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Modification of the β -adrenoceptor stimulation pathway in Zucker obese and obese diabetic rat myocardium

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Cheng Jiang. Modification of the β -adrenoceptor stimulation pathway in Zucker obese and obese diabetic rat myocardium. *Tissues and Organs [q-bio.TO]*. Université Pierre et Marie Curie - Paris VI; Université de Wuhan (Chine), 2015. English. NNT : 2015PA066726 . tel-01418221

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Université Pierre et Marie Curie
Université de Wuhan

Ecole Doctorale 394
UMR INSERM-UPMC 1166/Physiologie Physiopathologie et Thérapeutique

**Modification de la voie de la stimulation des récepteurs
 β -adrénergiques dans le myocarde des rats Zucker obèses
et obèses diabétiques**

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Thèse de doctorat de Physiologie et Physiopathologie

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Présentée et soutenue publiquement le 26 juin 2015

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Remerciements

To four outstanding teachers: Professor Bruno Riou, Professor Yan Zhao, Professor Julien Amour and Doctor Aude Carillion, for their trust, support and wise advice which enabled me to carry out this research.

To my rapporteurs, Professeur Jean-Emmanuel de LA COUSSAYE and Professeur Li-Bo CHEN who made me the honor to report my work.

To my important colleagues and friends in France: Ms. Na Na, Ms. Sarah Feldman, Ms. Beibei Wang, and Mr. Qiuji Wu.

To my colleagues and friends in China: Ms. Shan Jiang, Mr. Xianlong Zhou, and all the staffs in the Emergency Center of Zhongnan Hospital of Wuhan University. Thanks a lot for language review by Ms. Wenting Zhou.

To my family.

I want to especially thank the China Scholarship Council (CSC) for the State Scholarship Fund (File NO. 201206270014), the International Office in Wuhan University, and the International Relation Office in Université Pierre et Marie Curie - UPMC. Without their supports and help, I would not have the chance to study and make my research with those leader teams in France.

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Chapter One

Introduction

The prevalence of obesity around the world leads to a remarkable increase in the metabolic syndrome and the metabolic related disorders, including insulin resistance, hyperglycemia, dyslipidemia and hypertension^[1,2]. The risks of diabetes mellitus and the cardiovascular abnormalities associated with aforementioned factors are also increased. Patients of metabolic syndrome have an about five-fold increase in the risk of diabetes mellitus^[3,4]. Approximate 1 adult in 4 or 5 has metabolic syndrome, depending on different countries. In the United States of America, approximately 47 million (24%) adult suffered from the metabolic syndrome in 2002^[5]. The morbidity is increasing in both developed and developing countries^[5,6]. With the change of diet habits and physical exercise levels in modern life, together with the increasing morbidity of obesity at an early age, the World Health Organization (WHO) projections in 2005 have alarmed for obesity and obesity related diseases, and regarded obesity as a global public health problem^[2]. Estimated by WHO, in 2015, approximately 2.3 billion adults are overweight and among them, at least 700 million are obese (body mass index (BMI) > 30 g/m²)^[7]. Considering diabetes mellitus, WHO has stated that up to 347 million people worldwide suffered from diabetes mellitus in 2008, and the incidence is so rapidly increasing that estimating number will almost double by 2030^[8].

Obesity has been regarded as an independent predictor of left ventricular (LV) diastolic dysfunction and can cause depression of cardiac function^[9,10]. Central obesity is one independent risk factor for cardiovascular disease and is associated with metabolic syndrome^[11]. Obesity increases the risk of heart failure about two folds even when hypertension and other related risk factors are corrected^[12]. However, after the onset of heart failure, obesity will be a positive predictor for survival, which is considered as “obesity paradox”^[13-15]. This phenomenon has also been observed in the perivascular and epicardial white adipose tissues, which demonstrates a potential cardio - protective effects^[15]. Diabetes mellitus also increases the incidence of heart failure, despite correcting age, hypertension, obesity, hypercholesterolemia and coronary artery disease^[16]. The diabetic cardiomyopathy is characterized as diastolic dysfunction followed by the systolic dysfunction^[17]. From the echocardiography of the diabetic patients without known cardiac disease, the damage of early diastolic filling, prolonged isovolumic relaxation and increased atrial filling were observed^[18].

Metabolic syndrome, also as a major risk factor of cardiovascular disease, has been considered as a direct precursor of diabetes mellitus [19-23]. The National Cholesterol Education Program's Adult Treatment Panel III (ATPIII) report has identified six components of metabolic syndrome that related to cardiovascular disease, including abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance / glucose intolerance, and proinflammatory and prothrombotic states[24].

Pathophysiologically, the onset and progression of obesity, diabetes mellitus and metabolic syndrome are complicated and still not fully elucidated, among which the activation of sympathetic nervous system and a marked adrenergic hyperactivity are mostly observed [2,25-32]. Metabolic syndrome is associated with sympathetic activation, possibly attributed to the neural mechanism (direct activation of sympathetic nerve system and renal afferent nerve activation), genetic factors (β -adrenoceptor polymorphisms), metabolic factors (hyperinsulinemia, insulin resistance and dysregulated production and secretion of adipokines), reflex factors (an impairment to restrain the adrenergic cardiovascular drive in the baroreflex), psychological stress, oxidative stress, obstructive sleep apnea and inflammation^[32,33], among which insulin resistance (IR) is proposed as a “driving force” of the metabolic syndrome^[34,35]. In the obese normotensive individuals, elevation of sympathetic outflow was observed from the examinations of circulatory catecholamines, urine norepinephrine, muscle sympathetic nerve activity (MSNA) of postganglionic sympathetic nerve fibers, and renal norepinephrine spillover [36,37]. In obesity, the degree of the sympathetic activation paralleled closely to the increase of body mass index (BMI)^[31]. Most studies have demonstrated that the β -adrenoceptor polymorphisms accompanying the sympathetic hyperactivity are associated with hypertension, obesity and diabetes mellitus^[38,39]. In addition, the sympathetic overdrive can be observed in obese individuals predisposed to metabolic syndrome before hypertension happens^[32]. And there is also an evidence that obesity *per se* did not generalize sympathetic hyperactivity, but caused differential activation of tissues sympathetic activities [40,41]. The importance of the sympathetic nervous system in the cardiovascular disease has already been clarified from the cardiac arrhythmias, hypertension, cardiomyopathy and progressive heart failure to the final death [2,42].

Previous research about obesity-related cardiac dysfunction showed controversial results from different animals or experimental models, such as obese rats or rabbits induced by special dietary, genetic obese rats etc. [43-51]. There are complex alterations in cardiac structure and function which occur in obesity and obesity-related diseases, including the reduced myofilament calcium sensitivity, changed calcium handling proteins and calcium transients,

and changed β -adrenergic pathway^[50,52-54]. But the mechanism responsible for those changes has not been well understood. Several methods with different species have been used to explore the influences of diabetes on the cardiac function, such as echocardiography, isolated heart perfusion, *in situ* LV catheterization, LV papillary muscles and magnetic resonance imaging (MRI)^[16,55-60]. And many mechanisms were also studied to explain the cardiac dysfunction in diabetes mellitus, including the metabolic disorders and structural remodeling caused by hypertrophy, apoptosis, necrosis and fibrosis, changes in the cardiac autonomic neuropathy and calcium handling^[57,61-66]. Nevertheless, from those evidences, the responsible factors for the myocardial dysfunction in diabetes are still incompletely established.

Although obesity is associated with lower perioperative mortality (mentioned as “obesity paradox”), patients with the metabolic syndrome are exposed to a higher mortality risk during the perioperative period^[67]. Such obesity paradox has also been reported in critically ill patients^[68], including those with severe sepsis^[69]. However, several large clinical studies challenge the validity of the obesity paradox^[70] and obese trauma patients are at risk of higher mortality from persistent hemorrhage^[71]. In diabetic patients, diastolic dysfunction and a reduced response to β -adrenoceptor stimulation are observed which may contribute to hemodynamic instability during the perioperative period. Although metabolic syndrome is associated with increasing catecholamine levels and sympathetic activity that chronically stimulates β -adrenoceptors^[72], the β -adrenoceptor signaling pathway has been inadequately studied in this situation.

Alterations in the β -adrenoceptor signaling pathway have been observed in senescent^[73] and type 1 diabetic rats^[55]. While an increase in sympathetic nervous system activation is an important mechanism for maintaining cardiac output, the positive inotropic response to β -adrenoceptor stimulation is markedly altered in type 1 diabetic rats, in part, owing to the down-regulation of β_1 -adrenoceptor and the up-regulation of β_3 -adrenoceptor, source of a negative inotropic effect. Active efflux transporters, namely the multidrug resistance-associated protein 4 (MRP4), acts also as an independent endogenous regulator of intracellular cyclic nucleotide levels (3'-5'-cyclic adenosine monophosphate, cAMP),^[74] and has been recently shown to be involved in β -adrenoceptor dysfunction during aging^[75].

Zucker obese rat (fa/fa) is considered as a reliable model of metabolic syndrome.^[76] Moreover, this model enables us to study separately obesity and obesity associated with type 2 diabetic status^[77]. Zucker obese rats develop metabolic syndrome characterized by obesity because of the hyperphagia due to a mutation in the leptin receptor^[76,78,79], insulin resistance, hyperinsulinemia, hypertriglycemia and hypercholesterolemia^[80]. Zucker obese diabetic

(Zucker diabetic fatty, fa/fa) rats are originated from the selective breeding of Zucker rats with high glucose, which develop hyperphagia due to the nonfunctional leptin receptor, leading obesity and prediabetic state similar to humans^[81].

The aim of this study was to compare the β -adrenoceptor signaling pathway in Zucker lean, Zucker obese, and Zucker obese diabetic rats. This pathway was assessed both *in vivo* using echocardiography and *in vitro* using isolated LV papillary muscle. Our hypothesis was that metabolic syndrome is associated with β -adrenergic dysfunction to some degree, which is aggravated when associated with diabetes. We precisely assessed the effects of β -adrenergic stimulation on both contraction and relaxation, and particularly focussed on the following possible mechanisms involved, *i.e.* down-regulation of β_1 -adrenoceptors, up-regulation of β_3 -adrenoceptors, and up-regulation of MRP4^[55,73-75].

Chapter Two

Background

Heart works on the basis of the normal fundamental properties of the heart muscles and numerous modifying, protecting and controlling effects under the nerves, chemicals and mechanical mechanisms on them^[82]. Control mechanisms are very important to achieve and regulate the physiology of heart. These control mechanisms can be exerted at the central nervous system levels or at the periphery levels, or through the integrative signals transmitted by the local metabolites. Two divisions of the autonomic nervous system, along with their primary messengers / neurotransmitters and two major types of specific receptors, conduct different functions in the cardiovascular reactions^[83]. The sympathetic nervous system / adrenergic nervous system acts through the adrenergic receptors, while the parasympathetic system acts through the cholinergic receptors. The adrenergic receptors / adrenoceptors are associated with the strengthened contractility and heart rate (β -adrenoceptor) or the enhancement of the arteriole tone (α -adrenoceptor). The cholinergic receptors react to their primary messengers, acetylcholine, and exert the opposite effects of adrenergic stimulations. As Rockman, et al.^[84] concluded that “*Adrenergic receptors do not simply generate second messengers but rather activate a host of signaling proteins and pathways that control cardiac function, myocyte growth and cell death*”.

There are two main types of adrenergic receptors, α -adrenoceptors and β -adrenoceptors. The density of α_1 -adrenoceptor in human heart is only 10% - 15% of the density of β -adrenoceptor. The maximal positive inotropic effect by the stimulation of α_1 -adrenoceptor is far less than the effects under the stimulation to β -adrenoceptor^[85]. α_2 -adrenoceptor in human heart is still not fully understood. However, it has been observed that stimulation to α_2 -adrenoceptor could inhibit the norepinephrine release in the presynaptic component of sympathetic nerve endings in human heart^[85,86], thus affecting the plasma norepinephrine levels, especially in patients with heart failure. β -adrenergic receptors are class of G protein-coupled receptors, with three subtypes β_1 -adrenoceptor, β_2 -adrenoceptor and β_3 -adrenoceptor. β_1 -adrenoceptor subtype is the main cardiac β -adrenergic receptor, while most β_2 -adrenoceptors are noncardiac receptor^[87]. Although they have different distributions within different tissues, organs and species, they can co-exist in the same ventricular cell and exert the positive inotropic responses^[88]. In the human heart, β -adrenoceptors, such as β_1 -

adrenoceptor and β_2 -adrenoceptor, play important roles not only in the physiological status, but also in diseased conditions. Different cardiac receptors related pathways participate in the regulation of cardiac performances: some receptors work via the Gs protein - adenylyl cyclase pathway, such as β -adrenoceptors and histamine receptors; some work via the Gi protein - adenylyl cyclase pathway, such as muscarinic receptors and adenosine receptors; and some others work via $G_{q/11}$ protein - phospholipase C - protein kinase C pathway, including α_1 -adrenoceptor and angiotension II receptors^[85].

1 Physiological and pathophysiological β -adrenergic receptor signaling pathway in the heart

1.1 The physiological characteristics of β -adrenergic receptors signaling pathway in the heart of animals and human

In human heart, β_1 -adrenoceptor and β_2 -adrenoceptor co-exist, β_1 -adrenoceptor being predominant^[89]. Both β -adrenoceptor subtypes couple to Gs protein, increase the cAMP levels and induce the positive inotropic and chronotropic effects *in vivo*, and *in vitro*. In atria, stimulation to β_1 -adrenoceptor and β_2 -adrenoceptor can cause maximal physiological effects. But in ventricles, only stimulation to β_1 -adrenoceptor can evoke maximal effects, and stimulation to β_2 -adrenoceptor causes submaximal results^[89]. In rats and murines hearts, stimulation to β_1 -adrenoceptor can induce the positive inotropic and chronotropic effects, but facilitate the cardiomyocytes apoptosis as well^[90-94].

The post-receptor signaling pathway of β_1 -adrenoceptor is widely clarified to conduct the positive inotropic effects, the lusitropic / relaxant effects, the chronotropic effects and the dromotropic effects under the β -adrenergic stimulation^[82]. The cardiac inotropic effect is regulated mostly by the amount of calcium ions entry into cytosol during the process of activation. β -adrenergic stimulation can enhance the force of contraction (positive inotropic effect) and the rate of relaxation (lusitropic effect), then change the pattern of contraction and relaxation (Fig. 2-1)^[82].

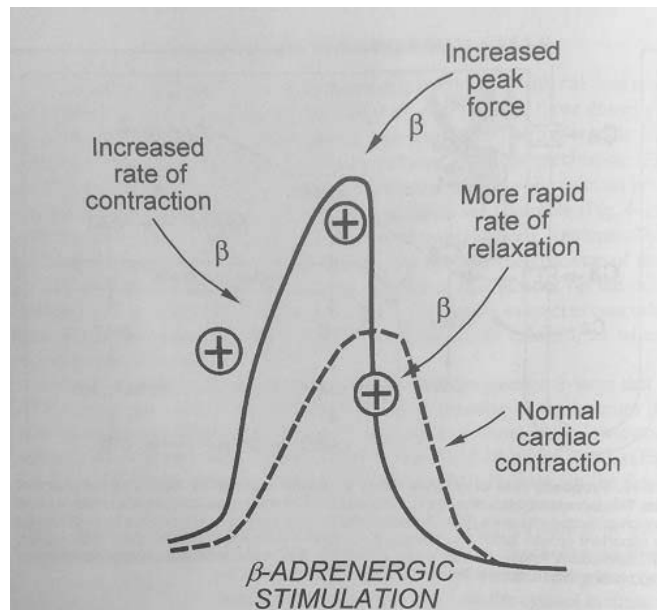


Fig. 2-1 The effects of β -adrenergic stimulation on the pattern of cardiac contraction.

Reproduced from Opie^[82]

β -adrenergic stimulation to β -adrenoceptor induces molecular changes, then the binding of Gs protein to guanosine 5' – triphosphate (GTP) catalyses subunit of adenylyl cyclase to produce cAMP from adenosine 5' – triphosphate (ATP). Positive inotropic effect in response to β -adrenergic stimulation involves some pathways: 1) β -adrenergic stimulation increases cAMP-mediated activation of protein kinase A (PKA) that subsequently increases the phosphorylation of sarcolemmal protein of the calcium channels by PKA; thereby increases the inward calcium current (I_{ca}), causing a great rate of calcium-induced calcium ions release through the ryanodine receptor (RyR). 2) β -adrenoceptor stimulation increases cAMP-mediated activation of PKA that subsequently increases the phosphorylation of phospholamban (PLB), enhancing re-uptake of calcium into the sarcoplasmic reticulum (SR); then, preloading the SR with more calcium ions will increase the amount of calcium ions released in response to any amount of trigger calcium; thus, the contractile response will be further stimulated^[95]. 3) increase of intracellular free calcium ions enhances de - inhibition of actin and myosin by interaction of calcium with troponin C, and promotes actin-myosin interaction; 4) increase in intracellular free calcium ions accelerates splitting of ATP by myosin ATPase to increase the rate of development of contractile forces; thus, β -adrenoceptor stimulation increases the crossbridge cycling^[82,96].

β -adrenergic stimulation can also enhance the rate of relaxation (lusitropic effect), then change the pattern of contraction and relaxation^[82]. Lusitropic effect is mostly induced at a

subcellular level by the increased activity of calcium pump in the SR in response to the phosphorylation of phospholamban by cAMP and PKA. Sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) is a calcium uptake pump of sarcoplasmic reticulum, constituting about 40% of the protein component of SR with the major mechanism of reducing the cytosolic calcium ion level and initiating diastole^[82]. In the rabbit myocardium, nearly 75% of the activator calcium is removed by calcium uptake pump of SR, and sodium / calcium exchanger (NCX) removes nearly 25%, and only about 1% is removed by the calcium pump of sarcolemma or transported into mitochondria^[97]. Thus, the most majority of calcium released from SR return to its origin site by the activity of SERCA. There are three different genes encoding for SERCA, among which the SERCA2a is predominately expressed in cardiac tissues (cardiac isoform, SERCA2a)^[98]. The activity of SERCA2a depends on the amount of SERCA2a proteins, and is normally inhibited by phospholamban^[99]. β -adrenergic stimulation can relieve this inhibition via cAMP and PKA phosphorylating phospholamban, therefore the calcium uptake is stimulated. Lusitropic effects of β -adrenergic stimulation involve several pathways: 1) β -adrenergic stimulation, via cAMP and PKA, phosphorylates phospholamban, increases the turnover and sensitivity of SERCA2a, and further increases the removal of calcium out of cytosol; 2) phosphorylation of plasma member sodium pump could increase the calcium efflux via sodium/calcium exchanger; 3) phosphorylation of troponin I could reduce the calcium binding through decreasing the calcium sensitivity to the troponin I complex, and further increase the rate of crossbridge detachment. 4) increased intracellular calcium level, via calmodulin and calmodulin-dependent kinase (CaMK), enhances the phosphorylation of phospholamban directly^[82,96]. Thus, the contraction and relaxation rates are both enhanced, which are the positive inotropic effects and the positive lusitropic effects. β_1 -adrenoceptor / Gs protein coupling pathway can also evoke PKA-independent, CaMK II-mediated apoptotic procedure. Figure 2-2 demonstrates the signaling pathway of β_1 -adrenoceptor and β_2 -adrenoceptor in cardiomyocytes^[100].

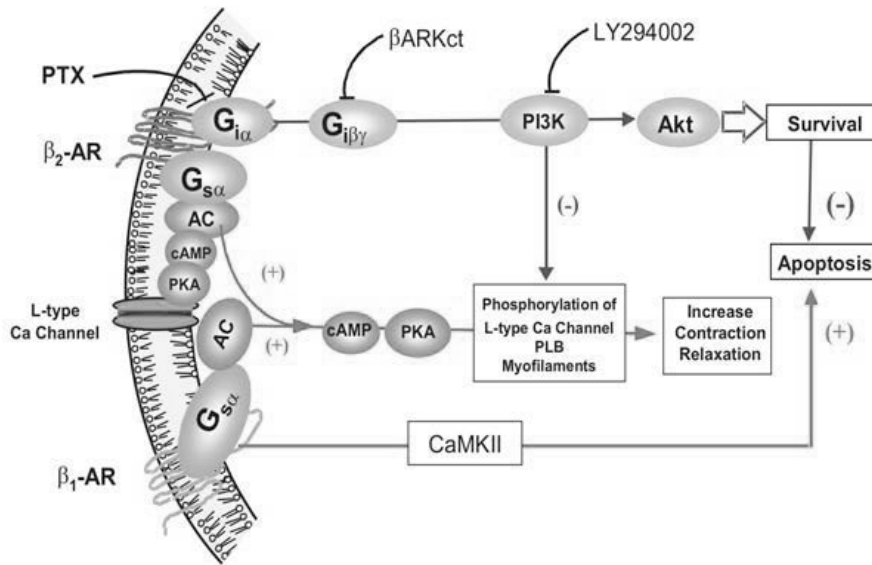


Fig. 2-2 Signaling pathway of β_1 -adrenoceptor and β_2 -adrenoceptor in cardiomyocytes.

β ARK-ct, a peptide inhibitor of $G_i\beta\gamma$ signaling; LY294002, a PI3K inhibitor. PTX: pertussis toxin; β ARK: β -adrenergic receptor kinase; PI3K: phosphatidylinositol 3-kinase; Akt: protein kinase B; AC: adenylyl cyclase; cAMP: 3',5'-cyclic adenosine monophosphate; PKA: protein kinase A; PLB: phospholamban; CaMK II : calmodulin-dependent kinase II .

Reproduced from Zheng et al. ^[100]

The role of cardiac β_2 -adrenoceptor has still not been fully understood. One early research, using transgenic mice of over - expressing human β_2 -adrenoceptor (TG4 mice), first confirmed the biochemical evidences that β_2 -adrenoceptor was coupled to both Gs protein and Gi protein ^[101]. The ventricular myocytes of wild - type (WT) and TG4 mice did not develop the increased contractile responses to the stimulation of β_2 -adrenoceptor agonists, unless the Gi protein activity was inhibited by pertussis toxin (PTX). That was to say that the activation of β_2 -adrenoceptor - Gi protein system would preclude the positive inotropic responses under the β_2 -adrenoceptor stimulation. In WT mice pretreated with β_1 -adrenoceptor blockade or in β_1 -adrenoceptor knock-out mice, stimulation of isoproterenol (a mixed agonist) could not induce positive inotropic responses ^[102,103]. These results indicate that the positive inotropic responses under isoproterenol were mostly attributed to β_1 -adrenoceptor stimulations in WT mice cardiac myocytes or *in vivo* ^[102,104,105], whereas β_2 -adrenoceptor - Gi protein coupling induced a negative feedback to β -adrenoceptor stimulations. Because of the concurrent coupling to Gi protein, β_2 -adrenoceptor seems dormant and shows no significant function in the regulation of contractions in cardiac myocytes or myocardiums of mice and other

mammalian species^[88,101,103,106]. Nevertheless, β_2 -adrenergic stimulation can still function via the Gs protein - adenylyl cyclase - cAMP - PKA pathway, and exert the positive inotropic and lusitropic effects similar to the β_1 -adrenoceptor stimulations. In all, β_2 -adrenoceptor is coupled to Gs protein and Gi protein in rats and murines, and can cause anti - apoptotic effects^[90-94,107-109]. In isolated human atrium and ventricular myocardium, activation of β_2 -adrenoceptor can induce similar effects as the stimulation to β_1 -adrenoceptor, including increased contractile force, and enhanced relaxation through cAMP dependent phosphorylation of phospholamban and troponin I^[110-113]. These evidences show that β_1 -adrenoceptor and β_2 -adrenoceptor in human heart are both coupled to Gs protein. However, whether β_2 -adrenoceptor is coupled to Gi protein as in rats and murines hearts, it is still in debate.

Researchers found that different β_2 -adrenoceptor agonists may activate different fashion of pathway, either β_2 -adrenoceptor - Gs protein pathway or β_2 -adrenoceptor - Gs protein and β_2 -adrenoceptor - Gi protein pathways^[114-116]. An early study found that nebulised β_2 -adrenoceptor agonist could cause different cardiovascular effects in healthy human heart, which showed that fenoterol induced significantly greater chronotropic electrocardiographic and inotropic effects than terbutaline did^[117]. In animal experiment, terbutaline was observed to couple to Gs protein and Gi protein, and fenoterol only coupled to Gs protein^[116]. Another research found that a β_2 -adrenoceptor antagonist (ICI118,551) worked as the agonist to Gi protein in ventricular cardiomyocytes of human failing heart (increased Gi protein activity in failing heart), which was the direct negative inotropic effects, whereas this did not occur in non-failing human heart (normal Gi protein activity in healthy heart)^[118]. Besides, β_2 -adrenoceptor coupled to the inhibitory G protein Gi can activate the phospholipase A2 / arachidonic acid pathway^[119,120]. The coupling of β_2 -adrenoceptor to the inhibitory protein Gi also activates the Gi protein - PI3K - Akt pathway, which can compartmentalize and counteract the Gs protein - adenylyl cyclase - cAMP - PKA signaling pathway^[100]. Thus cardiac β_2 -adrenoceptor - Gi protein pathway might be more important in rodents than in human. In the failing heart, the percentage of β_2 -adrenoceptor may double as that in the normal ventricle, while the β_1 -adrenoceptor down-regulates. It has been suggested that the Gi pathway is associated with an anti - apoptotic mechanism which benefits in the failing heart^[121,122]. Therefore, β_2 -adrenoceptor is regarded to work via both the stimulatory G protein Gs and the inhibitory G protein Gi, with the former dominating physiologically and the latter dominating pathologically (Fig. 2-2)^[100,108].

β_3 -adrenoceptor mainly functions in the adipose tissues, and also acts in the heart. Unlike β_1 -adrenoceptor and β_2 -adrenoceptor, the cardiac β_3 -adrenoceptor is lack of the PKA phosphorylation site and has fewer serine / threonine residues in the C - terminus tail^[123]. So, under the sustained adrenergic stimulations or high catecholamine levels, the β_3 -adrenoceptor may preserve the responses, whereas β_1 -adrenergic and β_2 -adrenergic responses may diminish them^[119,124]. In human, the cardiac β_3 -adrenergic stimulations were found to reduce the cardiac contractility and induce a negative inotropic response, but a positive chronotropic effect^[119,125,126]. However, this positive chronotropic effects *in vivo* by β_3 -adrenergic stimulation could be inhibited by β_1 -adrenoceptor and β_2 -adrenoceptor blockades, implying that these effects might be attributed to the baroreflex activation resulting from the vasodilation induced by β_3 -adrenergic stimulation^[127]. The concentration - dependent negative inotropic effects were observed under different β_3 -adrenergic stimulations in rabbit ventricular cardiomyocytes and rat Langendorff - perfused heart^[128-130]. Meanwhile, the β_3 -adrenergic stimulation was found to induce a negative lusitropic effect, and counteract the positive lusitropic effect by isoproterenol in rats^[130]. These suggest that the lusitropic effects by β_3 -adrenergic stimulation would counteract the excessive responses by β_1 -adrenergic and β_2 -adrenergic stimulation to keep a normal cardiac function. The cardiac effects of different β_3 -adrenergic stimulations are also variable among different tissues and species. The responses of contractility by β_3 -adrenergic stimulations are significantly different between atria and ventricles in rats, but not in human^[119,124]. Absence or very low effects of β_3 -adrenergic stimulations were observed in rats atria^[119]. In the neonatal rats cardiomyocytes, there are also lack of responses to β_3 -adrenergic stimulation^[131]. Almost no β_3 -adrenoceptor expression in heart was observed in rat myocardium^[132]. The attenuation of cardiac responses to the β_3 -adrenergic stimulations is suggested to involve the alterations in the excitation - contraction coupling and transmembrane ions channels activities^[119]. The post - receptor signaling pathway of β_3 -adrenoceptor in heart is not coupled to Gs protein, but the Gi protein (Fig. 2-3). In rodent adipocytes, β_3 -adrenoceptor is coupled to both Gs and Gi proteins^[133,134]. In human ventricular myocardium, the activation of Gi protein causes the activation of the nitric oxide (NO) pathway rather than the inhibition of adenylyl cyclase, suggesting that the β_3 -adrenoceptor - NO - guanylyl cyclase - cyclic guanosine monophosphate (cGMP) pathway may function as a negative feedback to the positive inotropic effects under the β -adrenergic stimulations in heart^[126,135]. And NO could also mediate the cardiac responses in a cGMP - independent way through the covalent modifications of the key proteins^[119]. Nevertheless, the

negative inotropy induced by the cardiac β_3 -adrenoceptor still remains subtle in healthy tissues^[136].

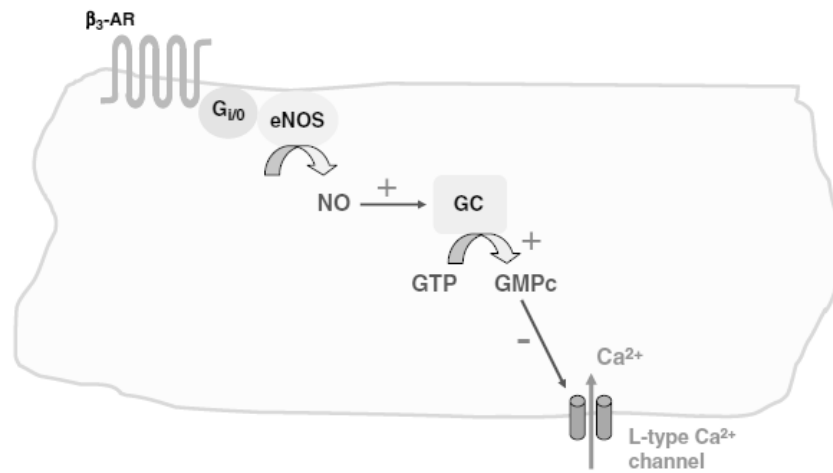


Fig. 2-3 Signaling pathway of β_3 -adrenoceptor in cardiomyocytes. NOS: nitric oxide synthase; NO: nitric oxide; GC: guanylate cyclase; cGMP: 3',5'-cyclic guanosine phosphate. Reproduced from Rozec et al. ^[119]

Additionally, a “putative β_4 -adrenoceptor”, identified as a low - affinity state of β_1 -adrenoceptor (β_{1L} -adrenoceptor), was found to exert positive inotropic effects and hastening relaxation through Gs protein - cAMP - PKA pathway under the stimulation of non - conventional partial agonist^[137-139]. These agonists, originally as β_1 -adrenoceptor and β_2 -adrenoceptor antagonists, induced the stimulant effects at a higher concentration than the blockade concentration to β_1 -adrenoceptor and β_2 -adrenoceptor^[137].

β -adrenoceptor genetic polymorphisms play important roles in cardiac function, with two functional important single nucleotide polymorphisms (SNPs) in β_1 -adrenoceptor gene: Ser49Gly and Arg389Gly; and three important SNPs in β_2 -adrenoceptor gene: Arg16Gly, Gln27Glu and Thr164Ile^[85]. Although still conflicting, it seems that they act as risk factors rather than disease causing genes in cardiovascular diseases, which could exert influences on the development and progress of disease.

1.2 The pathophysiological changes of β -adrenergic receptor signaling pathways in the heart of two common diseases

The state of aging or chronic heart failure (CHF) both facilitate sympathetic activity, which shows an increase of plasma norepinephrine / epinephrine levels but a diminished functional responses to β -adrenergic stimulations. The employment of β -blockers, including carvedilol,

metoprolol, bisoprolol and nebivolol, has shown beneficial effects on the survival and left ventricular remodeling in the chronic heart failure^[140,141]. It further confirms the state of enhanced adrenergic drive in the development of cardiac dysfunction. Although the exact mechanisms still remain unclear, functions of β -adrenoceptor signaling pathway might change as following under both diseased conditions: 1) deficiency of the excitation - contraction coupling mechanism; 2) down - regulation of β_1 -adrenoceptor, including reduced β_1 -adrenoceptor density; 3) changes in the β -adrenoceptor - G protein pathway, including uncoupling or reduced reaction to the Gs protein - adenylyl cyclase - cAMP pathway, and enhanced Gi protein; 4) an up - regulation of G protein-coupled receptor kinase (GRK) and β -adrenoceptor desensitization; 5) a decreased re-uptake of neuronal uptake transporter^[85,142].

As mentioned about Gi protein, at least in rats, stimulations to cardiac β -adrenoceptor can exert proapoptotic effects via β_1 -adrenoceptor - Gs protein pathway, and anti - apoptotic effects via β_2 -adrenoceptor - Gi protein pathway^[142,143]. Meanwhile, cardiac Gi protein might protect patients with chronic heart failure from catecholamine-related arrhythmias^[144,145]. However, the influences of Gi protein in patients with chronic heart failure are still under explorations.

In aging heart, the reduced responsiveness to β -adrenoceptor stimulation is associated with the deficiency in the β -adrenoceptor signaling pathways of both β_1 -adrenoceptor and β_2 -adrenoceptor, while the primary characteristics of excitation - contraction coupling is unchanged^[142]. Although conflicting results were observed in the selective reduction of myocardial β_1 -adrenoceptors and β_2 -adrenoceptor in aging heart, some research still confirmed non - selective reduced β -adrenoceptor density in aged rats ventricular myocytes and myocardium^[142,146]. In senescent rats, the down - regulation of β_1 -adrenoceptor and β_2 -adrenoceptor, and the up - regulation of β_3 -adrenoceptor have also been confirmed^[73,142]. This change is different from the condition in the failing heart, predominately down - regulation in β_1 -adrenoceptor but almost no change in β_2 -adrenoceptor density^[147-149]. Meanwhile, the age - related reduction in the cardiac adenylyl cyclase activity might also contribute to the decreased responses to agonists of both β -adrenoceptors. The sarcolemmal L - type calcium channel was same between ventricular myocytes of young and aged rats^[150]. Similar to the hypertrophic or failing heart, the reduced contractile responses to β_2 -adrenoceptor stimulations was observed due to the reduced amplification function of SR in the aged rats. Considering the changes of Gi protein, there were also different findings in rats, but some researchers still confirmed that age - related changes of β -adrenoceptor signaling system were not associated with the biochemical or functional alterations of pertussis toxin (PTX) -

sensitive G protein (Gi protein) ^[142,151-153]. This is also different from the changes in the failing heart, which demonstrated the elevated abundance and functional activity of Gi protein ^[154,155].

Additionally, β -adrenergic receptor kinase (a GRK), is up - regulated and can promote the binding of β -arrestins to the phosphorylated receptor in the failing hearts and hypertrophic hearts^[156-158]. This causes the uncoupling of β -adrenoceptor to Gs protein pathway and consequently the decreased responsiveness of β -adrenoceptor to the agonist stimulation^[85]. Actually, for most of the G protein coupled receptors, increased exposure to the stimulations by agonists would lead to a reduction in responsiveness of receptors, which is called desensitization. This desensitization might be initiated by the phosphorylation of GRK family to the activated receptors^[142]. Among which, GRK₂ is the most common isoform and crucial to myocardial function. However, the role of the GRKs is still not totally clarified in human heart. The major stimuli to activate GRK₂ are β -adrenoceptor stimulations. In fact, some animal and human experiments observed the decreases of GRK₂ activation under β -adrenoceptor blockades in failing heart^[89,159]. In animal models of heart failure, a C - terminal domain of GRK₂ (β ARKct) was found to inhibit GRK₂ activity, reduce cardiac hypertrophy and reduce the progressive worsening of myocardial function^[160,161]. However, in aging heart, no changes of GRK₂ and its activity were found^[142,162].

2 Obesity and diabetes mellitus related cardiomyopathy and the alterations in the β -adrenergic receptor signaling pathway

2.1 Obesity related cardiac dysfunctions and alterations in the β -adrenergic receptor signaling pathway

Obesity has been paid ever-increasing attentions not only for the obesity influences *per se*, but also for its prevalent co - morbidities, including diabetes mellitus and cardiovascular disease^[124,163]. In the obese human, there are cardiac hypertrophy, hyperkinetic circulation and cardiac dysfunction^[164-166]. Cardiovascular dysfunction in the clinically severe obesity was first reported in the obese volunteers through cardiac catheterization, who showed decreased left ventricular compliance and stroke work index, and increased LV end-diastolic pressure^[167]. These abnormalities are all correlated to the severity of obesity^[167]. And the correlations between obesity and left ventricular mass, as well as the correlations between body weight and diastolic dysfunction, have all been confirmed ^[168,169]. In the young healthy

obese women, the systolic function, diastolic function, and cardiac efficiency are all decreased^[170,171].

As in obese human, Zucker obese rats showed declined left ventricular functions^[172]. The contractile dysfunction has been observed in several studies in Zucker obese rats or obese rabbits^[43,54,173,174]. Zucker obese rats were shown to develop diastolic dysfunction with preserved left ventricular ejection fraction since 9 weeks of age^[175,176]. This is in accordance with the finding in isolated hearts of obese rabbits shown since a decrease in maximal rate of pressure rise (+ dp/dt) and the maximal rate of pressure decline (- dp/dt) in response to the stimulation of forskolin has been observed^[96]. In young Zucker obese rats (12 - 15 weeks of age), an increased LV end - systolic pressure^[81], or higher LV developed pressure has been observed^[177,178]. Some researchers observed unchanged LV fractional shortening, cardiac output, LV end - systolic pressure and LV end - systolic pressure - volume relation, along with increases in the LV end - diastolic pressure, LV relaxation constant Tau and LV end - diastolic pressure - volume relation in the Zucker obese rats at 24 weeks of age^[172]. These results demonstrate that the LV diastolic function is impaired but with relatively preserved systolic function during obesity. A recent study used hypercaloric - diet Wistar rats for 30 weeks which exhibit obesity, slight hyperglycemia, hyperinsulinemia and hyperleptinemia, but no differences in protein and lipid levels^[7]. Meanwhile, these obese Wistar rats showed no structural remodeling or changes in heart rate, systolic arterial blood pressure, and performances of LV papillary muscles after inotropic maneuvers (myocardial stiffness, postrest contraction, increase in extracellular calcium concentration, and change in heart rate). These results are similar with previous studies, including isolated hearts of obese rabbits with high - fat diet for 12 weeks and isolated cardiac myocytes of obese Sprague - Dauley rats with high - calorie diet for 14 weeks^[47,49], but different with diverse general characteristics and / or reduced myocardial mechanical functions in obese animals of other studies^[10,48,179-182]. Meanwhile, in the research of LV papillary muscles *in vitro*, there were also functional impairments in response to the progressively higher extracellular concentration of calcium and post-rest contraction stimulus, shown by the lower responses of peak developed tension and the lower maximum speed of the negative variation in developed tension (-dT/dt) in obese Wistar rats^[44]. In the obese hypertensive rabbits experiment, responses to β -adrenoceptor stimulation were decreased both in isolated heart and isolated papillary muscles^[43].

Cardiac remodeling has been proposed as an adaptive trait of obesity^[183]. It has been observed in many researches about obesity, especially in long - term obesity^[50,179,184,185]. It could be elicited directly by the increases in cardiac preload and afterload, and indirectly

through the cardio - metabolic changes related to obesity, including dyslipidemia, insulin resistance and diabetes^[186,187]. As a core abnormality of obesity, insulin resistance, along with hyperinsulinemia, could facilitate cardiac remodeling through the growth - promoting property of insulin, or through reduced anti - apoptotic signaling pathway (phosphatidylinositol 3 - kinase (PI3-K) / Akt pathway) evoked by the activation of insulin receptor^[188]. The cardiac remodeling in obesity may significantly influence the cardiovascular functions. Some animal experiments have shown a decreased or unaltered systolic function through echocardiography^[45,189,190]. One observation, in the long - term high - fat diet induced obese Wistar rats, showed elevated systolic function, including the increased endocardial and midwall fractional shortenings and posterior wall shortening velocity (PWSV) ^[179]. One explanation of these findings was the decreased afterload and left ventricular contractility improvement. The diastolic dysfunction in obesity could be found as a higher A wave and a lower E/A ratio using echocardiography, and the prolongation of isovolumic relaxation time (IVRT), which directly shows an impairment of cardiac filling^[191-196]. Diastolic filling includes the active relaxation associated with calcium handling, and the passive properties of myocardium related to the viscoelastic characteristics^[97,197-200]. The diastolic dysfunction might be attributed to the metabolic abnormalities (insulin resistance, hyperinsulinemia and hyperglycemia), hemodynamic changes, and / or calcium handling homeostasis in obesity^[201,202]. However, a diet with moderate fat in the obesity - prone Sprague - Dawley rats was only found to induce higher body weight, body fat gain, serum leptin and cholesterol^[45]. In these obesity - prone rats, no differences were observed in the arterial blood pressure, left ventricular systolic or diastolic function.

The basic mechanisms resulting in the cardiac dysfunction in obesity are still under debate, including a changed β -adrenergic signaling pathway. At first, researchers found that reduced β -adrenoceptor density was associated with the impaired contractile responses^[54]. This early experiment in Zucker obese rats aged 22 weeks showed that the impairment in response to β -adrenoceptor stimulation was associated with both reduction in the number of β -adrenoceptors and changed coupling between β -adrenoceptor and Gs protein in heart tissue membranes. Later, conflicting results were published, suggesting that the impaired inotropic responses in obesity occurred with unchanged β -adrenoceptor expression^[203,204]. Experiments in obese rabbits did not demonstrate differences in β -adrenoceptor density or affinity^[204]. In the cardiomyocytes of *ob/ob* mice, the abundance of β_1 -adrenoceptor and β_2 -adrenoceptor was not altered^[203]. In obese hypertensive rats experiments, a decreased response to β -adrenoceptor stimulation was observed together with a decreases in β -adrenoceptor number

and affinity^[174]. The decrease in β -adrenoceptor number might also be attributed to slight hypothyroidism in Zucker obese rats, since the thyroid hormone could elevate the β -adrenoceptor levels^[205]. Responses to β -adrenoceptor stimulation was reduced both in isolated heart and LV papillary muscles in the obese hypertensive rabbits^[43], and the possible defect site may lie in post- β -adrenoceptor signaling pathways^[96].

The post- β -adrenoceptor signaling pathways include series of components and cascade reactions. The switching of Gs and Gi proteins mediated the isoproterenol - induced cardiac hypertrophy in neonatal rats^[206]. In the heart of *ob/ob* mice, Gs protein and Gi protein levels were both reduced, among which only Gs α protein (52kDa) could be restored after leptin replenishments. It has been supported that, in the G proteins stimulating adenylyl cyclase, the long splice Gs α protein - 52kDa showed greater potency and more basic activity than the short splice Gs α protein - 45kDa^[207]. The activity of adenylyl cyclase of the cardiomyocytes in obesity shows different findings. The activity of adenylyl cyclase in the heart of diet - induced obese rabbit or *ob/ob* mice was unaltered^[203,204]. The findings in the *ob/ob* mice (10 weeks of age) demonstrated significantly impaired cardiac β -adrenergic inotropic responses as depressed responses to forskolin and dibutyl cAMP, whereas preserved adenylyl cyclase activity and reduced PKA activity were observed^[203]. Further, experiments in obese rabbits did not demonstrate differences in the adenylyl cyclase activity or coupling of the β -adrenoceptor to adenylyl cyclase activation^[204]. At the same time, the decreased response to β -adrenoceptor stimulation was also observed to be associated with reduction of cAMP production and SR calcium uptake in the obese hypertensive rats^[174]. The cardiac adenylyl cyclase activity of Zucker obese rats under the stimulation to the β -adrenoceptors exhibited a marked age - dependent reduction^[205]. One research^[174], using ventricular myocytes of female spontaneous hypertension-heart failure(SHHF)/*Mcc-cp* and JCR:LA-*cp* rats, found that obesity *per se* would not influence the accumulation of calcium in SR, but could reduce the cAMP production. Nevertheless, if obesity is associated to hypertension, SR calcium accumulation and cAMP production would both be depressed. PKA is very important in the regulation to the downstream of cAMP mediated phosphorylations in the β -adrenergic signaling pathway^[82], including the phosphorylation of PLB, which is pivotal for removing the inhibition of PLB on SERCA2a, allowing increases in calcium re-uptake, and consequently increased contractility. The PKA activity in the *ob/ob* mice was depressed, and the production of phospho - phospholamban (P-PLB) was reduced^[203]. Meanwhile, in the *ob/ob* mice, SR calcium stores were depressed even under the seemingly compensatory up - regulated SERCA2a expressions^[203].

Obesity related cardiac dysfunction is also related to the alterations in the intracellular calcium handling. Several studies in obese Wistar rats induced by hypercaloric diet for 15 weeks, showed cardiac dysfunction^[44,51]. This dysfunction suggested that obesity might impair the regulatory calcium channels in the myocardium, especially the sodium / calcium exchanger, L-type calcium channels of the sarcolemma, SR and myofilaments sensitivity to calcium^[44,163]. Besides, the decreased maximum speed of the positive variation in developed tension (+dT/dt) under the β -adrenergic stimulation was observed with a decreased phosphorylation of phospholamban via calcium - CaMK in obese Wistar rats^[44]. In high - calorie diet induced obese Sprague - Dawley rats, there was also a cardiac contractile depression due to the reduced phosphorylation of phospholamban, even though the increases of proteins expression in SERCA2a and phospholamban were found^[50]. Meanwhile, the unchanged proteins expression of SERCA2a, RyR2 and phospholamban was observed in non - obese rats with high - calorie diet^[48], which further confirmed the influences of obesity, not just high fat dietary, on the calcium related proteins expression. In hypercaloric - diet Wistar rats (for 30 weeks), no changes of responses under the inotropic stimulations was noted^[7]. The possible reasons for non - significant inotropic stimulation effects in those long - term obesity rats might be the unaffected intracellular calcium - cycling related proteins levels (L - type calcium channel, SERCA2a, calsequestrin), and further the preserved intracellular calcium entry and resequestration even when the down - regulation of phospholamban phosphorylation at Ser16 was observed^[179]. The cardiac β -adrenergic inotropic responses were impaired in the *ob/ob* mice at 10 weeks of age^[203]. This isolated cardiac myocytes model showed reduced sarcomere shortening and calcium transient under the stimulation of isoproterenol, and decreased calcium stores in SR in the obese mice. They also observed decreased expressions of G α protein, G α proteins and P-PLB, and increased expressions of SERCA2a. After replenishment of leptin to the cardiac myocytes, the inotropic responses, PKA activity, levels of P-PLB and SR calcium stores could be restored back to the control level^[203]. These results suggest that the role of PKA activity on the SR calcium cycling dysfunction, and the leptin - deficiency and resistance might be the cause of cardiac dysfunction in obesity. In the obese Wistar rats, the up - regulation of the gene expression of proteins in the left ventricles associated with calcium transport, SERCA2a, ryanodine receptor (RyR2) and phospholamban, were observed^[51]. While the gene expression of proteins related to sarcolemmal calcium transport, L-type calcium channel (*Cacna1c*) and sarcolemmal sodium / calcium exchanger, were unchanged. In the hypertrophied rat heart with pressure - overload, diastolic dysfunction is an early abnormality associated with the reduced

transcription of SERCA2 gene^[208]. Those results implied that the cardiac dysfunction in obesity is associated with changes in calcium transport related genes in the SR. Meanwhile, a pathological cardiac hypertrophy with pressure and volume overload presents a myosin isoform shift from V₁ (α - α dimer) type to V₃ (β - β dimer)^[209].

2.2 Diabetic related cardiac dysfunctions and alterations in the β -adrenergic receptor signaling pathway

The term “diabetic cardiomyopathy” was first defined as ventricular dysfunction which occurs in diabetic patients in absence of coronary artery disease and hypertension^[210,211]. Now it is characterized by diastolic dysfunction, with a high prevalence of 60%, even in the well - controlled type 2 diabetic patients^[212,213]. Diastolic dysfunction is the inability to relax and undergo appropriate filling during the diastolic phase of cardiac cycle. It is usually subclinical, but may result in diastolic heart failure in the presence of near - normal systolic function^[214]. The pathophysiological changes of diabetic cardiomyopathy has not been fully understood, and some factors, including metabolic disturbances, structural changes, autonomic dysfunctions, and changes in calcium handling have been proposed for the developments of diabetic cardiomyopathy^[61,63,215]. The pathological processes in the diastolic dysfunction include myocardium stiffening due to cross - linking and extracellular matrix deposition, hypertrophy, and neuronal abnormalities^[216]. In the clinical studies, the diastolic and systolic dysfunctions in the type 2 diabetic patients have been identified through Doppler imaging, echocardiography, radionuclide angiography and other techniques^[217]. These abnormalities include decreased LV ejection fraction, decreased myocardial velocity at early diastole, abnormal relaxation during the early filling phase, prolonged isovolumic relaxation, low peak systolic and early diastolic velocity, impaired diastolic relaxation and filling, and reduced peak filling rate depending on the age, time course and the severity of diabetes mellitus^[218-221].

The mechanisms responsible for cardiac dysfunctions in type 1 diabetes mellitus are still unclarified, and several factors have been evoked, including decreased myocardial adrenoceptor density, alterations in contractile proteins, or impaired calcium cellular movements^[222]. Streptozotocin (STZ) - induced type 1 diabetes models were observed to develop the systolic and diastolic dysfunctions as the duration of diabetes increased. From the echocardiography observations, researchers confirmed reduced rate of circumferential shortening and fractional shortening^[223,224]. In the analyses from LV catheterization, the decreased LV systolic pressure and rate of pressure rise or fall during systole and diastole (\pm dP/dt) were confirmed^[225,226]. However, in our laboratory, previous study found a preserved

systolic function in STZ induced diabetic rats as shown by the unaltered LV ejection fraction and fractional shortening during echocardiography^[55,56]. Diastolic dysfunction can be observed in many kinds of type 1 diabetic animals^[227], as shown by the prolongation of isovolumic relaxation time, increased E / A ratio, E / Ea ratio, and LV end-diastolic pressure in diabetic rats^[55,56,228]. The cardiac dysfunction observed in the STZ - induced type 1 diabetic rats has been regarded to be associated with an increased myocardial activity of inducible nitric oxide synthase (iNOS), iNOS protein levels and expression of iNOS mRNA levels^[55,222].

In the type 2 diabetic animals, cardiac dysfunction also shows diverse performances^[229-231]. The *ob/ob* mice exhibit cardiac hypertrophy with mild or no impairments in systolic function^[232]. The *db/db* (C57BL/Ks) mice exhibit cardiac hypertrophy, and marked contractile dysfunction^[233]. In *db/db* mice (18 weeks of age), which exhibit severe stage of diabetes, left ventricular systolic and diastolic functions were both preserved^[16]. Some studies of the Goto - Kakizaki (GK) rats also demonstrated cardiac dysfunctions, including decreased heart rate and LV ejection fraction, and the prolongation of contraction /relaxation^[234,235]. Nevertheless, a previous study performed in the Goto - Kakizaki rats and using isolated heart and the isolated cardiomyocytes, showed no significant differences compared to the controls^[236].

The cardiac function observed in Zucker diabetic (type 2) fatty rats shows highly different results^[81,237-241]. Basically, the characteristics and severity of cardiac dysfunction is age related. In the Zucker obese diabetic rats aged 10 weeks, researcher found the increased LV end - diastolic volume and end - systolic volume, reduced LV ejection fraction, and reduced velocity of circumferential shortening have been noted^[81]. In the young Zucker obese diabetic rats (14 weeks of age), LV dysfunction has been observed with increased end - diastolic and end - systolic volume, increased end - systolic wall stress, decreased LV ejection fraction and decreased velocity of circumferential shortening using echocardiography^[81]. In Zucker obese diabetic rats of 19 - 20 weeks of age, either impaired, improved, or unchanged baseline cardiac functions has been observed using echocardiography or left ventricular catheterization monitor^[222,241-243]. One research performed in aged Zucker obese diabetic rats (45 weeks of age), using LV magnetic resonance imaging and invasive catheterization, showed only slight impairment of LV diastolic function, whereas LV systolic function was preserved^[244]. Meanwhile, no cardiomyocyte hypertrophy was found in these aged Zucker obese diabetic rats. This result was consistent with the unchanged cardiomyocyte cross - sectional area, and unaltered mRNA expressions of hypertrophic markers brain natriuretic peptide (BNP) and α skeletal actin (α SKA)^[244]. This relatively slight cardiac dysfunction markedly contrasts with the severity of diabetic status in these aged Zucker obese diabetic rats. At the

same time, right ventricular (RV) dysfunction in Zucker obese diabetic rats was also studied, which was related to the systematic insulin sensitivity and the decreased insulin - stimulated glucose utilization in right ventricle^[245]. This is consistent with the findings of impaired RV systolic function in type 2 diabetic patients^[246,247]. The remodeling of ventricles in Zucker obese diabetic rats at 14 weeks of age showed left ventricular hypertrophy without ventricular dilation, whereas, in the right ventricle, dilation without hypertrophy was noted ^[245,248]. The changes in both ventricles and the systolic functions might result from ventricular interdependence and diabetes influences. The myocardial activity of iNOS, iNOS protein levels and nitrotyrosine (a biomarker for oxidative damage) were observed to be higher in the Zucker obese diabetic rats at 20 weeks of age, which could lead to the reduced response to β -adrenoceptor stimulation and decreased contractile function^[222]. This result suggests the role of iNOS in the pathophysiological developments of type 2 diabetes.

The basic mechanisms resulting in the diabetic cardiomyopathy are still under research, including the alterations in the β -adrenergic signaling pathway. In the diabetic patient, a blunt inotropic response was found under the stimulation of dobutamine, suggesting an impairment in the cardiac β -adrenergic pathway^[249]. These authors also confirmed the down - regulation of β_1 -adrenoceptor in diabetes mellitus^[250,251]. Some previous studies about diabetic rats have reported the markedly decreased positive inotropic effects of β -adrenoceptor stimulations *in vivo* and *in vitro* ^[55,252-256]. In Zucker obese rats associated with diabetes, β -adrenoceptor density and affinity did not change at 6 weeks of age or 10 weeks of age compared with age-matched Wistar rats, but a significant higher β -adrenoceptor affinity was observed at 20 weeks of age^[257]. A few studies suggested that the diabetic - related altered positive inotropic effects were associated with the down - regulation of β_1 -adrenoceptor, β_2 -adrenoceptor, and the up - regulation of β_3 -adrenoceptor^[252,258]. In the hearts of chronic diabetic rats, the density of β_1 -adrenoceptor and β_3 -adrenoceptor were decreased and increased respectively, as in heart failure^[259]. In our laboratory, previous studies have found a decreased expression of β_1 -adrenoceptor proteins, and an increased expression of β_3 -adrenoceptor proteins in type 1 diabetic rats^[55,56]. The *db/db* diabetic mice showed no significant changes of β_1 -adrenergic receptor^[16]. While some research showed the insignificant role of the cardiac β_2 -adrenoceptor in diabetic rats^[55,259]. In our previous studies of diabetic rats, our team already found that the diastolic dysfunction developed in early and evolved diabetes *in vivo*, whereas the positive lusitropic effects of β -adrenoceptor stimulation were preserved *in vitro*^[55,56,260,261]. β_3 -adrenoceptor is involved in this impaired positive inotropic response to β -adrenergic stimulations via the endothelial NOS₁ - derived NO pathway, and the positive lusitropic

effects were preserved in spite of the effects of β_3 -adrenoceptor in the early and evolved diabetic cardiomyopathy of rats *in vivo* and *in vitro*^[55,56]. These results suggest that the influence of β_3 -adrenoceptor on the cardiac dysfunction may vary in different stage of diabetes. At the early stage, the activation of β_3 -adrenoceptor may convey a protective effect against the catecholamine - induced remodeling^[124].

The post- β -adrenoceptor signaling pathways in diabetic cardiomyopathy also include a variety of components and interactions. The dual coupling of β_2 -adrenoceptor to Gs and Gi proteins can also be found in the isolated cardiomyocytes from STZ - induced type 1 diabetic rats^[262]. The early research about heart tissue membranes of two genetic obese diabetic animals (7-12 weeks of age), male obese *db/db* diabetic mice and obese diabetic CBA/Ca mice, showed no differences in the response to β -adrenergic stimulation, or adenylyl cyclase activities compared to their controls respectively^[54]. Meanwhile, this study did not observe any alteration of Gs protein levels or the functional reactions between Gs protein and catalytic unit of adenylyl cyclase. In the Zucker obese diabetic rats, adenylyl cyclase activity was not elevated with age, and no significant differences were observed in unstimulated or maximum forskolin stimulated activities of adenylyl cyclase between the two strains of rats^[257]. This result suggests that, although the adenylyl cyclase activity had the ability to be maximally stimulated in obese diabetic rats *in vitro*, the attenuation of enzyme activity still exists compared with control Wistar rats^[257]. Furthermore, the basal cAMP levels reduced progressively with age in obese diabetic rats, and were significantly lower at 20 weeks of age compared with control Wistar rats. However, cAMP production in response to isoproterenol in obese diabetic rats increased higher than in control rats, which suggests a defective regulation of cAMP- phosphodiesterase in obese diabetic rats^[257]. However, it has been known that the increase contractile response is not just determined by the high level of cAMP^[82]. As the comparison between healthy Wistar rats and STZ - induced Wistar rats, a down-regulation of β -adrenoceptor population and reduced cAMP response to β -adrenoceptor stimulation were observed^[257,263,264]. One *db/db* diabetic mice study showed no significant changes in the gene expression of the SERCA2a, or PKA - mediated target proteins, whereas a marked alteration in cardiac metabolic related gene expressions was observed^[16]. This research shows the role of metabolic changes involving the energy conversion in diabetic cardiomyopathy, especially when cardiac workload is increased. The reduced cardiac performance, whereas increased PKA mediated phosphorylation and the endogenous PKA activity, were observed in the STZ-induced diabetic rats^[265].

Diabetic cardiomyopathy associated with reduced contractility and impaired relaxation, results from the changes in intracellular calcium handling, including decreased expression and activity of SERCA2a, reduced NCX expression, decreased calcium release and sequestration in SR, and compromised mitochondrial calcium cycling^[227,266]. There are several conflicting researches about the SERCA2a or phospholamban of diabetic rats. In previous study, our team found a decreased expression of SERCA2a and increased expression of phospholamban in STZ - induced diabetic rats at 4 weeks or 12 weeks of age, which was associated with myocardial relaxation dysfunction *in vivo* and *in vitro*, whereas the positive lusitropic effects to the β -adrenoceptor stimulation was preserved^[56]. The reduced cardiac performance, decreased SR cycling proteins (including ryanodine receptor, SERCA2a and phospholamban), and the depressed calcium uptake and release in SR, were observed in the STZ-induced diabetic rats^[265]. Further insulin treatment could improve cardiac performance, SR calcium cycling proteins contents and calcium uptake / release in SR, which suggests the role of the reduced content of calcium cycling proteins in the SR dysfunction of diabetic heart. In a sucrose (SU) - fed insulin resistance rats model, normal SERCA2a protein content but impaired SERCA activity were observed^[267]. Further, the slow ventricular diastolic rate and reduced SERCA2a level were observed in *Otsuka - Long- Evans Tokushima Fatty* (OLETF) rats^[268,269]. One previous research in the *ob/ob* mice showed deficiency in calcium signaling, including decreased SR calcium stores and increased SERCA^[203]. The cardiomyocytes of *ob/ob* mice have increased intracellular resting calcium concentrations, prolonged intracellular calcium decay, reduced responsiveness to extracellular calcium, and reduced SERCA2a activities^[270]. The cardiac calcium handling in *db/db* mice exhibited that calcium transient, L - type calcium current and SR calcium load were all reduced^[233]. One research found that SERCA2a mRNA expression was not altered in Zucker obese diabetic rats^[231]. In young Zucker obese diabetic rats at aged of 9-13 weeks, longer time to maximum contraction and relaxation in the cardiomyocytes in spite of the normal calcium flow amplitude were observed, suggesting the reduction in the electric current density through L-type calcium channels which was related to the changes of genes expression in the synthesis of myosin heavy chain, L-type calcium channel, intracellular calcium transport and regulation proteins^[271]. This research also found that down - regulation of gene *Atp2a2* (encoding SERCA2) was accompanied with the preserved SR calcium transport^[271]. However, a study of Zucker obese diabetic rats at 19 weeks of age showed an increase of SERCA levels and decreased phospholamban levels. Furthermore, in the aged Zucker obese diabetic rats (30-34 weeks), researchers found unchanged amplitude and duration of myocyte shortening and

relaxation, and prolonged duration of intracellular calcium transient^[217]. This might be the consequence of the decreased L - type calcium current, even when the calcium content and transport in SR was unchanged in the Zucker obese diabetic rats. Meanwhile, the preserved contractility of cardiomyocytes might be associated with changing pattern in cardiac genes expressions, which encode calcium channel proteins, sodium / calcium exchanger protein, SR calcium transport proteins, cardiac muscle proteins and cardiac muscle contraction regulation proteins. A significant 50% reduction in the gene expression of SERCA2a was observed in Zucker obese diabetic rats at 45 weeks of age, which was in line with the diastolic dysfunction observed in severe diabetes^[244]. Despite the different strains and models of animals, these results suggest the subtle changes in calcium regulation might exist prior to the marked ventricular dysfunction and /or heart failure, and be common in many disorders involving insulin resistance^[272]. And in diabetes, the synthesis of β -myosin heavy chain increases and the contraction is slower^[209].

Because changes in contraction phase can correspondingly induce changes in relaxation phase, variations in contraction and relaxation need to be considered simultaneously to evaluate the changes in lusitropy under the β -adrenergic stimulation, which is named as contraction-relaxation coupling. Like our previous experiments about the LV papillary muscles, the coefficient R_1 (ratio of maximum shortening velocity and maximum lengthening velocity, $_{\max}Vc/_{\max}Vr$) and the coefficient R_2 (ratio of the peak of the positive force derivative at L_{\max} normalized by cross-sectional area and the peak of the negative force derivative at L_{\max} normalized by cross-sectional area, $+dF.dt_{\max}^{-1}.s^{-1} / -dF.dt_{\max}^{-1}.s^{-1}$) were used to measure contraction - relaxation coupling^[55,56,73,253,273,274]. R_1 reflects the contraction - relaxation coupling and thus lusitropy under low load, which tests sarcoplasmic reticulum uptake calcium function in rat myocardium. R_2 reflects the contraction - relaxation coupling and thus lusitropy under high load, which indirectly reflects the myofilament calcium sensitivity^[275-277]. A decrease in R_1 or R_2 demonstrates a positive lusitropic effect. In diabetic rats, R_1 was preserved in response to β -adrenergic stimulation under isotonic condition because of the significant prolonging duration of contraction^[56,253,274]. Under low load, the SR plays a major role in the regulation of relaxation. So the preservation of R_1 under β -adrenergic stimulation suggested the preservation of SR function of heart in diabetic rats. R_2 did not change in response to β -adrenergic stimulation under isometric condition, suggesting the lack of alterations in myofilament calcium sensitivity in diabetic rats, compared with control rats^[56,274]. It was also confirmed that myofilament sensitivity to calcium was unaltered in Zucker obese diabetic rats at ages of 9 to 13 weeks^[271]. And further, a previous study about

type 2 diabetic Goto - Kakizaki rats at ages of 18 months suggested the alteration of myofilament calcium sensitivity during the later stages of diabetes^[234].

3 Animal models of obesity, diabetes mellitus and metabolic syndrome

Metabolic syndrome is a group of health conditions caused by genetic and environmental factors. The main pathophysiological change of metabolic syndrome is insulin resistance, which can induce many syndromes ultimately related to the cardiovascular disease and coronary disease^[278]. For the clinical diagnosis of the metabolic syndrome, the U.S. National Health Institute made the definition as the following three or more risk factors: abdominal obesity, high triglyceride, low high density lipoprotein (HDL), high fasting glucose level and hypertension^[279]. It is a challenge to find a completely adequate animal model of metabolic syndrome to represent the different kinds of syndromes in patients with metabolic syndrome. Meanwhile, using animal models for the research about cardiomyopathy, there still exist some questions. For example, the length of cardiac cycle in mice is only one tenth of that in human, so differences certainly exist in some expression of ion channel and contractile protein isoforms^[227]. However, there are still some strains of animals, especially rats, exhibiting the characteristics similar to most of metabolic syndrome patients^[278]. Rodent animal models of obesity, insulin resistance and type 2 diabetes mellitus have been identified to exhibit LV hypertrophy, diastolic dysfunction, increased cardiac fatty acid uptake and utilization, reduced cardiac efficiency, impaired mitochondrial energetic, increased myocardial lipid storage, and impaired calcium handling^[63,280,281]. These animals can be used to evaluate the pathophysiological changes, medications intervention and lifestyle treatments within the metabolic syndrome.

The most reliable rat strain to study metabolic syndrome seems to be Zucker obese rats, which can also be used as an obesity animal model. Some other different experimental models can be used to study metabolic syndrome for their similar abnormalities aforementioned, mainly including rats derived from spontaneous hypertensive rats (SHR)^[278]. Meanwhile, some mice models, such as *Psammomys obesus*, leptin deficient mice (*ob/ob*), and apoE - deficient mice, could be studied for metabolic syndrome^[278]. Because of the changing lifestyle and increased intake of energy, high - fat / calorie induced obese animal models have also been used as an appropriate model to study obesity and obesity - related diseases for decades, which could reproduce the molecular, biochemical, morphological and functional alterations closely remodeling human^[45,282-284].

3.1 Zucker rats strains

3.1.1 Zucker obese rats

In 1961, L.M. Zucker and T.F. Zucker reported an autosomal recessive mutation in fatty (*fa*) gene, which was shown later to be the leptin receptor gene, on chromosome 5 in rats [76,285]. This *fa* mutation was found in a cross between Merck M strain and Sherman rats [76]. Zucker obese rats are homozygous for *fa* allele. The presentation in these animals is a mutation in the leptin - receptor [286-288]. Leptin, generated by adipose tissue, can interact with leptin - receptors in the brain, leading to the decrease in food intake and increase in energy consumption [289-295]. The result of the leptin - receptor deficiency in Zucker obese rats is the elevation of leptin level in the circulation [296,297]. This character, together with the increase in some classical orexigenic peptides, such as neuropeptide Y, galanin, orexins and melanin - concentrating hormones, determines obesity in Zucker obese rats [298-301].

Zucker obese rats show hyperphagia, deficient non - shivering thermogenesis and preferential deposition of energy in the adipose tissues since 17-day age [286,302]. These could influence the body weight and the proportions of body lipids in rats [79,303,304]. At the same time, some endocrinological abnormalities originated from insulin resistance can be observed as early as 3 to 4-week age in Zucker obese rats, which are dyslipidemia, mild hyperglycemia and hyperinsulinemia [79,303-310]. At 4 weeks of age, plasma insulin levels of Zucker obese rats are 4.6 times higher than that in Zucker lean rats [205]. But the plasma insulin levels will return to normal when this strain of rats live to 30 weeks of age [311]. Zucker obese rat expresses obesity with subcutaneous fat accumulation early from 5 weeks of age on [285].

One of the primary abnormalities attributed to *fa* gene is the increase of the activity of an enzyme, adipose tissue lipoprotein lipase. The change of its activity emerges and affects lipid filling of the adipocytes since 12 days age, long before the visual obesity in Zucker obese rats [312,313]. Their plasma fatty acid and cholesterol levels can be ten and four times more than control animals respectively [278]. Zucker obese rats show the high low-density lipoprotein (LDL) -cholesterol and HDL-cholesterol, with the latter to be a marker of good prognosis in cardiovascular disease [177]. Moreover, the plasma cholesterol levels show different classifications between male and female obese rats [314]. For the plasma glucose, Zucker obese rats only show normal or just slightly higher levels, even though some research found the vascular alterations like diabetes mellitus in rats [315]. The hypertensive state in Zucker obese rats still shows conflicting results [316-327]. Hypertension could be observed since 24 weeks age in Zucker obese rats [319]. The reason of the increased arterial blood pressure in Zucker obese rats is not the increased renal Na⁺ retention, but the elevations of the proportion of adipose

tissues, which could result in the increased production of angiotension II and reactive oxygen species^[319,328-330]. Those could promote hypertension and endothelial dysfunction.

Obesity and obesity co - morbidities may also be attributed to oxidative stress and inflammatory responses. Several factors can cause oxidative stress, such as hyperglycemia, hyperleptinemia, increased tissue lipid levels, deficiency of antioxidant defense, increased formation of free radical and chronic systematic inflammations^[331]. The analysis about plasma markers of oxidative stress did not show that Zucker obese rats at 13 weeks of age are more exposed to radical species, only the coronary vessels produced higher levels of superoxide anion^[177]. Zucker obese rats develop oxidative stress, as the oxidized lipids increased in serum, urine and liver since 14 weeks of age^[332-334]. The plasma antioxidant defense factors, such as glutathione peroxidase, was damaged^[333,335].

3.1.2 Zucker obese diabetic (Zucker diabetic fatty) rats

A phenotype of Zucker obese rats with marked diabetes was found and designated as Zucker obese diabetic rats^[285]. This strain of rats is an inbred strain generated from the outbred Zucker obese rats. Zucker obese diabetic rats develop obesity, insulin resistance, hyperinsulinemia, hyperglycemia, hyperleptinemia, and hyperlipidemia. In the Zucker obese diabetic rats, obesity could be observed since 3 weeks of age and develop severely by 5 weeks of age^[295]. Zucker obese diabetic rats demonstrate a higher body weight compared to the controls when they are young (9-13 weeks of age)^[271]. But the research about aged Zucker obese diabetic rats at 30-34 weeks of age showed the similar body weight as the controls^[217]. This is in line with the development in the diabetic patients, that is, with the age and severity of diabetes progressing and the complications worsening, diabetic individual would become more reliant on the utilization of lipids and lipid reserves to meet the metabolic requirements^[217]. Meanwhile, the female Zucker obese diabetic rats are less prone to develop metabolic syndrome.^[336,337] Although having high insulin level, Zucker obese diabetic rats have been reported to become diabetics after 10 weeks of age^[175,257]. The pancreatic β -cell mass in Zucker obese diabetic rats increases from 6 weeks to 16 weeks of age, and begin to decline thereafter because of apoptosis. So plasma insulin levels increase rapidly from 6 weeks to 8 weeks of age, then decline thereafter that to the levels similar to that in 6 weeks of age^[338,339]. Zucker obese diabetic rats have a limit life span, and the mortality rises after 50 weeks of age^[340,341]. The exact cause of the death is not clearly understood, but it has been observed that the increased mortality is associated with the increased blood urea nitrogen and renal insufficiency^[340].

Although the model of type 2 diabetes mellitus has many similar features as in human type 2 diabetes mellitus, the presence of the leptin receptor mutation is not common in human. However, this genetic animals is considered as a reliable model of metabolic syndrome for the similarity to the development and maintenance of metabolic syndrome in human^[175]. Rats developing obesity with or without the onset of type 2 diabetes have insulin resistance, which was observed in the two Zucker rat strains. Insulin resistance is associated with increased adrenergic activity, either as a cause of the increase or the consequence^[82]. The increased sympathetic activity causes an increase in afterload and heart rate. And because of the greater blood volume in obese rats, the preload is also increased. Thus the cardiac output tends to increase in obese rats and obese diabetic rats. At the same time, Zucker obese diabetic rats do not develop atherosclerosis and hypertension, which are usually present in human type 2 diabetes mellitus^[244]. Thus, the strain of Zucker obese diabetic rats is also a good animal model of diabetic cardiomyopathy without other co - morbidities.

3.2 Obese spontaneous hypertensive rats

Spontaneous hypertensive (SHR) rats, a good model to study hypertension or insulin resistance, also show hypertriglyceridemia, obesity, and hypertension^[342,343]. Another different strains of SHR rats, obese SHR rats, seems to be a better model of metabolic syndrome, such as Koletsky rats, SHR/N-corpulent rats and SHR/NDmc- corpulent rats.

3.2.1 Koletsky rats

Koletsky rats can also be named as obese SHR or SHROB rats. This strain of rats has monogenetic obesity superimposed on hypertensive genetic background^[317,344,345]. In this strain of rats, the corpulent (*cp*) phenotype was first recognized from an autosomal recessive mutation on the *Lepr* gene^[317]. The mutation is a Thr2349Ala transversion, causing a premature stop codon in leptin - receptors of extracellular domain. The obese SHR has two *cp* alleles and shows leptin resistance. No matter what kind of diet, these animals show hyperphagia, hyperlipidemia (significantly high triglyceridemia and moderate elevation of cholesterol), hyperinsulinemia, hypertension, premature vascular disease and nephropathy^[295]. Meanwhile, since the intake of calorie had a link with sympathetic activity, refeeding after dietary restriction to obese SHR exhibited another animal model similar to essential hypertension in obese human, which is refeeding obese SHR^[316]. This strain of rats also develops increased sympathetic activity, hyperinsulinemia, insulin resistance, increase in the ventricular wall thickness and kidney diseases. They could be used to study the effects of fluctuations in the nutrition intake on the obese hypertension.

3.2.2 N-corpulent rats

These rats are a substrain of Koletsky rats, characterized as non - insulin - dependent diabetes mellitus^[295,346]. Obesity in this strain of rats is evident by 5-6 weeks of age^[347,348]. These animals show hyperinsulinemia, hypercholesterolemia, hyperglycemia after oral glucose load or postprandially, glycosuria and proteinuria. They develop impaired glucose regulation from 2 months of age, and the glucose tolerance improves with aging^[349,350]. The hyperinsulinemic levels in this strain of rats develop from 4 weeks of age, and reach 6 times higher than controls in 5 months of age^[347,351]. However, there exist sex differences in the nature of the strain of rats. Female SHR / N-*cp* rats are less obese than males, but serum triglyceride levels are higher than male rats, which might be associated with increased hepatic lipogenic enzyme activities^[351].

3.2.3 NDmc-corpulent rats

These rats are an inbred subline of SHR/N-corpulent rats and homozygous of the *cp* gene (*cp/cp*). These animals exhibit hyperphagia, obesity, hypertension, hyperlipidemia, insulin resistance and hyperinsulinemia, and diabetes mellitus^[352,353].

3.2.4 Spontaneous hypertension - heart failure (SHHF) / Mcc - *cp* rats

SHHF / Mcc - *cp* rats carry the recessive *cp* or corpulent gene and develop obesity and the spontaneous hypertension, consequently causing cardiac dysfunction and heart failure^[174]. There exist sex differences in this strain of rats. Obese male rats develop obesity, hypertension and non - insulin - dependent - diabetes mellitus, and emerge dilated cardiomyopathy since 10 to 12 months of age. Male rats would finally evolve to heart failure after 3 years old. However, obese female rats only have slight glucose tolerance abnormality and develop fatal dilated cardiomyopathy between 14 and 16 months of age^[174,354].

3.2.5 JCR: LA - *cp* rats

This strain of rats carry *cp* gene^[174]. They develop obesity, dyslipidemia, insulin resistance, which are much more extreme than Zucker rats^[355]. This strain of rats develop slight hyperinsulinemia at 3 weeks of age, and rapidly progress to an evident level much more severe than Zucker rats after 5 weeks of age, in which the insulin levels are higher in male rats than that in females^[356,357]. At 12 weeks of age, they develop so severe insulin resistance that no insulin - mediated glucose uptake by skeletal muscle occurs. Meanwhile, they do not

appear hyperglycemia due to islet β -cell insulin hypersecretion, so the major drawback of this rat as type 2 diabetes model is the absence of fasting hyperglycemia^[357]. Elevation of triglyceride levels can be observed from 4 weeks of age exclusively attributed to the increased hepatic very low-density lipoprotein (VLDL) levels^[358]. HDL levels increase in this strain of rats^[359]. But hyperlipidemia in female rats is more severe than that in males^[356]. They develop early atherosclerotic lesions in the major blood vessels and occlusive coronary thrombi at later stages of disease^[236].

3.3 Stroke - prone - spontaneous hypertensive fatty rats

Stroke - prone spontaneous hypertensive rats (SHRSP) are an animal model developing marked hypertension and hypertension related abnormalities, including atherosclerosis, cardiac hypertrophy and nephropathy, and finally dying from stroke^[360]. The stroke - prone SHR rats exhibit insulin resistance, yet no obesity, higher total cholesterol or non - esterified fatty acid (NEFA) compared with the control animals. Consequently, a new strain of rats was produced under the genetic background of stroke - prone SHR rats to be a model of metabolic syndrome^[361]. This strain of rats, SHRSP fatty rats, is derived by crossing stroke - prone SHR rats of the Izumo strain to Zucker obese (fa/fa) rats and has a missense mutation in leptin - receptor gene. These rats show hypertension and obesity. And the plasma leptin concentration, glucose, insulin, total cholesterol and triglyceride levels are significantly increased in these animals.

3.4 Sterol - regulatory element - binding protein - spontaneously hypertensive rats

This strain of rats came from a consideration that non - alcoholic fatty liver disease may be one feature of metabolic syndrome^[362]. Through the transgenic over - expression of a sterol - regulatory element - binding protein in SHR rats, a non - obese model of rats with hypertension, fatty liver, and several characteristics of metabolic syndrome, including hyperinsulinemia, hyperglycemia, and hypertriglyceridemia, is established.

3.5 Wistar Ottawa Karlsburg W rats

Wistar Ottawa Karlsburg W (WOKW) rats were produced as a new inbred strain of rats model in 1995. This strain of rats is a good animal model of metabolic syndrome because of their polygenic pathogenetic backgrounds similar to human conditions^[363]. These animals show hyperphagia, and exhibit almost complete traits of metabolic syndrome with obesity, moderate hypertension, dyslipidemia, hyperinsulinemia and impaired glucose tolerance

compared with their control animals, the dark agouti rats^[364-366]. Those manifestations could emerge between 8 and 10 weeks of age in rats^[367]. Meanwhile, the metabolic syndrome in WOKW also showed relations to coronary dysfunction^[368].

3.6 Low - capacity runner rats

This strain of rats was selectively bred according to their abilities to make a treadmill endurance running^[369]. Those who could only run relatively short distance are classified as low - capacity runner (LCR) rats and selectively bred eleven generation. Then the LCR rats exhibit hypertension, endothelial dysfunction, insulin resistance, hyperinsulinemia, visceral adiposity, hypertriglyceridemia, and high NEFA.

3.7 The streptozotocin (STZ) model

The STZ model is the most commonly used uncontrolled type 1 diabetes model. STZ treatment intraperitoneal causes β -cell toxicity and necrosis, and finally insulin deficiency^[370]. According to the recommendations from the Animal Models of Diabetic Complications Consortium (AMDCC), low - dose of STZ (50 mg/kg) administered five times consecutively can induce hyperglycemia in the rodents within 7 to 14 days after the first injection. These rodents exhibit increased serum fatty acid, triglyceride and cholesterol levels, whereas the insulin levels decrease progressively with the development of diabetes^[371]. This model can be easily induced in different genetic background strains of animal and at different ages of animals. One limitation of STZ induced model is the potential extrapancreatic genotoxic effects^[372]. STZ may directly damage the cardiac contractile function through p38 mitogen activated protein (MAP) kinase - dependent oxidative stress mechanism^[373]. And the disease severity in this model may be diverse, with some developing ketosis, whereas others not.

3.8 Type 2 diabetic Goto - Kakizaki rats

The Goto - Kakizaki (GK) rats are generated by the selective inbreeding of glucose intolerant Wistar rats^[374,375]. They have impaired insulin secretion at birth^[81]. They could develop type 2 diabetes within the first few weeks of age, with mild hyperglycemia, hyperinsulinemia, impaired glucose induced insulin secretion, marked glucose intolerance, hepatic glucose overproduction and insulin resistance, but without obesity, hypertension or significant hyperlipidemia^[374-376]. It has been recognized as one of the best characterized genetic animal model for the research of type 2 diabetes, without the associated influences of obesity or hypertension^[236,377]. Meanwhile, type 2 diabetes mellitus is developed under a

complex combination of environments and genetic factors. In the GK rats, at least six independent genetic loci are responsible for the deficiency in glucose and insulin metabolism^[374]. So this polygenic animal model of spontaneous type 2 diabetes mellitus is invaluable for the research of type 2 diabetes mellitus.

3.9 DahlS.Z-*Lep^{fa}/Lep^{fa}* (Dahl salt - sensitive (DS)/obese) rats

Researchers established an animal model of metabolic syndrome by crossing Dahl salt - sensitive (DS) rats and Zucker rats^[378,379]. The DS / obese rats develop similar features to the human of metabolic syndrome, including obesity, hypertension, dyslipidemia, insulin resistance and type 2 diabetes mellitus. They also develop LV hypertrophy, LV fibrosis, LV diastolic dysfunction, cardiac oxidative stress and inflammation, and renal and liver damages^[378,379].

3.10 The *ob/ob* mouse

In 1940s, diabetes was identified in *ob/ob* mouse, which has a single autosomal recessive mutation on the obese gene (*ob*, leptin encoding gene on chromosome 6, also known as *Lep^{ob}*)^[227,295]. This strain of mice has a high levels of leptin mRNA in adipocytes, whereas completely lack of functional leptin^[289,380]. Because of the leptin deficiency, this strain of mice develops obesity and diabetes mellitus. The strain of mice has hyperphagia, increased body fat content, decreased body temperature and energy expenditure. From 4 weeks of age, these mice on the C57BL/6 background develop moderate obesity, hyperinsulinemia and impaired glucose tolerance, but not diabetes yet^[381]. Since 15 weeks of age, they become severely obese. They have increased serum fatty acid and triglyceride levels dependent on the nutritional state and age. These mice die at about 14 months of age^[382]. The administration of leptin can correct several diabetic manifestations in this strain of mice. The corrections of hyperinsulinemia and hyperglycemia occur before the correction of obesity^[383-385], which suggested that obesity in this model does not play an important role in the pathogenesis of diabetic manifestations.

As a hallmark of type 2 diabetes, hyperglycemia in this strain of rats can only be observed from 1 month of age, and begins to decline after 3 month of age^[386,387]. This is in significant contrast to human diabetes, in which hyperglycemia progresses slowly and severely. The reasons of the difference might be the different causes of hyperglycemia in *ob/ob* mice and human diabetes. In the former one, hyperglycemia is caused by leptin deficiency, and the persist hyperinsulinemia limits the severity of hyperglycemia, but leads to obesity^[388,389]. In

the latter one, hyperglycemia develops when compensatory hyperinsulinemia in response to insulin resistance cannot control blood glucose. The lack of leptin results in the high levels of neuropeptide Y and increased cortisol levels, which underlies the muscular insulin resistance^[390]. The metabolic syndrome in human develops hyperleptinemia and leptin resistance, which has been described as a key driver of obesity - related cardiovascular dysfunction^[391]. Thus, this strain of mice could be a good model for studying the effects of leptin resistance in human obesity^[227].

3.11 The *db/db* mouse

The *db/db* mice have a single autosomal recessive mutation in the leptin receptor gene on chromosome 4 (*Lepr^{db}*), resulting in the abnormal mRNA splicing and subsequent production of a non - functional Ob-Rb protein^[227,295]. The deficiency of leptin receptor causes the over - production of extracellular leptin, whereas lack of intracellular leptin action through Ob-Rb. This strain of mice develops severe type 2 diabetes since 8 weeks of age and obesity equal to *ob/ob* mice^[381]. These mice have early hyperinsulinemia and increased serum fatty acid and triglyceride levels^[381,392]. The leptin receptor - deficient *db/db* mice develop hyperglycemia since 2 months of age, but not all of them develop hyperglycemia^[393]. At 10 weeks of age, their fasting blood glucose levels can reach about 4 times to that of the control mice^[394]. Plasma insulin begins to rise at 10 - 14 days and reaches to peak at 3 months of age^[394,395]. This severe hyperinsulinemia is in contrast to the moderate conditions in human type 2 diabetes. And this strain of mice dies at about 10 months of age, with female living longer^[396]. Insulin receptor tyrosine kinase is involved in the cellular insulin signaling process, which is markedly reduced in the muscle and liver of type 2 diabetes patients and associated with the developing of insulin resistance^[397-399]. However, in the *db/db* mice, the activity of the enzyme in muscle is not altered^[400]. Furthermore, the *db/db* mice do not develop atherosclerotic lesions despite the presences of obesity, hyperlipidemia, advanced kidney disease and cardiomyopathy^[216].

Chapter Three

Experimental Studies

We performed two experiments about β -adrenoceptor stimulation pathway in different pathophysiological status. The first experiment is to explore the role of multidrug resistance protein 4 (MRP4) in the regulation of intracellular cyclic adenosine monophosphate (cAMP) concentration and the influence on β -adrenergic dysfunction in the senescent rat (24-month age) heart. We observed the echocardiography and isolated cardiomyocyte under baseline and stimulation of isoproterenol, along with or without the pretreatment by MK571, a specific MRP4 inhibitor. MRP4 was quantified in left ventricular homogenates by Western blotting. We confirmed that the MRP4 overexpression contributes to the decrease of positive inotropic response to β -adrenoceptor stimulation in the senescent heart. The second experiment is to compare the β -adrenoceptor signaling pathway in Zucker lean, Zucker obese, and Zucker obese diabetic rats, the reliable rat models of metabolic syndrome. The effects of β -adrenoceptor stimulation were investigated with echocardiography (baseline and isoproterenol stimulation) and in isolated left ventricular papillary muscles (baseline, and stimulations under isoproterenol, forskolin and dibutyryl cAMP). The expressions of β_1 -, β_2 -, β_3 -adrenoceptors and MRP4 in left ventricular muscles were detected by Western Blotting. We concluded that the positive inotropic effect of β -adrenoceptor stimulation is slightly decreased in Zucker obese rats and is more markedly decreased in Zucker obese diabetic rats. These decreases are mainly related to β_1 - and β_2 -adrenoceptors down-regulation.

Study No. 1

Overexpression of cyclic adenosine monophosphate effluent protein MRP4 induces an altered response to β -adrenergic stimulation in the senescent rat heart. Carillion A, Feldman S, Jiang C, Atassi F, Na N, Mougnot N, Besse S, Hulot JS, Riou B, Amour J. *Anesthesiology*, 2015, 122(2): 334-342.

Overexpression of Cyclic Adenosine Monophosphate Effluent Protein MRP4 Induces an Altered Response to β -Adrenergic Stimulation in the Senescent Rat Heart

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ABSTRACT

Background: In the senescent heart, the positive inotropic response to β -adrenoceptor stimulation is reduced, partly by dysregulation of β 1- and β 3-adrenoceptors. The multidrug resistance protein 4 (MRP4) takes part in the control of intracellular cyclic adenosine monophosphate concentration by controlling its efflux but the role of MRP4 in the β -adrenergic dysfunction of the senescent heart remains unknown.

Methods: The β -adrenergic responses to isoproterenol were investigated *in vivo* (stress echocardiography) and *in vitro* (isolated cardiomyocyte by Ionoptix[®] with sarcomere shortening and calcium transient) in young (3 months old) and senescent (24 months old) rats pretreated or not with MK571, a specific MRP4 inhibitor. MRP4 was quantified in left ventricular homogenates by Western blotting. Data are mean \pm SD expressed as percent of baseline value.

Results: The positive inotropic effect of isoproterenol was reduced in senescent rats *in vivo* (left ventricular shortening fraction $120 \pm 16\%$ vs. $158 \pm 20\%$, $P < 0.001$, $n = 16$ rats) and *in vitro* (sarcomere shortening $129 \pm 37\%$ vs. $148 \pm 35\%$, $P = 0.004$, $n = 41$ or 43 cells) as compared to young rats. MRP4 expression increased 3.6-fold in senescent compared to young rat myocardium ($P = 0.012$, $n = 8$ rats per group). In senescent rats, inhibition of MRP4 by MK571 restored the positive inotropic effect of isoproterenol *in vivo* ($143 \pm 11\%$, $n = 8$ rats). *In vitro* in senescent cardiomyocytes pretreated with MK571, both sarcomere shortening ($161 \pm 45\%$ vs. $129 \pm 37\%$, $P = 0.007$, $n = 41$ cells per group) and calcium transient amplitude ($132 \pm 25\%$ vs. $113 \pm 27\%$, $P = 0.007$) increased significantly.

Conclusion: MRP4 overexpression contributes to the reduction of the positive inotropic response to β -adrenoceptor stimulation in the senescent heart. (ANESTHESIOLOGY 2015; 122:334-42)

ELDERLY patients are exposed to a higher mortality risk during the perioperative period.¹ In the senescent heart, diastolic dysfunction²⁻⁴ and a reduced response to β -adrenoceptor stimulation are observed which may contribute to hemodynamic instability during the perioperative period.⁵ Down-regulation of β 1- and β 2-adrenoceptors impair the positive inotropic effect of β -adrenergic stimulation,²⁻⁶ whereas overexpression of β 3-adrenoceptor increases nitric oxide production *via* nitric oxide synthase 1, activates protein kinase G, and then promotes the increased hydrolysis of cyclic adenosine monophosphate (cAMP) by activation of phosphodiesterases.³ However, these abnormalities only partly contribute to the alteration of the β -adrenergic pathway.

A complementary mechanism of regulation of cAMP has been recently described, the multidrug resistance protein 4 (MRP4).^{7,8} This channel was first described in resistance to chemotherapy as it allows malignant cells to extrude the drug and survive⁹ but further studies revealed its role as transporter of cyclic nucleotides.¹⁰⁻¹² In cardiomyocytes

What We Already Know about This Topic

- The multidrug resistance protein MRP4 plays an important role in regulation of intracellular cyclic adenosine monophosphate and β -adrenergic stimulated heart performance
- Elderly patients are at significant risk of perioperative cardiovascular compromise; however, the role of MRP4 in the senescent heart has never been investigated

What This Article Tells Us That Is New

- MRP4 is overexpressed in the senescent rat heart and is involved in the limited positive inotropic response of the senescent heart to β -adrenergic stimulation
- MRP4 may be a therapeutic target for altering the inotropic reserve of elderly patients

and vascular smooth cells, MRP4 is located at the plasma membrane level⁷ and extrudes cAMP.^{7-9,11} *In vitro*, inhibition of MRP4 increased intracellular cAMP level and protein kinase A activity,⁸ leading to a greater positive inotropic

Submitted for publication January 27, 2014. Accepted for publication September 2, 2014. From the Sorbonne Universités UPMC Univ Paris 06, UMR INSERM-UPMC 1166, Paris, France (A.C., S.F., C.J., F.A., N.M., J.-S.H., B.R., J.A.); Department of Anesthesiology and Critical Care Medicine (A.C., J.A.), Department of Emergency Medicine and Surgery (N.N., B.R.), and Department of Pharmacology (J.-S.H.), Hôpital Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France; Université de Reims Champagne-Ardenne and Department of Anesthesiology and Critical Care Medicine, Robert Debré Hospital, Reims, France (A.C.); Wuhan University, Wuhan, The People's Republic of China (C.J.); UMR INSERM 902 Université Evry Val d'Essonne, Evry, France (S.B.); and Université Paris-Descartes, Paris, France (S.B.).

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effect of β -adrenoceptor stimulation. In mice with genetic deletion of MRP4, an increase in cAMP has been observed in cardiomyocytes after adenylyl cyclase stimulation.⁷ However, the involvement of MRP4 in the senescence-induced β -adrenergic dysfunction had not yet been studied.

This study tested the hypothesis that an overexpression of MRP4 contributes to the altered response of the β -adrenoceptor stimulation in the senescent heart.

Materials and Methods

Animals

Experiments were conducted in an authorized laboratory under supervision of an authorized researcher (J. Amour, A-75-20-81). The project has been submitted to the relevant Animal Care Committee through the French Ministry of High Education and Research (Comité Régional d'Ethique en Expérimentation Animale Paris-comité 3, Paris, France). Wistar male rats have been studied in two groups: senescent rats (24 months old) and young adult rats (3 months old). Animals were purchased from Janvier (Le Genest St Isle, France) and cared according to the *Guiding Principles in the Care and Use of Animals* in a labeled housing place (B-75-13-08) with food and water *ad libitum*. The heart was removed during anesthesia after the measurements of arterial blood pressure and weighed, except for the hearts used for the isolation of cardiomyocytes, in order to shorten the time of ischemia. Then, the left ventricle was carefully dissected and frozen in liquid nitrogen for Western blotting experiments.

Stress Echocardiography

Transthoracic stress echocardiography was performed on anesthetized rats under 1 to 2% isoflurane using a General Electric Vivid 7 instrument (Aulnay-sous-Bois, France) as previously described.^{3,13,14} Systolic function and inotropy were studied with left ventricular shortening and ejection fractions using a modified version of Simpson's analysis on parasternal short-axis and long-axis views in M mode. These variables were measured in basal conditions and under β -adrenergic stimulation using isoproterenol ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$, continuous intravenous administration) with or without intravenous pretreatment with MK571 (30 mg/kg, Enzo Life Sciences, Villeurbanne, France) a specific inhibitor of MRP4^{7,15} or the same volume of NaCl 0.9% as control. Stress values were determined after stabilization of heart rate (HR) 6 min after each drug administration.

Arterial Pressure Measurements

In vivo, at least 2 days after the echocardiographic assessment, the rats were anesthetized using pentobarbital (50 mg/kg intraperitoneally) to measure arterial blood pressure. Pressure transducer catheter (size 2F, Millar Micro-tip catheter transducer, model SPR-407; Millar Instruments, Inc., Houston, TX), was introduced into the right carotid artery and connected to a pressure transducer (Gould Electronic, Cleveland, OH). After stabilization, the arterial systolic,

mean arterial pressure and diastolic pressures were recorded. From the arterial blood pressure tracings, the HR and the maximum positive values of first derivative of arterial pressure (+dP/dt) were quantified. The cardiac variables were recorded under baseline conditions and under β -adrenergic stimulation using isoproterenol ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$, continuous intravenous administration although the tail vein) with or without pretreatment with MK571 (intravenous administration of 30 mg/kg 6 min before through the tail vein)^{7,15} or the same volume of normal saline as a control. Stress values were determined after stabilization of HR.

Measurements of Intracellular Calcium Transient and Contractile Function in Isolated Cardiomyocytes

Ventricular cardiomyocytes were isolated from rat hearts on a Langendorf apparatus using enzymatic digestion by collagenase A (Roche Diagnostics, Meylan, France) as previously described.¹⁶ Under intravenous anesthesia (pentobarbital 65 mg/kg, intraperitoneally) the chest was opened, the heart was removed and washed in buffer then connected through the aorta to the Langendorf perfusion cannula. An anterograde perfusion *via* the coronary circulation was applied to the heart with a HEPES buffer (117 mM NaCl, 5.7 mM KCl, 1.5 mM KH_2PO_4 , 4.4 mM NaHCO_3 , 1.7 mM MgCl_2 , 11.7 mM glucose, 10 mM creatine, 21 mM HEPES, and 20 mM taurine, all from Sigma-Aldrich, L'Isle d'Abeau Chesnes, Saint-Quentin Fallavier, France) bubbled with oxygen and maintained at 37°C and pH 7.40. The cardiac digestion was performed by the perfusion of the same buffer with collagenase A (1.2 to 1.4 mg/ml), 100 μM EGTA and 240 μM CaCl_2 (both from Sigma-Aldrich). After 60 to 80 min, the heart was removed and the atria excised. A careful mechanical dissection completed the digestion. The cells were filtered and resuspended in the native calcium-free buffer with bovine serum albumin (Sigma-Aldrich). Calcium was progressively added to the suspension to reach an extracellular calcium concentration of 0.5 mM. Freshly isolated cardiomyocytes were used in the same day. The ventricular cardiomyocytes were loaded for 20 min at room temperature with Fura2-AM (1 μM , Molecular Probes, Invitrogen, Saint-Aubin, France) and then resuspended in the HEPES buffer with 0.5 mM calcium. Contractility and calcium transient of the cardiomyocytes were assessed on each cell with a Ionoptix[®] platform (Ionoptix Corporation, Milton, MA).¹⁷ Only rod-shaped cardiomyocytes with sharp edges were studied. Cardiomyocytes with spontaneous contraction or sarcolemal blebs were avoided. Myocytes were electrically stimulated at 1 Hz and 8 V in a room at 25°C. The contractile properties of the cardiac myocytes were analyzed with the IonWizard[®] software (Ionoptix[®], Ionoptix Corporation) from the trace of the sarcomere shortening by peak shortening (PS), time to PS normalized to PS and the maximum shortening velocity ($-\text{dL}/\text{dt}$) for the shortening phase and time to 90% relengthening and the maximum relengthening velocity ($+\text{dL}/\text{dt}$) for the relengthening phase were recorded.

Myocytes were alternatively exposed to a light of 340 and 380 nm by the Fluorescence System Interface with Hyper-switch (Ionoptix Corporation). Fura2-AM is a fluorescent dye whose emission wavelength depends on the calcium concentration and the ratio 340/380 of fluorescence of the Fura2-AM is correlated to the intracellular calcium level.¹⁷ The changes in Fura2-fluorescence intensity (FFI) reflect the variation of the intracellular calcium level during the cardiac cycle, also known as calcium transient. The calcium transient was described in our study by the Δ FFI, amplitude of the FFI increase after twitch (peak FFI-baseline FFI) and the time course of the fluorescence constant decay (τ). The inhibition of MRP4 was provided by a pretreatment with MK571 (10^{-7} M). The contraction and fluorescence ratio of the cardiomyocyte were recorded continuously at basal condition and after adjunction of isoproterenol (10^{-6} M). Since calibration was not performed, only percent changes with isoproterenol and baseline values of Δ FFI could be compared between groups. Stress values were evaluated at maximal cell contraction. As MK571 could also inhibit leukotrienes receptors CysLT1 and CysLT2 and thus influence the response to β -adrenoceptor stimulation, we performed additional experimentation in isolated cardiomyocytes using BAY-u9773 (10^{-7} M) (Cayman Chemical Bertin Pharma, Montigny-le-Bretonneux, France), a specific inhibitor of CysLT1 and CysLT2 receptors.¹⁸ Then, β -adrenoceptor stimulation was performed as described in the experiment with MK571.

Western Blotting

The left ventricles were removed from anesthetized rats and frozen in liquid nitrogen. The samples were homogenated in a lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% triton, phosphatase and protease-inhibitor cocktail, Sigma-Aldrich). The protein concentration was determined by the Bradford protein assay (Bio-Rad, Marnes-la Coquette, France). After denaturation in Laemmli buffer, 60 μ g of total protein extract was loaded in each lane for separation in a sodium dodecyl sulfate 9% polyacrylamide electrophoresis gel and transferred on a nitrocellulose membrane (Hybond, Amersham, GE Healthcare, Velizy, France). After saturation in milk, each membrane was incubated overnight at 4°C with primary antibodies (anti-MRP4 1/200: M4I-80 (Abcam, Paris, France), anti-CysLT1 Receptor 1/10,000 or anti-CysLT2 receptor 1/5,000 (both from Cayman Chemical), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) 1/1,500 (ab9485, Abcam)).⁸ The day after, membranes were washed with a Tris-saline buffer with Tween and incubated with appropriate secondary antibody (anti-rat 1/2,500, anti-rabbit 1/2,500, all from Cell Signaling, Ozyme, Saint-Quentin en Yvelines, France). Relative quantification of the targeted protein was achieved by fluorescence recording on EthanDIGE reader with an ECL[®] detection system (GE Healthcare). MRP4 was detected at 150 kDa and GAPDH at 37 kDa. MRP4 expression was

quantified using Image J software (NIH, Bethesda, MA) and normalized *versus* GAPDH expression to ensure no variation in protein gel loading. In addition, Ponceau S staining was performed to confirm that GAPDH expression did not differ between young and senescent rats. Two membranes were generated and comparison was performed on ratio of MRP4/GAPDH expression normalized on the mean expression in control samples in the concerning gel.

Statistical Analysis

Data are expressed as mean \pm SD. We used absolute values to compare baseline characteristics between young and senescent rats and delta percent changes from baseline to compare the pharmacological effects, as previously described.^{3,13,14,16,19,20} As a matter of fact, SD of delta percent changes represents the variation of the pharmacological effect we are measuring, whereas the SD of absolute values mainly reflects interindividual differences. Moreover, some variables were expected to significantly differ at baseline because we compared young and senescent rats. The main criteria of our study was sarcomere shortening, especially PS. Assuming a baseline value of PS of $10.7 \pm 2.3\%$, an alpha risk of 0.05 and a beta risk of 0.20, we determined that a sample size of at least $n = 35$ cells per group would enable us to detect a 15% change in PS (PASS 11 software, Statistical Solutions Ltd., Cork, Ireland). Young and senescent rats or cells were studied alternatively. Experiments could not be blinded because young and senescent rats look particularly different. For *in vivo* experiments, treatment was allocated before anesthesia by manual randomization. For *in vitro* experiments, cells were parted in aliquots. Inhibitor or suspension buffer was added after manual randomization before being examined. Means were compared using the Student *t* test or one-way ANOVA with *post hoc* test Newman-Keuls. All *P* values were two-tailed and a *P* value of less than 0.05 was considered as significant. Statistical analysis was performed using NCSS 7.0 software (Statistical Solutions Ltd.).

Results

We studied a total of 21 young and 20 senescent rats. Two young and four senescent rats exhibited ventricular fibrillation or asystole during invasive arterial pressure measurement and were excluded from the analysis.

Baseline Characteristics

Baseline characteristics of rats are reported in table 1. As expected, senescent rats had significantly higher body weight and heart weight than young rats but the heart to body weight ratio was not significantly different between groups. Using echocardiography, HR in senescent rats was significantly lower in senescent than that in young rats. Both left ventricular shortening and ejection fractions were not significantly different between senescent and young rats. Under baseline conditions *in vivo*, the HR was significantly lower in senescent rats but the mean arterial pressure was

Table 1. Characteristics of Young and Senescent Rats

	Young	Senescent
General characteristics (no. of rats)	(n = 21)	(n = 20)
Body weight (g)	430 ± 60	566 ± 50*
Heart weight (mg)	(n = 12) 872 ± 104	(n = 12) 1,294 ± 274*
Heart weight/body weight (mg/g)	3.2 ± 0.3	3.1 ± 0.5
Echocardiography (no. of rats)	(n = 16)	(n = 16)
Heart rate (beats/min)	366 ± 33	331 ± 13*
LV ejection fraction (%)	81 ± 7	80 ± 3
LV shortening fraction (%)	47 ± 6	44 ± 4
Arterial catheterization (no. of rats)	(n = 16)	(n = 16)
HR (beats/min)	420 ± 35	355 ± 33*
Systolic arterial pressure (mmHg)	130 ± 16	135 ± 19
Diastolic arterial pressure (mmHg)	105 ± 12	105 ± 16
Mean arterial pressure (mmHg)	113 ± 13	115 ± 17
+dP/dt (mmHg/s)	2,680 ± 501	2,250 ± 665*
Sarcomere kinetics (no. of cells)	(n = 43)	(n = 41)
PS (%)	10.7 ± 2.3	9.5 ± 3.0*
TPS (ms)	11.8 ± 3.0	20.6 ± 9.6*
-dL/dt (mm/s)	-3.0 ± 0.8	-2.1 ± 1.0*
+dL/dt (mm/s)	2.7 ± 1.0	1.7 ± 1.1*
TR90 (ms)	261 ± 83	340 ± 102*
Calcium transient (no. of cells)	(n = 43)	(n = 41)
ΔFFI (arbitrary units)	0.53 ± 0.19	0.37 ± 0.27†
Tau (ms)	124 ± 25	125 ± 28

Data are mean ± SD.

* $P < 0.05$ vs. Young. †Because calibration was not performed, these values could not be compared between young and senescent rats.

-dL/dt = maximum shortening velocity; +dL/dt = maximum relengthening velocity; +dP/dt = first derivative of arterial pressure; ΔFFI = changes in Fura-2 fluorescence intensity; HR = heart rate; LV = left ventricular; PS = peak shortening; TPS = time to peak shortening; TR90 = time to 90% relengthening.

not significantly different between groups. *In vitro*, the basal characteristics of sarcomere shortening of the cardiomyocytes revealed a moderate alteration of contractility and relaxation parameters in the senescent cardiomyocytes. The cardiomyocytes from senescent rats displayed reduced PS and -dL/dt and increased time to PS. The +dL/dt was significantly lower and time to 90% relengthening was significantly higher in the senescent cardiomyocytes (table 1).

Effects of MK571, a Specific Inhibitor of MRP4

Since MK571 may also inhibit leukotrienes receptors, we verified that inhibition of CysLT1 and CysLT2 receptors by BAY-u9773 did not significantly modify the response to isoproterenol on sarcomere shortening in isolated cardiomyocytes from young rats (data not shown). Moreover, CysLT1 and CysLT2 receptors protein expression were not significantly different between young and in senescent rats (1.0 ± 0.7 vs. 1.0 ± 0.8 arbitrary units, $P = 0.99$ and 1.0 ± 0.8 vs. 1.0 ± 0.7 arbitrary units, $P = 0.90$, respectively).

Administration of saline did not significantly modify any echocardiographic variables in the senescent or young groups. In senescent rats, administration of MK571 had no significant effect on any echocardiographic variable. In contrast, in young rats, a slight but significant increase in left ventricular shortening and ejection fractions was observed (table 2). During arterial catheterization, administration

of saline or MK571 induced no significant effect on HR, mean arterial pressure, or dP/dt in any group. Nevertheless, in young rats during echocardiography, the isoproterenol-induced increase in HR seems to be blunted by MK571 (table 2), an effect not observed during arterial catheterization (table 2). We did not test the direct effect of MK571 on sarcomere shortening and calcium transient since isolated cells were incubated with or without MK571. Nevertheless, we did not observe any significant difference in the groups incubated with or without MK571 (table 2).

Effects of MK571 on β -Adrenoceptor Stimulation

In vivo, using echocardiography, the positive inotropic effect of β -adrenoceptor stimulation was altered in senescent rats as compared with young rats. Pretreatment with MK571 restored the positive inotropic effect in the senescent group to the level observed in young rats (fig. 1).

During arterial catheterization, the increase in HR and the decrease in mean arterial pressure induced by isoproterenol were not significantly different between senescent and young rats. Pretreatment with MK571 restored the positive inotropic effect in the senescent group to the level observed in young rats (fig. 1).

In vitro, the senescent cardiomyocytes exhibited a reduced positive inotropic response to isoproterenol compared with young cardiomyocytes (fig. 2). Pretreatment with MK571 restored the positive inotropic response of sarcomere shortening in senescent cardiomyocytes returning to the level observed in young cardiomyocytes, whereas it had no significant effect in young cardiomyocytes (table 2). Calcium transient increased with β -adrenoceptor stimulation. In senescent group, calcium transient increase was more important in cardiomyocytes pretreated with MK571 than in non-pretreated cardiomyocytes (fig. 3).

Expression of MRP4

In senescent rats, MRP4 protein expression increased 3.6-fold in comparison to young rats ($P = 0.012$) (fig. 4). Using Ponceau staining, we confirmed the absence of significant variation in GAPDH expression in senescent compared to young rats (203 ± 9 vs. 211 ± 15 arbitrary units, $P = 0.70$).

Discussion

In the current study, we confirmed that the positive inotropic response to β -adrenoceptor stimulation is altered in senescent hearts. We observed that the expression of MRP4 was 3.6-fold increased in left ventricle of senescent rats and inhibition of MRP4 by MK571 restored the positive inotropic effect of β -adrenoceptor stimulation in senescent rats both *in vivo* and *in vitro*. In parallel, the calcium transient was improved by MK571 pretreatment in the senescent cardiomyocytes. Consequently, these results strongly support the role of MRP4 in the altered β -adrenergic response in the senescent heart.

In vivo, our results confirm that the systolic function was preserved (left ventricular shortening and ejection

Table 2. Comparison of the Response to β -Adrenergic Stimulation in Young and Senescent Rats with or without Pretreatment by MK571

	Baseline	Saline	Isoproterenol	Baseline	MK	Isoproterenol
	Young			Young with MK571		
Echocardiography (no. of rats)	(n = 8)			(n = 8)		
HR (beats/min)	361 ± 40	358 ± 20	421 ± 30*	371 ± 28	358 ± 17	387 ± 29
LV ejection fraction (%)	79 ± 9	78 ± 7	96 ± 3*	83 ± 5	87 ± 5*	97 ± 2*
LV shortening fraction (%)	46 ± 6	45 ± 7	72 ± 8*	47 ± 5	53 ± 8*	71 ± 5*
Arterial catheterization (no. of rats)	(n = 6)			(n = 4)		
HR (beats/min)	440 ± 17	450 ± 33	530 ± 23*	389 ± 33	384 ± 41	525 ± 50*
Mean arterial pressure (mmHg)	116 ± 15	110 ± 11	61 ± 8*	109 ± 11	107 ± 12	60 ± 8*
+dP/dt (mmHg/s)	2,800 ± 566	2,600 ± 860	6,733 ± 1,115*	2,500 ± 383	2,933 ± 787	5,800 ± 1,649*
Sarcomere kinetics (no. of cells)	(n = 43)			(n = 41)		
PS (%)	10.7 ± 2.3	NA	15.4 ± 2.5	NA	9.5 ± 3.0	15.3 ± 2.4
TPS (ms)	11.8 ± 3.0		6.9 ± 1.7		20.6 ± 9.6	7.0 ± 2.6
-dL/dt (mm/s)	-3.0 ± 0.8		-5.1 ± 1.5		-2.1 ± 1.0	-5.5 ± 2.2
+dL/dt (mm/s)	2.7 ± 1.0		3.7 ± 1.4		1.7 ± 1.1	3.9 ± 1.3
TR90 (ms)	261 ± 83		214 ± 83		340 ± 102	211 ± 59
	Senescent			Senescent with MK571		
Echocardiography (no. of rats)	(n = 8)			(n = 8)		
Heart rate (beats/min)	327 ± 13	322 ± 10	333 ± 13	335 ± 12†	320 ± 23	341 ± 21
LV ejection fraction (%)	81 ± 4	80 ± 3	89 ± 3	78 ± 2	79 ± 3	92 ± 3
LV shortening fraction (%)	46 ± 4	44 ± 3	54 ± 4	42 ± 2	43 ± 2	61 ± 5
Arterial catheterization (no. of rats)	(n = 4)			(n = 4)		
HR (beats/min)	351 ± 30	376 ± 19	424 ± 36*	360 ± 33	360 ± 33	446 ± 19*
Mean arterial pressure (mmHg)	118 ± 16	113 ± 28	73 ± 44*	112 ± 20	113 ± 30	71 ± 13*
+dP/dt (mmHg/s)	1,950 ± 661	2,150 ± 412	3,070 ± 1,429*	2,550 ± 598	2,750 ± 1,370	4,900 ± 744*
Sarcomere kinetics (no. of cells)	(n = 41)			(n = 41)		
PS (%)	9.5 ± 3.0	NA	11.7 ± 3.3†	NA	8.8 ± 2.9	13.2 ± 2.3
TPS (ms)	20.6 ± 9.6		14.1 ± 5.7†		20.9 ± 8.3	11.0 ± 3.2
-dL/dt (mm/s)	-2.1 ± 1.0		-3.0 ± 1.3†		-1.8 ± 0.8	-3.7 ± 1.2
+dL/dt (mm/s)	1.7 ± 1.1		1.9 ± 1.1†		1.7 ± 1.2	2.6 ± 1.5
TR90 (ms)	340 ± 102		316 ± 65†		316 ± 67	290 ± 74

Data are mean ± SD. Δ is % of baseline value.

* $P < 0.05$ vs. baseline; † $P < 0.05$ vs. Young.

-dL/dt = maximum shortening velocity; +dL/dt = maximum relengthening velocity; +dP/dt = first derivative of arterial pressure; Δ FFI = changes in Fura-2 fluorescence intensity; HR = heart rate; LV = left ventricular; NA = not applicable; PS = peak shortening; TPS = time to peak shortening; TR90 = time to 90% relengthening.

fractions) in senescent hearts, as previously observed.³ *In vitro*, the cardiomyocytes extracted from senescent rats exhibited an impaired sarcomere shortening with prolonged duration of shortening (time to peak shortening) and time to 90% relengthening consistent with the literature.^{2,4} The positive inotropic response to β -adrenoceptor stimulation was reduced in senescent rats both *in vivo* and *in vitro*. The senescent heart displays different mechanisms to preserve itself from an excessive work such as the reduction of positive chronotropic and inotropic effects of the β -adrenergic stimulation. β -Adrenergic dysfunction may contribute to long-term saving of heart function and senescence adaptation. Unfortunately during the perioperative period, this β -adrenergic dysfunction limits cardiac output adaptation and favors hemodynamic instability in aging patients.³ Although β -blockers have been shown to improve long-term survival in patients with chronic heart failure and may be also beneficial during the perioperative

period, deleterious effects have also been clearly demonstrated in the perioperative period when bleeding or postoperative complications occur.²¹

Several modifications in the β -adrenergic signaling pathway have been described in the senescent heart.^{2,4} Due to decreased expression of β 1- and β 2-adrenoceptors, less cAMP is produced in the senescent heart after isoproterenol. The effect of direct stimulation of adenylyl cyclase is also reduced, but the effect of a cAMP analog is preserved, confirming the crucial role of intracellular cAMP level in the β -adrenergic response.⁶ β 3-adrenoceptor induces the production of nitrite oxide by nitrite oxide synthase 1 that activates protein kinase G which increases the catabolism of cAMP by phosphodiesterase activation.³ An increase in β 3-adrenoceptor expression and activity in the senescent heart³ contributes to the reduction of the positive inotropic effect of β -adrenergic stimulation as we previously observed in the diabetic cardiomyopathy.¹³

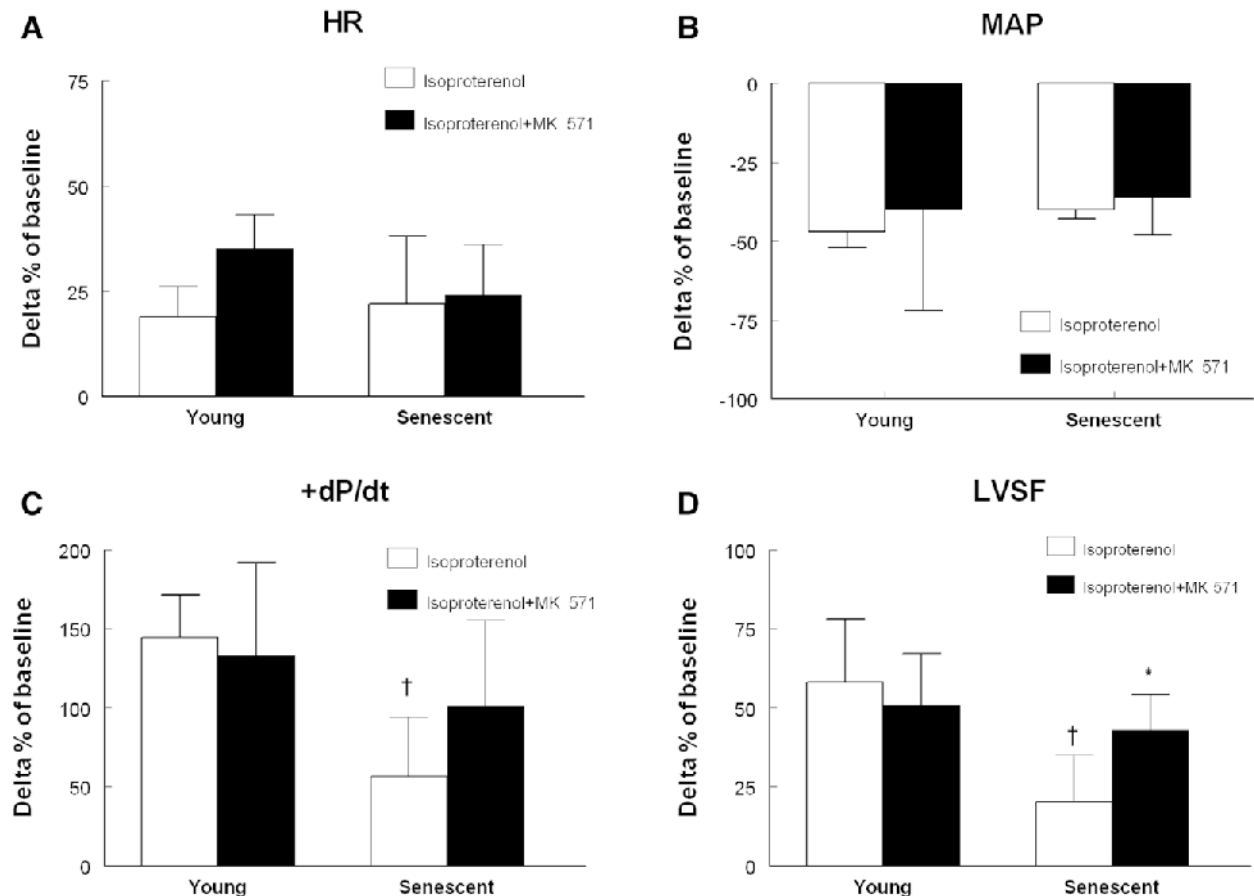


Fig. 1. Comparison of the effect of isoproterenol on heart rate (HR) (A), mean arterial blood pressure (MAP) (B), positive first derivative for maximal rates of arterial pressure development (+dP/dt) (C), and left ventricular shortening fraction (LVSF) (D) in young and senescent rats with and without MK571. HR, MAP, and +dP/dt were obtained during invasive catheterization (n = 6 and 4, respectively), and LVSF was obtained during echography (n = 8 in each group). Data are mean \pm SD. **P* < 0.05 versus group without MK571; †*P* < 0.05 versus Young group.

The transmembrane protein MRP4 is known as an effluent pump of cyclic nucleotides in platelets,²² hepatic,⁹ and renal cells.²³ MRP4 is overexpressed in the liver of female aging mice.²⁴ In vascular smooth cells^{8,11,25} and cardiomyocytes,⁷ MRP4 is known to control the efflux of cAMP. In cultured cells, MRP4 could regulate the submembrane pool of cAMP by efflux and interaction with phosphodiesterases.¹⁰ In this context, the large MRP4 overexpression observed in senescent rats suggests a pathway for increased removal from the myocyte of cAMP that was synthesized after β -adrenoceptor stimulation. Such cAMP elimination from the cell could markedly decrease the positive inotropic effect of β -adrenergic stimulation.

MK571 has been used as a specific inhibitor of MRP4.^{7,8,11,12} The effect of MK571 is rapid and constant after intravenous administration.^{15,26} Inhibition of MRP4 by MK571 induces an elevation in cAMP intracellular concentration in vascular smooth cells^{8,11} and cardiomyocytes.¹² Combined inhibition of MRP4 and phosphodiesterases induces an elevation of cAMP more important than with isolated inhibition of MRP4 suggesting that the two mechanisms may be additive.¹¹ Inhibition of MRP4

by MK571 enhanced the chronotropic effect of isoproterenol in neonatal mice cardiomyocytes.¹² In our study, MK571 had no or little effect on *in vivo* or *in vitro* contractility before β -adrenergic stimulation. This result is consistent with the fact that MK571 is not a direct agonist of β -adrenoceptors and MRP4 only affects submembrane compartment of cAMP produced after β -adrenergic stimulation.⁷ The elevation of cAMP after isoproterenol stimulation of isolated cardiac myocytes from 9-month-old mice is increased by silencing of MRP4.⁷ In agreement with these results, we demonstrated here that MRP4 inhibition restored the positive inotropic effect of β -adrenoceptors stimulation both *in vivo* and *in vitro* in the senescent rats. MRP4 may extrude the cAMP produced by β -adrenoceptor stimulation by isoproterenol and may limit the increase in intracellular second messenger concentration. The intracellular calcium level closely reflects the contractility of a muscle as it is directly involved in the actin/myosin interaction and muscle shortening. The β -adrenergic stimulation induces a positive inotropic effect *via* cAMP production which activates the protein kinase A. Activation of protein

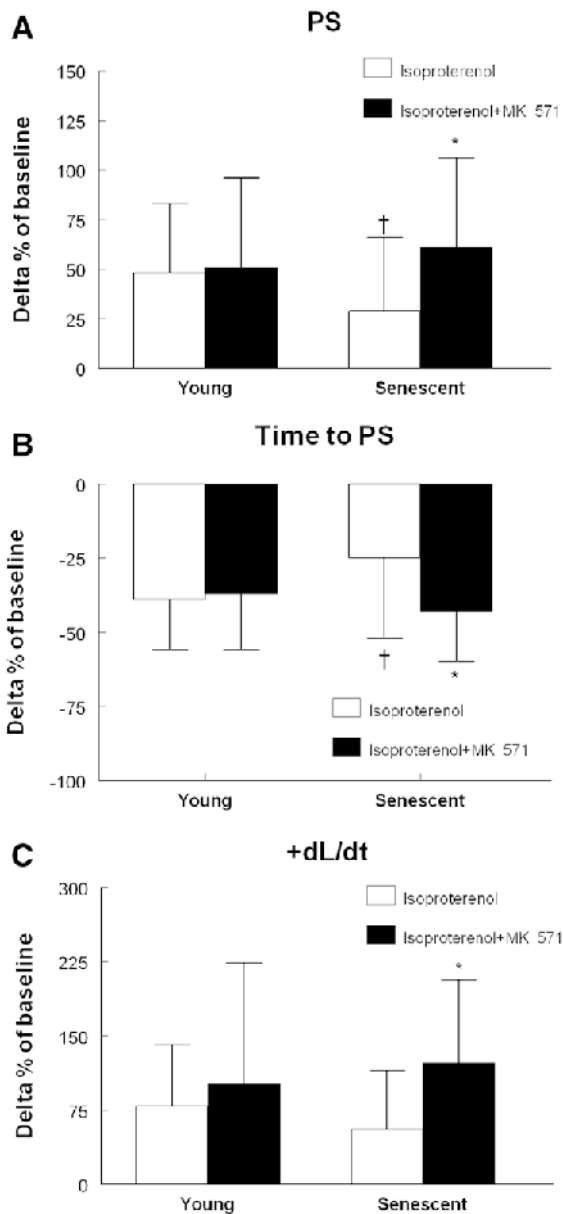


Fig. 2. Sarcomere kinetics: comparison of the effect of isoproterenol on the peak shortening (PS) amplitude (A), time to PS (B), and maximum velocity of shortening ($-dL/dt$) (C) in young and senescent rats with and without MK571 ($n = 41$ to 43 cells in each group). Data are mean \pm SD. * $P < 0.05$ versus group without MK571; † $P < 0.05$ versus Young group.

kinase A increases calcium transient after phosphorylation of targeted proteins (calcium channel, ryanodine receptor, sarco-endoplasmic reticulum ATPase, and troponin). Since calcium transient was altered in senescent cardiomyocytes and since we observed an increase in calcium transient amplitude and sarcomere shortening induced by β -adrenergic stimulation after MK571 pretreatment, we can conclude that MRP4 overexpression plays a role in the altered inotropic response of β -adrenergic stimulation in the senescent heart.

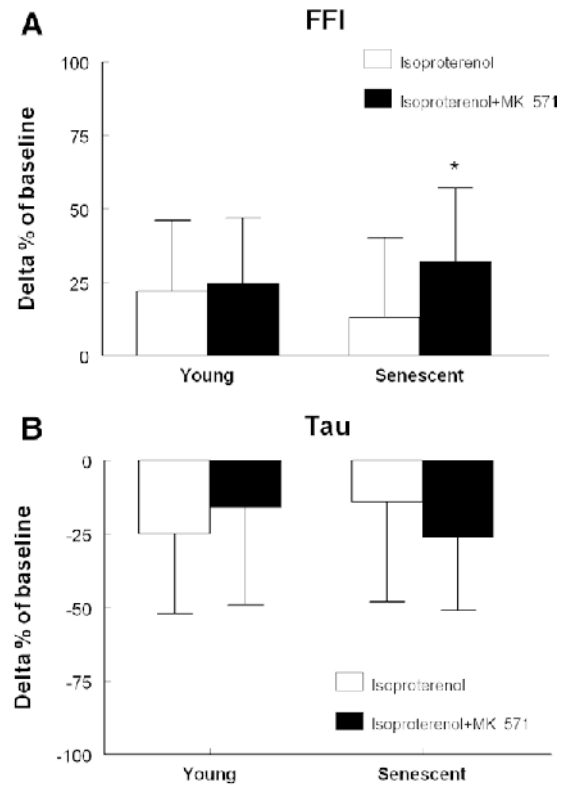


Fig. 3. Calcium transient: comparison of the effect of isoproterenol (10^{-6} M) on the amplitude of calcium transient (changes in Fura2-fluorescence intensity [ΔFFI]) (A) and time decay constant of the calcium transient (tau) (B) in isolated cardiomyocytes from young and senescent rats with and without MK571 ($n = 41$ to 43 cells in each group). Data are mean \pm SD.

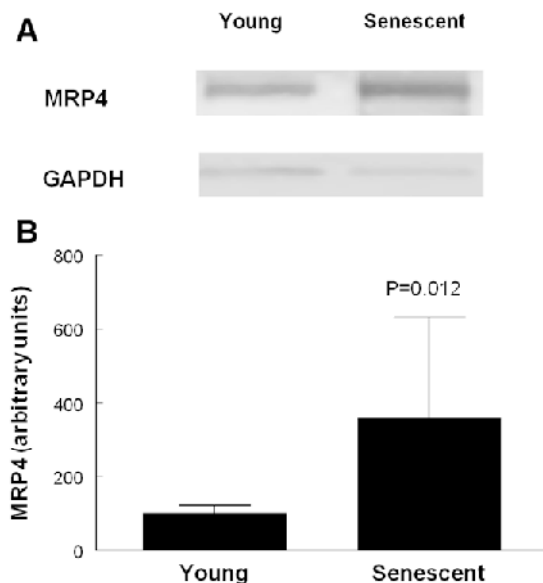


Fig. 4. Representative Western blot (A) and normalized densitometric data (B) showing left ventricular expression of multidrug resistance protein 4 (MRP4) compared with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in young and senescent rats ($n = 8$ in each group). Data are mean \pm SD.

The following points have to be considered to assess the clinical relevance of our results. First, these experiments were conducted in rats and the results may not be generalized to humans as rat myocardium exhibits several differences with human myocardium, including MRP4 expression and function.²⁷ Further experiments are mandatory to confirm the increased expression and the role of MRP4 in the human senescent heart. Second, *in vivo* studies require anesthetized animals. We used isoflurane inhalation for echocardiography and pentobarbital for arterial blood pressure recordings. Halogenated agents are known to interfere with β -adrenergic stimulation in different cardiomyopathies,^{19,20} but our results are consistent, regardless of the anesthetic technique used. Since the isoproterenol-induced increase in HR seems to be blunted by MK571 in young rats during echography (*i.e.*, with isoflurane) but not during arterial catheterization (*i.e.*, with pentobarbital), we cannot rule out the hypothesis that an interaction between MK571 and baroreflex activity was differentially altered by anesthetics *in vivo*. Third, *in vitro* experiments may be affected by a selection bias as cardiomyocytes surviving to cell isolation may differ from the more disabled ones present in the total heart. Fourth, we elected not to directly measure cAMP or cyclic guanosine monophosphate concentrations since these messenger molecules are compartmentalized within the cell, and average cellular concentrations may not reflect critical concentrations at near relevant kinase mediators within the cell. Furthermore, MRP4 has been located in caveolae near from β -adrenoceptors and we think it could effectively act on this pool of cAMP, not necessarily on the whole cell cAMP mean concentration.⁷ Fifth, we used MK571 to selectively inhibit MRP4. This drug is also an inhibitor of the CysLT1 receptor for leukotrienes D4.^{15,28} The inhibition of CysLT1 and CysLT2 receptors by MK571 could alleviate the negative inotropic effect due to leukotrienes D4 in heart and be confusing in interpretation of our results.^{17,29} However, inhibition of CysLT1 and CysLT2 receptors by BAY-u9773 did not modify the response to isoproterenol in young cardiomyocytes and CysLT1 and CysLT2 receptor expressions were not significantly modified in senescent hearts. In the same manner, that we cannot rule out the possibility that the *in vivo* effects of MK571 could also be modulated by CysLT1 receptors in vascular smooth muscle.³⁰ Sixth, in a therapeutic view, chronic inhibition of MRP4 may alter other organ function or may be compensated by an increase in phosphodiesterases.

In conclusion, we observed that MRP4 is overexpressed in the heart of senescent rats and plays an important role in the altered positive inotropic response to β -adrenoceptor stimulation.

Acknowledgments

The authors thank David Baker, D.M., F.R.C.A. (Department of Anesthesiology and Critical Care, Hôpital Necker-Enfants Malades, Paris, France), for reviewing the manuscript

and Anne-Marie Lompré, Ph.D. (UMR INSERM 1166, Paris, France), for critical reading of the manuscript.

Dr. Carillion was the recipient of a research grant from the Fondation pour la Recherche Médicale (Paris, France; FRM grant no. DEA20090616244). Dr. Jiang was the recipient of a doctoral grant from the People's Republic of China (State Scholarship Fund by China Scholarship Council; grant no. 201206270014).

Competing Interests

The authors declare no competing interests.

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Study No. 2

Modification of the β -adrenoceptor stimulation pathway in Zucker obese and obese diabetic rat myocardium. Jiang C, Carillion A, Na N, de Jong A, Feldman S, Lacorte JM, Bonnefont-Rousselot D, Riou B, Amour J. Crit Care Med, 2015, in press.

Modification of the β -Adrenoceptor Stimulation Pathway in Zucker Obese and Obese Diabetic Rat Myocardium*

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Objectives: Although metabolic syndrome is associated with increased sympathetic activity that chronically stimulates β -adrenoceptors, the β -adrenoceptor signaling pathway has been poorly studied in this situation. We studied the β -adrenoceptor signaling pathway in Zucker lean, obese, and obese diabetic rats.

*See also p. 1552.

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Dr. Jiang did the statistics. Drs. Jiang, Riou, and Amour conceived the study and wrote the article. Drs. Jiang, Carillion, Na, and Feldman were involved in the experiments. Drs. Lacorte and Bonnefont-Rousselot performed the biochemical analysis. All authors read, corrected, and approved the final article.

Supported solely by institutional and/or departmental sources.

Dr. Jiang was the recipient of a doctoral grant from the People's Republic of China (the State Scholarship Fund by China Scholarship Council, file No. 201206270014). Dr. Carillion received grant support from the Fondation pour la Recherche Médicale. Dr. Feldman received grant support from Bourse Pierre Viars recherche 2012 and received support for article research from the Association pour la recherche clinique et expérimentale en anesthésie-réanimation de la Pitié-Salpêtrière. Dr. Riou served as a board member for LFB (Les Ulis, France); provided expert testimony for ThermoFisher (Clichy, France) and Crossject (Paris, France); lectured for ThermoFisher (Clichy, France), Sanofi (Paris, France), and LFB (Les Ulis, France); and received support for travel from ThermoFisher (Clichy, France) (all unrelated to this research). The remaining authors have disclosed that they do not have any potential conflicts of interest.

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DOI: 10.1097/CCM.0000000000000999

Design: Experimental, prospective study.

Setting: University medical research laboratory.

Subjects: Adult male Zucker lean (control), obese, and obese diabetic rats.

Interventions: The effects of β -adrenoceptor stimulation were investigated in vitro in isolated left ventricular papillary muscles in control, obese, and obese diabetic rats. β_1 -, β_2 -, and β_3 -adrenoceptors and multidrug resistance-associated protein 4 were quantified by Western Blotting. Triglyceride, cholesterol, leptin, adiponectin, and C-peptide plasma concentrations were measured. Data are mean \pm SD.

Measurements and Main Results: Hyperlipidemia, high leptin, and C-peptide concentrations were observed in obese and obese diabetic strains, whereas hyperglycemia occurred only in the diabetic strain. The positive inotropic effect of isoproterenol was slightly reduced in obese rats (183% \pm 11% of baseline; $p = 0.003$; $n = 7$) and markedly reduced in obese diabetic rats (137% \pm 18% of baseline; $p < 0.001$; $n = 10$) when compared with control rats (210% \pm 17% of baseline; $n = 9$). β_1 -adrenoceptors were down-regulated in obese (-41%; $p = 0.02$) and diabetic (-54%; $p = 0.003$) when compared with control rats, whereas β_3 -adrenoceptors and multidrug resistance-associated protein expression remained unchanged. Direct stimulation of adenylate cyclase with forskolin or administration of 3',5'-cyclic adenosine monophosphate suggests that subtle impairments also occurred beside the down-regulation of β_1 -adrenoceptor.

Conclusions: The positive inotropic effect of β -adrenoceptor stimulation is slightly decreased in Zucker obese rats and was more markedly decreased in Zucker diabetic rats. These decreases are mainly related to β_1 -adrenoceptor down-regulation. (*Crit Care Med* 2015; 43:e241–e249)

Key Words: β -adrenoceptor; cardiac muscle; catecholamines; diabetes; heart; obesity

There is a worldwide increasing burden of diabetes, obesity, and related cardiovascular diseases. Metabolic syndrome (obesity, hypertension, and diabetes) is known to be associated with increased cardiovascular risk, leading to

higher morbidity and mortality. The key elements of metabolic syndrome are obesity, impaired glucose tolerance, insulin resistance, dyslipidemia, hypertension, and increased sympathetic activity, all factors that may lead to cardiac dysfunction and ultimately congestive heart failure (1). Although obesity is associated with lower perioperative mortality (known as “obesity paradox”), patients with the metabolic syndrome are exposed to a higher mortality risk during the perioperative period (2). Such obesity paradox has also been reported in critically ill patients (3), including those with severe sepsis (4). However, several large clinical studies challenge the validity of the obesity paradox (5), and obese trauma patients are at risk of higher mortality from persistent hemorrhage (6).

In diabetic patients, diastolic dysfunction and a reduced response to β -adrenoceptor stimulation are observed, which may contribute to hemodynamic instability during critical care and the perioperative period (7). Although metabolic syndrome is associated with increased catecholamine levels and increased sympathetic activity that chronically stimulates β -adrenoceptors (8), the β -adrenoceptor signaling pathway has been poorly studied in this situation. Conflicting results have been obtained in obese swine and rabbit concerning cardiac β -adrenoceptor expression (9–11). The Zucker obese rat is considered as a reliable model of metabolic syndrome (12, 13). Furthermore, this model enables the separate study of obesity and obesity associated with type 2 diabetic status (14). We have previously observed alterations in β -adrenoceptor signaling pathway in type 1 diabetic (15) and senescent rats (16). Although an increase in sympathetic nervous system activation is an important mechanism for maintaining cardiac output, the positive inotropic response to β -adrenoceptor stimulation is altered in type 1 diabetic rats, in part, owing to the down-regulation of β_1 -adrenoceptor and the up-regulation of β_3 -adrenoceptor, which is the source of a negative inotropic effect. Active efflux transporters, namely the multidrug resistance-associated protein 4 (MRP4), also act as an independent endogenous regulator of intracellular cyclic nucleotide levels (3',5'-cyclic adenosine monophosphate, cAMP) (17) and has been recently shown to participate in β -adrenoceptor dysfunction during aging (18).

The aim of this study was to compare the effects of β -adrenoceptor stimulation in Zucker lean (as the control group), Zucker obese (also known as “Zucker fatty rats”), and Zucker obese diabetic (also known as “Zucker diabetic fatty rats”) rats *in vitro* using isolated left ventricular papillary muscle. Our hypothesis was that metabolic syndrome is associated with some degree of β -adrenergic dysfunction, which is aggravated when associated with diabetes. We precisely assessed the effects of β -adrenergic stimulation on both contraction and relaxation and particularly focused on the following possible mechanisms involved, that is, down-regulation of β_1 and up-regulation of β_3 -adrenoceptors (15, 16) and up-regulation of MRP4 (17, 18).

MATERIALS AND METHODS

Experiments were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985) in an authorized laboratory under supervision of an

authorized researcher (J.A.; A-75-20-81). The project had been approved by the relevant Animal Care Committee through the French Ministry of Higher Education and Research (Comité Régional d’Ethique en Expérimentation Animale Paris-Comité 3, Paris, France). Animals were purchased from Charles River (Saint Germain sur l’Arbresle, France) and cared in a labeled housing place (agreement number B-75-13-08) with food and water *ad libitum*. Animals were fed with normal rat chow containing 10% of calories from fat, 67% from carbohydrates, and 23% from proteins, 2.791 kcal/g (A04-10, SAFE, Augy, France). Three groups of 15-week-old male rats were studied: 1) Zucker lean (fa/–) rats; 2) Zucker obese (fa/fa) rats; and 3) Zucker obese (fa/fa) diabetic rats (12, 14). Because of the obvious morphological differences between groups, blinding was not possible.

Biological Measurements

Total cholesterol and triglyceride concentrations were determined by automated enzymatic methods (19, 20), and glucose concentration was assayed by hexokinase-mediated reaction (21) on a Modular P chemistry analyzer (Roche Diagnostics, Meylan, France). C-peptide was measured by quantitative enzyme-linked immunosorbent assay (ELISA) kit provided by Mercodia France (Paris, France). Rat leptin and rat total adiponectin levels were quantified using ELISA kits provided by BioVendor (Eurobio, Courtabeuf, France) and Alpco (Eurobio), respectively, and measurements were performed according to the manufacturer’s instructions.

Isolated Left Ventricle Papillary Muscle

Shortly after induction of general anesthesia with pentobarbital, the heart was removed *in bloc*, dissected, and weighed. The left ventricular papillary muscles were carefully excised and suspended vertically in a Krebs-Henseleit bicarbonate buffer solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.1 mM KH₂PO₄, 25 mM NaHCO₃, 2.5 mM CaCl₂, and 4.5 mM glucose) maintained at 29°C with a thermostatic water circulator and bubbled with 95% oxygen and 5% CO₂ as previously described (22). The papillary muscles were stimulated at 12 pulses/min for 60-minute stabilization period at the initial muscle length (L_{max}) at the apex of length–active isometric tension curve. The electromagnetic lever system has been described previously (22). All analyses were made from digital records of force and length obtained with a computer. Conventional mechanical variables at L_{max} were calculated from three twitches. The first twitch was isotonic and loaded with the preload corresponding to L_{max} . The second twitch was rapidly clamped to zero load just after the electrical stimulus with a critical damping. The third twitch was fully isometric at L_{max} . We determined the maximum unloaded shortening velocity (V_{max}) using the zero-load technique, and we determined maximum shortening ($_{max}Vc$) and lengthening ($_{max}Vr$) velocities and time to peak shortening (TPS) of the twitch with preload only. In addition, the maximum isometric active force normalized for cross-sectional area (AF), the peaks of the positive (+dF/dt) and the negative (–dF/dt) force derivatives at L_{max} normalized for cross-sectional area,

and the time to peak force (TPF) from the isometric twitch were recorded. Because changes in the contraction phase induce coordinated changes in the relaxation phase, indices of contraction–relaxation coupling have been developed to study lusitropy (23). The R1 coefficient ($R1 = \frac{Vc}{Vr}$) studies the coupling between contraction and relaxation under low load and thus lusitropy, in a manner that is independent of inotropic changes. R1 tests sarcoplasmic reticulum (SR) calcium uptake function (23). The R2 coefficient ($+dF/dt/-dF/dt$) studies the coupling between contraction and relaxation under high load and thus lusitropy, in a manner that is less dependent on inotropic changes, and reflects the myofilament calcium sensitivity (23). The cross-sectional area was calculated from the length and weight of papillary muscle, assuming a density of 1.

Since the contractility is nearly maximum at a calcium concentration of 2.5 mM in the rat myocardium, the extracellular calcium concentration was decreased from 2.5 to 0.5 mM to assess the inotropic response to β -adrenoceptor stimulations, as previously described (23). A decrease in AF between 45% and 65% of baseline was required when calcium concentration was decreased. β -adrenoceptor stimulation was induced with increasing concentrations of isoproterenol (10^{-8} to 10^{-4} M), a nonselective β -adrenoceptor agonist, in the presence of phentolamine (10^{-6} M) to block α -adrenoceptors (13). The effect of isoproterenol was expressed by the percentage of baseline value of the maximal effect of isoproterenol on AF and V_{\max} (Eff_{\max}) and the concentration of isoproterenol producing 50% of the maximal effect (C_{50}) (23).

We also studied the effects of stimulation of adenylate cyclase using forskolin (5×10^{-5} M) and the direct effect of dibutyryl cAMP (5×10^{-4} M) as previously described (24). All drugs were purchased from Sigma-Aldrich Chimie (l'Isle d'Abeau-Chesnes, France) and added volumes never exceed 2% of the total.

Immunoblotting

The left ventricles were removed from anesthetized rats and frozen in liquid nitrogen. Total proteins were extracted in a Triton 1% buffer with anti-phosphatase/protease inhibitor (Sigma-Aldrich). All protein concentrations were determined using Bradford reagent (BioRad, Marne-La-Coquette, France). After denaturation in Laemmli buffer, a fixed amount of mixed proteins was loaded in each lane of a sodium dodecyl sulfate polyacrylamide gel electrophoresis (9–15%). Proteins are separated by electrophoresis in a migration buffer and then transferred to a nitrocellulose membrane (Hybond, GE Healthcare, Vélizy, France). After saturation in milk or bovine serum albumin, each membrane was incubated overnight at 4°C with primary antibodies: anti-sarcoplasmic reticulum Calcium ATPase (SERCA-2a) (1:2,500, Abcam, Paris, France), antiphospholamban (1:1,000, Thermo Fisher Scientific, Rockford, IL), anti- β_1 -adrenoceptor (1:1,000, Santa Cruz Biotechnology, Le Perray en Yvelines, France), anti- β_3 -adrenoceptor (1:1,000, Santa Cruz Biotechnology), anti- β_2 -adrenoceptor (1:1,000, Santa Cruz Biotechnology), and anti-MRP4 (multidrug-resistance protein 4) (1:200, Abcam), respectively. The following day, membranes were washed with Tris-buffered saline Tween and incubated

with appropriate secondary antibody. Relative quantification of targeted protein was achieved by fluorescence recording on EthanDIGE reader with an ECL detection system (GE Healthcare, Vélizy, France). The SERCA 2a protein was detected at 110 kDa, phospholamban at 25 kDa, β_1 -adrenoceptor at 65 kDa, β_2 -adrenoceptor at 56–85 kDa, β_3 -adrenoceptor at 44 kDa, and MRP4 at 150 kDa. All Western blot experiments were quantified using Image J software (NIH, Bethesda, MD) and normalized versus glyceraldehyde 3-phosphate dehydrogenase (37 kDa), ensuring no variation in protein gel loading.

Statistical Analysis

Data are expressed as mean \pm SD. Comparison of means was performed using the repeated-measures analysis of variance and Newman-Keuls test. All *p* values were two-tailed, and a *p* value of less than 0.05 was considered significant. Statistical analysis was performed using NCSS 2007 software (Statistical Solutions, Cork, Ireland). Concentration response curves were determined by fitting the data to the Hill sigmoid pharmacological model according to the following equation: $\text{Eff}_o = \text{Eff}_{\max} (1 + (C_{50} C^{-1})^n)^{-1}$ in which Eff_o is the observed effect, Eff_{\max} is the maximum effect, C_{50} is the concentration that results in 50% of Eff_{\max} , *C* is the studied concentration, and *n* is the Hill coefficient. Iterative nonlinear regression curve fitting was used to obtain the best fit (Matlab 1.2c software; The MathWorks, South Natick, MA). The main endpoint of our study was the Eff_{\max} of the concentration-response curve of AF with isoproterenol. Assuming a value of Eff_{\max} of 179% \pm 15% in the control group (15), an α risk of 0.05, and a β risk of 0.20, we determined that a sample size of at least *n* = 7 papillary muscle per group would enable us to detect a 15% change in Eff_{\max} (PASS 11 software, Statistical Solutions).

RESULTS

Characterization of the Experimental Models

Obese rats had significantly higher body weight and heart weight than control rats, whereas obese diabetic rats did not significantly differ from control rats. The heart weight to body weight ratio was significantly decreased in obese rats mainly due to the greater increase in body weight than in heart weight (Table 1). Blood glucose levels were elevated (four times) only in obese diabetic rats. The C-peptide level, which reflects the insulin secretion, was markedly increased in obese rats while it was moderately increased in obese diabetic rats despite high blood glucose levels, suggesting β -cell dysfunction. Total cholesterol and triglycerides levels in both obese and obese diabetic rats were significantly higher than control rats. The leptin levels were significantly increased in both obese and obese diabetic rats. Adiponectin levels were not significantly modified in both obese and obese diabetic rats when compared with control rats (Table 1).

We measured the baseline characteristics of left ventricular papillary muscles in the different group of rats (Table 1). Although V_{\max} was not significantly modified, a significant increase in AF was observed in obese and obese diabetic rats. Prolongation of the duration of contraction was observed in

TABLE 1. General Characteristics of Zucker Lean (Control), Obese, and Obese Diabetic Rats

Variables	Control	Obese	Obese Diabetic
General characteristics (no. of rats)	<i>n</i> = 21	<i>n</i> = 21	<i>n</i> = 18
Body weight (g)	358 ± 25	498 ± 41 ^a	346 ± 23 ^b
Heart weight (mg)	921 ± 61	1,031 ± 77 ^a	888 ± 50 ^b
Heart weight/body weight (mg/g)	0.26 ± 0.01	0.21 ± 0.01 ^a	0.26 ± 0.01 ^b
Biological measurement (no. of rats)	<i>n</i> = 10	<i>n</i> = 10	<i>n</i> = 9
Blood glucose (mmol/L)	8.6 ± 1.6	8.6 ± 1.2	36.1 ± 4.9 ^{ab}
Total cholesterol (mmol/L)	2.58 ± 0.22	6.26 ± 0.71 ^a	4.64 ± 0.42 ^{ab}
Triglycerides (mmol/L)	1.39 ± 0.69	3.73 ± 1.38 ^a	3.00 ± 1.59 ^a
	<i>n</i> = 8	<i>n</i> = 9	<i>n</i> = 9
Leptin (μg/L)	5.2 ± 1.9	61.0 ± 11.8 ^a	10.0 ± 1.4 ^{ab}
Adiponectin (mg/L)	3.2 ± 0.4	3.3 ± 0.5	4.3 ± 1.0
C-peptide (μmol/L)	1.29 ± 0.51	6.59 ± 2.25 ^a	1.85 ± 0.53 ^{ab}
Mechanical properties (no. of muscles)	<i>n</i> = 28	<i>n</i> = 25	<i>n</i> = 24
<i>L</i> _{max} (mm)	4.2 ± 1.5	3.9 ± 1.8	3.1 ± 1.2 ^{ab}
Cross-sectional area (mm ⁻²)	0.55 ± 0.13	0.54 ± 0.20	0.43 ± 0.16 ^{ab}
Maximal unloading isotonic shortening velocity (<i>L</i> _{max} /s)	2.35 ± 0.38	2.43 ± 0.37	2.41 ± 0.50
Active force normalized per cross-sectional area during isometric contraction (mN/mm ²)	51 ± 8	59 ± 11 ^a	67 ± 13 ^{ab}
Time to peak shortening (ms)	164 ± 9	173 ± 12 ^a	196 ± 13 ^{ab}
Time to peak force (ms)	152 ± 11	160 ± 13 ^a	179 ± 16 ^{ab}
Ratio of maximum shortening to maximum shortening velocity to maximum lengthening velocity (i.e., contraction relaxation coupling under low load)	0.55 ± 0.10	0.57 ± 0.16	0.60 ± 0.11
Ratio of the peak of positive force derivative to the peak negative force derivative (i.e., contraction relaxation coupling under low load)	1.71 ± 0.23	1.87 ± 0.28	2.08 ± 0.40 ^a

*L*_{max} = initial muscle length.

^a*p* < 0.05 versus control group.

^b*p* < 0.05 between obese and obese diabetic rats.

Data are mean ± SD.

obese and obese diabetic rats as shown by the prolongation of TPS and TPE. No significant difference was observed in contraction-relaxation coupling under low load (R1, Table 1). By contrast, contraction-relaxation coupling under high load (R2) was significantly increased in obese diabetic rats (Table 1).

β-Adrenergic Stimulation

β-adrenoceptor stimulation induced a marked positive inotropic effect in control rats (Fig. 1 and Table 2). A slight decrease in this inotropic response was observed in obese rats and a marked decrease was observed in obese diabetic rats in low (*V*_{max}) and high (AF) loading conditions (Fig. 1 and Table 2).

In control rats, β-adrenoceptor stimulation induced a positive lusitropic effect under low load (R1) but not under high load (R2) (Table 2). Comparable effects were observed in control, obese, and obese diabetic rats, and there was no significant difference between strains (Table 2).

Figure 2 shows the comparison of the stimulation of β-adrenoceptors by isoproterenol, the stimulation of adenylate cyclase by forskolin, or direct stimulation by dibutyryl cAMP. The results obtained with forskolin were consistent with those obtained with isoproterenol, indicating a moderate impairment in the positive inotropic response in obese rats and a marked impairment in obese diabetic rats. By contrast, using dibutyryl cAMP, the degree of impairment in the positive inotropic response did not significantly differ between obese and obese diabetic rats (Fig. 2).

Immunoblotting

In agreement with the functional changes observed in the papillary muscle experiments, we found that the abundance of proteins for β₁-adrenoceptor were significantly reduced by 40% (*p* = 0.02) in obese rats and 54% (*p* = 0.003) in obese diabetic rats compared with levels measured in control rats

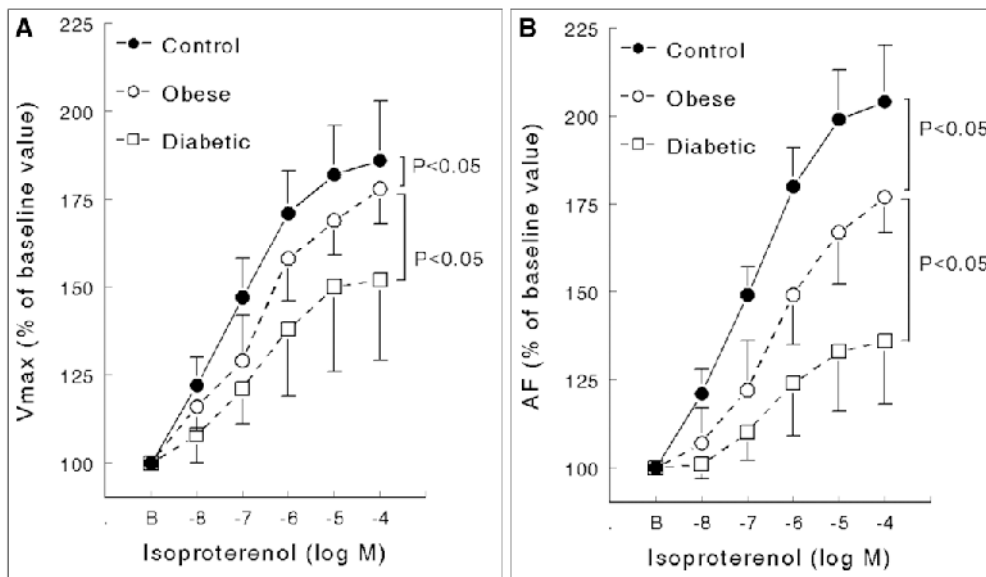


Figure 1. Inotropic response to β -adrenoceptor stimulation (isoproterenol) in control ($n = 9$), obese ($n = 7$), and obese diabetic ($n = 10$) Zucker rats under low (A) and high (B) loads. Data are mean percentage of baseline value \pm SD. The p values refer to the comparison of the maximum effect reported in Table 2. AF = isometric active force normalized by cross-sectional area, V_{max} = maximum shortening velocity.

(Fig. 3A). A similar decrease was observed in β_2 -adrenoceptor protein (Fig. 3B). By contrast, β_3 -adrenoceptors were not significantly modified in obese and obese diabetic rats ($p = 0.97$) (Fig. 3C). Consequently, the β_1/β_3 and β_2/β_3 ratios were significantly decreased in obese rats (2.4 ± 1.3 , $p = 0.01$ and 1.8 ± 1.3 ,

DISCUSSION

In the present study, we observed a slight decrease in the positive inotropic effect of β -adrenoceptor stimulation in obese rats. In obese diabetic rats, the decrease was more pronounced but not as severe as that previously observed in type 1 diabetic

($p = 0.03$, respectively) and in obese diabetic rats (2.0 ± 1.0 , $p = 0.003$ and 1.3 ± 0.8 , $p = 0.02$, respectively) when compared with control rats (4.2 ± 1.3 and 4.8 ± 4.0 , respectively). No significant changes were noted for phospholamban ($p = 0.71$) (Fig. 3D) and MRP4 ($p = 0.64$) (Fig. 3F). The abundance of SERCA 2a protein was significantly reduced by 40% ($p = 0.03$) in obese rats but not significantly modified in obese diabetic rats ($p = 0.67$) (Fig. 3E). But the SERCA 2a/phospholamban ratio was not significantly decreased ($p = 0.61$) in obese rat (3.5 ± 1.8) and obese diabetic rats (3.5 ± 1.8) when compared with control rats (3.9 ± 2.5).

TABLE 2. Comparison of the Inotropic Response to β -Adrenoceptor Stimulation (Isoproterenol) in Zucker Lean (Control), Obese, and Obese Diabetic Rats

Variables	Control ($n = 9$)	Obese ($n = 7$)	Obese Diabetic ($n = 10$)
Maximum unloaded shortening velocity			
Eff _{max} (% of baseline)	193 \pm 18	182 \pm 14	156 \pm 26 ^{a,b}
C ₅₀ (μ mol/L)	0.05 \pm 0.05	0.27 \pm 0.41	0.20 \pm 0.09
Maximum isometric active force normalized by cross-sectional area			
Eff _{max} (% of baseline)	210 \pm 17	183 \pm 11 ^a	137 \pm 18 ^{a,b}
C ₅₀ (μ mol/L)	0.11 \pm 0.11	0.53 \pm 0.63 ^a	0.53 \pm 0.32 ^a
Ratio of maximum shortening velocity to maximum lengthening velocity (i.e., contraction relaxation coupling under low load)			
Eff _{max} (% of baseline)	74 \pm 8	73 \pm 13	64 \pm 10
C ₅₀ (μ mol/L)	0.15 \pm 0.30	0.14 \pm 0.26	0.39 \pm 0.48
Ratio of the peak of positive force derivative to the peak of negative force derivative (i.e., contraction relaxation coupling under high load)			
Eff _{max} (% of baseline)	90 \pm 17	100 \pm 27	89 \pm 25
C ₅₀ (μ mol/L)	ND	ND	ND

C₅₀ = concentration of isoproterenol producing 50% of Eff_{max}; Eff_{max} = maximum effect in percentage of baseline value, n = number of papillary muscles, ND = not determined.

^a $p < 0.05$ versus control.

^b $p < 0.05$ between obese and obese diabetic rats.

Data are mean \pm SD.

C₅₀ was not determined when Eff_{max} was not significantly different from baseline values.

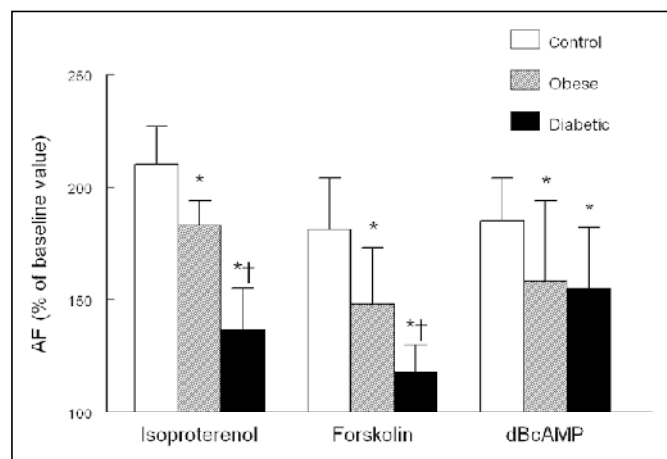


Figure 2. Comparison of the positive inotropic effects of isoproterenol (10^{-4} M), forskolin (5×10^{-5} M), and dibutyryl cyclic adenosine monophosphate (dBCcAMP; 5×10^{-4} M) in left ventricular papillary muscles from Zucker control, obese, and obese diabetic rats. Data are mean percentage of baseline value \pm SD ($n = 9-10$ per group). * $p < 0.05$ versus control; † $p < 0.05$ versus obese. AF = isometric active force normalized by cross-sectional area.

rats (11) (Fig. 4). The main mechanism involved is probably the down-regulation of β_1 - and β_2 -adrenoceptors, which occurred without up-regulation of β_3 -adrenoceptor and without modification in MRP4.

In Zucker obese rats, a single mutation A to C at nucleotide 880 (called "fa") in the leptin receptor gene on chromosome 5 causes a Gln to Pro substitution at residue 269 of the leptin receptor, leading to nonfunctional receptor. Zucker obese diabetic strain is a substrain selectively inbred for hyperglycemia, which carries an autosomal recessive defect in pancreatic β -cell transcription that is inherited independently of the fa mutation (25). The gene involved in this diabetic profile has not been yet identified but requires the fa mutation to induce diabetes. The Zucker obese rat model is associated with moderate hypertension without significant cardiac dysfunction, whereas the Zucker obese diabetic model is associated with no hypertension but mild diastolic cardiac dysfunction. We observed important metabolic changes with a different profile in obese and obese diabetic rats. These two strains are known to be associated with hyperlipidemia due to high low-density lipoprotein and high-density lipoprotein cholesterol, increased lipoprotein lipase activity, hyperinsulinemia, and insulin resistance, whereas hyperglycemia is observed only in the obese diabetic strain (male only) (25).

We did not observe noticeable myocardium dysfunction in obese and obese diabetic rats. AF was significantly increased in both obese and obese diabetic rats and associated with a prolongation of the contraction phase (increase in TPS and TPF). This prolongation of contraction has been previously reported in type I diabetic rats (15) and has been related to a slower cross-bridge cycling rate, a slower Ca^{2+} release from the SR, and an alteration of I_{to} potassium current.

A slight decrease in the positive inotropic response of β -adrenergic stimulation was observed in obese rats and a marked decrease in obese diabetic rats (Fig. 1 and Table 2).

Conflicting results have been obtained in obese swine and obese rabbit (high-fat diet model) concerning cardiac β -adrenoceptor signaling pathway (9–11). Lima-Leopoldo et al (26) concluded that no change occurs in cardiac function after β -adrenoceptor stimulation in obese rats, but a significant decrease was observed only at the highest concentration of isoproterenol, and the magnitude of the effect was comparable to our results. Our results suggest that the impairment in β -adrenergic signaling pathway is less severe in obese than in obese diabetic rats and less severe in obese diabetic than that previously observed in type 1 diabetic rats (15).

The observed decreases in the responses to β -adrenoceptor stimulation in both obese and obese diabetic Zucker rats are consistently explained by the down-regulation of β_1 - and β_2 -adrenoceptors (Fig. 3A). In Zucker obese rats, two earlier studies reported a decrease in β -adrenoceptor density but without separating β -adrenoceptor subtypes (27, 28). Our results markedly differ from those previously observed in type 1 diabetic rats (streptozotocin-induced diabetes) in which β_1 -adrenoceptor down-regulation is associated with β_3 -adrenoceptor up-regulation (15). This important difference may explain why the decrease in β -adrenoceptor stimulation was moderate in obese and obese diabetic Zucker rats in comparison to type 1 diabetic rats (15). It is well established now that the stimulation of β_3 -adrenoceptors results in nitric oxide synthase-derived nitric oxide which is at the beginning of cyclic guanosine monophosphate production. Thereafter, cyclic guanosine monophosphate activates inhibitory G proteins and phosphodiesterases that increase the catabolism of the cAMP produced by the β_1 - and β_2 -adrenoceptors stimulation (29) and thus results in a negative inotropic effect (15, 30). The down-regulation of β_1 - and β_2 -adrenoceptors in the heart of obese rats is consistent with the chronic sympathetic activation associated with the metabolic syndrome (31) and the catecholamine resistance of adipose tissue (32). In contrast with type 1 diabetes (15), the lack of β_3 -adrenoceptor overexpression observed in obese and obese diabetic rats did not impact the inotropic effect induced by β_1 - and β_2 -adrenoceptors stimulation. In this way, we still observed a decreased response with dibutyryl cAMP administration in both obese and obese diabetic rats.

However, down-regulation of β_1 - and β_2 -adrenoceptors is probably not the sole mechanism involved in the decreased response to β -adrenoceptor stimulation. When the papillary muscles were directly stimulated by dibutyryl cAMP, we still observed a decreased response in both obese and obese diabetic rats, which were not significantly different between these two strains (Fig. 2). This suggests that some abnormality occurs downstream the adenylate cyclase level. Further studies are required to elucidate that mechanism, although our study clearly eliminates the role of MRP4 (Fig. 3E), which has been recently demonstrated to be overexpressed during aging and to contribute to the decrease in β -adrenoceptor stimulation observed in old rats (19). In the present study, the lack in overexpression of MRP4 and the modest decrease in cardiac contractile responsiveness observed in obese rats (Fig. 1) suggest

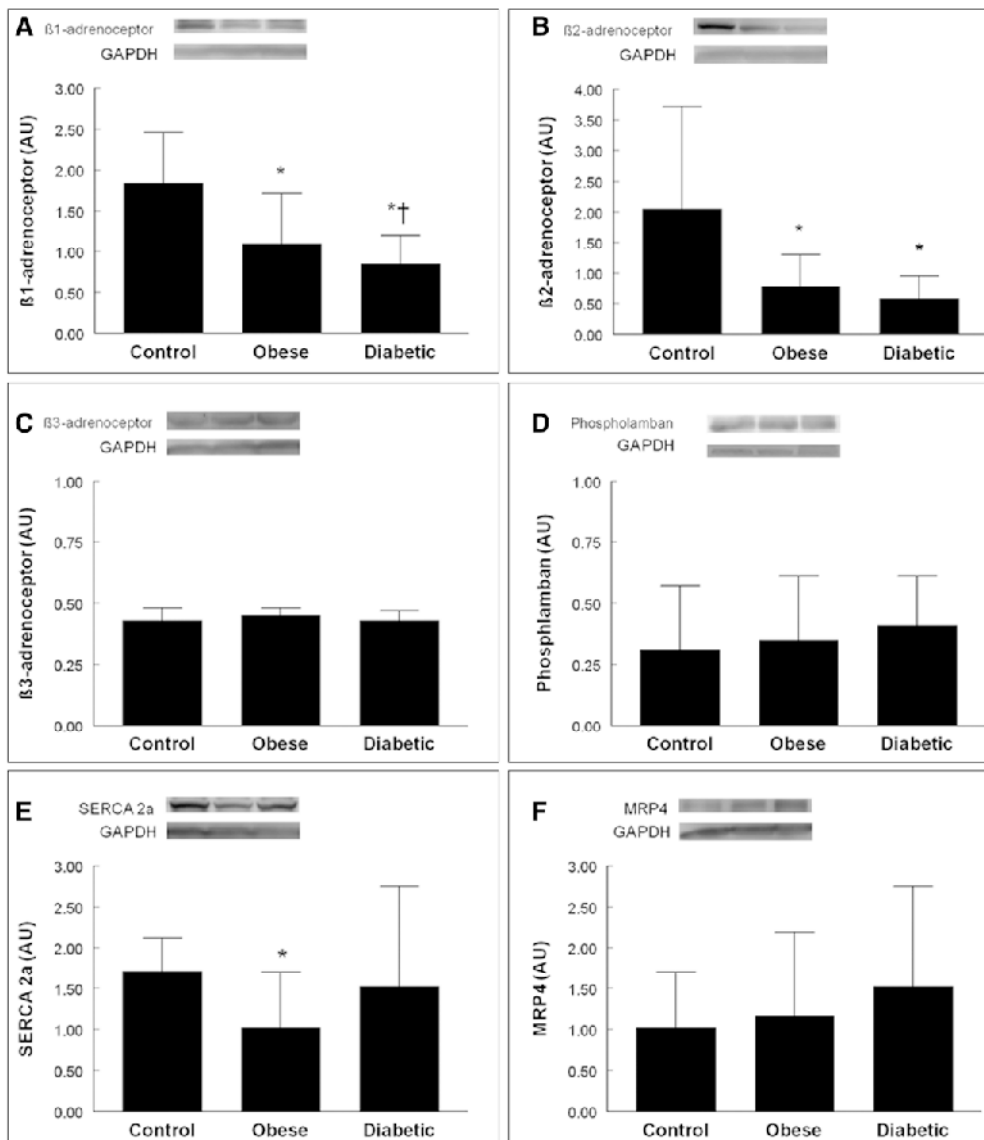


Figure 3. Representative Western blot and densitometric analysis reflecting β_1 -adrenoceptor (A), β_2 -adrenoceptor (B), β_3 -adrenoceptor (C), phospholamban (D), SERCA 2a (E), and multidrug resistance-associated protein (MRP4) (F) protein expression in Zucker control, obese, and obese diabetic rats ($n = 7$ in each group). Data are mean \pm sd. * $p < 0.05$ versus control; † $p < 0.05$ versus obese. GAPDH = glyceraldehyde 3-phosphate dehydrogenase, SERCA2a = sarcoplasmic reticulum calcium ATPase.

that this mechanism is of limited importance. To support this conclusion, when the production of cAMP by adenylyl cyclase was directly stimulated using forskolin (Fig. 2), we still observed a decreased response in both obese and obese diabetic rats, which was more pronounced in obese diabetic rats. These results contrast with those obtained in isolated membrane of heart cells in Zucker obese rats (25). This discrepancy may be explained by the use of isolated membrane preparation and not intact papillary muscle. Thus, it is likely that several other mechanisms participate in the impairment in the β -adrenergic signaling pathway, one situated at the level of adenylyl cyclase and the other downstream, beside the down-regulation of β_1 - and β_2 -adrenoceptors.

Lusitropy plays an important role in the maintenance of cardiac output. The lusitropic effect of β -adrenoceptor stimulation

was not modified in either obese or obese diabetic rats. The decrease in SERCA 2a, which occurred without significant changes in the SERCA 2a/phospholamban ratio, did not impact the positive lusitropic effect of β -adrenergic stimulation. These results are consistent with those reported in type 1 diabetic rats in which the lusitropic effects of β -adrenoceptor stimulation were preserved, despite diabetic cardiomyopathy (33). Such discrepancies between inotropic and lusitropic effects have been reported in other situations (24) and are probably related to the fact that smaller concentrations of cAMP are required to induce a maximal lusitropic effect (34).

Catecholamines are widely used in critically ill patients but considerable intra- and inter-individual variability exists in the response. Most previous studies have tried to elucidate pharmacokinetic and pharmacodynamic differences in catecholamine response (35, 36), but very few have considered differences which may be linked to the baseline characteristics of the patient. Recently, Bauman et al (37) demonstrated that ethnic differences may be associated with significant difference in vasopressor requirements in patients with septic shock, but these authors did not look

at genetic or phenotypic characteristics that might explain these differences. This study reported that the required doses of vasopressors were higher in Afro-American patients compared with white patients. However, the proportion of diabetic patients was also markedly different (68% vs 32%), and it is likely that the proportion of obese patients may also have been different, although this was not stated in the article. Future clinical research on the catecholamine requirements in the ICU should focus not only on genetic and phenotypic profile and catecholamine pharmacokinetics (35, 36) but also on the pathophysiologic conditions that may modify adrenoceptor signaling pathway.

The following limitations should be considered when assessing the clinical relevance of our results. First, this study was performed in rat myocardium, which differs from human myocardium.

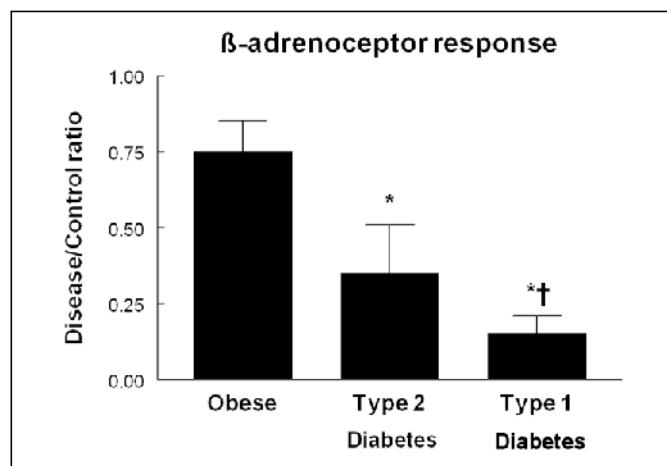


Figure 4. Comparison of the β -adrenergic stimulation (isoproterenol) response in Zucker obese, Zucker obese diabetic (type 2), and Wistar diabetic (type 1) rats. The ratio is the ratio of Eff_{max} in the disease strain to Eff_{max} in their respective control strain. Eff_{max} is the maximum effect on active isometric force of the concentration-effect curve of left ventricular papillary muscles exposed to increasing concentrations of isoproterenol. Data from type 1 diabetic rats (streptozotocin-induced diabetes) were obtained from Amour et al (15). Data are mean \pm sd. * $p < 0.05$ versus Zucker obese; † $p < 0.05$ versus Zucker obese diabetic rats.

Second, no animal model of obesity and diabetes completely mimicks diseases observed in humans, and thus, it is important to acknowledge the limitations of the leptin/leptin receptor-based rodent model. For example, Zucker obese rats have only moderate hypertension, do not develop premature atherosclerosis, and their endothelium-dependent relaxation is not impaired, and Zucker obese diabetic rats do not develop sympathetic neuroaxonal dystrophy (25). Thus, further studies in humans are required to validate our results. Third, we did not study changes observed after treatment of diabetes or food restriction in obesity. For example, it has been shown that food restriction may improve β -adrenergic stimulation in obese rats (28).

In conclusion, we observed a slight decrease in the positive inotropic effect of β -adrenoceptor stimulation in Zucker obese rats, which was more pronounced in Zucker obese diabetic rats and mainly related to the down-regulation of β_1 - and β_2 -adrenoceptors.

ACKNOWLEDGMENT

We thank Dr. David Baker, DM, FRCA (Department of Anesthesiology and Critical Care, Hôpital Necker-Enfants Malades, Paris), for reviewing the article and Dr. Michèle Guerre-Millo (Research Director, UMR-S INSERM 1166, IHU ICAN, Sorbonne Universités UPMC Univ Paris 06, Paris, France) for scientific advice.

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Chapter Four

Limitations

In our study, several limitations should be taken into consideration. Firstly, our study was conducted in rat myocardium. It differs from human myocardium. In rat myocardium, a negative staircase (increase in stimulation frequency decreases force) is observed, and the SR is more developed than in human.^[273] Thus, there would be some difficulties if applying our results to human myocardium. Secondly, no animal model of obesity or diabetes mellitus completely mimic diseases observed in human and thus it is important to acknowledge the limitations of the leptin/leptin receptor-based rodent models. Zucker obese rats have only moderate hypertension, do not develop premature atherosclerosis, and their endothelium-dependent relaxation is not impaired. Zucker diabetic rats do not develop sympathetic neuroaxonal dystrophy^[295]. Thus further studies in humans are required to validate our results. Thirdly, we did not study changes observed after treatment of diabetes or food restriction in obesity. For example, it has been shown that food restriction may improve β -adrenergic stimulation in obese rats^[205]. Fourthly, our study about rat myocardium *in vitro* was conducted at 29 °C because of the stability of the mechanical properties of the papillary muscles at this temperature. Meanwhile, this isolated left ventricular papillary muscle experiment can only observe the muscle performances without the inter-regulation of heart rates, loads, or wall geometry of heart *in vivo*^[401]. Fifthly, echocardiography was conducted under isoflurane anesthesia, and previous studies reported that the halogenated anesthetic agents can interfere with β -adrenergic stimulation in healthy rats and many kinds of cardiomyopathy^[253,275,402]. Although the potentiation of β -adrenoceptor stimulation was reported to be preserved with isoflurane in diabetic rats, our difference was still considered as the adjustment of neurohumoral compensatory mechanisms.

Chapter Five

Perspectives

Although the extrapolation of our experimental research into clinical practice should be considered with caution, due to several limitations, these experiments shed a new light on the cardiac dysfunction of obesity with or without type 2 diabetes mellitus. Our experiment demonstrates one of the basic mechanisms related to the cardiac dysfunction in obesity and diabetes mellitus, that is the alteration in the β -adrenoceptor signaling pathway. There is a slight decrease in the positive inotropic effects under the β -adrenergic stimulation in the myocardium of obese rats, and the decrease is more significant in the myocardium of type 2 diabetic rats, which are related to the down - regulation of β_1 - and β_2 -adrenoceptor proteins. Meanwhile, previous researches in our laboratory already showed that the down - regulation of β_1 -adrenoceptor and up - regulation of β_3 -adrenoceptor are associated with the decreased positive inotropic responses in type 1 diabetes mellitus^[55,56], which is mediated by NOS₁-derived NO.

A recent study has shown the years of life lost from diabetes and cardiovascular disease in the very obese people could be up to 8 years, which was 6 years in the obese people and 3 years in the overweight ones^[403]. In addition, the worst prognosis was in those who gained weight at young ages. So, it is urgent to further study the relative treatments to prevent the obesity and metabolic syndrome. In our experiment, we have not studied the influences on the cardiovascular functions after treatment to obesity or diabetes. It is worthwhile to continue the researches of the cardiac β -adrenergic signalling pathway under appropriate treatments.

Since the sympathetic nervous hyperactivity is one of the basic driving forces in human obesity and obesity - related disease, treatments to inhibit hyperactivity of sympathetic nervous system would be a useful method, including diet adjustments, physical exercises and some pharmacological treatments. The dietary weight loss and physical exercises are still the first line of therapy^[2]. It has been shown that food restriction may improve β -adrenergic stimulation in obese rats^[205]. The proportion of dietary components for the optimal cardiac health is still under research. The unsaturated fat is conventionally regarded as a “healthier” fat than saturated fat^[46]. However, in the human with metabolic syndrome, researchers recently found that high intake of saturated fat in the context of a low carbohydrate intake still did not generate the accumulation of plasma saturated fat acid, which is a predictor of the

increasing risk of diabetes and heart diseases^[404]. Secondly, physical exercises, regardless of low or high training volumes, have shown no effects on the decreased β -adrenoceptor responsiveness in the obese animals^[405,406], even though the improvements in body fat, circulatory norepinephrine and hemodynamic status could be observed. In one experiment on the STZ - induced diabetic rats, researcher found that the exercise trainings initiated after the onset of diabetes could delay the deterioration of myocardial contractility, reduce the loss of β_1 -adrenoceptor, and improve the responsiveness to the β -adrenergic stimulations *in vivo*^[251]. Thirdly, some drugs with neuroadrenergic effects showed different results in the metabolic syndrome and heart diseases. For an instance, antihypertensive drug diuretics might worsen the insulin resistance status and exacerbate the sympathetic hyperactivity in metabolic syndrome^[33,407]. The angiotensin - converting enzyme inhibitor (ACEI) or angiotensin receptor blockade (ARB) can improve insulin sensitivity and cause sympathoinhibitory effects^[407]. Statins and some hypoglycemic drugs were also found to exert sympathoinhibitory properties^[408]. The β -blockers, including carvedilol, metoprolol, bisoprolol and nebivolol, showed the beneficial influences on the survival and left ventricular remodeling in the chronic heart failure^[140,141]. Specific agonist or antagonist to β_3 -adrenoceptor might be one strategy for different stage of heart failure^[124]. As in our previous study, β_3 -adrenoceptor inhibitor could partially restore the decreased positive inotropic effects under the β -adrenergic stimulations in diabetic rats *in vitro*^[55].

Catecholamines are widely used in critically ill patients but considerable intra- and inter-individual variability exists in the response. Most previous studies tried to elucidate pharmacodynamics and pharmacodynamics difference in catecholamines response^[409,410], but very few considered differences linked to baseline characteristics of the patient. Recently, Bauman et al.^[411] demonstrated that ethnic differences may be associated with significant difference in vasopressor requirements in patients with septic shock, but overlooked the genetic or phenotypic characteristics that may explain these differences. This study reported that the required doses of vasopressors were higher in Afro-American patients compared with white patients. However, the proportion of diabetic patients was also markedly different (68 vs 32%), and it is likely that the proportion of obese patients may also have been different, although this was not illustrated in the article. Future clinical research in ICU should focus not only on the catecholamine requirements according to both genetic and phenotypic profile, but also on the pathophysiological conditions that may both modify adrenoceptor signaling pathway and catecholamine pharmacokinetics^[409,410].

Chapter Six

Conclusions

In the present study, we mainly confirmed the following conclusions:

1. According to the baseline values of transthoracic echocardiography (*in vivo*) and isolated left ventricular papillary muscles (*in vitro*), no alteration was observed in the baseline cardiac function of Zucker obese and obese diabetic rats.
2. The positive inotropic effects in response to β -adrenergic stimulations demonstrated a slight impairment in Zucker obese rats. This decrease in the positive inotropic effects to β -adrenergic stimulations was more pronounced in Zucker obese diabetic (type 2) rats. Comparing with the previous study of our laboratory, Zucker obese diabetic (type 2) rats showed less severe change in the positive inotropic effects than that previously observed in type 1 diabetic rats.
3. Those modifications were mainly associated with the down-regulation of β_1 - and β_2 -adrenoceptor proteins, and without up-regulation of β_3 -adrenoceptor protein.
4. Subtle impairments also occur upstream and downstream of the adenylate cyclase level of the β -adrenergic stimulation pathway in Zucker obese and obese diabetic rats, besides the down-regulation of β_1 - and β_2 -adrenoceptor.
5. Those modifications were not due to the over-expression of MRP4.

Chapter Seven

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Appendix

Other manuscripts published during the PhD. Candidate period

1. **Jiang C**, Jiang S, Zhao Y, et al. Dominant hand position improves the quality of external chest compression: A manikin study based on 2010 CPR guidelines. *J Emerg Med*, 2015, 48(4): 436-444.
2. **Jiang C**, Zhao Y. Reply to letter: Video recording and feedback of resuscitation. *Resuscitation*, 2012, 82(9): e181.



Selected Topics: Prehospital Care



DOMINANT HAND POSITION IMPROVES THE QUALITY OF EXTERNAL CHEST COMPRESSION: A MANIKIN STUDY BASED ON 2010 CPR GUIDELINES

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Abstract—Background: The 2010 cardiopulmonary resuscitation (CPR) guidelines increased the importance of external chest compression. However, the best hand position to be the compressing one has not been identified. **Objectives:** To investigate the effects of dominant or nondominant external chest compression hand position during CPR. **Methods:** Medical students performed five cycles of conventional CPR and completed one questionnaire. The CPR performances were manually evaluated, and detailed aspects of the external chest compression quality were assessed via the SimMan® Essential system (Laerdal China Ltd., Hangzhou, China). **Results:** One hundred fifty-seven students participated in the nondominant hand (NH) group, and 68 students participated in the dominant hand (DH) group. The manual evaluations revealed no differences between the two groups. The proportion of chest compressions “above 100 cpm [compressions per minute]” was higher in the DH group than in the NH group (97% vs. 92%, respectively, $p = 0.002$). The frequency distributions of the chest compression rates were also significantly different between the two groups ($p < 0.0001$). The distribution of the NH group was concentrated within “130–139” cpm, whereas this distribution was concentrated within “140–149” cpm in the DH group. The chest compression depth of the DH group was deeper than that of the NH group ($p = 0.001$). The depth of the fifth cycle was significantly decreased compared with those of cycles 1, 2, and 3 in the NH group. A greater number of full chest recoils were observed in the NH group ($p = 0.02$).

Conclusion: The dominant hand position during CPR was associated with a higher chest compression rate, a greater chest compression depth, and delayed fatigue. © 2015 Elsevier Inc.

Keywords—cardiopulmonary resuscitation (CPR); dominant hand; chest compression; fatigue; manikin

INTRODUCTION

External chest compression is the core of cardiopulmonary resuscitation (CPR). For over 40 years, researchers have been seeking the best manipulations for increasing the quality of CPR during sudden cardiac arrest, and the International Liaison Committee on Resuscitation renews its guidelines every 5 years (1,2). The American Heart Association (AHA) 2010 CPR guidelines recommend that the CPR sequence be changed to compression-airway-breathing (C-A-B), which increases the attention placed on the importance of chest compression (3). Moreover, the AHA also recommends “pushing hard and pushing fast.”

The guidelines for external chest compression suggest “placing the heel of one hand on the center (middle) of the victim’s chest and the heel of the other hand on top of the first so that the hands are overlapped and parallel” (3). Which hand should be used as the compressing hand (i.e., the lower hand that is in contact with the sternum)

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has not been identified. Kundra et al. showed that chest compressions are performed with fewer errors (i.e., the number of correct chest compressions is higher, and the compression depths and hand locations are better according to the 1998 European Resuscitation Council guidelines) when the dominant hand is in contact with the sternum of the Laerdal Skillmeter Resusci Anne (Laerdal China Ltd., Hangzhou, China) (4). Nikandish et al. found that positioning the dominant hand in contact with the sternum may increase the total number of the correct chest compressions during 5 min of hands-only CPR according to the European Resuscitation Council or AHA 2005 CPR guidelines when CPR is practiced on a recording Resusci Anne, but this difference was not statistically significant (5). Both of these studies were conducted based on the older CPR guidelines, which recommended that providers compress at a rate of approximately 100 compressions per minute (cpm), produce a compression depth of 40–50 mm, and that the compression to ventilation ratio be 15:2 (1998 guidelines) or 30:2 (2005 guidelines). Using an advanced manikin, the SimMan® Essential (Laerdal China Ltd.), we sought to record the parameters of CPR quality more objectively than the manikins used in the two previous studies. And meanwhile, we tried to explore whether the position of the dominant hand would influence the quality of external chest compression according to the new CPR guidelines using this objective simulation system and to learn more about the position of the dominant hand during CPR.

MATERIALS AND METHODS

Participants

All the medical students in the clinical department of our university who took a course in “Emergency Medicine” participated during the 2013/2014 academic year in their first standard CPR courses. The students had no previous experience using the SimMan Essential manikin. The study was approved by the Clinical Department of our university.

Study Procedures

As shown in Figure 1, the participants had received training in CPR skills via a formal classroom lecture that introduced the basic knowledge and skills with a PowerPoint presentation (Microsoft Corporation, Redmond, WA) and video, and repeatedly practiced their skills on a Resusci Anne (Laerdal China Ltd.) under the supervision of AHA-certificated Basic Life Support (BLS) instructors. During the courses, the instructors taught the hand placement according to the recommendations of the guidelines.

Within 1 week of the training, all participants took a CPR test that was based on the criteria of the CPR skill test in the Objective Structural Clinical Examination of our school. The participants were tested in ascending order of their student ID numbers and were presented with a simulated scenario of witnessing an adult collapse in the out-of-hospital setting. The manipulations were performed on the SimMan Essential. The participants were all asked to complete five cycles of complete conventional single-rescuer CPR on the manikin’s right side of approach.

The performances of the participants were manually evaluated by an AHA-certificated BLS instructor according to the CPR Objective Structural Clinical Examination criteria (a standard template) at the scene. Each performance was exhibited as a score (the full mark of the criteria is 100). Simultaneously, two observers recorded the results on the SimMan Essential tablet personal computer remotely in another room. The computer showed objective parameters of the CPR performances. We focused on the chest compression-related parameters, including chest compression rate, chest compression depth, and the chest recoil of every cycle. The students, monitors, and observers were all blind to the aims of the study. Finally, the students were required to complete one questionnaire that included nine single-choice questions. The participants indicated their general characteristics, dominant hands, their choices of compressing hands, and self-assessments of the quality of the CPR they had given (see Appendix). During the whole procedure of the study, the organizer of the study was at the scene to monitor and observe the test flow, including the occurrence of changing hand in contact with the sternum during CPR.

Next, two researchers collected the scores, the manikin results, and the questionnaires. The participants were then divided into two groups. A nondominant hand group (NH group) included those who had used their nondominant hand as the compressing hand (i.e., the lower hand that was in contact with the sternum). A dominant hand group (DH group) included those who used their dominant hand as the compressing hand.

Statistical Analyses

The data are expressed as the means \pm the SD and numbers or percentages. Evaluations of the distributions of the variables were performed using Levene’s test for homogeneity of variances. Comparisons of two means were performed using *t*-tests, comparisons of several means were performed using analyses of variance and least-significant difference tests, and comparisons of several percentages were performed using chi-squared tests or Fisher’s exact tests, as appropriate. Statistical analyses were performed using SPSS software (SPSS 13.0,

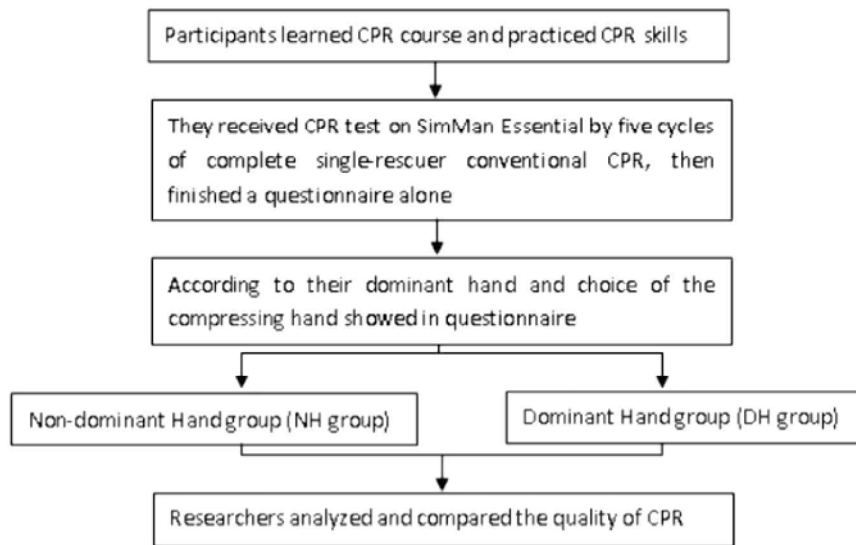


Figure 1. Schema of the study. CPR = cardiopulmonary resuscitation.

SPSS Inc., Chicago, IL). All *p* values were two-tailed, and *p* values < 0.05 were considered significant.

RESULTS

General Characteristics and Evaluations by the BLS Instructor

There were 228 students involved in the CPR course and test. There was no one changing hand in contact with the sternum during CPR. After the final collection of all of the data, three students were excluded because they

completed only two cycles of CPR. One of these students was pregnant, one experienced carpalitis, and one refused to perform all five cycles of CPR. Thus, we included totals of 157 participants in the NH group and 68 participants in the DH group. Some data were missing: the general characteristics of two cases in the NH group and three cases in the DH group were missing, no answer to question 3 was provided by one case in the NH group, and no answer was provided for question 8 by one case in the DH group.

There were no significant between-group differences in the main characteristics, with the exception of height (Table 1). There were also no significant differences in the manual scores (Table 2).

Table 1. General Characteristics of the NH and DH Groups

	NH Group (n = 155)	DH Group (n = 65)
Age, years		
18–24	110 (71%)	48 (74%)
25–31	44 (28%)	17 (26%)
>31	1 (1%)	0 (0%)
Gender		
Male	59 (38%)	30 (46%)
Female	96 (62%)	35 (54%)
Height (cm)		
150–159	38 (25%)	12 (19%)
160–169	90 (58%)	27 (42%)
170–179	23 (15%)	22 (34%)
180–189	4 (2%)	4 (6%)
Weight (kg)		
40–49	31 (20%)	14 (22%)
50–59	67 (43%)	22 (34%)
60–69	39 (25%)	17 (26%)
70–79	17 (11%)	10 (15%)
80–89	1 (1%)	2 (3%)

NH = nondominant hand; DH = dominant hand. The data are presented as numbers (percentages). Note: some of the items were missing: two cases in the NH group and three cases in the DH group did not provide all general characteristics.

Table 2. CPR Scores from the BLS Instructor and Total Chest Compression Qualities from the SimMan Essential of the NH and DH Groups

	NH Group (n = 157)	DH Group (n = 68)	<i>p</i> Value (Means)	<i>p</i> Value (Variance)
Scores	88 ± 4	88 ± 3	0.98	0.31
Rate (cpm)			0.002	NA
<100	61 (8%)	10 (3%)		
≥100	724 (92%)	330 (97%)		
Depth (mm)			0.001	NA
<50	630 (80%)	241 (71%)		
≥50	155 (20%)	99 (29%)		
Recoil (%)			0.02	NA
<100	118 (15%)	70 (21%)		
=100	667 (85%)	270 (79%)		

CPR = cardiopulmonary resuscitation; BLS = basic life support; NH group = nondominant hand group; DH group = dominant hand group; cpm = compressions per minute; NA = unavailable or not performed.

The data are presented as the means ± the SDs or as numbers (percentages).

Chest Compression Rate

The chest compression rates were 131 ± 21 cpm for the NH group and 133 ± 18 cpm for the DH group ($p = 0.18$). There were no differences between the cycles across the two groups or across the five cycles within the NH or DH group. We also analyzed the frequency distributions of the chest compression rates of each group. The frequency of chest compressions that were “above 100 cpm” was greater in the DH group than in the NH group ($p = 0.002$, Table 2). Moreover, the distributions were significantly different between the two groups ($p < 0.0001$; Figure 2). The highest proportion of the distribution of the NH group was “130–139” cpm, but in the DH group, this value was “140–149” cpm.

Chest Compression Depth

The chest compression depths were 43 ± 8 mm in the NH group and 44 ± 8 mm in the DH group ($p = 0.001$). There were no differences between any cycles across the two groups. In the NH group, the depth in cycle 5 (41 ± 8 mm) was significantly less than those of cycle 1 (44 ± 7 mm, $p = 0.002$), cycle 2 (43 ± 7 mm, $p = 0.006$), and cycle 3 (43 ± 8 mm, $p = 0.04$). However, in the DH group, there were no significant differences between the cycles (Table 3). The frequency of depths > 50 mm in the DH group was higher than that of the NH group ($p = 0.001$), as shown in Table 2.

Chest Recoil

The chest recoils were $98\% \pm 8\%$ in the NH group and $97\% \pm 10\%$ in the DH group ($p = 0.13$). There were no differences between any cycle between the two groups or

Table 3. Chest Compression Depths and Chest Recoils of the NH and DH Groups

	NH Group (n = 157)	DH Group (n = 68)	p Value (Means)	p Value (Variance)
Depth (mm)	43 ± 8	44 ± 8	0.001	0.37
Cycle 1	$44 \pm 7^*$	45 ± 8		
Cycle 2	$43 \pm 7^*$	45 ± 7		
Cycle 3	$43 \pm 8^*$	44 ± 8		
Cycle 4	42 ± 8	44 ± 8		
Cycle 5	41 ± 8	43 ± 8		
Recoil (%)	98 ± 8	97 ± 10	0.17	0.02
Cycle 1	99 ± 5	97 ± 7		
Cycle 2	98 ± 8	97 ± 11		
Cycle 3	98 ± 9	97 ± 11		
Cycle 4	98 ± 9	97 ± 11		
Cycle 5	98 ± 8	98 ± 9		

NH = nondominant hand; DH = dominant hand.
The data are presented as the means \pm the SDs.
* $p < 0.05$ vs. cycle 5 in the NH group.

across the five cycles within the NH group or DH group (Table 3). More of the chest recoils were full in the NH group ($p = 0.02$, Table 2).

CPR Training Questionnaire

Across all of the questionnaires, one participant in the NH group did not complete question 3, and one participant in the DH group responded to question 8 with “no fatigue” rather than choosing a single answer.

Within each group, most of the students were right-handed (98% in the NH group and 65% in the DH). Most students in the NH group believed that the use of the nondominant hand would optimize CPR quality (78% with the left hand and 9% with the right hand). In the DH group, the students primarily believed that the use of the dominant would be better (26% with the left hand and 46% with the right hand). In the NH and DH groups, 13% and 28% of the students, respectively, believed that the identity of the compression hand did not influence CPR quality.

Regarding chest compression rates, 66% of the students in the NH group and 69% in the DH group believed that they had achieved compression rates above 100 cpm. Regarding chest compression depth, 62% of the students in the NH group and 72% in the DH group believed that they had achieved chest compression accuracies above 76%. Regarding chest recoil, 32% of the students in the NH group and 32% in the DH group believed that they had achieved complete chest recoil. Regarding chest compression location, 78% of students in the NH group and 79% in the DH group believed that they had achieved accuracies above 76%. Regarding fatigue, the students felt fatigue at different time points (43% in the NH and 40% in the DH groups after the fourth cycle, and 39% in the NH and 45% in the DH groups after the fifth cycle).

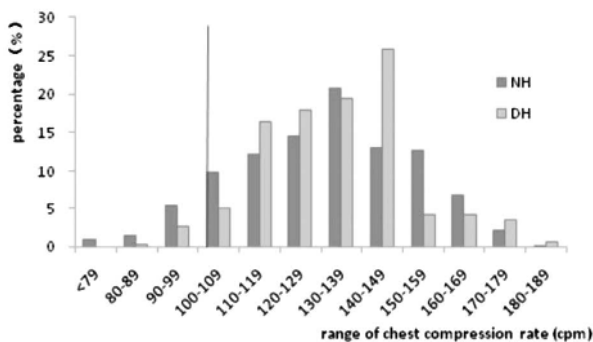


Figure 2. Frequency distributions of the chest compression rates of the NH and DH groups. There were significant differences in the distributions between the two groups ($p < 0.0001$). The line indicates the chest compression rate of 100 cpm, which is the minimum recommended chest compression rate according to the American Heart Association 2010 CPR guidelines. NH = nondominant hand; DH = dominant hand; cpm = compressions per minute.

Ninety-nine percent of the students in each of the NH and DH groups believed that they had nearly or completely mastered CPR during their training.

DISCUSSION

Chest compression is an especially important component of CPR. Chest compression provides sufficient blood flow when sudden cardiac arrest occurs. To optimize the quality of CPR, the AHA 2010 CPR guidelines changed the points of emphasis regarding chest compression. The newer guidelines stipulate that the adult chest should be compressed at a rate of at least 100 cpm and that the compression depth should be at least 50 mm to allow for complete chest recoil and to minimize the frequency and duration of interruptions in compressions (3). Several factors can affect the quality of chest compression, such as the position of victim, the position of rescuer, hand position, the depth and rate of compression, recoil, and the duty cycle (6). We found having the dominant hand in contact with the sternum during compression resulted in the following: 1) trends toward greater chest compression rates and deeper chest compressions; 2) a trend toward incomplete chest recoil; and 3) the delaying of fatigue compared to the use of the nondominant hand.

Kundra et al. showed that external chest compression is performed with fewer errors when the dominant hand of the rescuer is placed in contact with the sternum (4). This result was mainly due to the significant alteration in the magnitude of the force that is applied to the manikin (4). Nikandish et al. also found a trend toward an increasing incidence of correct chest compressions when the dominant hand is positioned to be the lower one (5). Our study used the SimMan Essential, which is a realistic full-body adult wireless patient simulator that offers comprehensive clinical functionality for the practice of airway, breathing, cardiac, and circulation skills. Some researchers have reported clinical improvements or equal performances when advanced realistic manikins are used, rather than less technologically advanced manikins, in clinical education (7–10). The AHA 2010 CPR guidelines also state that the evidence is insufficient to advocate for or against the routine use of more realistic manikins (11). However, the advantage of the manikin used here is its clear, objective, and easy-to-read demonstration of CPR performance, which provides a more convenient method for monitoring, analysis, and feedback. We found that the evaluation scores that were based on traditional evaluation methods and given by the BLS instructor were not different between the two groups. However, the analyses of each detailed performance from the SimMan Essential revealed important differences.

We wanted to obtain the primary parameters of chest compression quality from the SimMan Essential. Thus, we did not analyze the hand location during the compressions, which are shown by the computer only as hands that emerge in or outside of a “correct location circle” and not as a number or percentage. Regarding chest compression rates, both groups achieved the recommended rate (at least 100 cpm), and a higher proportion among the DH group achieved this rate. Comparisons revealed that a higher proportion of the DH group compressed more rapidly (140–149 cpm) (Figure 2). In both groups, the chest compression depths were less than the recommended depth (at least 50 mm), but a lower proportion of the DH group failed to meet this recommended depth. Although they were not deep enough, the compressions of the DH group were still 1 mm deeper than those of the NH group. Regarding chest recoil, neither group achieved complete recoil, but the proportion of participants that achieved 100% recoil in the DH group was lower than that of the NH group.

Hand dominance refers to the preference of one hand for performing fine and gross motor skills. Humans may have instinctive tendencies to perform tasks with the dominant hand to ensure that the required force, direction, and precision are applied. Our study was not designed to interfere with the choices of the participants. Thus, we did not ask the participants to perform CPR twice using each hand as the compression hand, as was done in the studies by Kundra et al. and Nikandish et al. (4,5). Therefore, we believe that we observed instinctual CPR performances, which may help us to identify the primary differences between the use of the dominant and nondominant hands. Similar to the results reported by Kundra et al., we observed superior chest compression performances in terms of compression rate and depth when the dominant hand was used (4). However, the proportion of full chest recoils was lower in the DH group. We believe that the faster and harder chest compressions might not have allowed for sufficiently complete chest relaxation. The depths of both groups were < 50 mm, and they performed CPR on the manikin’s right side. You et al. reported that a significant decrease in mean compression depth is observed when rescuers perform on the right side of the manikin with the left hand in contact with the sternum, regardless of hand dominance (12). Geddes et al. found that trained CPR rescuers exert more force than do laypersons, and thus perform more effective CPR (13). We presume that the students involved in our study could improve their chest compression depths via further training.

We compared the participants’ questionnaire results to the actual performances on the manikin. The assessments were approximately the same in terms of the accuracy of the chest compression rates. The students strongly

overestimated the accuracies of their chest compression depths, because only 20% of the students in the NH group and 29% in the DH group produced sufficient chest compression depths. However, the students underestimated their performances in terms of chest recoil. In the NH and DH groups, 85% and 79% of the students, respectively, achieved complete chest recoil, and these percentages were higher than those reported in the students' own assessments (32% in the NH and 32% in the DH groups). These results again verify the benefits of the use of advanced manikin examination analyses and feedback in CPR training. Moreover, these results may help instructors adjust future training courses.

Significant fatigue and shallow compressions are common after 1 min of CPR, although rescuers may not recognize that fatigue is present for more than 5 min (14). Our participants felt fatigue mostly from the fourth or fifth cycle on. The manikin data revealed that the participants in the NH group made significantly shallower compressions beginning in the fifth cycle, which is an obvious indicator of fatigue. However, no such difference was observed in the DH group. It seems intuitive that the dominant hand would be capable of sustaining the proper force and control for a longer period of time than the nondominant hand.

With the exception of height, there were no differences between the two groups in terms of general characteristics. Ødegaard et al. used a manikin to show that compression depth does not depend on rescuer gender, height, or weight (15). Nikandish et al. and Sandroni et al. showed that the rescuer's weight and height do not influence compression quality (5,16). We also analyzed the correlations between chest compression parameters (i.e., rate, depth, and recoil) and general characteristics (i.e., gender, height, and weight). We found that chest compression quality exhibited nearly no correlation with gender, height, or weight (data not shown).

Limitations

First, although we used an advanced realistic manikin, we did not examine real clinical resuscitations of patients. The feeling of the compressions, and the controls and motivations vary to some extent between humans and manikins, and this variation may have influenced the students' CPR performances. Secondly, the subjects of the study were students. Although our previous study showed that students are able to perform CPR as well as the medical staffs of emergency departments, we believe that it remains necessary to observe CPR performances in terms of the position of the dominant hand among different populations, such as skillful health care providers and the public (17). Third, we did not obtain objective results

regarding chest compression location, which is also an important component of chest compression.

CONCLUSIONS

Our study showed that the use of the dominant hand in the compression position during CPR can produce higher-quality CPR via greater chest compression rates, deeper chest compressions, and delayed fatigue.

Acknowledgments—We would like to thank all of the staff of the American Heart Association Emergency Cardiovascular Care Training Center (Zhongnan Hospital of Wuhan University) and the Emergency Diagnostic & Therapeutic Center of Central China. Their supports made this study possible.

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APPENDIX:

CARDIOPULMONARY RESUSCITATION (CPR) TRAINING QUESTIONNAIRE

Hello, dear student!

Thank you for attending the CPR training course. This survey is to help us learn more about your CPR studies and is not a test. Please choose one answer for each question with a “√”.

Thank you for your honest replies.

Name	Age	Sex	Height cm	Weight Kg
<p>1、 Which statement best describes you?</p> <p>A. Right-hand dominant B. Left-hand dominant</p> <p>2、 Which statement best describes your CPR performance just now?</p> <p>A. Right hand in contact with the chest, left hand overlapping the right hand</p> <p>B. Left hand in contact with the chest, right hand overlapping the right hand</p> <p>3、 The placement of which hand in contact with the chest would optimize the quality of CPR?</p> <p>A. Left hand B. Right hand C. No difference between the left and right</p> <p>4、 What is your opinion about the accuracy of your chest compression location during the CPR you performed just now?</p> <p>A. <25% B. 26-50% C. 51-75% D. ≥76%</p> <p>5、 What is your opinion about the accuracy of the chest compression depth of the CPR you performed just now?</p> <p>A. <25% B. 26-50% C. 51-75% D. ≥76%</p> <p>6、 What is your opinion about the chest compression rate of the CPR you performed just now?</p> <p>A. <80 cpm B. 80—90 cpm C. 90—100 cpm D. >100cpm</p> <p>7、 What is your opinion about the chest recoil of the CPR you performed just now?</p> <p>A. No recoil B. Less recoil C. Most recoil D. Complete recoil</p> <p>8、 In which cycle did you feel fatigue?</p> <p>A. 1 B. 2 C. 3 D. 4 E. 5</p> <p>9、 Please give yourself a score regarding your learning of CPR.</p> <p>A. 1 (no mastery) B. 2 (unsure mastery) C. 3 (nearly mastery) D. 4 (complete mastery)</p> <p>This is the end of the questionnaire. Thank you for your attendance and support!</p>				

ARTICLE SUMMARY

1. Why is this topic important?

According to the 2010 CPR guideline, external chest compression becomes the core of cardiopulmonary resuscitation (CPR). Although many factors can affect the quality of chest compression, hand choice to be the lower compressing one has not been clearly identified.

2. What does this study attempt to show?

We designed to explore the dominant hand position influences according to the new CPR guidelines, through an advanced manikin system.

3. What are the key findings?

We found that the dominant hand position in contact with the sternum during compression resulted in the following: 1) trends toward greater chest compression rates and deeper chest compressions; 2) a trend toward incomplete chest recoil; and 3) the delaying of fatigue compared to the use of the nondominant hand.



Reply to Letter to the Editor

Reply to Letter: Video recording and feedback of resuscitation

Sir,

We are grateful for the opportunity to reply to the helpful comments about our study made by Dr Ma and colleagues.

In our series of cardiac arrests, a total of 16 cases which were caused by haemorrhage (trauma, extrauterine pregnancy, cerebral haemorrhage). The treatment protocols for traumatic arrests include not just the ABCs, but also specific surgical interventions and coordinated team work. However, the aim of our study was to describe how recording of CPR performed in our emergency department (including all the adult cases) with real-time video and regular feedback learning may improve CPR – our emphasis was the quality of CPR. We still contend that we can improve the outcome of resuscitation from traumatic cardiac arrest by using video feedback.

The methods used to review the video recordings were described in our paper. The reviewers were trained to watch videos in the same mode, to record the time period, number of chest compressions and activities needed in a prospectively designed template. Both reviewers were blind to the date, time and other information in the video. If an assessment of one recording was different between two observers, a third expert physician was consulted to give the final decision. The lead investigator devised the indicators used in our study, which mainly reflected CPR quality. The feedback of the video recording to our resuscitation teams was undertaken every week. The attendants watched the video recording and discussed their performance according to our main indicators of CPR quality: (1) the initiation time of CPR; (2) instantaneous rates of chest compression; (3) hands-off time; and (4) intubation time.

We recorded the duration of each CPR session and calculated for other indicators such as average chest compression count in every minute (count/manual compression resuscitation Time (T_1)). We analyzed the T_1 for three groups: 13.17 ± 10.10 (min) (group 1), 19.67 ± 11.01 (min) (group 2) and 14.49 ± 10.61 (min) (group 3), which showed no significant difference among groups ($F = 1.582$, $P = 0.218$).

The hands-off time definition varies with different studies: Abella et al. recommended 1.5 s,¹ Wang et al. recommended periods longer than 2 s,² and another paper by Abella et al. recommended periods longer than 4 s.³ In our study, we did not want unnecessary hands-off time, so we defined it as longer than 1 s.

Conflict of interest statement

None.

Acknowledgment

We would like to thank Peng Yaqiong, lecturer of Foreign language department, Shanghai Institute of Electric Power, for the language editing to this paper.

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4 January 2012

RÉSUMÉ

Le système nerveux sympathique (SNS) a été identifié à être progressivement activé dans de nombreuses maladies cardio-vasculaires, du processus chroniques différents, y compris l'hypertension et de cardiomyopathie, à l'insuffisance cardiaque congestive^[82,156,412-414]. L'hyperactivité du système nerveux sympathique peut modifier le nombre, la fonction et la voie de signalisation en aval des récepteurs β -adrénergiques. Bien qu'il n'y ait pas de mécanisme unifié pour interpréter ces découvertes divergentes, des anomalies de calcium ont été reconnu comme une cause fondamental de la fonction systolique défectueuse du ventricule gauche^[82]. Le niveau de calcium intracellulaire est directement impliqué dans l'interaction entre l'actine et la myosine, qui contrôle la contractilité du muscle. Les stimulations des récepteurs β -adrénergiques peuvent induire des réponses inotropes / lusitropes positifs par la production d'adénosine monophosphate cyclique (AMPC) et l'activation de la protéine kinase A (PKA). Le calcium de type transitoire est augmenté suite à la phosphorylation des protéines ciblées (canaux calciques, récepteurs de la ryanodine, SERCA2a et troponine). Dans les conditions physiologique, la dysfonctionnement des récepteurs β -adrénergiques peut être un mécanisme commun de la réduction de la fonction cardiaque. Dans ce contexte, nous avons effectué des expériences au sujet de la voie de stimulation β -récepteurs adrénérgiques en deux états physiopathologiques différents, cardiomyopathie du syndrome métabolique et de personnes âgées.

La première expérience est menée au sein de rats sénescents. Au coeur sénescents, un dysfonctionnement diastolique et une réponse réduite à la stimulation β -adrénergiques ont été identifiés, qui sont associés à une sous-expression des récepteurs β_1 - et β_2 -adrénergiques, ainsi qu'à une sur-expression des récepteurs β_3 -adrénergiques.^[73,415,416] Ces changements permettent de réduire la production d'AMPC ou facilitent l'hydrolyse de l'AMPC. Ainsi, un mécanisme complémentaire de la régulation de l'AMPC a été impliqué dans le cœur. La protéine de multirésistance 4 (MRP4) joue un rôle important dans la régulation de l'AMPC intracellulaire et les réponses cardiaques stimulées par les récepteurs β -adrénergiques. Mais le rôle de MRP4 au coeur sénescents n'a jamais été étudié. Dans ce fait, nous avons mené de l'expérience pour étudier l'expression de MRP4 et son influence sur le dysfonctionnement β -adrénérgique dans le cœur sénescents. L'expression de MRP4 a été quantifiée dans ventriculaires gauches par Western Blotting. Les réponses des récepteurs β -adrénérgiques à l'isoprotérénol ont été étudiés *in vivo* (échocardiographie de stress) et *in vitro* (raccourcissement des sarcomères et de calcium de type transitoire dans les cardiomyocytes isolés par Ionoptix®) chez les rats jeunes (âgés de 3 mois) et les rats sénescents (âgés de 24 mois) prétraités ou non avec MK571, un inhibiteur spécifique de MRP4. En conséquence, nous avons confirmé que la sur-expression de MRP4 contribue à la diminution de la réponse inotrope positive du coeur sénescents à la stimulation de la β -adrénorécepteurs. La deuxième expérience est menée au sein des rats du syndrome métabolique. Bien que le syndrome métabolique est associé à une augmentation de l'activité sympathique qui stimule chroniquement les récepteurs β -adrénérgiques, la voie de signalisation de ces récepteurs impliqués dans cette situation a été très peu étudiée. En utilisant le modèle des rats Zucker témoins, obèses et obèses diabétiques (de type 2), nous avons étudié la voie de signalisation de ces récepteurs β -adrénérgique. Nous avons comparé la voie de signalisation des β -récepteurs adrénérgiques dans les rats Zucker témoins, Zucker obèses, et Zucker obèses diabétiques, qui sont les modèles de rats fiables du syndrome métabolique. Les effets de la stimulation des récepteurs β -adrénérgiques ont été évalué *in vivo* avec l'échocardiographie transthoracique et *in vitro* dans les muscles papillaires ventriculaires gauches des rats Zucker témoins, obèses et obèse diabétiques. L'expression des récepteurs β_1 -, β_2 -, et β_3 -adrénérgiques et de la protéine 4 associée aux résistances multidrogues dans les muscles ventriculaires

gauches a été quantifiée par Western Blotting. Les concentrations du triglycéride, du cholestérol, de la leptine, de l'adiponectine, et du peptide-C dans le plasma sanguin ont été mesurées avec la méthode enzymatique, ou la méthode immuno-enzymatique quantitative ELISA. Les données sont présentées en moyenne \pm SD. L'hyperlipidémie et des concentrations élevées de la leptine et du peptide-C ont été observés dans des souches obèses diabétiques et obèses, alors que l'hyperglycémie n'est présente que dans la souche diabétique. Aucune différence significative a été observée entre les souches *in vivo* en échocardiographie. *In vitro*, l'effet inotrope positif de l'isoprotérénol est légèrement réduite chez les rats obèses ($183\% \pm 11\%$ de la valeur de base, $P = 0,003$; $n = 7$) et nettement réduite chez les rats obèses diabétiques ($137\% \pm 18\%$ de la valeur de base, $P < 0,001$; $n = 10$) par rapport aux rats témoins ($210\% \pm 17\%$ de la valeur de base; $n = 9$). L'expression du récepteur β_1 -adrénergique est diminuée chez les rats obèses (-41% , $P = 0,02$) et les rats diabétiques (-54% , $P = 0,003$) par rapport aux rats témoins, et une diminution similaire a été observée en l'expression du récepteur β_2 -adrénergique. Alors que l'expression des récepteurs β_3 -adrénergiques et la protéine 4 associée aux résistances multidrogues reste inchangée. L'effet lusitrope de l'isoprotérénol n'est pas modifié entre les souches. L'effet inotrope positif de la forskoline est légèrement diminué chez les rats obèses ($148\% \pm 25\%$ de la valeur de base, $P = 0,005$) et nettement diminué chez les rats obèses diabétiques ($118\% \pm 12\%$ de la valeur de base, $P < 0,001$) par rapport aux rats témoins ($181\% \pm 23\%$ de la valeur de base). Lors de la stimulation de la 3', 5'-adénosine monophosphate cyclique par le dibutyryl cAMP, l'effet inotrope positif chez les rats témoins ($185\% \pm 19\%$ de la valeur de base) est significativement plus élevée que celui chez les rats obèses et chez les rats obèses diabétiques ($158\% \pm 36\%$ et $155\% \pm 27\%$ de la valeur de base; $P = 0,043$ et $P = 0,031$, respectivement). Outre une diminution de l'expression du récepteur β_1 - et β_2 -adrénergique, la stimulation directe de l'adénylate cyclase par la forskoline et l'administration de la 3', 5'-adénosine monophosphate cyclique suggère une déficience subtile s'est produite également au-dessus et au-dessous du niveau de voie des β -adrénergiques chez les rats obèses et diabétiques. En somme, l'effet inotrope positif de la stimulation des récepteurs β -adrénergiques est légèrement diminué dans les rats Zucker obèses et diminué plus nettement dans les rats Zucker obèses diabétiques. Ces diminutions sont principalement liées à une sous-expression des récepteurs β_1 - et β_2 -adrénergiques.

Mots clés récepteurs β -adrénergiques; muscle cardiaque; catécholamines; l'obésité; le diabète

ABSTRACT

The sympathetic nervous system (SNS) has been identified to be progressively activated in many cardiovascular diseases, from different chronic process including hypertension and cardiomyopathy, to congestive heart failure ^[82,156,412-414]. The hyperactivity of sympathetic nervous system may change the number, function and downstream mechanisms of β -adrenoceptors. Although there is no unified mechanism to interpret those divergent findings, calcium abnormalities has been recognized to be fundamental in the defective systolic function in left ventricle ^[82]. The intracellular calcium level is directly involved in the interaction of actin and myosin, thus reflects the contractility of muscle. β -adrenergic stimulation can induce the positive inotropic / lusitropic responses via the production of cyclic adenosine monophosphate (cAMP) and the activation of protein kinase A (PKA). Thus calcium transient is increased after phosphorylation of serials targeted proteins (calcium channel, ryanodine receptor, SERCA2a, and troponin). Under the pathophysiological condition, β -adrenergic dysfunction may be a common mechanism of decreased cardiac function. So, we performed experiments about β -adrenoceptor stimulation pathway in two different pathophysiological status, cardiomyopathy of the elderly and metabolic syndrome.

The first experiment is conducted within senescent rat. In the senescent heart, diastolic dysfunction and reduced response to β -adrenergic stimulation have been identified, which are associated with the down-regulation of β_1 - and β_2 -adrenoceptors, along with the up-regulation of β_3 -adrenoceptor. ^[73,415,416] These changes either reduce the cAMP production or facilitate the hydrolysis of cAMP. Meanwhile, a complementary mechanism of the regulation of cAMP has been involved in heart. The multidrug resistance protein 4 (MRP4) plays an important role in the regulation of intracellular cAMP and β -adrenergic stimulated cardiac responses. But the role of MRP4 in the senescent heart has never been studied. Thus, we conducted the experiment to study the MRP4 expression and its influence on β -adrenergic dysfunction in the senescent rat heart. MRP4 was quantified in left ventricular homogenates by Western blotting. The β -adrenergic responses to isoproterenol were investigated *in vivo* (stress echocardiography) and *in vitro* (sarcomere shortening and calcium transient of isolated cardiomyocyte by Ionoptix®) in young (3-month age) and senescent (24-month age) rats pretreated or not with MK571, a specific MRP4 inhibitor. As a result, we confirmed that the MRP4 overexpression contributes to the decrease of positive inotropic response to β -adrenoceptor stimulation in the senescent heart. The second experiment is conducted within metabolic syndrome rats. Although metabolic syndrome is associated with increased sympathetic activity that chronically stimulates β -adrenoceptors, the β -adrenoceptor signaling pathway has been poorly studied in this situation. We studied the β -adrenoceptor signaling pathway in Zucker lean, obese, and obese diabetic (type 2) rats. We compared the β -adrenoceptor signaling pathway in Zucker lean, Zucker obese, and Zucker obese diabetic rats, the reliable rat models of metabolic syndrome. The effects of β -adrenoceptor stimulation were investigated *in vivo* with transthoracic echocardiography and *in vitro* in isolated left ventricular papillary muscles in Zucker lean (control), obese, and obese diabetic rats. The expressions of β_1 -, β_2 -, β_3 -adrenoceptors and multidrug resistance-associated protein 4 in left ventricular muscles were detected by Western Blotting. The plasma concentrations of triglyceride, cholesterol, leptin, adiponectin, and C-peptide were measured using automated enzymatic method, or quantitative enzyme-linked immunosorbent assay (ELISA) kits. Data are presented as mean \pm SD. Hyperlipidemia, high leptin, and C-peptide concentrations were observed in obese and obese diabetic strains, whereas hyperglycemia occurred only in the diabetic strain. No significant difference among strains was observed in echocardiography *in*

vivo. *In vitro*, the positive inotropic effect of isoproterenol was slightly reduced in obese rat ($183\% \pm 11\%$ of baseline, $P=0.003$; $n=7$) and markedly reduced in obese diabetic rats ($137\% \pm 18\%$ of baseline, $P < 0.001$; $n=10$) when compared with control rats ($210\% \pm 17\%$ of baseline; $n=9$). β_1 -adrenoceptor protein expression were down-regulated in obese (-41% , $P=0.02$) and diabetic rats (-54% , $P=0.003$) when compared with control rats, and a similar decrease was observed in β_2 -adrenoceptors protein expression. But β_3 -adrenoceptor and multidrug resistance-associated protein 4 expressions remained unchanged. The lusitropic effect of isoproterenol was not modified among strains. The positive inotropic effect of forskolin slightly decreased in obese rats ($148\% \pm 25\%$ of baseline value, $P=0.005$) and markedly decreased in obese diabetic rats ($118\% \pm 12\%$ of baseline value, $P < 0.001$) when compared with control rats ($181\% \pm 23\%$ of baseline value). Upon stimulation of 3',5'-cyclic adenosine monophosphate by dibutyryl cAMP, the positive inotropic effect in control rats ($185\% \pm 19\%$ of baseline value) was significantly higher than that in obese rats or obese diabetic rats ($158\% \pm 36\%$, $155\% \pm 27\%$ of baseline value; $P=0.043$, $P=0.031$, respectively). Direct stimulation of adenylate cyclase with forskolin and administration of 3',5'-cyclic adenosine monophosphate suggests that subtle impairments also occur upstream and downstream of the adenylate cyclase level of the β -adrenergic pathway in obese and diabetic rats, besides the down-regulation of β_1 - and β_2 -adrenoceptor. In conclusion, the positive inotropic effect of β -adrenoceptor stimulation is slightly decreased in Zucker obese rats and is more markedly decreased in Zucker obese diabetic rats. These decreases are mainly related to β_1 - and β_2 -adrenoceptors down-regulation.

Key words β -adrenoceptors; cardiac muscle; catecholamines; obesity; diabetes mellitus

中文摘要

在许多心血管疾病中，包括从高血压、心肌病在内的各种慢性病到最终的充血性心力衰竭，交感神经系统进行性地被激活已经得到确认。^[82,156,412-414] 交感神经系统的高活性可以改变 β -肾上腺素受体的数量、功能以及其下游机制。尽管目前尚无简单统一的机制可以解释这些不同的发现，钙离子活动的异常却已经被认为是左心室收缩功能不全的一大基础原因^[82]。细胞内钙离子的水平直接影响着肌动蛋白与肌球蛋白的相互作用，从而影响着心肌收缩性。 β -肾上腺素能刺激可以通过刺激生成环磷酸腺苷（cAMP），并激活蛋白激酶 A（PKA），介导产生正性肌力/松弛力效应。随后，一系列目标蛋白（钙离子通道，兰尼碱受体，肌浆网钙泵，以及肌钙蛋白）紧接着被磷酸化后，钙离子瞬变得以增强。在病理生理状态下， β -肾上腺素能功能不全是心功能减低的一类常见机制。因此，我们分别针对衰老和代谢综合征相关心肌病，进行了有关 β -肾上腺素受体刺激通路的两项研究。

第一项研究针对衰老大鼠进行。在衰老心脏中，存在舒张功能不全和对 β -肾上腺素能刺激的效应下降。这些与 β_1 -和 β_2 -肾上腺素受体的下调，以及 β_3 -肾上腺素受体的上调有关。^[73,415,416] 这些改变要么减少 cAMP 的产生，要么促进 cAMP 的水解。同时还有其他调节 cAMP 的相关机制也在心脏中起到作用。多药耐药相关蛋白 4（MRP4）在心肌细胞内 cAMP 的调节和 β -肾上腺素能刺激效应方面起到了重要作用。但是 MRP4 在衰老心脏中的作用尚无相关研究。因此，我们进行了本研究，目的是观察衰老心脏中 MRP4 的表达和其对 β -肾上腺素能功能不全的影响。在年轻大鼠（3 月龄）和衰老大鼠（24 月龄）中，心脏对异丙肾上腺素刺激后的 β -肾上腺素能效应通过在体（超声心动图）和离体（离体心肌细胞通过 Ionoptix® 观察肌节的缩短和钙瞬变）。其效应的研究将在是否使用 MK571（一种特异性 MRP4 抑制剂）进行预处理之间进行对比。左心室组织的 MRP4 蛋白表达将通过免疫印迹法进行测定。实验结果显示，我们确认了 MRP4 蛋白的过度表达是导致衰老心脏中 β -肾上腺素能刺激后正性肌力反应下降的一个原因。第二项研究针对代谢综合征大鼠进行。尽管由于代谢综合征存在的交感神经活动性增加，会对 β -肾上腺素受体产生慢性刺激，但是， β -肾上腺素能受体信号传导通路在这种情况下的研究仍较少。因此，我们研究了 Zucker 瘦，肥胖和肥胖糖尿病（2 型糖尿病）大鼠的 β -肾上腺素能受体信号传导通路的变化。利用 Zucker 瘦，肥胖和肥胖糖尿病（2 型糖尿病）大鼠， β -肾上腺素能的反应性得以研究。我们研究在体超声心动图下 β -肾上腺素受体的刺激效应，还进行了离体左心室乳头肌的反应性研究，并对 β_1 -、 β_2 -和 β_3 -肾上腺素受体和多药耐药相关蛋白 4 进行免疫印迹定量研究。同时，利用自动酶标法或定量酶联免疫吸附测定试剂盒，我们测定血浆中甘油三酯、胆固醇、瘦素、脂联素以及 C-肽的浓度。数据以平均值 \pm 标准差表达。在 Zucker 肥胖和肥胖糖尿病大鼠中，我们观察到高脂血症，高瘦素和高 C-肽浓度，但是，只有肥胖糖尿病大鼠可以观察到高血糖。在体实验中，各种系大鼠的超声心动图表现无显著性差异。离体实验中，与对照组（基线的 $210\% \pm 17\%$ ， $n=9$ ）相比，异丙肾上腺素的正性肌力作用在肥胖大鼠中略有减少（基线的 $183\% \pm 11\%$ ， $P=0.003$ ； $n=7$ ），而在肥胖糖尿病大鼠中则显著降低（基线的 $137\% \pm 18\%$ ， $P<0.001$ ； $n=10$ ）。 β_1 -肾上腺素受体蛋白表达在肥胖大鼠中减少（ -41% ， $P=0.02$ ），在肥胖糖尿病大鼠中也显著减少（ -54% ， $P=0.003$ ）； β_2 -肾上腺素受体蛋白具有与 β_1 -肾上腺素受体相似的表达减少；但 β_3 -肾上腺素受体和多药耐药相关蛋白 4 的表达保持不变。各种系动物在异丙肾上腺素诱导中的松弛性影响均无显著性差异。与对照组（基线的 $181\% \pm 23\%$ ）相比，Forskolin 刺激产生的正性肌力作用在肥胖大鼠中略有减少（基线的 $148\% \pm 25\%$ ， P

=0.005)，而在肥胖糖尿病大鼠中出现显著减少（基线的 $118\% \pm 12\%$, $P < 0.001$ ）。当直接用 3',5'-环化腺苷酸类似物刺激时，在对照组大鼠中产生的正性肌力效应（基线的 $185\% \pm 19\%$ ）显著高于肥胖大鼠或肥胖糖尿病大鼠（基线的 $158\% \pm 36\%$, $155\% \pm 27\%$ ；分别 $P = 0.043$, $P = 0.031$ ）。用 Forskolin 刺激腺苷酸环化酶，或用 3',5'-环化腺苷酸类似物直接刺激的结果显示：除了存在 β_1 -和 β_2 -肾上腺素受体的下调，肥胖和肥胖糖尿病大鼠的 β -肾上腺素能通路中，腺苷酸环化酶水平的上游和下游，均有可能存在轻微的受损。Zucker 肥胖大鼠中， β -肾上腺素受体激动的正性肌力效应略有下降，而 Zucker 肥胖糖尿病大鼠的反应性则更为显著地下降。这些效应主要与 β_1 -和 β_2 -肾上腺素受体的下调有关。

关键词： β -肾上腺素受体；心肌；儿茶酚胺；肥胖；糖尿病