The Sympathetic Nervous System in Heart Failure: Physiology, Pathophysiology, and Clinical Implications
Filippos Triposkiadis, George Karayannis, Grigorios Giamouzis, John Skoularigis, George Louridas, and Javed Butler

*J. Am. Coll. Cardiol.* 2009;54;1747-1762
doi:10.1016/j.jacc.2009.05.015

*This information is current as of November 7, 2009*

The online version of this article, along with updated information and services, is located on the World Wide Web at:
[http://content.onlinejacc.org/cgi/content/full/54/19/1747](http://content.onlinejacc.org/cgi/content/full/54/19/1747)
Heart failure is a syndrome characterized initially by left ventricular dysfunction that triggers countermeasures aimed to restore cardiac output. These responses are compensatory at first but eventually become part of the disease process itself leading to further worsening cardiac function. Among these responses is the activation of the sympathetic nervous system (SNS) that provides inotropic support to the failing heart increasing stroke volume, and peripheral vasoconstriction to maintain mean arterial perfusion pressure, but eventually accelerates disease progression affecting survival. Activation of SNS has been attributed to withdrawal of normal restraining influences and enhancement of excitatory inputs including changes in:

1) peripheral baroreceptor and chemoreceptor reflexes;
2) chemical mediators that control sympathetic outflow; and
3) central integratory sites.

The interface between the sympathetic fibers and the cardiovascular system is formed by the adrenergic receptors (ARs). Dysregulation of cardiac beta1-AR signaling and transduction are key features of heart failure progression. In contrast, cardiac beta2-ARs and alpha1-ARs may function in a compensatory fashion to maintain cardiac inotropy. Adrenergic receptor polymorphisms may have an impact on the adaptive mechanisms, susceptibilities, and pharmacological responses of SNS. The beta-AR blockers and the inhibitors of the renin-angiotensin-aldosterone axis form the mainstay of current medical management of chronic heart failure. Conversely, central sympathectomies have proved harmful, whereas sympathomimetic inotropes are still used in selected patients with hemodynamic instability. This review summarizes the changes in SNS in heart failure and examines how modulation of SNS activity may affect morbidity and mortality from this syndrome. (J Am Coll Cardiol 2009;54:1747–62) © 2009 by the American College of Cardiology Foundation

Heart failure is a clinical syndrome that develops in response to an insult resulting in a decline in the pumping capacity of the heart. This is subsequently characterized by the continuous interaction between the underlying myocardial dysfunction and the compensatory neurohumoral mechanisms that are activated. Among many, these include the sympathetic nervous system (SNS), the renin-angiotensin-aldosterone axis, and the cytokine system (1). These systems are initially able to compensate for the depressed myocardial function and preserve cardiovascular homeostasis. However, their long-term activation has deleterious effects on cardiac structure and performance, leading to cardiac decompensation and heart failure progression.

SNS and Normal Cardiac Function

The SNS has a wide variety of cardiovascular actions, including heart rate acceleration, increase in cardiac contractility, reduction of venous capacitance, and constriction of resistance vessels. On the contrary, the parasympathetic nervous system affects the cardiovascular system by slowing heart rate through vagal impulses (Fig. 1). The cardiac sympathetic nerve fibers are located subepicardially and travel along the major coronary arteries representing the predominant autonomic component in the ventricles. The parasympathetic fibers run with the vagus nerve subependocardially after it crosses the atrioventricular groove and are mainly present in the atrial myocardium and less abundantly in the ventricular myocardium (2). The ventricular sympathetic innervation is characterized by a gradient from base to apex (3). The cardiac neuronal system is made up of spatially distributed cell stations comprising afferent, efferent, and interconnecting neurons behaving as a control system (4). The neurons are in constant communication with each other and each neuronal cell station is involved in cardio-cardiac reflexes that control spatially organized cardiac regions.

The sympathetic outflow to the heart and peripheral circulation is regulated by cardiovascular reflexes. Afferent fibers are usually carried toward the central nervous system by autonomic nerves, whereas efferent impulses travel from the central nervous system toward different organs either in autonomic or...
Cardiovascular Adrenergic Receptors (ARs)

The sympathetic transmitters NE and EPI bind to specific ARs, which are specialized macromolecules embedded in the cell membrane. Approximately 80% of NE released by the sympathetic nerve terminals is recycled by the NE transporter 1, whereas the remainder clears into circulation (8). Both NE and EPI exert their biological actions via activation of 9 different AR subtypes, 3 alpha1-receptors (alpha1A, alpha1B, and alpha1D), 3 alpha2-receptors (alpha2A, alpha2B, and alpha2C), and 3 beta-receptors (beta1, beta2, and beta3) (9). All ARs have 7 transmembrane receptors that signal primarily via interaction with heterotrimERIC G proteins.

The human heart contains beta1, beta2, and beta3 receptors (10). The beta1- and beta2-AR subtypes are expressed at a ratio of 70:30, and their stimulation increases cardiac contractility (positive inotropic effect), frequency (positive chronotropic effect), and rate of relaxation (lusitropic effect) as well as impulse conduction through the atrioventricular node (positive dromotropic effect). Beta2-ARs are predominantly inactive during normal physiologic conditions (11); however, their stimulation seems to produce a negative inotropic effect opposite to that induced by beta1- and beta2-ARs, involving the nitric oxide synthase pathway (12), acting as a “safety valve” during intense adrenergic stimulation (13). Agonist-induced activation of beta-ARs catalyzes the exchange of guanosine triphosphate for guanosine diphosphate on the G\sub\alpha -subunit of G proteins, resulting in the dissociation of the heterotrimer into active G\sub\alpha- and G\sub\beta\gamma -subunits, which are competent to signal independently (14). The heterogeneity of G-protein alpha subunit, of which there are ~20 subtypes (G\sub{a1}, G\sub{a2}, G\sub{a3}, and so on), is the central basis of G-protein coupled receptor signaling.

In the human heart, activation of beta1- and beta2-ARs is the most powerful physiologic mechanism to acutely increase cardiac performance. Beta1-ARs activate G\sub{s} proteins whereas beta2-ARs use both G\sub{s} and G\sub{i} proteins. G\sub{s} signaling acts as a “receptor-accelerator,” and G\sub{i} signaling as a “receptor-brake” (15). G\sub{i} signaling stimulates the effector enzyme, adenyl cyclase, resulting in dissociation of adenosine triphosphate (ATP) into the second messenger adenosine 3',5'-cyclic monophosphate (cAMP), which in turn binds to cAMP-dependent protein kinase A. Targets of protein kinase A-induced phosphorylations in the AR signaling pathway are: 1) the L-type calcium channels and ryanodine receptors, both leading to an increase in Ca\sup{2+} entry into the cell (16); 2) the hyperpolarization-activated cyclic nucleotide-gated channels, which generate the hyperpolarization-activated cation inward current (I\sub{f}) affecting the initiation and modulation of rhythmic activity in cardiac pacemaker cells (17); 3) phospholamban, a modulator of the sarcoplasmic reticulum associated ATP-dependent calcium pump, which accelerates Ca\sup{2+} reuptake by the sarcoplasmic reticulum accelerating cardiac relaxation (18); 4) troponin I and myosin binding protein-C, which reduce myofilament sensitivity to Ca\sup{2+} accelerating the relaxation of myofilaments (18); and 5) phospholemman, a subunit of Na+/K+-ATPase, relieving its inhibitory influence and resulting in the stimulation of the sodium pump (19) (Fig. 2).

Moreover, protein kinase A phosphorylates beta-ARs, resulting in partial uncoupling and desensitization of the receptor to further agonist stimulation (heterologous desensitization). G\sub{i} signaling decreases cAMP levels, activates mitogen-activated protein kinase, and contributes to the regulation of receptor signaling and activation of nuclear transcription.

The human heart also expresses alpha1A- and alpha1B-ARs at lower levels (~20%) than those of beta-ARs (20). It is unknown whether cardiac alpha1-ARs play a major role under physiologic conditions. Moreover, the alpha1-ARs heavily populate major arteries (including the aorta, pulmonary arteries, mesenteric vessels, and coronary arteries) and activation of these receptors by NE and EPI is a major contributor to the regulation of blood flow by vasconstriction (21). Both alpha1A- and alpha1B-AR subtypes couple to the G\sub{q} family of heterotrimeric G proteins, which, in turn, activate phospholipase C\sub{1}, Phospholipase C\sub{2} hydrolyzes phosphatidylinositol (4,5) bisphosphate to generate the second messengers inositol [1,4,5]-trisphosphate and 2-diacylglycerol. Inositol [1,4,5]-trisphosphate contributes to the regulation of intracellular Ca\sup{2+} responses, whereas 2-diacylglycerol activates some of the isomers of protein kinase C along with some of the transient receptor potential channels (Fig. 3). Stimulation of vascular alpha2B-ARs...
causes a transient vasoconstriction, whereas activation of central alpha2A-ARs leads to a decrease in blood pressure by inhibiting central sympathetic outflow (22). The release of NE from sympathetic nerves is controlled by pre-synaptic alpha2A- and alpha2C-ARs. Both pre-synaptic alpha2-Ars are essential, as deletion of alpha2A- and alpha2C-ARs leads to cardiac hypertrophy and failure due to chronically enhanced catecholamine release.

Assessment of SNS Activity

Activity of SNS is difficult to evaluate in the clinical setting. Plasma NE measurement represents a crude guide to assess sympathetic neural function because it depends on the rate of immediate NE reuptake as well as NE clearance from circulation (23). Likewise, frequency analysis of heart rate variability signals, although easily performed noninvasively, has been shown to have limitations (24). Indeed the low-frequency spectral power (approximately 0.05 to 0.15 Hz), in addition to cardiac sympathetic nerve traffic, depends on other factors, including multiple neural reflexes, cardiac AR sensitivity, post-synaptic signal transduction, and electrochemical coupling (25). Two state-of-the-art techniques that best quantify sympathetic nerve activity in humans are radiotracer measurements of regional NE spill-over and microneurography (microelectrode direct measurements of post-ganglionic sympathetic nerve activity—the

---

**Figure 1** The Sympathetic Nervous System

The most important level of integration of sympathetic nervous system efferent activities to the cardiovascular system resides in the dorsolateral reticular formation of the medulla. The hypothalamus modifies the activity of the medullary centers and is important in stimulating cardiovascular responses to emotion and stress. The motor outflow of the sympathetic nervous system is formed by 2 serially connected sets of neurons. The first set (pre-ganglionic neurons) originates in the brain stem or the spinal cord, and the second set (post-ganglionic neurons) lies outside the central nervous system in collections of nerve cells called sympathetic ganglia. The sympathetic pre-ganglionic neurons originate in the lateral horns of the 12 thoracic and the first 2 or 3 lumbar segments of the spinal cord (thoracolumbar outflow). The axons of these neurons (short, myelinated) exit the spinal cord in the ventral roots and then synapse on either sympathetic ganglion cells or chromaffin cells in the adrenal gland that release epinephrine (EPI). The sympathetic ganglia can be divided into 2 major groups: 1) the paravertebral (3 in the cervical region including the right and left stellate ganglia, 10 to 11 in the thoracic region, 4 in the lumbar region, 4 in the sacral region, and a single, unpaired ganglion lying in front of the coccyx), which lie on each side of the vertebrae and are connected to form the sympathetic chain or trunk; and 2) the prevertebral (pre-aortic), which provide axons that are distributed with the 3 major gastrointestinal arteries arising from the aorta. The predominant neurotransmitter of the sympathetic pre-ganglionic neurons is acetylcholine, whereas the predominant neurotransmitter of most sympathetic post-ganglionic neurons is norepinephrine. Sympathetic activity is attenuated (–) by the arterial baroreflex and the cardiopulmonary reflex and increased (+) by the cardiac sympathetic afferent reflex (CSAR) and the arterial chemoreceptor reflex. H = hypothalamus; M = medulla.
proximate neural stimulus to NE release) (26,27). These assessments allow discrimination between the central or peripheral contribution of increased plasma NE levels and precise estimation of the regional sympathetic neural function, both under physiological and pathological conditions.

Neural imaging techniques allow direct visualization of sympathetic innervation of human organs, thus providing information on the in vivo metabolism of NE in different cardiovascular beds. Cardiac neuronal distribution and function can be imaged with standard gamma-cameras and positron emission tomography using radiolabeled analogs of NE (28); whereas, post-synaptic beta-AR distribution and density can be determined using positron emission tomography (29). Although technical improvements have allowed for a more precise assessment of human adrenergic function, no technique so far available can be viewed as the “gold standard” (30). Limitations of the various techniques may be reduced if these methods are seen as being complementary and are employed in combination.

Cardiac sympathetic neuronal activity or its pharmaceutical inhibition can also be noninvasively assessed by the use of 123I-metaiodobenzylguanidine (MIBG), an analogue of NE (31), using semiquantitative analyses, namely early heart-to-mediastinum ratio, late heart-to-mediastinum ratio, and myocardial washout. Beta-blockade and renin-angiotensin-aldosterone inhibition are associated with an increase in 123I-MIBG uptake and a reduced washout. Data from a systematic meta-analysis suggest that patients with decreased late heart-to-mediastinum or increased myocardial 123I-MIBG washout have a worse prognosis than those patients with normal semiquantitative myocardial MIBG parameters (32). A prospective study that compared the predictive value of cardiac 123I-MIBG imaging for sudden cardiac death with that of the signal-averaged electrocardiogram, heart rate variability, and QT dispersion in patients with mild-to-moderate heart failure demonstrated that 123I-MIBG was the only powerful predictor of sudden cardiac death independently of left ventricular ejection fraction (33). Finally, the recently presented ADMIRE-HF (AdreView Myocardial Imaging for Risk Evaluation in Heart Failure) trial demonstrated that 123I-MIBG cardiac imaging carries additional independent prognostic information for risk-

Figure 2  Beta-AR Signaling

The major intracellular effect of the sympathetic transmitters norepinephrine and epinephrine is mediated by formation of 3',5'-cyclic monophosphate (cAMP), which increases the activity of the cAMP-dependent protein kinase A (PKA). PKA mediates a series of phosphorylations in diverse intracellular substrates, including the L-type Ca++ channels (LTCC), hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, sarcoplasmic ryanodine receptors (RyR), phospholamban (PLB), myofibrillar proteins troponin I (TnI), cardiac myosin-binding protein C (MyBPC), and phospholemman (PLM). AC = adenylyl cyclase; AR = adrenergic receptor; ATP = adenosine triphosphate; CNBD = cyclic nucleotide-binding domain; Galpha-i and Galpha-s = G protein alpha-subunit subtypes; SERCA = sarcoendoplasmic reticulum.

1750 Triposkiadis et al.
Sympathetic Nervous System in Heart Failure
November 3, 2009:1747–62
stratifying heart failure patients on top of commonly used markers such as left ventricular ejection fraction and B-type natriuretic peptide (34).

Heart Failure and SNS Hyperactivity

Myocardial systolic dysfunction is associated with neurohumoral hyperactivity aiming at preservation of cardiac output. The neuronal limb of this response is represented by SNS, whereas the humoral limb is represented by the increased secretion of certain hormones, the most important being those forming the renin-angiotensin-aldosterone axis (35). Sympathetic hyperactivity is evidenced by increased plasma NE levels, central sympathetic outflow, and NE plasma spillover from activated sympathetic nerve fibers (36). Indeed, application of isotope dilution methods for measuring cardiac NE plasma release indicate that in untreated heart failure patients, cardiac NE spillover is increased as much as 50-fold, similar to levels seen in healthy hearts during maximal exercise (37). There is limited information regarding chronic SNS activation in heart failure with preserved left ventricular ejection fraction (diastolic heart failure) (38). However, the findings of a recent study indicate that, in patients with hypertension, SNS hyperactivity (increased muscle sympathetic nerve traffic) may contribute to the development of left ventricular diastolic dysfunction and account for the increased cardiovascular risk (39).

In contrast to the increased muscle sympathetic nerve activity and NE spillover, patients with systolic heart failure may have decreased neuronal density, decreased neuronal function resulting in decreased NE concentration within the cardiomyocytes, or a combination, besides a reduction in post-synaptic beta-receptor density (40). The mechanism for decreased intracellular NE concentration is unclear. Experimental studies suggest reduced neuronal NE transporter expression and/or activity in association with heightened release (41). Moreover, complementary animal and human studies demonstrated a reduction in the myocardial content and rate of release of the nerve growth factor in

Figure 3  Alpha1-AR Signaling

Agonist-induced stimulation of alpha1-ARs activates Gq and phospholipase C (PLC), resulting in hydrolysis of phosphatidylinositol bisphosphate (PIP2) to generate inositol trisphosphate (IP3) and diacylglycerol (DAG). DAG, in turn, activates protein kinase C (PKC) to initiate a series of phosphorylations that alter channel activity and induce transcriptional changes. Moreover, IP3 interacts with perinuclear inositol trisphosphate receptors (IP3R) inhibiting growth-related gene transcription. Both PIP2 and DAG increase the permeability of the transient receptor potential (Trp) channel to Ca2+, which enters the cell and activates calcineurin to initiate downstream growth signaling pathways. Ca2+ entry through transient receptor potential channels may also act on myofilaments enhancing contractile responses. The alpha1-AR also transactivates epithelial growth factor receptors, resulting in formation of phosphoinositide 3-kinase (PI3K) and phosphatidylinositol trisphosphate (PIP3), activation of the Akt pathway, and initiation of cell-survival signaling pathways. Abbreviations as in Figure 2.
heart failure, which in turn modifies the expression of NE transporter in cell culture models (42).

**Mechanisms of SNS hyperactivity in heart failure.** The sympathetic hyperactivity observed in heart failure is closely related to abnormalities in cardiovascular reflexes. The sympathoinhibitory cardiovascular reflexes such as the arterial baroreceptor reflex are significantly suppressed, whereas the sympathoexcitatory reflexes, including the cardiac sympathetic afferent reflex and the arterial chemoreceptor reflex, are augmented (43). Moreover, in heart failure, the cardiac neuronal hierarchy undergoes remodeling and the spatially organized reflexes acting in isolation may destabilize efferent neuronal control of regional cardiac electrical and/or mechanical events. Also, the central nervous system may receive input from a variety of sources in the body and activate mechanisms that play a central role in progressive cardiac remodeling and dysfunction (Fig. 4) (44,45). For example, it has been demonstrated that the angiotensin-II and aldosterone produced locally in the brain affect SNS activation and progression of systolic heart failure (46,47). It seems that angiotensin-II initiates a positive feedback mechanism leading to up-regulation of the angiotensin II type 1 receptor, nitric oxide inhibition, and increased production of superoxide anion through the action of reduced nicotinamide adenine dinucleotide phosphate oxidase, leading to increased sympathetic outflow and disease progression (48,49).

**SNS hyperactivity and cardiac ARs.** Prolonged SNS activation adversely affects excitation-contraction coupling (50) and enhances apoptotic pathways (51), playing a central role in the progression of chronic heart failure. A striking feature of heart failure is a characteristic set of molecular alterations in the components of the beta-AR signaling pathway, including a decrease in beta 1-AR density and messenger ribonucleic acid, uncoupling of beta1-AR from Gs, increased Gi protein and messenger ribonucleic acid, and impaired compartmentalization of cAMP/protein kinase A signaling (52–54). Beta1-AR abnormalities have been attributed to the recruitment of both beta-AR kinase 1 and phosphoinositide 3-kinase to the ligand-activated receptor complex (55,56). In addition, the levels of both Gs signaling proteins and the G-protein receptor kinases are increased. It seems that beta-AR desensitization is a predominantly protective adaptation, which follows the increase in NE plasma levels that keeps intracellular cAMP concentration constant (57). This is further supported by the fact that overexpression of human beta1-ARs in transgenic mice initially augments cardiac function but eventually leads to pathologic hypertrophy and heart failure (58).

**Figure 4**  **Sympathetic Activation in Systolic Heart Failure**

(1) An insult causes cardiac dysfunction and decreases cardiac output. (2) Attenuation of inhibitory sympathetic cardiovascular reflexes and augmentation of excitatory sympathetic cardiovascular reflexes is associated with increased sympathetic input in the central nervous system. (3 and 4) Central facilitation of the augmented cardiovascular sympathetic afferent reflex mediated by an increase in angiotensin II and cytokines and a decrease in nitric oxide (NO) contributes to tonic increases in sympathetic output. (5) The chronic increase in sympathetic output is associated with structural and functional changes in the cardiomyocytes and the interstitium leading to left ventricular (LV) dilation and systolic dysfunction (LV remodeling), m = medulla; RAAS = renin-angiotensin-aldosterone system.
The role of beta2-ARs in heart failure has not been delineated clearly. There is no significant change in the levels of beta2-ARs in the failing heart, and studies in transgenic animals have demonstrated that, in contrast to the early cardiomyopathy resulting from low-level (≈5-fold) cardiac overexpression of beta2-ARs, even a 100-fold overexpression of beta2-ARs in the mouse heart significantly increases cardiac contractile force without any cardiomyopathic consequences; extremely higher levels of overexpression (up to 350-fold) are needed to induce pathological changes (59). Moreover, because beta2-AR signaling contributes to an increase in the levels of G_{i}, it has been suggested that this may activate a protective antiapoptotic pathway in the setting of catecholamine excess. Indeed, in a recent experimental study, selective inhibition of G_{i}-signaling in response to myocardial ischemia was associated with a marked enhancement of myocardial infarct size and apoptotic signaling (60). The role of beta1-ARs in heart failure has not been elucidated. However, it has been proposed that in heart failure there is an excess of beta2-AR signaling, which exerts a negative inotropic effect by increasing nitric oxide production and inhibiting calcium transients (61,62).

Alpha1-ARs may function in a compensatory fashion to maintain cardiac inotropy in the heart involved in both developmental cardiomyocyte growth as well as pathological hypertrophy (63). In the presence of pressure overload or with myocardial infarction, activation of alpha1-ARs, particularly the alpha1A-subtype, also appears to produce important pro-survival effects at the level of the cardiomyocyte and to protect against maladaptive cardiac remodeling and decomposition to heart failure (64,65). Recent evidence suggests that alpha2-AR deficiency is associated with elevated catecholamine levels, cardiac hypertrophy, fibrosis, and eventually heart failure (66).

**Catecholamine cardiotoxicity.** Catecholamine-induced cardiotoxicity is well known. Intravenous infusions of isoproterenol or NE result in acute contraction band lesions attributed to relative hypoxia, increased sarcolemmal permeability, calcium overload, elevation of cAMP, activation of alpha-ARs, activation of beta-ARs, and formation of oxidative catecholamine metabolites (67,68). Chronic catecholamine administration in rats causes interstitial fibrosis, reduces beta-AR-mediated inotropic responses, promotes myocyte apoptosis, and induces pump dysfunction primarily through left ventricular dilation (69,70). Moreover, exposure to NE of both adult and neonatal rat cardiac myocytes stimulates apoptosis via the adrenergic and the reactive oxygen species–tumor necrosis factor–caspase signaling pathways (71,72). Of note, catecholamines may induce oxidative damage through reactive intermediates resulting from their auto-oxidation, irrespective of their interaction with ARs, thus representing an important factor in the pathogenesis of catecholamine-induced cardiotoxicity (73).

Catecholamine surge has been implicated in the pathogenesis of stress (Takotsubo) cardiomyopathy or apical ballooning (74). It has been hypothesized that stress cardiomyopathy is a form of myocardial stunning, produced by the high levels of circulating EPI, which trigger a negative inotropic switch in intracellular signal trafficking in ventricular cardiomyocytes, from G_{i} protein to G_{i} protein signaling via the beta2-AR (75). This effect is magnified at the apical myocardium, where beta-AR density is greatest (76).

**AR Polymorphisms**

Marked variability in disease phenotype and response to treatment suggests a potential disease-modifying role for common genetic variants in heart failure. Functional polymorphisms in beta- and alpha-AR genes, which have been associated with heart failure phenotypes and interaction with beta-blockers, are under pharmacogenomics, the study of variations of deoxyribonucleic acid and ribonucleic acid characteristics as related to drug response, and pharmacogenetics investigations. Common polymorphisms of the beta2-ARs are: 1) the arginine to glycine switch at codon 389 (Arg389Gly), associated with significantly lower adenylyl cyclase activity in response to agonist than the Arg389 variant (77); and 2) the serine to glycine switch at codon 49 (Ser49Gly), associated with increased agonist-promoted down-regulation and adrenergic coupling than the Ser49 variant (78). Likewise, common polymorphisms of the beta1-ARs are: 1) the glycine to arginine switch at codon 16 (Gly16Arg), resulting in reduced agonist-promoted down-regulation (79); 2) the glutamine to glutamic acid switch at codon 27 (Gln27Glu), which is more resistant to receptor down-regulation; and 3) the threonine to isoleucine switch at codon 164 (Thr164Ile), demonstrating impaired receptor coupling and reduced adenylyl cyclase activation (80). Finally, a deletion mutation of amino acids 322 to 325 (alpha2Cdel322-325) in alpha2-AR gene leads to increased NE release (81).

A lower prevalence of ventricular arrhythmias has been attributed to the Gly389 allele (82) and improved survival has been reported for African-American heart failure patients with the G-protein receptor kinase 5–Leu41 variant (83).

**Therapeutic impact of beta-AR polymorphisms.** Many studies have examined the impact of the aforementioned polymorphisms in response to beta-blockade among heart failure patients. Preliminary studies in patients treated with various beta-blockers have demonstrated a survival benefit (84) and a significant reduction in left ventricular end-diastolic diameter (85) for those who carried the Gly49 allele as compared to the Ser49 homozygous patients. A similar reversal of the remodeling process has been reported for Arg389-homozygous heart failure patients treated with carvedilol as compared with Gly389–homozygous (86) and Arg389Gly-heterozygous patients (87). However, neutral results have also been reported. For example, no survival benefit was observed for the Ser49Gly or the Arg389Gly patients treated with carvedilol (79,88), bisoprolol (79), or...
metoprolol (88), and for the Arg389-homozygous or Gly389 patients treated with metoprolol CR/XL (89). Finally, a dose-dependent response to beta-blockade has been reported, whereby low doses in patients carrying the Gly49 allele portend worse outcomes than in Ser49-homozygous patients, whereas in higher doses, genotype-dependent differences are not observed (90).

More recent data, however, suggest improvement in the therapeutic effect of bucindolol by pharmacogenetic targeting. In the deoxyribonucleic acid substudy of the BEST (Beta Blocker Evaluation of Survival Trial) (91), patients carrying the Arg389 genotype had a greater agonist-promoted ventricular contractility and improved age-, sex-, and race-adjusted survival than the Gly389 carriers (92). Combinations of the beta1-AR (codon 389 Arg→Gly) and alpha2c-AR (322–325 WT→Del) polymorphisms were recently used to stratify patients according to the clinical response to bucindolol into “very favorable,” “favorable,” and “unfavorable” genotypes (Table 1) (93). Finally, a combination of 3 genetic polymorphisms (the Arg389 allele included) acted as a predictor of appropriate shock therapies included) acted as a predictor of appropriate shock therapies in patients with and without heart failure carrying an implantable cardioverter-defibrillator, thus identifying those at high risk for sudden cardiac death (94). In this respect, the use of genetic polymorphisms as potential tools to guide therapeutic strategies seems a promising new approach.

**Therapeutic Implications**

Inhibition of SNS activity. **BETA-BLOCKERS.** Beta-blockers can be broadly classified into generations: 1) *first generation*, which are nonselective and competitively block both the beta1- and beta2-AR (propranolol, nadolol, timolol); 2) *second generation*, with much higher affinity for the beta1- than for the beta2-AR (atenolol, metoprolol, bisoprolol); and 3) *third generation*, which may be selective (celiprolol, nebivolol) or nonselective (bucindolol, carvedilol, labetalol) but all cause peripheral vasodilation mediated via alpha1-AR blockade (bucindolol, carvedilol, labetalol), beta2-AR agonism (celiprolol), or nitric oxide synthesis (nebivolol) (95). Cardioselectivity of beta-blockers is dose-dependent and decreases with larger doses. Both selective and nonselective agents have negative chronotropic and inotropic effects. Selective agents have a less inhibitory affect on the beta2-receptors and are less likely to cause peripheral vasodilatation (96). Exercise performance may be impaired to a lesser extent by beta1-selective agents, partially because beta2-blockade tends to blunt the exercise-induced increase in skeletal muscle blood flow. Finally, there are beta-blockers that at low concentrations antagonize the cardiostimulant effects of catecholamines but at high concentrations cause cardiostimulation. These cardiostimulant beta-blockers (e.g., pindolol, alprenolol, oxprenolol), widely known as nonconventional partial agonists, antagonize the effects of catecholamines through a high-affinity site (beta1HAR), but cause cardiostimulation mainly through a low-affinity site (beta1LAR) of the myocardial beta-,AR (97). Nonconventional partial agonists are considered potentially arrhythmogenic and should not be used for heart failure treatment.

The majority of beta-blockers are partially or totally metabolized by CYP2D6, whose gene has more than 90 different variants (98). Individuals homozygous for nonfunctional alleles are considered poor metabolizers. Several inconsistencies have been reported regarding the genotype-phenotype correlations for intermediate or extensive metabolizers (99). Poor metabolizers treated with metoprolol have a 5-fold higher risk for adverse effects (100), whereas the adverse effects of carvedilol and bisoprolol are less influenced by the genetic background (101,102).

Among all beta-blockers, bisoprolol (except in U.S.), carvedilol, and metoprolol succinate (except in Canada) are almost universally approved for the treatment of chronic heart failure (Table 2) (103–114). Chronic beta-blocker therapy improves left ventricular performance and reverses left ventricular remodeling, reduces risk of hospitalization, and improves survival. Various protective mechanisms linked with SNS antagonism have been attributed to beta-blockers, namely: 1) inhibition of catecholamine cardiotoxic effects; 2) beta1-AR up-regulation (carvedilol is an exception); 3) attenuation of neurohumoral vasoconstrictive, growth-promoting, and pro-apoptotic systems (renin-angiotensin-aldosterone system, endothelin); 4) subendocardial coronary flow enhancement (as a result of diastolic prolongation); 5) restoration of the reflex control on the heart and circulation; and 6) improved myocardial performance (by reducing heart rate and oxygen demand) (115).

**ALPHA-BLOCKERS.** In the Veterans Administration Cooperative Study (116), heart failure patients receiving the alpha1-blocker prazosin experienced worse outcomes than did those receiving the combined vasodilator therapy of hydralazine and isosorbidine dinitrate. Prazosin chronic use has been reported to increase catecholamine

---

**Table 1**

Outcomes by Beta1 and Alpha2c Genotype in the BEST Genetic Substudy

<table>
<thead>
<tr>
<th>End Point</th>
<th>Beta389Arg/Arg + Alpha2c322-325 WT or DEL Carrier (n = 493)</th>
<th>Beta389Gly Carrier + Alpha2c322-325 WT/WT (n = 413)</th>
<th>Beta389Gly Carrier + Alpha2c322-325 DEL Carrier (n = 134)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td>0.62 (0.39–0.99)</td>
<td>0.75 (0.48–1.17)</td>
<td>1.04 (0.43–2.54)</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td>0.52 (0.31–0.88)</td>
<td>0.60 (0.36–0.97)</td>
<td>1.11 (0.45–2.78)</td>
</tr>
<tr>
<td>Heart failure hospitalization</td>
<td>0.56 (0.39–0.82)</td>
<td>0.77 (0.53–1.13)</td>
<td>0.73 (0.35–1.53)</td>
</tr>
</tbody>
</table>

Data presented as relative risk (95% confidence interval). *p < 0.05; †p < 0.01.

Arg = arginine; BEST = Beta-Blocker Evaluation of Survival Trial; DEL = deletion; Gly = glycine; WT = wild type.
levels, diminishing any potentially beneficial action mediated through inhibition of the alpha3-receptor (117). In the ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) study (118), the doxazosin arm was terminated early because of the higher heart failure incidence.

Centrally acting agents. Central alpha2-ARs are possible targets of treatment, because their excitation inhibits SNS activation (119). Clonidine is a centrally acting agent with alpha2-agonist actions. In modest doses, it significantly attenuates cardiac and renal sympathetic tone in heart failure patients. Interestingly, chronic clonidine administration exerts marked sympathoinhibitory effects without further clinical deterioration (120). Large clinical trials, however, are needed to evaluate the prospective of its broader use in chronic heart failure.

The centrally acting sympatholytic agent moxonidine has also been used in heart failure patients. Moxonidine acts through both alpha2 and imidazoline receptors (121,122). It causes a marked dose-related reduction in plasma NE (123),

---

**Table 2: Large Randomized Trials With Beta-Blockers and the ACC/AHA Guidelines Recommended Doses**

<table>
<thead>
<tr>
<th>Beta-Blocker</th>
<th>Trial(s)</th>
<th>Year</th>
<th>n</th>
<th>Benefit</th>
<th>Initial Daily Dose(s) (103)</th>
<th>Maximum Dose(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoprolol</td>
<td>MDC (104)</td>
<td>1993</td>
<td>383</td>
<td>All-cause mortality or morbidity was 34% lower in the metoprolol than in the placebo group (HR: 0.66; 95% CI: 0.62 to 1.06; p = 0.058). The change in LVEF from baseline to 12 months was significantly greater with metoprolol than with placebo (0.13 vs. 0.06; p = 0.0001)</td>
<td>5–10 mg twice</td>
<td>100 mg twice</td>
</tr>
<tr>
<td>Metoprolol CR/XL</td>
<td>MERIT-HF (105)</td>
<td>1999</td>
<td>3,991</td>
<td>All-cause mortality was 34% lower in the metoprolol CR/XL group than in the placebo group (7.2% vs. 11.0%; HR: 0.66; 95% CI: 0.53 to 0.81; p = 0.00009)</td>
<td>12.5–25 mg once</td>
<td>200 mg once</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>U.S. Carvedilol HF Study Group (106)</td>
<td>1996</td>
<td>1,094</td>
<td>All-cause mortality was 65% lower in the carvedilol than in the placebo group (3.2 vs. 7.8%; HR: 0.66; 95% CI: 0.39 to 0.80; p = 0.001)</td>
<td>3.125 mg twice</td>
<td>25 mg twice</td>
</tr>
<tr>
<td></td>
<td>Australia/New Zealand HF Research Collaborative Group (107)</td>
<td>1997</td>
<td>415</td>
<td>All-cause mortality or morbidity was 26% lower in the carvedilol than in the placebo group (104 vs. 131; HR: 0.74; 95% CI: 0.57 to 0.95)</td>
<td>1.25 mg once</td>
<td>10 mg once</td>
</tr>
<tr>
<td></td>
<td>CAPRICORN (108)</td>
<td>2001</td>
<td>1,959</td>
<td>All-cause mortality was lower in the carvedilol than in the placebo group (12% vs. 15%; HR: 0.77; 95% CI: 0.60 to 0.98; p = 0.03)</td>
<td>1.25 mg once</td>
<td>10 mg once</td>
</tr>
<tr>
<td></td>
<td>COPERNICUS (109)</td>
<td>2001</td>
<td>2,289</td>
<td>Carvedilol reduced the combined risk of death or hospitalization for a cardiovascular reason by 27% (p = 0.00002) and the combined risk of death or HF hospitalization by 31% (p = 0.000004)</td>
<td>1.25 mg twice</td>
<td>10 mg twice</td>
</tr>
<tr>
<td></td>
<td>COMET (110)</td>
<td>2003</td>
<td>3,029</td>
<td>All-cause mortality was lower in the carvedilol than in the metoprolol group (34% vs. 40%; HR: 0.83; 95% CI: 0.74 to 0.93; p = 0.0017)</td>
<td>1.25 mg once</td>
<td>10 mg once</td>
</tr>
<tr>
<td>Bisoprolol</td>
<td>CIBIS (111)</td>
<td>1994</td>
<td>641</td>
<td>All-cause mortality did not reach statistical significance: 67 patients died on placebo, 53 on bisoprolol (HR: 0.80; 95% CI: 0.56 to 1.15; p = 0.22). Bisoprolol reduced HF hospitalization (p &lt; 0.01) and improved the functional status</td>
<td>1.25 mg once</td>
<td>10 mg once</td>
</tr>
<tr>
<td></td>
<td>CIBIS II (112)</td>
<td>1999</td>
<td>2,647</td>
<td>All-cause mortality was 34% lower with bisoprolol than on placebo (11.8% vs. 17.3%; HR: 0.66; 95% CI: 0.54 to 0.81; p = 0.0001)</td>
<td>1.25 mg once</td>
<td>10 mg once</td>
</tr>
<tr>
<td></td>
<td>CIBIS III (113)</td>
<td>2005</td>
<td>1,010</td>
<td>This study demonstrated that it may be as safe and efficacious to initiate treatment for CHF with bisoprolol as with enalapril</td>
<td>1.25 mg once</td>
<td>10 mg once</td>
</tr>
<tr>
<td>Nebivolol*</td>
<td>SENIORS (114)</td>
<td>2005</td>
<td>2,128</td>
<td>All-cause mortality or cardiovascular hospital admission occurred in 332 patients (31.1%) on nebivolol compared with 375 (35.3%) on placebo (HR: 0.86; 95% CI: 0.74 to 0.99; p = 0.039)</td>
<td>1.25 mg once</td>
<td>10 mg once</td>
</tr>
</tbody>
</table>

*Not endorsed in the ACC/AHA 2005 heart failure guidelines.

ACC/AHA = American College of Cardiology/American Heart Association; CAPRICORN = Carvedilol Post-Infarct Survival Controlled Evaluation; CHF = chronic heart failure; CI = confidence interval; CIBIS = Cardiac Insufficiency Bisoprolol Study; COMET = Carvedilol or Metoprolol European Trial; COPERNICUS = Carvedilol Prospective Randomized Cumulative Survival; HF = heart failure; HR = hazard ratio; LVEF = left ventricular ejection fraction; MDC = Metoprolol in Dilated Cardiomyopathy; MERIT-HF = Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure; SENIORS = Study of the Effects of Nebivolol Intervention on Outcomes and Rehospitalisation in Seniors with Heart Failure.
accompanied by evidence of reverse remodeling. However, in clinical trials, it led to increased mortality (124). Most likely, the harmful effects of sympatholysis are observed in hemodynamically unstable heart failure patients, who may need the adrenergic drive and, therefore, are pre-disposed to the adverse effects of adrenergic withdrawal (125). Beta-AR inhibition can be easily reversed by NE competition, which allows recruit adrenergic drive when needed to support cardiac function.

**Renin-angiotensin-aldosterone system modulating agents.** The renin-angiotensin-aldosterone system is activated in heart failure and the degree of activation correlates with prognosis. Angiotensin-II and aldosterone production enhances the release and inhibits the uptake of NE at nerve endings (126,127). Angiotensin-converting enzyme inhibitors have a predictable effect in increasing plasma renin and decreasing angiotensin-II and aldosterone levels, whereas NE and vasopressin reduction is attributed to the hemodynamic improvement (128). Plasma aldosterone levels may be elevated as high as 20-fold in patients with heart failure, primarily due to increased production by the adrenal glands following stimulation by the high plasma angiotensin-II concentrations. In addition to its electrolyte and metabolic effects, aldosterone promotes the development of myocardial fibrosis, facilitating the remodeling process and disease progression. Besides decreasing NE uptake, aldosterone has a negative effect on endothelial function and increases plasminogen activator inhibitor-1 levels. Two trials have shown benefit with aldosterone antagonists in heart failure patients and may be partially related to their effect on NE (129,130).

**SNS stimulation.** Dobutamine and milrinone represent the most commonly used inotropes targeting increase in cAMP levels (131). Dobutamine stimulates cAMP production by adenylate cyclase through the beta-adrenergic pathway, whereas the phosphodiesterase inhibitor milrinone prevents cAMP breakdown. Increased intracellular cAMP leads to an increase in endomyocyte calcium release. Elevated calcium levels enhance cardiac contractility through optimizing actin-myosin binding. However, increased calcium levels are also responsible for inotropic therapy side effects, especially arrhythmogenesis. Both inotropes produce a vasodilatory effect and can cause a reduction in arterial pressure; this is more prominent with milrinone (132). Finally, the effects of dobutamine are blunted when the patients are already on beta-blocker therapy (133).

Despite the clinical rationale for using inotropes for the failing heart, clinical trials conducted in chronic heart failure demonstrated disappointing results, with inotropic use significantly increasing mortality (134–136). For acute heart failure, the OPTIME-CHF (Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure) study compared 48-h intravenous infusion of milrinone with placebo in patients without absolute indication for inotropic therapy; milrinone was associated with increased morbidity and similar mortality rate compared with placebo (137). In the PROMISE (Effects of Oral Milrinone on Mortality in Severe Chronic Heart Failure) trial (136), 1,088 patients with severe chronic systolic heart failure were assigned to double-blind treatment with 40-mg oral milrinone daily or placebo, on top of conventional treatment including digoxin, diuretics, and an angiotensin-converting enzyme inhibitor. Despite its beneficial hemodynamic actions, long-term therapy with oral milrinone increased the morbidity and mortality of patients with severe chronic systolic heart failure. Likewise, the ADHERE (Acute Decompensated Heart Failure National Registry) database (138) showed a worse clinical course in acute heart failure patients receiving inotropes compared to vasodilators. In accordance with these findings, inotrope use should only be considered for the subgroup of acute heart failure patients with clinically evident hypoperfusion or shock, or as a bridge to more definitive treatment, such as revascularization or cardiac transplantation. However, this subgroup represents the minority of those with acute heart failure (≈10%).

**Combined SNS inhibition and stimulation.** Combined beta1-inhibition with beta2-stimulation with clenbuterol has been proposed as a treatment modality to achieve sustained reversal of severe heart failure in selected patients requiring left ventricular assist devices (139). The rationale for this approach was based on experimental studies demonstrating that clenbuterol treatment, alone or in combination with mechanical unloading, improved left ventricular function at the whole-heart and cellular levels by affecting cell morphology, excitation-contraction coupling, and myofilament sensitivity to calcium (140). After optimization of medical therapy and achieving a constant left ventricular size for at least 2 weeks, clenbuterol was administered at an initial dose of 40 μg twice daily, then at a dose of 40 μg 3 times daily, and finally at a dose of 700 μg 3 times daily. The dose was adjusted to maintain resting heart rate at a level below 100 beats/min. Prior to clenbuterol initiation, carvedilol was replaced by the selective beta1-blocker bisoprolol.

It should be taken into consideration, however, that in a recent small, randomized controlled study, clenbuterol use in patients with chronic heart failure was associated with a significant increase in both lean mass and lean/fat ratio as well as in muscle strength, and a decrease in exercise duration (141). Thus, the effectiveness of clenbuterol in heart failure patients should be further evaluated in larger prospective trials.

**Exercise.** Exercise intolerance is a characteristic of patients with chronic heart failure, and skeletal myopathy contributes to the limitation of functional capacity in heart failure (142). SNS activation and myogenic reflex engagement moderate the heart and muscle vasculature to maintain adequate blood pressure during exercise (143). However, sympathetic overactivity contributes to the skeletal myop-
Parasympathetic modulation. There is a complex, often antagonistic, interaction between the parasympathetic nervous system and the SNS mediated partially by the second messenger's cAMP and cyclic guanosine monophosphate. Indeed, vagus nerve afferent activation, originating peripherally, can modulate efferent sympathetic and parasympathetic function centrally and at the baroreceptor level. Moreover, efferent vagus nerve activation can have tonic and basal effects that inhibit sympathetic activation and release of NE at the pre-synaptic level. Cardiovascular effects of parasympathetic activation include heart rate reduction (indirectly by inhibition of the SNS and directly by hyperpolarization of sinus node cells) and vasorelaxation (through nitric oxide synthesis) or vasoconstriction (direct activation of smooth muscle) (150). The activity and physiological effects of the parasympathetic nervous system are attenuated in heart failure, including lack of attenuation of SNS activity (151). Clinical and experimental data suggest that beta-blockade in heart failure can augment reflex vagus nerve control of heart rate, by blocking cardiac sympathetic pre-junctional beta_2-AR that facilitate NE release (152), and by increasing the density of M_2 receptors, especially in endocardial tissues of the left ventricle free wall (153), resulting in improved heart rate variability measurements (154). Finally, there are experimental studies that suggest that repeated exposure with a nicotinic agonist during the development of heart failure results in not only preserved but also supranormal effects of parasympathetic stimulation on the sinus node and that vagus nerve stimulation therapy, when combined with chronic beta-blockade, elicits an improvement in left ventricular function and remodeling that is additive to that achieved with beta-blockade alone (155,156).

Other treatment modalities. DIGOXIN. Digoxin increases cardiac contractility in heart failure patients by reversibly inhibiting the alpha subunit of the sodium-potassium ATPase, reducing the transmembrane sodium gradient and indirectly inhibiting the sodium-calcium exchanger. Thus, it allows calcium to accumulate in cardiac myocytes and be taken up by the sarcoplasmic reticulum (157). In addition, digoxin improves baroreceptor function, decreases sympathetic tone, and increases parasympathetic tone, favorably influencing autonomic balance in heart failure (158–160). In heart failure patients with atrial fibrillation, the combination of digoxin and a beta-blocker controls heart rate, and for heart failure patients in sinus rhythm, digoxin should be considered as an adjunct therapy for uncontrolled symptomatology (161).

POSITIVE AIRWAY PRESSURE. Continuous or bi-level positive airway pressure has been employed for the treatment of obstructive or central sleep apneas, which are highly prevalent in heart failure. Obstructive sleep apnea may affect heart failure through mechanical, adrenergic, and vascular mechanisms, whereas central sleep apnea likely arises as a consequence of heart failure (162,163). Treatment of heart failure patients with coexisting obstructive sleep apnea by continuous positive airway pressure improves baroreflex sensitivity during wakefulness, augments left ventricular ejection fraction, and lowers blood pressure and heart rate (164). The result may be even better with the use of bi-level positive airway pressure (165). Evidence also supports the use of positive airway pressure for improving several parameters of cardiovascular function in heart failure patients with coexisting central sleep apnea. Studies in this patient population have shown that continuous positive airway pressure attenuates central sleep apnea, improves nocturnal oxygenation, increases left ventricular ejection fraction, lowers NE levels, and increases the 6-min walking distance (166). Moreover, its early institution reduces the frequency of central sleep apnea episodes and may prolong heart transplant-free survival (167).

Conclusions

The role of activated SNS in maintaining hemodynamic stability in the face of acute injury to the cardiovascular
system is well known. Similarly, its role in heart failure progression and the benefits of beta-blocker therapy are well documented. Several newer developments related to the SNS are at various stages of research and clinical application. Genetic polymorphisms in the ARs may prove to be one of the earliest clinical applications for personalized medicine, both in terms of risk prediction, and pharmacogenomic application and drug response. There has been a growing interest in the use of SNS activity assessment by 123I-MIBG imaging leading to an ongoing clinical trial. Role of alpha ARs in modulating cardiac function is unfolding and has potential therapeutic applications. Newer drugs that may modify the SNS, such as combined beta1-inhibition and beta2-stimulation with clenbuterol has sparked interest in patients with advanced heart failure requiring mechanical assist device support. Understanding SNS functioning in further details and its modulation will continue to provide pathophysiologic and therapeutic insights for heart failure management.

Reprint requests and correspondence: Dr. Javed Butler, Emory University Hospital, 1365 Clifton Road, NE, Suite AT430, Atlanta, Georgia 30322. E-mail: javed.butler@emory.edu.

REFERENCES

16. Zhao XL, Gutierrez LM, Chang CF, Hosey MM. The alpha 1-subunit of skeletal muscle L-type Ca channels is the key target for regulation by A-kinase and protein phosphatase-1C. Biochem Biophys Res Commun 1994;198:166–73.


113. Willenheimer R, van Veldhuisen DJ, Silke B, et al., on behalf of the CIBIS III Investigators. Effect on survival and hospitalization of initiating treatment for chronic heart failure with bisoprolol followed by enalapril, as compared with the opposite sequence: results of the randomized Cardiac Insufficiency Bisoprolol Study (CIBIS III). Circulation 2005;112:2426–35.


Key Words: heart failure • sympathetic nervous system • adrenergic receptor • beta-blockers • sympatholytics • sympathomimetic inotropes.
The Sympathetic Nervous System in Heart Failure: Physiology, Pathophysiology, and Clinical Implications
Filippos Triposkiadis, George Karayannis, Grigorios Giamouzis, John Skoularigis, George Louridas, and Javed Butler
*J. Am. Coll. Cardiol.* 2009;54;1747-1762
doi:10.1016/j.jacc.2009.05.015

This information is current as of November 7, 2009

<table>
<thead>
<tr>
<th>Updated Information</th>
<th>including high-resolution figures, can be found at:</th>
</tr>
</thead>
<tbody>
<tr>
<td>&amp; Services</td>
<td><a href="http://content.onlinejacc.org/cgi/content/full/54/19/1747">http://content.onlinejacc.org/cgi/content/full/54/19/1747</a></td>
</tr>
<tr>
<td>References</td>
<td>This article cites 166 articles, 95 of which you can access for free at:</td>
</tr>
<tr>
<td></td>
<td><a href="http://content.onlinejacc.org/cgi/content/full/54/19/1747#BIBL">http://content.onlinejacc.org/cgi/content/full/54/19/1747#BIBL</a></td>
</tr>
<tr>
<td>Rights &amp; Permissions</td>
<td>Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:</td>
</tr>
<tr>
<td></td>
<td><a href="http://content.onlinejacc.org/misc/permissions.dtl">http://content.onlinejacc.org/misc/permissions.dtl</a></td>
</tr>
<tr>
<td>Reprints</td>
<td>Information about ordering reprints can be found online:</td>
</tr>
<tr>
<td></td>
<td><a href="http://content.onlinejacc.org/misc/reprints.dtl">http://content.onlinejacc.org/misc/reprints.dtl</a></td>
</tr>
</tbody>
</table>