovas Res	707	107-116	2006
Asanuma.H (2 人略 4 番目),Kim.J, (10 人略 15 番目)Tomoike.H, **Kitakaze M.**	Blockade of histamine H_2 receptors protects the heart against ischemia and reperfusion injury in dogs.	J Mol Cell Cardiol	40(5)	666-674	2006
Kubota.D (5 人略 7 番目)**Kitakaze M.**(最終略)	Tricyclic pharmacophore-based molecules as novel Integrin avb3 antagonists. IV. Preliminary control of avb3 selectivity by meta-oriented substitution.	Bioorganic Medicinal Chemistry	14	4158-4181	2006
Takahama.H (13 人略 15 番目)**Kitakaze M.**	Granulocyte colony-stimulating factor mediates cardioprotection against ischemia/reperfusion injury via phosphatidylinositol-3-kinase/Akt pathway in canine hearts.	Cardiovasc Drugs Ther	20(3)	159-165	2006

Iwahashi.M (4 人略 last author)**Kitakaze M.**	Acute improvement in myocardial function assessed by myocardial strain and strain rate after aortic valve replacement for aortic stenosis.	Journal of the American Society of Echocardiography	19	1238-1244	2006
Kotake.T (4 人略 6 番目) **Kitakaze M.** (最終略)	Heart failure elevates serum levels of cibenzoline in arrhythmic patients.	Circ J	70(5)	588-592	2006
Shintani.Y (10 人略 12 番目) **Kitakaze M,**Hori.M.	Glycosaminoglycan modification of Neuropilin-1 modulates VEGFR2 signaling.	EMBO Journal	25	3045-3055	2006
Kim.J (7 人略 9 番目)Kitamura.S, Tomoike.H, **Kitakaze M.**	Impact of blockade of histamine H_2 receptors on chronic heart failure revealed by retrospective and prospective randomized studies.	J Am Coll Cardiol	48(7)	1378-1384	2006
Fujita.M(7 人省略 9 番目) Tomoike.H, **Kitakaze M.**	Blockade of angiotensin II receptors reduces the expression of receptors for advanced glycation end products in human endothelial cells.	Arterioscler osis, Thrombosis, and Vascular Biology	26(10)	e138-e142	2006
Kanzaki.H (4 人略 6 番目) **Kitakaze M.**	Impaired systolic torsion in dilated cardiomyopathy: Reversal of apical rotation at mid-systole characterized with magnetic resonance tagging method.	Basic Res Cardiol	101(6)	465-70	2006
Kim.J (7 人略 9 番目)Tomoike.H ,**Kitakaze M.**	Abnormal glucose tolerance contributes to the progression of chronic heart failure in patients with dilated cardiomyopathy.	Hypertension Res(in press)			

Fujita.M, Asanuma.H, Kim.J (4 人略 8 番目)Hori.M,(2 人略 11 番目 **Kitakaze M.**	Impaired glucose tolerance: A possible contributor to left ventricular hypertrophy and diastolic dysfunction.	Internatinal J Cardiol			2006
Soyama.A (7 人略 9 番目)**Kitakaze M,** Tomoike.H, (以下 2 人略).	Sequence-based analysis of the *CYP2D6*36-CYP2D6*10* tandem-type arrangement, a major *CYP2D6*10* haplotype in the Japanese population.	Drug Metab Pharmacoki net	21(3)	208-16	2006
Sai.k(9 人略 11 人目) **Kitakaze M,** Tomoike.H,(以下 13 人略)	Genetic variations and haplotype structures of the ABCB1 gene in a Japanese population: an expanded haplotype block covering the distal promoter region, and associated ethnic differences.	Ann Hum Genet	70(Pt 5)	605-62 2	2006
Wakeno.M,(10 人略 12 人目)Kim.J (1 人略 14 番目) **Kitakaze M.**	Chronic stimulation of adenosine A2b receptors begun after myocardial iInfarction prevents cardiac remodeling in rats.	Circulation	114(8)	1923-1 932	2006
Kinugawa.T (7 人略 9 番目) **Kitakaze M.**	Catabolism of adenine nucleotides favors adenosine production following exercise in patients with chronic heart failure.	Journal of Cardiac Failure	12(9)	720-72 5	2006
Mizuta.E (11 人略 13 番目) **Kitakaze M,** (以下 3 人略).	Delayed Onset of Beating and Decreased Expression of T-type Ca^{2+} Channel in Mouse ES Cell-Derived Cardiocytes Carrying Human Chromosome 21.	Biochem Biophys Res Commun	351(1)	126-13 2	2006

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Cardiovascular Research 70 (2006) 107 – 116

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

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Received 16 August 2005; received in revised form 13 January 2006; accepted 24 January 2006
Available online 28 February 2006
Time for primary review 23 days

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-glycosidase 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 ($p47^{phox}$). Glucose dose-dependently increased both neonatal rat myocyte protein synthesis and the expression of $p47^{phox}$ 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.

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.

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doi:10.1016/j.cardiores.2006.01.021

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=volume and D=the echocardiographically measured internal dimension [24]. Accordingly, LV end-diastolic volume (LVEDV)=$7(\text{LVEDd})^3/(2.4+\text{LVEDd})$, LV end-systolic volume (LVESV)=$7(\text{LVESd})^3/(2.4+\text{LVESd})$, stroke volume (SV)=LVEDV−LVESV, LV ejection fraction (LVEF %)=SV/LVEDV×100. LV mass was calculated according to cube assumptions and modified with Teichholz formula: LV mass (mg)=$1.0557[7(\text{LVEDd}+\text{LVPWd}+\text{VSTd})^3/(2.4+\text{LVEDd}+\text{LVPWd}+\text{VSTd})-\text{LVEDV}]$, 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 model 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 $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, and $gp91^{phox}$ (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 ^{3}H-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

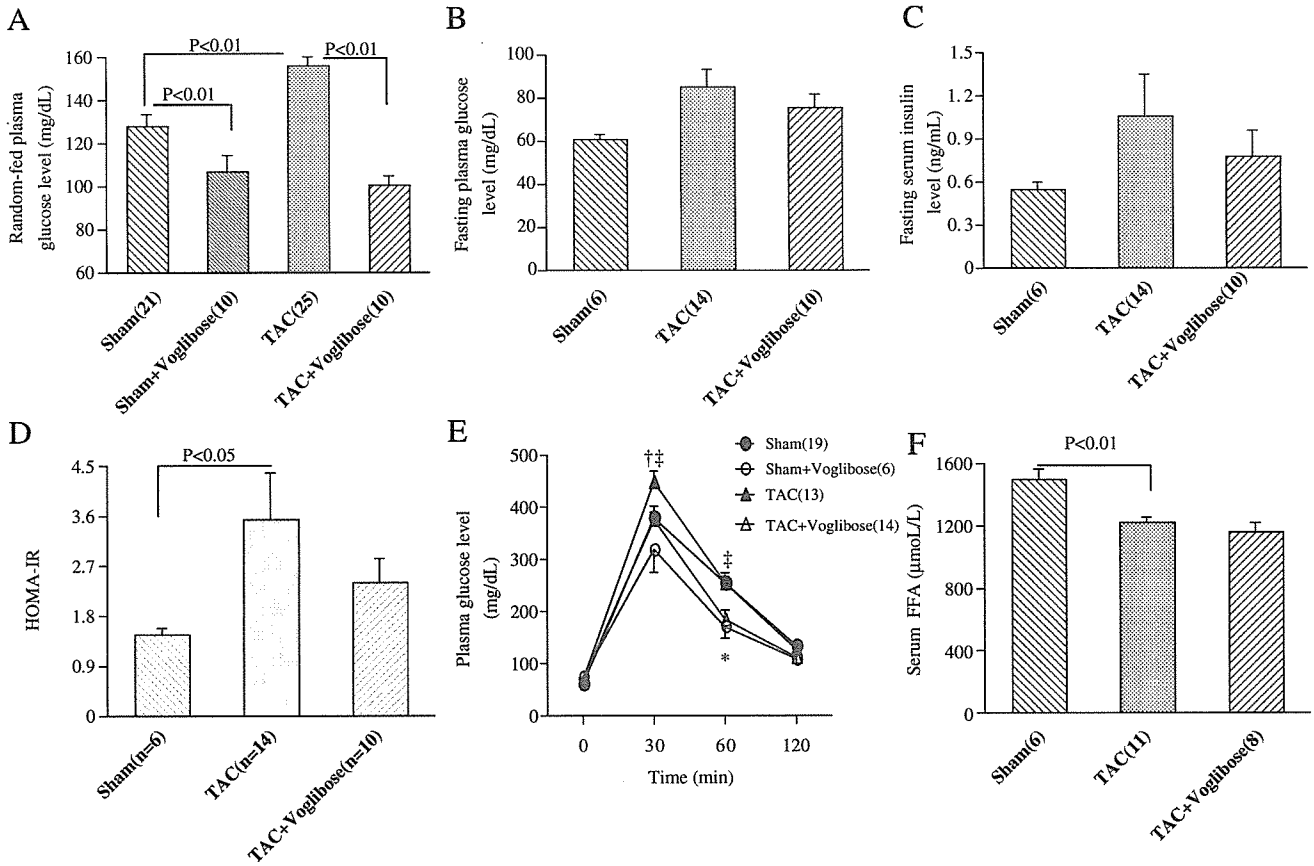

Fig. 1. Glucose and fatty acid metabolism in hypertrophic and failing hearts. (A) Random plasma glucose concentrations at 3 weeks after transverse aortic constriction (TAC) or a sham operation. (B) Fasting plasma glucose concentration at 4 weeks. (C) Fasting serum insulin concentration at 4 weeks. (D) The insulin resistance index HOMA-IR at 4 weeks. A *t*-test was performed after log-transformation. (E) Intraperitoneal glucose tolerance test. $^{\dagger}P<0.05$ vs. sham, $^{\ddagger}P<0.05$ vs. voglibose-treated mice, $^{*}P<0.05$ vs. sham. (F) Serum free fatty acid at 4 weeks (FFA).

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 d*P*/d*t* 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
Left ventricular hemodynamics at 4 weeks after TAC or sham operation

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

TAC, transverse aortic constriction; HR, heart rate; LVSP, maximum left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; Max d*P*/d*t*, the steepest slope during the upstroke of the pressure curve; Min d*P*/d*t*, the steepest slope during the downstroke of the pressure curve; Contractility index: Max d*P*/d*t* divided by the pressure at the time of Max d*P*/d*t*; Tau, the exponential time constant of relaxation; Sham+V, sham+voglibose 10 mg/kg/day; TAC+V, TAC+voglibose 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.

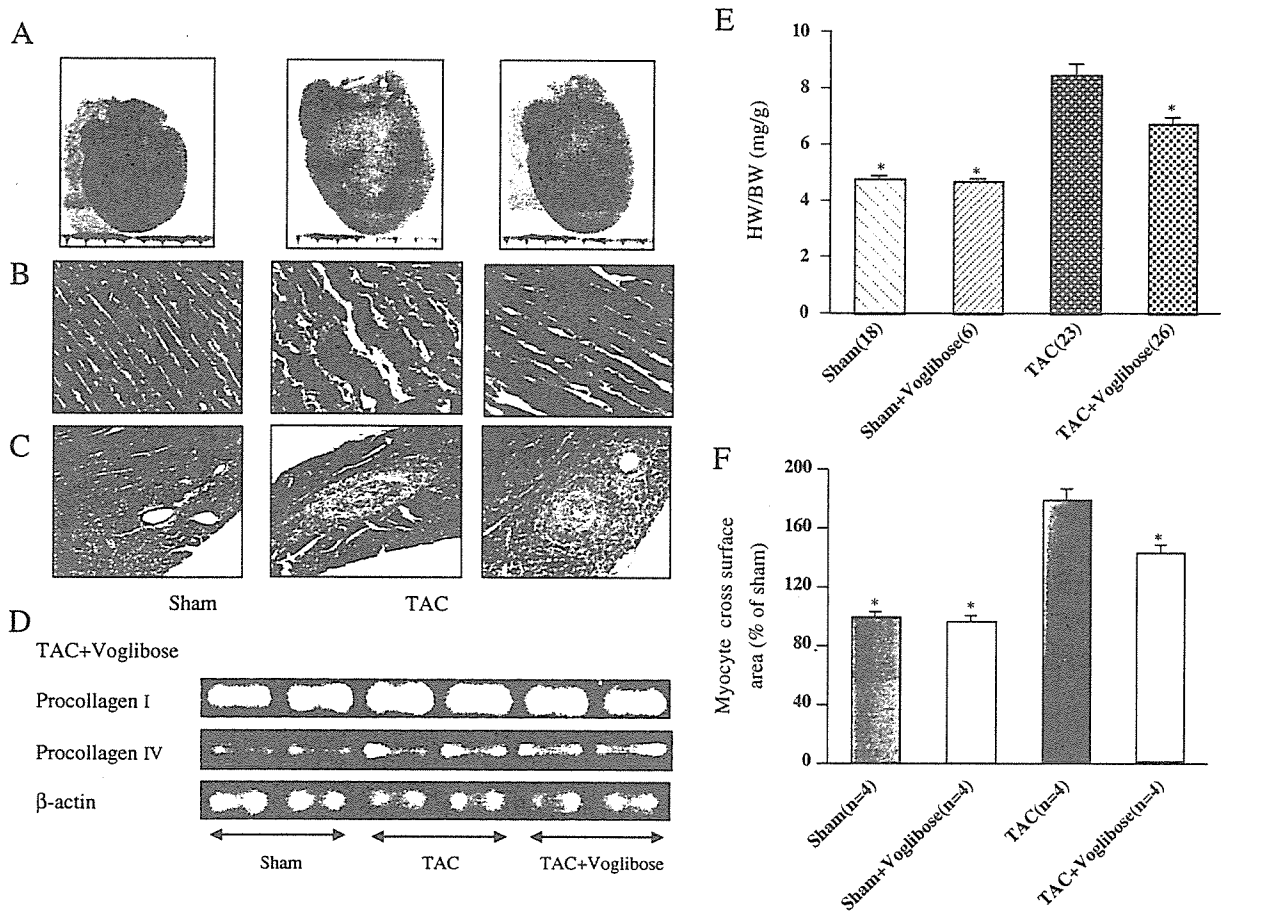

Fig. 2. Results of cardiac hypertrophy and fibrosis at 4 weeks. (A) Representative images of the whole heart. (B) Long-axis view of cardiac myocytes (HE stain, ×200). (C) Cardiac fibrosis with Azan staining (×100). (D) Expression of collagen I and collagen IV genes detected by RT-PCR with β-actin as internal control. The heart weight-to-body weight ratio (HW/BW) (E) and cardiac myocyte cross-sectional area (F) were decreased in voglibose-treated mice. *$P<0.01$ vs. TAC.

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 ^{3}H-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

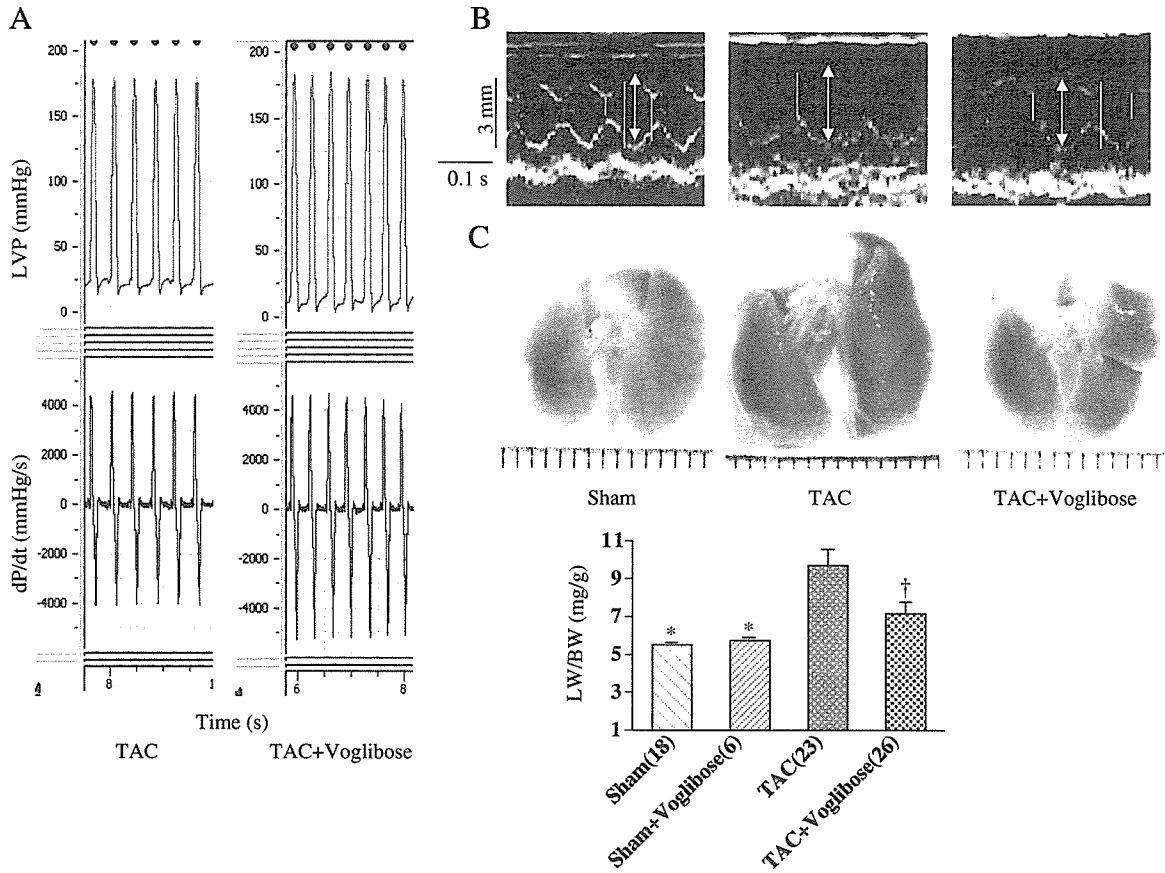

Fig. 3. Improvement in heart function by voglibose treatment. (A) Representative graphs of left ventricular pressure and its rate of change (dP/dt) showed a lower diastolic pressure and higher minimum dP/dt. (B) Representative M-mode echocardiographic images. (C) Pulmonary congestion was ameliorated 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.

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 ($p22^{phox}$, $p47^{phox}$, $p67^{phox}$) and gp 91 was determined by western blotting. Neonatal rat myocyte expression of $p47^{phox}$ was upregulated by culture in high glucose medium (Fig. 5A) and was

Table 2
Echocardiographic findings at 4 weeks after TAC or sham operation

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

TAC, transverse aortic constriction; LVEDd, left ventricular end-diastolic dimension; LVPWd, left ventricular diastolic posterior wall thickness; LVESd, left ventricular end-systolic 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<0.05, compared with TAC.

† P<0.01, compared with TAC.

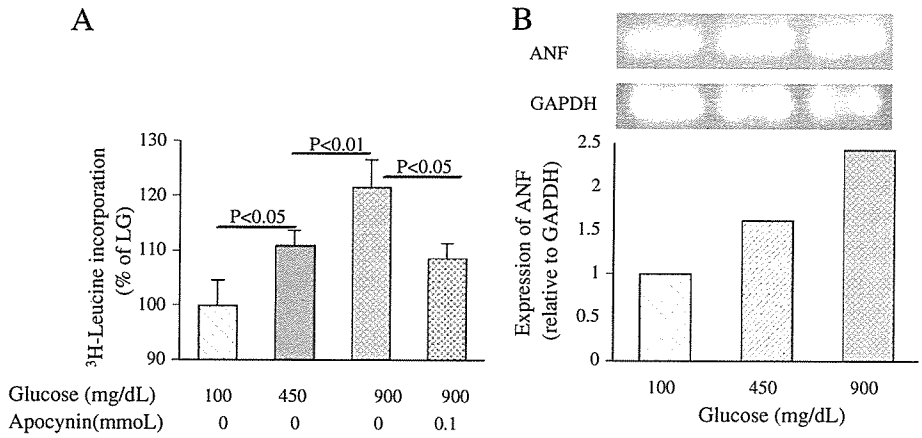

Fig. 4. High glucose culture enhances protein synthesis by neonatal rat cardiac myocytes. (A) Glucose caused a concentration-dependent increase of protein synthesis in cardiac myocytes, which was blocked by an NADPH oxidase inhibitor (apocynin). Each experiment was repeated at least 3 times. (B) A representative result of atrial natriuretic factor (ANF) detected by RT-PCR using GAPDH as an internal control. ANF gene was upregulated in high-glucose culture medium.

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 p47^{phox} expression ($P<0.05$ vs. TAC mice, Fig. 5B).

To confirm that the increase in p47^{phox} 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.

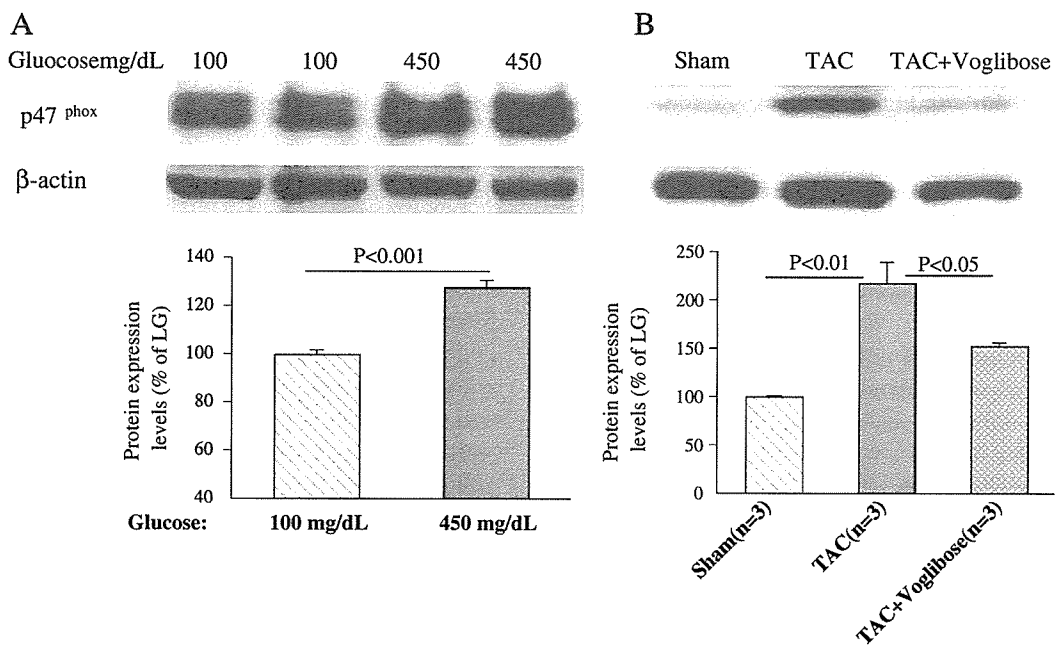

Fig. 5. Western blotting of NADPH oxidase subunits. (A) High glucose culture increased the expression of p47^{phox} protein in neonatal rat myocytes. LG: low glucose (100 mg/dL). (B) Voglibose decreased myocardial expression of p47^{phox} protein in TAC mice.

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, alpha$_1$-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 $p47^{phox}$ 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 $p47^{phox}$ 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, $p47^{phox}$ 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 $\leq$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.

Acknowledgements

This work was supported by grants (H13-21seiki (seikatsu)-23) from the Ministry of Health, Labour and Welfare, Japan. Dr. Liao is supported by a grant from the Japan Society for the Promotion of Science (P05228).

References

[1] Kosiborod M, Rathore SS, Inzucchi SE, Masoudi FA, Wang Y, Havranek EP, et al. Admission glucose and mortality in elderly patients hospitalized with acute myocardial infarction: implications for patients with and without recognized diabetes. Circulation 2005;111: 3078–86.

[2] Tenenbaum A, Motro M, Fisman EZ, Leor J, Freimark D, Boyko V, et al. Functional class in patients with heart failure is associated with the development of diabetes. Am J Med 2003;114:271–5.

[3] Suskin N, McKelvie RS, Burns RJ, Latini R, Pericak D, Probstfield J, et al. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. Eur Heart J 2000;21:1368–75.

[4] Tenenbaum A, Fisman EZ. Impaired glucose metabolism in patients with heart failure: pathophysiology and possible treatment strategies. Am J Cardiovasc Drugs 2004;4:269–80.

[5] He J, Ogden LG, Bazzano LA, Vupputuri S, Loria C, Whelton PK. Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. Arch Intern Med 2001; 161:996–1002.

[6] Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 1999;22:233–40.

[7] Ceriello A, Hanefeld M, Leiter L, Monnier L, Moses A, Owens D, et al. Postprandial glucose regulation and diabetic complications. Arch Intern Med 2004;164:2090–5.

[8] Doehner W, Rauchhaus M, Ponikowski P, Godsland IF, von Haehling S, Okonko DO, et al. Impaired insulin sensitivity as an independent risk factor for mortality in patients with stable chronic heart failure. J Am Coll Cardiol 2005;46:1019–26.

[9] Shiomi T, Tsutsui H, Ikeuchi M, Matsusaka H, Hayashidani S, Suematsu N, et al. Streptozotocin-induced hyperglycemia exacerbates left ventricular remodeling and failure after experimental myocardial infarction. J Am Coll Cardiol 2003;42:165–72.

[10] Paolisso G, Tagliamonte MR, Rizzo MR, Gambardella A, Gualdiero P, Lama D, et al. Prognostic importance of insulin-mediated glucose uptake in aged patients with congestive heart failure secondary to mitral and/or aortic valve disease. Am J Cardiol 1999;83:1338–44.

[11] Prasad A, Quyyumi AA. Renin–angiotensin system and angiotensin receptor blockers in the metabolic syndrome. Circulation 2004;110: 1507–12.

[12] Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelias L, et al. Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. Circulation 2004;110:955–61.

[13] D'Hahan N, Taouil K, Dassouli A, Morel JE. Long-term therapy with trimetazidine in cardiomyopathic Syrian hamster BIO 14:6. Eur J Pharmacol 1997;328:163–74.

[14] Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA 2003;290:486–94.

[15] Kim J, Washio T, Yamagishi M, Yasumura Y, Nakatani S, Hashimura K, et al. A novel data mining approach to the identification of effective drugs or combinations for targeted endpoints—application to chronic heart failure as a new form of evidence-based medicine. Cardiovasc Drugs Ther 2004;18:483–9.

[16] Ceriello A, Quagliaro L, Piconi L, Assaloni R, Da Ros R, Maier A, et al. Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. Diabetes 2004;53:701–10.

[17] Marfella R, Quagliaro L, Nappo F, Ceriello A, Giugliano D. Acute hyperglycemia induces an oxidative stress in healthy subjects. J Clin Invest 2001;108:635–6.

[18] Tsujimoto I, Hikoso S, Yamaguchi O, Kashiwase K, Nakai A, Takeda T, et al. The antioxidant edaravone attenuates pressure overload-induced left ventricular hypertrophy. Hypertension 2005;45:921–6.

[19] Maack C, Kartes T, Kilter H, Schafers HJ, Nickenig G, Bohm M, et al. Oxygen free radical release in human failing myocardium is associated with increased activity of rac1-GTPase and represents a target for statin treatment. Circulation 2003;108:1567–74.

[20] Liao Y, Ishikura F, Beppu S, Asakura M, Takashima S, Asanuma H, et al. Echocardiographic assessment of LV hypertrophy and function in aortic-banded mice: necropsy validation. Am J Physiol Heart Circ Physiol 2002;282:H1703–8.

[21] Liao Y, Takashima S, Asano Y, Asakura M, Ogai A, Shintani Y, et al. Activation of adenosine A1 receptor attenuates cardiac hypertrophy and prevents heart failure in murine left ventricular pressure-overload model. Circ Res 2003;93:759–66.

[22] Odaka H, Shino A, Ikeda H, Matsuo T. Antiobesity and antidiabetic actions of a new potent disaccharidase inhibitor in genetically obese-diabetic mice, KKA(y). J Nutr Sci Vitaminol (Tokyo) 1992; 38:27–37.

[23] Kurisu S, Ozono R, Oshima T, Kambe M, Ishida T, Sugino H, et al. Cardiac angiotensin II type 2 receptor activates the kinin/NO system and inhibits fibrosis. Hypertension 2003;41:99–107.

[24] Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic–angiographic correlations in the presence of absence of asynergy. Am J Cardiol 1976;37:7–11.

[25] Liao Y, Takashima S, Maeda N, Ouchi N, Komamura K, Shimomura I, et al. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. Cardiovasc Res 2005;67:705–13.

[26] Chang MS, Oh MS, Kim DR, Jung KJ, Park S, Choi SB, et al. Effects of Okchun-San, a herbal formulation, on blood glucose levels and body weight in a model of Type 2 diabetes. J Ethnopharmacol 2005.

[27] Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356–62.

[28] Privratsky JR, Wold LE, Sowers JR, Quinn MT, Ren J. AT1 blockade prevents glucose-induced cardiac dysfunction in ventricular myocytes: role of the AT1 receptor and NADPH oxidase. Hypertension 2003;42:206–12.
[29] Asakura M, Kitakaze M, Takashima S, Liao Y, Ishikura F, Yoshinaka T, et al. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. Nat Med 2002;8:35–40.
[30] Allard MF, Schonekess BO, Henning SL, English DR, Lopaschuk GD. Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts. Am J Physiol 1994;267:H742–50.
[31] Heymes C, Bendall JK, Ratajczak P, Cave AC, Samuel JL, Hasenfuss G, et al. Increased myocardial NADPH oxidase activity in human heart failure. J Am Coll Cardiol 2003;41:2164–71.
[32] MacCarthy PA, Grieve DJ, Li JM, Dunster C, Kelly FJ, Shah AM. Impaired endothelial regulation of ventricular relaxation in cardiac hypertrophy: role of reactive oxygen species and NADPH oxidase. Circulation 2001;104:2967–74.
[33] Paolisso G, Gambardella A, Galzerano D, D'Amore A, Rubino P, Verza M, et al. Total-body and myocardial substrate oxidation in congestive heart failure. Metabolism 1994;43:174–9.
[34] Chu CA, Galassetti P, Igawa K, Sindelar DK, Neal DW, Burish M, et al. Interaction of free fatty acids and epinephrine in regulating hepatic glucose production in conscious dogs. Am J Physiol Endocrinol Metab 2003;284:E291–301.
[35] Viollet B, Andreelli F, Jorgensen SB, Perrin C, Geloen A, Flamez D, et al. The AMP-activated protein kinase alpha2 catalytic subunit controls whole-body insulin sensitivity. J Clin Invest 2003;111:91–8.
[36] Lind L, Lithell H. Decreased peripheral blood flow in the pathogenesis of the metabolic syndrome comprising hypertension, hyperlipidemia, and hyperinsulinemia. Am Heart J 1993;125:1494–7.
[37] Sack MN, Harrington LS, Jonassen AK, Mjos OD, Yellon DM. Coordinate regulation of metabolic enzyme encoding genes during cardiac development and following carvedilol therapy in spontaneously hypertensive rats. Cardiovasc Drugs Ther 2000;14:31–9.
[38] Giugliano D, Acampora R, Marfella R, De Rosa N, Ziccardi P, Ragone R, et al. Metabolic and cardiovascular effects of carvedilol and atenolol in non-insulin-dependent diabetes mellitus and hypertension. A randomized, controlled trial. Ann Intern Med 1997;126:955–9.
[39] Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000;342:145–53.
[40] Julius S, Kjeldsen SE, Weber M, Brunner HR, Ekman S, Hansson L, et al. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomised trial. Lancet 2004;363:2022–31.
[41] Buse JB, Tan MH, Prince MJ, Erickson PP. The effects of oral anti-hyperglycaemic medications on serum lipid profiles in patients with type 2 diabetes. Diabetes Obes Metab 2004;6:133–56.
[42] Matsumoto K, Yano M, Miyake S, Ueki Y, Yamaguchi Y, Akazawa S, et al. Effects of voglibose on glycemic excursions, insulin secretion, and insulin sensitivity in non-insulin-treated NIDDM patients. Diabetes Care 1998;21:256–60.
[43] Stanley WC, Chandler MP. Energy metabolism in the normal and failing heart: potential for therapeutic interventions. Heart Fail Rev 2002;7:115–30.
[44] Wallhaus TR, Taylor M, DeGrado TR, Russell DC, Stanko P, Nickles RJ, et al. Myocardial free fatty acid and glucose use after carvedilol treatment in patients with congestive heart failure. Circulation 2001;103:2441–6.
[45] Chandler MP, Stanley WC, Morita H, Suzuki G, Roth BA, Blackburn B, et al. Short-term treatment with ranolazine improves mechanical efficiency in dogs with chronic heart failure. Circ Res 2002;91:278–80.
[46] Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH, Taegtmeyer H. Metabolic gene expression in fetal and failing human heart. Circulation 2001;104:2923–31.
[47] Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. Circulation 1996;94:2837–42.
[48] Chandler MP, Kerner J, Huang H, Vazquez E, Reszko A, Martini WZ, et al. Moderate severity heart failure does not involve a down-regulation of myocardial fatty acid oxidation. Am J Physiol Heart Circ Physiol 2004;287:H1538–43.
[49] Takemoto M, Node K, Nakagami H, Liao Y, Grimm M, Takemoto Y, et al. Statins as antioxidant therapy for preventing cardiac myocyte hypertrophy. J Clin Invest 2001;108:1429–37.
[50] Li JM, Gall NP, Grieve DJ, Chen M, Shah AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. Hypertension 2002;40:477–84.
[51] Date MO, Morita T, Yamashita N, Nishida K, Yamaguchi O, Higuchi Y, et al. The antioxidant *N*-2-mercaptopropionyl glycine attenuates left ventricular hypertrophy in in vivo murine pressure-overload model. J Am Coll Cardiol 2002;39:907–12.
[52] Bersin RM, Wolfe C, Kwasman M, Lau D, Klinski C, Tanaka K, et al. Improved hemodynamic function and mechanical efficiency in congestive heart failure with sodium dichloroacetate. J Am Coll Cardiol 1994;23:1617–24.
[53] Hermann HP, Pieske B, Schwarzmuller E, Keul J, Just H, Hasenfuss G. Haemodynamic effects of intracoronary pyruvate in patients with congestive heart failure: an open study. Lancet 1999;353:1321–3.
[54] Masoro EJ, McCarter RJ, Katz MS, McMahan CA. Dietary restriction alters characteristics of glucose fuel use. J Gerontol 1992;47:B202–8.
[55] Garber AJ, Moghissi ES, Bransome Jr ED, Clark NG, Clement S, Cobin RH, et al. American College of Endocrinology position statement on inpatient diabetes and metabolic control. Endocr Pract 2004;10:77–82.