Age-related difference in the effect of acute hyperglycemia on myocardial ischemia-reperfusion injury

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Age-related difference in the effect of acute hyperglycemia on myocardial ischemia-reperfusion injury

Directed by Professor Yon Hee Shim

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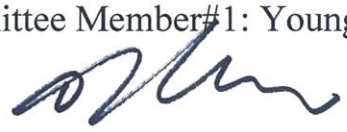

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Sung Yeon Ham

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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Background

Age and acute hyperglycemia are known risk factors for myocardial ischemia-reperfusion injury and combined frequently in clinical circumstances. However, there are limited data to examine myocardial ischemia-reperfusion injury in animal models exhibiting these co-morbidities concomitantly. Thus, we investigated the effect of acute hyperglycemia on myocardial ischemia-reperfusion injury in different age-groups and possible mechanisms.

Methods

Three different age groups of male Sprague Dawley rats were included (young-aged, 3 months; middle-aged, 10-12 months; and old-aged, 20-22 months). Rats received 1.2 g/kg of dextrose

(hyperglycemic group) or same volume of normal saline (normoglycemic group) according to the group. Rats were subjected to coronary artery occlusion for 45 min followed by reperfusion for 240 min. By measuring the infarct size and ejection fraction, we estimated the susceptibility to ischemia-reperfusion injury in rat. Proteins related to apoptosis (C-PARP, Bcl-2, Bax, and cytochrome C) and autophagy (Bnip3, Beclin-1, Atg5r, and LC3B-II) were evaluated by western blot assay.

Results

Infarct size was increased by acute hyperglycemia in young- and middle-aged rats but not in old-aged rats, while reduction of ejection fraction after ischemia-reperfusion was aggravated by acute hyperglycemia in all age groups. Acute hyperglycemia increased expression of Bnip3 and Beclin-1 after ischemia-reperfusion in young- and middle-aged rats but not in old-aged rats. Also, increased expression of Bax, Cytochrome C, Atg5, and LC3B-II by acute hyperglycemia occurred only in young- or middle-aged rats.

Conclusion

The results of current study demonstrated that acute hyperglycemia did not aggravate myocardial ischemia-reperfusion injury in old-aged rats, unlike young and middle-aged rats. This heterogeneity may be a

consequence of attenuation in changes of apoptotic and autophagic signalling after ischemia-reperfusion injury under acute hyperglycemia in old-aged heart.

Key words : myocardium, ischemia-reperfusion injury, hyperglycemia, age, apoptosis, autophagy

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I. INTRODUCTION

Hyperglycemia commonly accompanied in patients with myocardial infarction[1], which leads to increase mortality and morbidity by reducing myocardial blood flow reserve and cardiac function.[2,3] Several studies in experimental animals have shown that acute hyperglycemia per se, irrespective of the presence of diabetes, increases the susceptibility to ischemia-reperfusion injury.[4-6] Hyperglycemia is common complication and associated with high mortality in the elderly patients (≥ 65 yr) with myocardial infarction.[7] Aging is another independent risk factor for ischemic heart disease and poor clinical outcomes following ischemia-reperfusion injury to the heart.[8-10] Both aging and hyperglycemia enhance susceptibility to ischemia-reperfusion injury by increased oxidative stress which results in altered expression and activity of its downstream signaling proteins for survival and apoptosis. The preclinical study revealed the increased susceptibility to ischemia-reperfusion injury in the aged, and diabetic heart.[11] However, recent retrospective analysis of 2,207 patients with acute myocardial infarction shows that hyperglycemia has less impact on in-hospital mortality in elderly patient than in younger patient (<50 yr).[12] These results

suggest the impact of hyperglycemia on myocardial ischemia-reperfusion injury may be heterogeneous across age-groups and different mechanisms may be involved.

Myocardial infarction after ischemia and reperfusion was demonstrated to be caused not only by necrosis, but also by apoptosis and autophagy.[13,14] Apoptosis is a physiologic process resulting from genetic programs related to development and morphogenesis, finally leading to cell death.[15] Autophagy is a protein degradation system to destroy and recycle unnecessary or damaged components. Either apoptosis or autophagy is increasing with aging.[9,16]

Thus, we hypothesized that the impact of acute hyperglycemia on myocardial ischemia-reperfusion injury may be heterogeneous across age-groups. Based on the data from this initial experiment, we further investigated possible mechanisms such as apoptosis and autophagy related to age-related differences.

II. MATERIALS AND METHODS

All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine and conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, National Academy Press, Washington DC). The Association for Assessment and Accreditation of Laboratory Animal Care International accredits Yonsei University College of Medicine.

Male Sprague Dawley rats were studied. They were kept in laboratory until they reach 3, 10-12 and 22-24 months of age. These ages correspond to

approximately young adult and middle age around 50 years and old age older than 75 human years, respectively.[17] The rats were divided into 12 experimental groups. Using a computerized program, rats of 3 groups according to the age were randomly assigned to two groups, respectively: normoglycemia (NG) and hyperglycemia (HG): (1) Y/NG: young normoglycemic sham; (2) Y/HG: young hyperglycemic sham; (3) Y/NG+IR: young normoglycemic rats undergoing ischemia-reperfusion injury; (4) Y/HG+IR: young hyperglycemic rats undergoing ischemia-reperfusion injury (5) M/NG: middle normoglycemic sham; (6) M/HG: middle hyperglycemic sham; (7) M/NG+IR: middle normoglycemic rats undergoing ischemia-reperfusion injury; (8) M/HG+IR: middle hyperglycemic rats undergoing ischemia-reperfusion injury; (9) O/NG: old normoglycemic sham; (10) O/HG: old hyperglycemic sham; (11) O/NG+IR: old normoglycemic rats undergoing ischemia-reperfusion injury; (12) O/HG+IR: old hyperglycemic rats undergoing ischemia-reperfusion injury.

Rats were anesthetized with zoletil (30 mg/kg, i.p.) and received additional doses of rompun (10 mg/kg) to ensure that pedal and palpebral reflexes were absent throughout the experimental protocol. A tracheostomy was performed by intubating the trachea with a cannula connected to Havard rodent ventilator Model 683 (Havard Apparatus, INC., Massachussettes, USA), and the lungs were ventilated with 2-3 cmH_2O positive end-expiratory pressure and an air-oxygen mixture (fractional inspired oxygen concentration=0.5). Respiratory rate was adjusted to maintain P_{CO2} at 35 ± 5 mmHg. Body temperature was maintained at 37 ± 2℃ using a heating pad and radiant warmer. Baseline hemodynamics and blood glucose level were recorded 30 min after instrumentation.

In the hyperglycemic group, the rats received 1.2 g/kg dextrose (50%

dextrose) intravenously for 5 minutes. We checked blood glucose levels 30 minutes before the experiment to confirm increase of blood glucose concentration over 300 mg/dL as previously described by Kersten et al.[18]

1.Experimental protocol: ischemia-reperfusion (Fig.1)

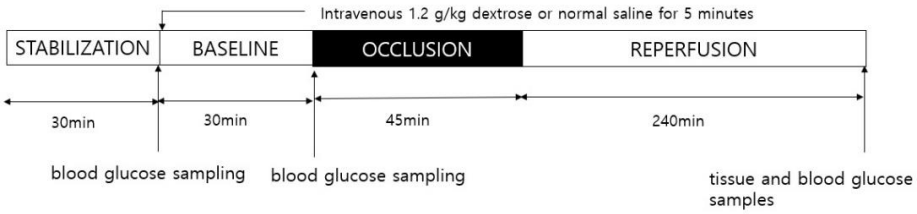


Figure 1. Experimental protocol. Rats were divided into 3 groups according to the age (3, 10-12 and 20-24 months of age). Each age group of rats were randomly assigned to 4 groups (a) normoglycemia (sham), (b) hyperglycemia (sham), (c) normoglycemia + ischemia-reperfusion injury, (d) hyperglycemia + ischemia-reperfusion injury. Rats of (c) and (d) were underwent 45 minutes of ischemia followed by 240 minutes of reperfusion.

A thoracotomy was performed in the left fifth intercostal space, and the pericardium was opened. A 6-0 prolene ligature was placed around the proximal left anterior descending coronary artery (LAD) in the area immediately below the left atrial appendage. The ends of the suture were threaded through a propylene tube to form a snare. Coronary artery occlusion was produced by clamping the snare onto the epicardial surface with a hemostat and was confirmed by the appearance of epicardial cyanosis and ST elevation on EKG. Reperfusion was achieved by loosening the snare and was verified by observing an epicardial hyperemia.

2. Hemodynamic monitoring

Rats were instrumented for the measurement of systemic hemodynamics as previously described.[19] Hemodynamic data were continuously recorded on a data acquisition system (Power Lab; AD Instruments, Colorado Springs, CO, USA). Briefly, heparin-filled catheters were inserted into the right jugular vein and the right carotid artery for fluid administration and measurement of mean arterial pressure (MAP), respectively. This pressure-volume catheter was inserted to measure left ventricular ejection fraction (LVEF). Heart rates (HR) were monitored.[20]

3. Determination of infarct size

For the determination of infarct size, regional ischemia was induced for 45 minutes by tightening of a snare around the LAD, followed by 240 minutes of reperfusion. Then after re-tightening of the snare, 0.25% Evans blue dye was infused through the jugular vein to delineate the anatomic area at risk. Then the hearts were sectioned into 4 or 5 cross-sectional pieces of 2 mm thickness. And the pieces of heart were incubated at 37℃ for 15 minutes in triphenyltetrazolium chloride solution (TTC) in 0.1M phosphate buffer adjusted to pH 7.4. TTC reacted with intracellular dehydrogenases and form a brick red color precipitate, leaving the dead tissue off white. Then infarcted and non-infarcted myocardium within area at risk (AAR) were separated and weighed. Infarct size was expressed as a percentage of the AAR.[18,21]

4. Protein analysis

Myocardium tissue samples were immediately freeze-clamped in liquid

nitrogen and frozen at -80℃. Frozen tissue was homogenized in cold buffer (4℃) containing 20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Trition X-100, complete protease inhibitor cocktails (one tablet per 10ml; Roche Diagnostics Corporation, Indianapolis, IN) and phosphate inhibitor cocktail set II (EMD biosciences, Darmstadt, Germany). And then, homogenates were centrifuged at 12,000 rpm for 30 minutes at 4℃. Protein concentration was determined using modified Bradford assay (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as a standard. And equal volumes of heart homogenate were added to Laemmli buffer (Bio-Rad Laboratories, Hercules, CA) and was denatured by heating to 100℃ for 5 minutes. Samples containing equal amounts were separated on a 10% SDS-polyacrylamide gels, and then proteins were transferred to polyvinylidene difluoride membrane. The membranes were incubated with the blocking buffer containing 5% bovine serum albumia in tris-buffred saline containing 0.1% Tween 20 for 1 hour under room temperature, and then membranes were incubated with C-PARP, Bcl-2, Bax, Cytochrome C, Bnip3, Beclin1, Atg5, and LC3B-II overnight at 4℃. Primary antibodies were visualized by using the EZ-western Lumi Femto kit (DOGEN, Korea) and using appropriated secondary antibodies conjugated to horseradish peroxidase .

5. Statistical analysis

Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA). All results were expressed as mean±standard deviation (SD). The statistical analysis was performed using one-way analysis of variance (ANOVA) or Student's t test followed by post hoc analysis. The P-values of post hoc tests were adjusted using Bonferroni's method. Values of $P<0.05$ were considered significant.

III. RESULTS

1. Body weight and blood glucose level in rats

Body mass were 352±19 g in young aged rats, 535±43 g in middle aged rats, and 581±43 g in old aged rats. Blood glucose levels are shown in Table 1. Blood glucose levels were significantly higher in the hyperglycemic (HG) group than normoglycemic (NG) group.

Table 1. Blood glucose levels (mg/dL)

		Baseline	before Ischemia	Reperfusion 240 min
Young	NG	152±23	144±30*	115±32*
	HG	141±11	314±70*	213±30*
Middle	NG	154±26	143±27*	174±30*
	HG	165±17	430±44*	352±43*
Old	NG	166±17	136±16*	151±36*
	HG	141±35	416±63*	342±106*

Values are means ± SD.

$p < 0.05$, * NG vs. HG

NG, normoglycemia; HG, hyperglycemia,

2. Perioperative hemodynamics

Table 2 shows hemodynamic data before and after ischemia-reperfusion according to the different age groups. Age-related differences of MAP were seen between Y/NG+IR vs. M/NG+IR and M/NG+IR vs. O/NG+IR. Acute hyperglycemia worsened the reduction of LVEF after ischemia-reperfusion

injury in all age groups (Figure 2).

Table 2. Heart rate and mean arterial pressure

		Baseline	before Ischemia	Reperfusion 240 min
Heart rate				
Young	NG	232±62	222±42	277±50†
	HG	262±21	205±22	287±34*†
Middle	NG	222±25	200±25	233±44
	HG	202±29	188±84	249±30*‡
Old	NG	218±41	197±34	223±34†
	HG	211±66	197±32	206±32†‡
Mean arterial pressure				
Young	NG	90±11	65±10	79±7*
	HG	83±7	64±6	86±8
Middle	NG	91±10	65±7	88±10*‡
	HG	86±11	70±10	81±5
Old	NG	98±12	64±6	76±4‡
	HG	92±8	67±5	80±8

Values are means ± SD.

$p < 0.05$, * Young vs. Middle, †Young vs. Old, ‡ Middle vs. Old

NG, normoglycemia; HG, hyperglycemia

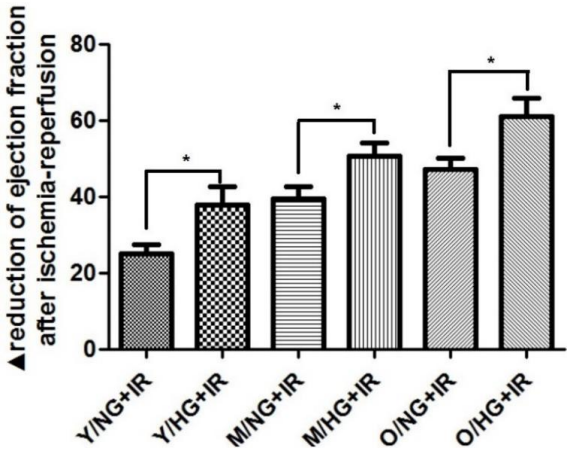


Figure 2. Changes of left ventricular ejection fraction (LVEF) after ischemia-reperfusion. Change of ejection fraction was defined as reduction of LVEF after ischemia-reperfusion/baseline LVEF * 100 (%). * $p < 0.05$. Y/NG+IR, young normoglycemic rats undergoing ischemia-reperfusion injury; Y/HG+IR, young hyperglycemic rats undergoing ischemia-reperfusion injury; M/NG+IR, middle normoglycemic rats undergoing ischemia-reperfusion injury; M/HG+IR, middle hyperglycemic rats undergoing ischemia-reperfusion injury; O/NG+IR, old normoglycemic rats undergoing ischemia-reperfusion injury; O/HG+IR, old hyperglycemic rats undergoing ischemia-reperfusion injury.

3. Myocardial infarction size

There were no significant differences in AAR among the groups (Figure 3). Hyperglycemia increased the infarct size in young and middle-aged rats, but not in old aged rats. Furthermore, when comparing infarct size in HG group according to the different age groups, infarct size was significantly smaller in O/HG+IR rats than in M/HG+IR rats, while there were no significant

differences between Y/HG+IR and M/HG+IR and Y/HG+IR and O/HG+IR (Figure 3).

(a)

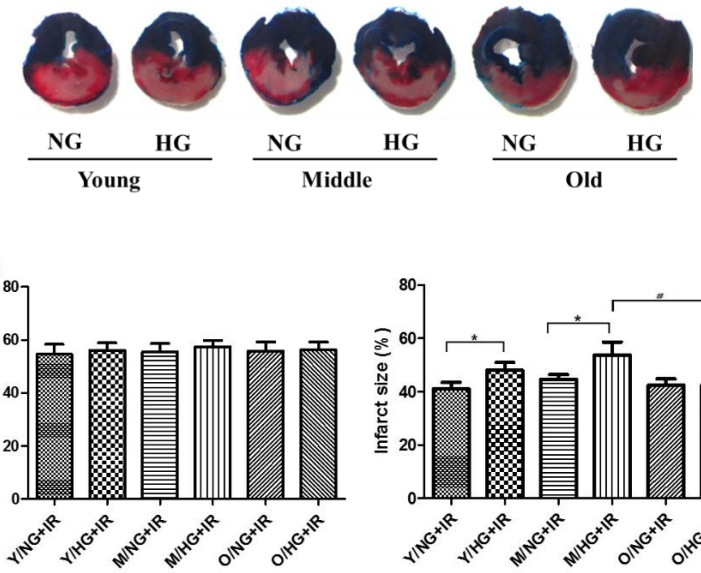
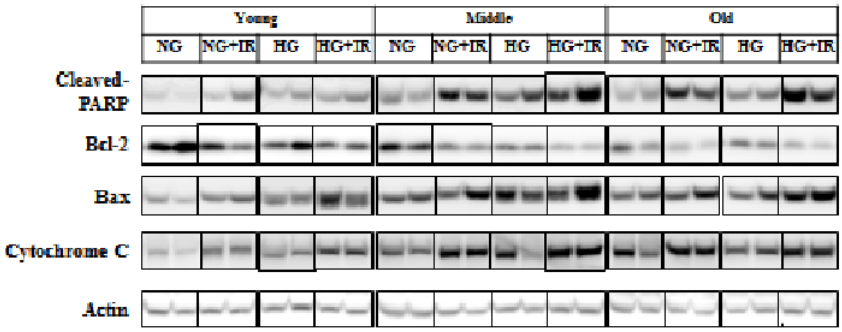


Figure 3. Myocardial infarct size. (a) Representative pictures of left ventricle sections after TTC staining. (b) Infarct size expressed as a percentage of left ventricular area and area at risk, respectively. * $p < 0.05$ *vs* HG+IR at each age group. $^{\#}$ $p < 0.05$ between different age groups. NG, normoglycemia; HG, hyperglycemia; Y/NG+IR, young normoglycemic rats undergoing ischemia-reperfusion injury; Y/HG+IR, young hyperglycemic rats undergoing ischemia-reperfusion injury; M/NG+IR, middle normoglycemic rats undergoing ischemia-reperfusion injury; M/HG+IR, middle hyperglycemic rats undergoing ischemia-reperfusion injury; O/NG+IR, old normoglycemic rats undergoing ischemia-reperfusion injury; O/HG+IR, old hyperglycemic rats undergoing ischemia-reperfusion injury

4. Protein expressions related to apoptosis and autophagy



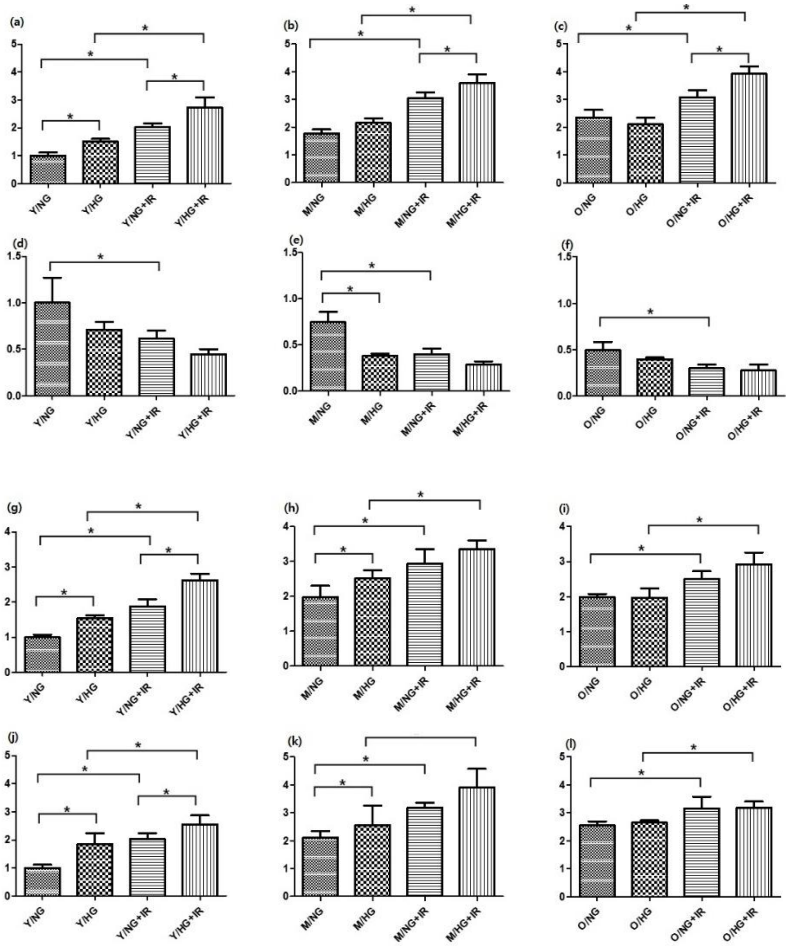
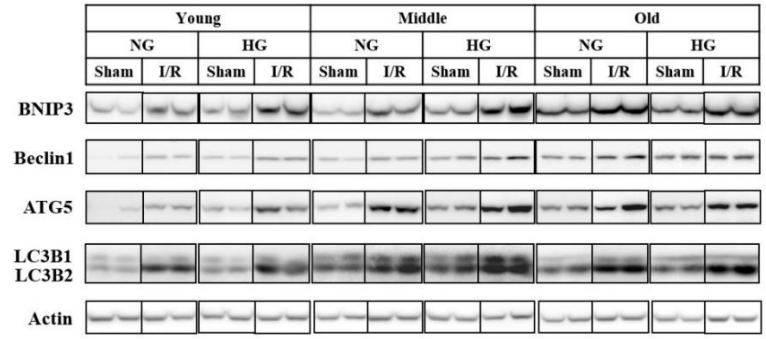


Figure 4. The expression of proteins related to apoptosis. (a,b,c) C-PARP, (d,e,f) Bcl-2, (g,h,i) Bax, (j,k,l) Cytochrome C. Data are shown as mean ± SD. * $p < 0.05$. Y/NG, young normoglycemic sham; Y/HG, young hyperglycemic sham; Y/NG+IR, young normoglycemic rats undergoing ischemia-reperfusion injury; Y/HG+IR, young hyperglycemic rats undergoing ischemia-reperfusion injury; M/NG, middle normoglycemic sham; M/HG, middle hyperglycemic sham; M/NG+IR, middle normoglycemic rats undergoing ischemia-reperfusion injury; M/HG+IR, middle hyperglycemic rats undergoing ischemia-reperfusion injury; O/NG, old normoglycemic sham; O/HG, old hyperglycemic sham; O/NG+IR, old normoglycemic rats undergoing ischemia-reperfusion injury; O/HG+IR, old hyperglycemic rats undergoing ischemia-reperfusion injury.

Figure 4 shows the expression of proteins related to apoptosis after ischemia-reperfusion under normoglycemia (NG) and hyperglycemia (HG) according to the age groups. C-PARP was increased by ischemia-reperfusion injury under normoglycemia and hyperglycemia in all age groups ($p<0.001$, $p<0.001$, $p=0.005$, in young, middle, and old-aged rats in normoglycemia, and $p<0.001$, $p<0.010$, $p<0.001$, in young, middle, and old-aged rats in hyperglycemia, respectively). In addition, hyperglycemia intensified the increased expression of C-PARP by ischemia-reperfusion in all age groups ($p=0.001$, $p=0.010$, and $p=0.001$, respectively). However, expression of Bcl-2 was decreased by ischemia-reperfusion injury under normoglycemia, but not under hyperglycemia ($p=0.007$, $p<0.001$, and $p=0.002$, in young, middle, and old-aged rats in normoglycemia, and $p=0.087$, $p=0.294$, and $p=0.052$, in young, middle, and old-aged rats in hyperglycemia, respectively). Expression of Bax was increased by ischemia-reperfusion in all age groups ($p<0.001$,

$p<0.001$, and $p=0.044$, in young, middle, and old-aged rats in normoglycemia, and $p<0.001$, $p=0.003$, and $p<0.001$, in young, middle, and old-aged rats in hyperglycemia, respectively), but hyperglycemia augmented the expression of Bax only in young ischemia-reperfusion heart ($p<0.001$).

Expression of Bnip3 ($p=0.008$, $p<0.001$, and $p<0.001$, in young, middle, and old-aged rats in normoglycemia, and $p<0.001$, $p<0.001$, and $p<0.001$, in young, middle, and old-aged rats in hyperglycemia, respectively), and Beclin-1 ($p=0.006$, $p=0.001$, and $p=0.002$, in young, middle, and old-aged rats in normoglycemia, and $p<0.001$, $p<0.001$, and $p=0.103$, in young, middle, and old-aged rats in hyperglycemia, respectively) was increased by ischemia-reperfusion injury under normoglycemia and hyperglycemia (Fig.5). And hyperglycemia intensified ischemia-reperfusion induced increases in expression of Bnip3 ($p=0.006$, $p=0.005$, and $p=1.000$, respectively), and Beclin-1 ($p=0.004$, $p=0.009$, and $p=1.000$, respectively) in young and middle-aged hearts, but not in old aged-heart. Increased expression of Atg5 ($p=0.069$, $p=0.016$, and $p=1.000$, respectively) and LC3B2 ($p=0.566$, $p=0.001$, and $p=0.921$, respectively) by ischemia-reperfusion injury was augmented under hyperglycemia only in middle-aged hearts.



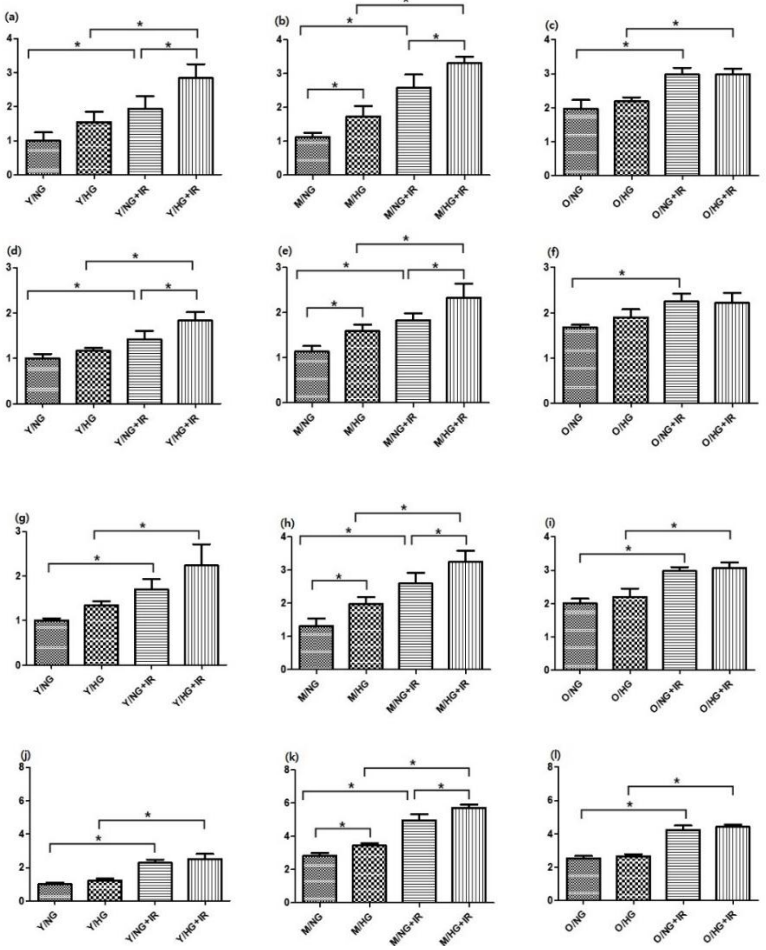


Figure 5. The expression of proteins related to autophagy. (a,b,c) Bnip3, (d,e,f) Beclin-1, (g,h,i) Atg5, (j,k,l) LC3BII. Data are shown as mean ± SD. * $p < 0.05$. Y/NG, young normoglycemic sham; Y/HG, young hyperglycemic sham; Y/NG+IR, young normoglycemic rats undergoing ischemia-reperfusion injury; Y/HG+IR, young hyperglycemic rats undergoing ischemia-reperfusion injury; M/NG, middle normoglycemic sham; M/HG, middle hyperglycemic sham; M/NG+IR, middle normoglycemic rats undergoing ischemia-reperfusion injury; M/HG+IR, middle hyperglycemic rats undergoing ischemia-reperfusion injury; O/NG, old normoglycemic sham; O/HG, old hyperglycemic sham; O/NG+IR, old normoglycemic rats undergoing ischemia-reperfusion injury; O/HG+IR, old hyperglycemic rats undergoing ischemia-reperfusion injury.

IV. DISCUSSION

In this preclinical study addressing the age-related differences of the impact of hyperglycemia on myocardial ischemia-reperfusion injury, we demonstrated less impact of acute hyperglycemia on infarct size in old aged rats comparing to young and middle-aged rats. However, reduction of EF after ischemia-reperfusion was aggravated by acute hyperglycemia in all age groups. Altered expression of proteins related to apoptosis and autophagy by acute hyperglycemia after ischemia-reperfusion was not evident in old aged rats.

Aging exacerbated myocardial ischemia-reperfusion injury in previous experimental studies.[9,22] These studies divided patients or rats into two groups according to the age. However, as the average life span increases, the number of extreme old age patients has increased, and thus the dichotomy of this age

may be difficult to apply to clinical practice in these days. We divided rats into three groups correspond to approximately young and middle aged about around 50 years and old age older than 75 human years. A previous study reported that functional deterioration by ischemia-reperfusion was aggravated even in middle-aged hearts.[10] However, comparison of middle-aged rats and old aged rats was not conducted in that study.

Several experimental studies have shown that acute hyperglycemia increases the susceptibility to myocardial ischemia-reperfusion injury.[4-6] In this study, acute hyperglycemia increased infarct size in young and middle-aged rats. However, infarct size in old aged rats was similar between under normoglycemia and under hyperglycemia. Literatures regarding age-related differences in the effect of hyperglycemia on ischemia-reperfusion injury were scarce. A previous study addressed that aged, diabetic rats were more susceptible to sub-lethal ischemia compared to younger diabetic rats.[11] However, these younger diabetic rats were 3-month GK rat and thus 'pre-diabetic'. It can be thought that the result is due to the absence of diabetes in young aged rats, rather than an increase in susceptibility to ischemia-reperfusion injury due to aging. Therefore, the strength of this study is to compare the effect of acute hyperglycemia on ischemia-reperfusion injury with aging.

Acute hyperglycemia aggravated the functional decline expressed as the reduction of LVEF after ischemia-reperfusion in all-age groups, although hyperglycemic effect on myocardial infarct size was attenuated in old-aged rats. Previous study revealed that older patients do not have larger infarcts, although both advanced age and infarct size were associated with higher mortality.[23] From this result, it can be inferred that functional decline in old aged heart, irrespective to infarct size, might be correlated with poor

prognosis. Ventricular function is determined by infarct size and residual function of spared myocardium. And myocardial perfusion reserve even had stronger correlation with LVEF than infarct size.[24] Even in a previous clinical trial, there was a negative linear relation between infarct size and LVEF in patients with moderate to large infarcts.[25] Taken together, it is necessary to estimate the ventricular function in addition to infarct size when evaluating myocardial ischemia-reperfusion injury. So, it is the strength of the current study that we revealed that altered response by acute hyperglycemia to ischemia-reperfusion injury expressed as infarct size and LVEF might be different according to the different age groups.

Myocardial infarction after ischemia and reperfusion was demonstrated to be caused not only by necrosis, but also by apoptosis and autophagy.[13,14] Apoptosis is programmed cell death and removal without activation of an inflammatory process, based on DNA and cellular fragmentation.[26] Previous study revealed increased plasma apoptotic marker levels in elderly patients than adult patients and higher myocardial apoptotic ratio in the older rat group thus concluded that aging exacerbated myocardial ischemia-reperfusion injury in humans and rats.[9] There were several reports regarding increased or decreased autophagic protein expression with aging.[27,28] We investigated the expression of apoptotic and autophagic proteins according to the different age groups in both normoglycemia and hyperglycemia. In accordance with the age-related differences of impact of acute hyperglycemia on infarct size, expression of proteins related to apoptosis or autophagy (Bnip3, Beclin1, Atg5, LC3B-II) after ischemia-reperfusion injury was not different between acute hyperglycemia and normoglycemia in old aged rats, which was significantly different in young or middle-aged rats.

Cleaved poly-ADP-ribose polymerase (C-PARP) is an abundant DNA-

binding enzyme that detects and signals DNA strand breakages.[29] And presence of C-PARP is used to detect apoptosis in many cell types.[30] Previous study conducted in a rat model of aging-associated cardiovascular dysfunction demonstrated that acute inhibition of PARP by PARP-inhibitor decreases the age-related myocardial and vascular PARP-activation, resulting in improvement of left ventricular contractility and enhanced endothelium-dependent vasorelaxation.[31] This result indicates that C-PARP is a consequence of apoptosis and is associated with cardiovascular dysfunction following aging. In the present study, acute hyperglycemia exaggerated the increased expression of C-PARP by ischemia-reperfusion in all age groups, which correlated with the functional decline of left ventricle.

Bcl-2 is well known inhibitor of apoptosis.[32] Overexpression of Bcl-2 in transgenic mouse effectively reduced myocardial ischemia-reperfusion injury.[33,34] In a previous study, aging decreased antiapoptotic Bcl-2 levels.[35] Acute hyperglycemia reduced Bcl-2 expression only in middle-aged rats.

Bax is a proapoptotic protein and have been shown to be upregulated in response to chronic pressure overload, cardiomyocyte-specific apoptosis.[36] Bax showed age-associated differences in rats underwent coronary artery occlusion.[37] Old aged rats exhibited higher level of Bax at baseline and greater decline of Bax after coronary artery occlusion than young aged rats in that study. However, Bax expression was elevated after ischemia-reperfusion injury on the contrary to previous report. This discrepancy might be due to different experimental design. Previous study elucidated the change of Bax protein after coronary artery occlusion, while we compared the expression of Bax between control and after ischemia-reperfusion injury. The relationship between age and expression of Bax under hyperglycemia have not been extensively characterized. In our study, we found that hyperglycemia did not

alter the level of Bax after ischemia-reperfusion injury in middle and old aged rats compared to young aged rats.

Mitochondrial cytochrome c release initiates apoptosis.[38] Previous study demonstrated that mitochondrial cytochrome c release were associated with hyperglycemia induced myocardial apoptosis.[39] However, age-related differences of release of cytochrome C under hyperglycemia have not been elucidated before. The differences of expression of cytochrome C induce by ischemia-reperfusion under different glycemic conditions was evident only in young aged rats.

Bnip3 is a hypoxia-regulated member of the Bcl-2 family of proteins that is implicated in apoptosis, programmed necrosis, autophagy and mitochondrial autophagy.[40,41] Previous study demonstrated that Bnip3 induced post-ischemic apoptosis[42] and diabetes-induced Bnip3 expression compromised cardiac cell survival and cardiac function.[43] Also, up-regulation of Beclin-1 by ischemia-reperfusion injury stimulated autophagy[44] and activation of Beclin-1 type 2 diabetes increased cardiomyocyte autophagy. [45] In the current study, expression of Bnip3, and Beclin-1 was increased by ischemia-reperfusion injury under normoglycemia and hyperglycemia in all age groups. And hyperglycemia intensified the increased expression of Bnip3, and Beclin-1 by ischemia-reperfusion in young and middle-aged hearts, but not in old aged-heart. These age-related differences of Bnip3 and Beclin-1 under hyperglycemia were paralleled with the difference in myocardial infarct size according to the age.

Cardiac specific loss of Atg5 caused cardiomyopathy in mice.[46] Another study demonstrated that silencing Atg5 aggravated cardiac hypertrophy.[47] Thus upregulation of autophagy may be an adaptive response for protecting cells.[46] Atg5 was also upregulated by hyperglycemia and ischemia-reperfusion

injury in the current study. Moreover, this upregulation of Atg5 showed age-related differences. The impact of hyperglycemia on ischemia-reperfusion injury was prominent only in middle aged rats. LC3B-II, an autophagy associated protein was increased during reperfusion in fibrillated mouse hearts, compared to non-fibrillated mouse hearts.[48] Accordingly, the expression of LC3B-II was increased in hyperglycemia and after ischemia-reperfusion injury. These upregulated expressions also exhibited age-related differences that upregulation in hyperglycemia than normoglycemia after ischemia-reperfusion injury was only seen in middle aged rats.

Taken together, there were age-associated differences of the impact of hyperglycemia on myocardial ischemia-reperfusion injury. And possible related mechanisms such as apoptosis and autophagy were elucidated. There was a trend toward lesser impact of acute hyperglycemia after ischemia-reperfusion injury on infarct size and apoptotic, autophagic protein expression in old aged rats. In accordance with these results, recent retrospective analysis of 2,207 patients with acute myocardial infarction shows that hyperglycemia as independent risk factor for in-hospital mortality has less impact in the elderly patient than in younger patient (<50 yr).[12] Based on these results, it can be seen that we need to make a difference according to age in blood glucose control in patients with coronary artery disease. Even though the benefits of glycemic control are well established in reducing the risk of microvascular disease, the possibility of acute hypoglycemia increases with the tight glycemic management especially among the elderly patients. The disappointing results of recent clinical trials of tight glycemic control in seniors with type 2 diabetes highlight the potential cardiovascular risks of hypoglycemia and hyperglycemia and hypoglycemia as a risk factor for poor prognosis is a topic of increasing interest.[1,49-52]

There are several limitations in the current study. The focus of this experiment was to evaluate age-related differences of protein expression associated with apoptosis and autophagy. Although our results demonstrated age-associated differences of protein expression, relationship between cardiac function such as hemodynamics and ventricular response and protein expression might be ambiguous. Secondly, the interaction between apoptosis and autophagy was not investigated. Previous studies addressed regulation of interplay between autophagy and apoptosis.[53] Lastly, the meaning of change in expression of apoptosis/autophagy remains elusive. Previously, upregulation of autophagy in failing heart was demonstrated as an adaptive response for protecting cells from hemodynamic stress.[46]

V. CONCLUSION

We demonstrated that acute hyperglycemia aggravate myocardial ischemia-reperfusion injury in young and middle-aged rats. The effect of acute hyperglycemia on myocardial ischemia-reperfusion injury was attenuated in old rats. This heterogeneity was paralleled with the attenuation in change of apoptotic and autophagic signalling such as Bcl-2, Bnip3, and Beclin-1 in aged myocardium. We suggest that the value of acute hyperglycemia as a risk factor for myocardial ischemia-reperfusion is less in aged myocardium, possibly due to differential activation in apoptotic and autophagic signalling. This finding may have clinical implications regarding the strategy of glucose control in patients with myocardial infarction according to the patient's age.

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ABSTRACT (IN KOREAN)

급성 고혈당증이 심근의 허혈-재관류 손상에 미치는 영향의 연령에 따른 차이

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함성연

고령과 급성 고혈당은 각각 심근 허혈-재관류 손상의 위험 인자이며, 임상에서 흔히 동반된다. 그러나 급성 고혈당이 심근의 허혈-재관류 손상을 악화시킨다는 연구는 대부분 젊은 동물들을 대상으로 이루어져 있고 노화된 심근에 미치는 영향에 대한 연구는 드물다. 따라서 본 논문에서는 연령에 따라 급성 고혈당이 심근의 허혈-재관류 손상에 미치는 영향이 어떻게 달라지는지 알아보고, 그 기전을 밝히고자 하였다.

Sprague Dawley계 수컷 쥐를 연령에 따라 세 군으로 나누었다 (젊은 쥐, 3개월령; 중년 쥐, 10-12개월령; 고령 쥐, 20-22개월령). Dextrose (1.2 g/kg)를 주입하여 고혈당을 유발하였으며, 좌전하행관상동맥을 45분간 폐쇄하였다가 240분간 재관류하여 심근손상을 유도하였다. 경색 크기와

좌심실 박출률을 측정하여 허혈-재관류 손상에의 민감도를 측정하였다. 또한 western blot assay를 통해 세포 자멸(apoptosis)과 연관된 단백질(C-PARP, Bcl-2, Bax, cytochrome C)과, 자가소화작용 (autophagy)과 연관된 단백질(Bnip3, Beclin-1, Atg5, LC3B-II)의 발현을 관찰 하였다.

젊은 쥐와 중년 쥐에서는 급성 고혈당에 의해 심근경색의 크기가 증가하였으나, 고령 쥐에서는 영향이 없었다. 그러나 허혈-재관류 이후 좌심실 박출률 감소는 전 연령 군에서 고혈당에 의해 악화되었다. 젊은 쥐와 중년 쥐의 심근에서는 허혈-재관류 손상으로 인해 Bnip3와 Beclin-1의 발현이 증가되는 것을 고혈당이 증폭시켰으나, 노화된 심근에서는 그렇지 않았다. 또한 재관류 후 Bax, Atg5와 LC3B-II 발현 역시 젊은 쥐나 중년 쥐에서만 고혈당에 의해 영향을 받았다.

급성 고혈당이 젊은 쥐와 중년 쥐에서는 심근의 허혈-재관류 손상을 악화시켰지만 고령 쥐에서는 영향이 적었다. 고령 쥐에서는 Bnip3, Beclin-1과 bax 같이 허혈-재관류 손상 후 증가하는 단백질들이 고혈당에 의해 영향을 적게 받았음을 고려할 때, 이러한 세포 자멸 및 자가소화작용과 관련된 단백질 발현이 연령에 따라 급성 고혈당의 영향이 다른데 기여했을 것으로 여겨진다.

핵심되는 말 : 심근, 허혈-재관류 손상, 고혈당, 연령, 세포 자멸, 자가소화작용