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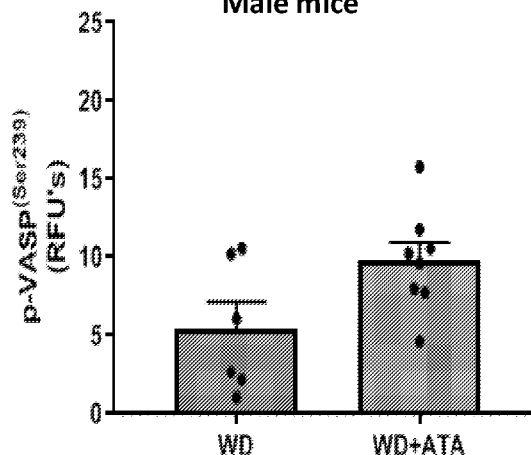
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(54) Title: METHODS AND MATERIALS FOR GENDER-DEPENDENT TREATMENT OF CARDIOVASCULAR DYSFUNCTION

FIG. 1A

Male mice



(57) Abstract: This document provides methods and materials involved in treating cardiovascular conditions such as calcific aortic valve stenosis. For example, methods and materials for using sGC agonists or a combination of sGC agonists and PDE5A inhibitors to reduce calcification of heart valves and/or vessels or to slow progression of aortic sclerosis to calcific aortic valve stenosis are provided.

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METHODS AND MATERIALS FOR GENDER-DEPENDENT TREATMENT OF CARDIOVASCULAR DYSFUNCTION

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims benefit of priority from U.S. Provisional Application
Serial No. 62/832,139, filed April 10, 2019.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

10 This invention was made with government support under TR000954 and
HL092235 awarded by the National Institutes of Health. The government has certain
rights in the invention.

BACKGROUND

1. Technical Field

15 This document relates to methods and materials involved in treating
cardiovascular conditions such as calcific aortic valve stenosis. For example, this
document provides methods and materials for using soluble guanylate cyclase (sGC)
agonists of the oxidized or non-oxidized form of sGC, or a combination of sGC
agonists and cGMP-specific phosphodiesterase 5A (PDE5A) inhibitors, to reduce
20 calcification of heart valves and/or vessels or to slow progression of aortic sclerosis to
calcific aortic valve stenosis in male patients.

2. Background Information

25 Calcific aortic valve stenosis is a disease in which the opening of the aortic
valve is narrowed. The symptoms of calcific aortic valve stenosis can vary depending
on the degree of valve stenosis. Patients with mild to moderate calcific aortic valve
stenosis may lack symptoms, which typically appear in patients with severe calcific
aortic valve stenosis. Symptoms can include progressive shortness of breath on
exertion, syncope, chest pain, and sudden death.

30

SUMMARY

 This document provides methods and materials involved in treating
cardiovascular conditions such as calcific aortic valve stenosis in mammals based on

their gender. For example, this document provides methods and materials for using sGC agonists or a combination of sGC agonists and PDE5A inhibitors in male patients to reduce calcification of heart valves and/or vessels or to slow progression of aortic sclerosis to calcific aortic valve stenosis. As described herein, sGC agonists such as ataciguat can be used to slow progression of aortic sclerosis to calcific aortic valve stenosis, but such treatment is more effective in males than in females. Having the ability to effectively slow progression of aortic sclerosis to calcific aortic valve stenosis can allow male patients to live longer and happier lives. In some cases, selectively slowing progression of aortic sclerosis to calcific aortic valve stenosis in male patients can allow female patients to avoid unnecessary treatment with sGC agonists. This document also provides methods and materials for using anti-fibrotic agents to treat females identified as having aortic sclerosis. In some cases, such as when calcification is observed after treatment with an anti-fibrotic agent, females can be treated with one or more sGC agonists. The methods disclosed herein also can be used to reduce blood pressure in mammals who have, or who are at risk of having, hypertension, particularly in a supine position. In addition, the methods disclosed herein can include treatment of diastolic dysfunction, mitigation of elevated pulse pressure, and reduction of blood pressure, particularly in a supine position; in some cases, these methods can be carried out on a gender-dependent basis in which males are treated and females are excluded from treatment.

In one aspect, this document features a method for treating a mammal on the basis of the mammal's gender, where the method includes (a) identifying a mammal as having, or as being at risk of developing, heart valve calcification or vessel calcification, (b) identifying the mammal as a male mammal or a female mammal, and (c) when the mammal is a male mammal, administering a sGC agonist to the male mammal, thereby slowing progression of calcification of a heart valve or vessel within the male mammal, and when the mammal is a female mammal, not administering a sGC agonist to the female mammal and, optionally, administering a treatment that is not a sGC agonist to the female mammal to slow progression of calcification of a heart valve or vessel within the female mammal. The mammal can be a human. The sGC agonist can be ataciguat (5-chloro-2-[[5-chloro-2-thienyl)sulfonyl]amino]-N-[4-(4-morpholinylsulfonyl)phenyl]-benzamide; HMR1766), YC-I (5-[1-(phenylmethyl)-1H-indazol-3-yl]-2-furanmethanol), BAY 58-

2667 (4-[[[(4-carboxybutyl)(2-[(4-phenethylbenzyl)oxy] phenethyl)amino] methyl[benzoic]acid hydrochloride), BAY 41-2272 (3-(4-amino-5-cyclopropylpyrimidin-2-yl)-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridine), BAY-41-8543 (2-[1-[(2-fluorophenyl)methyl]-1H-pyrazolo[3,4-b]pyridin-3-yl]-5-(4-morpholinyl)-4,6-pyrimidinediamine), BAY 63-2521 (methyl (4,6-diamino-2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-yl)(methyl)carbamate), CFM-1571 (3-[3-(dimethylamino)propoxy]-N-(4-methoxyphenyl)-1-(phenylmethyl)-1H-pyrazole-5-carboxamide hydrochloride), A-350619 (3-[2-(4-chlorophenylthio)phenyl]-N-(4-dimethylaminobutyl)acrylamide), vericiguat (methyl (4,6-diamino-2-(5-fluoro-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-yl)carbamate), praliguat (1,1,1,3,3,3-hexafluoro-2-[(5-fluoro-2-[1-(2-fluorobenzyl)-5-(1,2-oxazol-3-yl)-1H-pyrazol-3-yl]pyrimidin-4-yl} amino)methyl]propan-2-ol), olinciguat ((2R)-3,3,3-trifluoro-2-[(5-fluoro-2-[1-(2-fluorophenyl)methyl]-5-(1,2-oxazol-3-yl)-1H-pyrazol-3-yl]pyrimidin-4-yl)amino]methyl}-2-hydroxypropanamide), bis-heteroaryl pyrazole IWP-051 (5-fluoro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4(3H)-one), IW-6463, GSK2181236A (1-(6-{2-[(3-methyl-4'-(trifluoromethyl)oxy]-4-biphenyl]methyl)oxy]phenyl}-2-pyridinyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid), IWP-550, IWP-854 (4-(5-fluoro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-yl)-1-(3-methyl-3H-diazirin-3-yl)-N-(37-oxo-41-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-3,6,9,12,15,18,21,24,27,30,33-undeca-36-azahentetracontyl)-7,10,13,16-tetraoxa-4-azanonadecan-19-amide), IWP-953, nelociguat (methyl (4,6-diamino-2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-yl)carbamate), MGV354 ((S)-1-(6-(3-((4-(1-(cyclopropanecarbonyl)piperidin-4-yl)-2-methylphenyl)amino)-2,3-dihydro-1H-inden-4-yl)pyridin-2-yl)-5-methyl-1H-pyrazole-4-carboxylic acid), BI 703704 ((1R,5S,8s)-3-(4-(5-methyl-2-((2-methyl-4-(piperidine-1-carbonyl)benzyl)oxy)phenyl)thiazol-2-yl)-3-azabicyclo[3.2.1]octane-8-carboxylic acid), S3448 (2-[[[(4-chlorophenyl)sulfonyl]amino]-4,5-dimethoxy-N-[4-(4-thiomorpholinyl)sulfonyl]phenyl]benzamide), or BAY 60-2770 (4-[[[(4-carboxybutyl)[2-[5-fluoro-2-[[4'-(trifluoromethyl)[1,1'-biphenyl]-4-yl]methoxy]phenyl]ethyl]amino]methyl]benzoic acid).. The method can further include administering a PDE5A inhibitor to the male mammal. The method can

further include identifying the male mammal as having an elevated plasma level of lysophosphatidic acid (LPA). The method can include administering an anti-fibrotic agent to the female mammal to slow progression of calcification of a heart valve or vessel within the female mammal.

5 In another aspect, this document features a method for treating a population of mammals identified as having, or as being at risk for developing, heart valve calcification or vessel calcification, wherein the population includes at least one male mammal and at least one female mammal, and where the method includes administering a sGC agonist to the at least one male mammal, thereby slowing
10 progression of calcification of a heart valve or vessel within the at least one male mammal, and not administering the sGC agonist to the at least one female mammal. The mammals can be human. The sGC agonist can be ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliciguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463,
15 GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MG3354, BI 703704, S3448, or BAY 60-2770. The method can further include administering a PDE5A inhibitor to the at least one male mammal. The at least one male mammal also can be identified as having an elevated plasma level of LPA.

In another aspect, this document features a method for treating a population of
20 mammals based on gender, where the method includes (a) identifying the gender of mammals in a population determined to have, or to be at risk for developing, heart valve calcification or vessel calcification, and (b) administering a sGC agonist to mammals identified as being male mammals and not administering a sGC agonist to mammals identified as being female mammals and, optionally, administering a
25 treatment that is not a sGC agonist to the female mammals. The mammals can be human. The sGC agonist can be ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliciguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MG3354, BI 703704, S3448, or BAY 60-2770. The
30 method can further include administering a PDE5A inhibitor to the mammals identified as being male mammals. The mammals identified as being male mammals also can be determined to have an elevated plasma level of LPA. The method can

include administering an anti-fibrotic agent to the mammals identified as being female mammals.

In still another aspect, this document features a method for avoiding the unnecessary use of a sGC agonist to treat a mammal having, or at risk of developing, heart valve calcification or vessel calcification, where the method includes identifying the mammal as being a female mammal and as having, or at risk of developing, the heart valve calcification or vessel calcification, and excluding the female mammal from treatment with the sGC agonist based, at least in part, on the female gender of the female mammal. The mammal can be a human. The sGC agonist can be ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MG354, BI 703704, S3448, or BAY 60-2770.

This document also features a method for treating a mammal on the basis of the mammal's gender, where the method includes (a) identifying a mammal as having, or as being at risk of developing, aortic sclerosis, (b) identifying the mammal as a male mammal or a female mammal, and (c) when the mammal is a male mammal, administering a sGC agonist to the male mammal, thereby slowing progression of aortic sclerosis to calcific aortic valve stenosis within the male mammal, and when the mammal is a female mammal, not administering a sGC agonist to the female mammal and, optionally, administering a treatment that is not a sGC agonist to the female mammal to slow progression of aortic sclerosis to calcific aortic valve stenosis within the female mammal. The mammal can be a human. The sGC agonist can be ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MG354, BI 703704, S3448, or BAY 60-2770. The method can further include administering a PDE5A inhibitor to the male mammal. The method can further include identifying the male mammal as having an elevated plasma level of LPA. The method can include administering an anti-fibrotic agent to the female mammal.

In another aspect, this document features a method for treating a population of mammals identified as having, or as being at risk for developing, aortic sclerosis,

wherein the population includes at least one male mammal and at least one female mammal, where the method includes administering a sGC agonist to the at least one male mammal, thereby slowing progression of aortic sclerosis to calcific aortic valve stenosis within the at least one male mammal, and not administering the sGC agonist to the at least one female mammal. The mammals can be human. The sGC agonist can be vericiguat, praliciguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770. The method can further include administering a PDE5A inhibitor to the at least one male mammal. The method can further include identifying the at least one male mammal as having an elevated plasma level of LPA.

In another aspect, this document features a method for treating a population of mammals based on gender, where the method includes (a) identifying the gender of mammals in a population determined to have, or to be at risk for developing, aortic sclerosis, and (b) administering a sGC agonist to mammals identified as being male mammals and not administering a sGC agonist to mammals identified as being female mammals and, optionally, administering a treatment that is not a sGC agonist to the female mammals. The mammals can be human. The sGC agonist can be ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliciguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770. The method can further include administering a PDE5A inhibitor to the mammals identified as being male mammals. The mammals identified as being male mammals can further have been determined to have an elevated plasma level of LPA. The method can include administering an anti-fibrotic agent to the mammals identified as being female mammals.

In yet another aspect, this document features a method for avoiding the unnecessary use of a sGC agonist to treat a mammal having, or at risk of developing, aortic sclerosis, where the method includes identifying the mammal as being a female mammal and as having, or at risk of developing, the aortic sclerosis, and excluding the female mammal from treatment with the sGC agonist based, at least in part, on the female gender of the female mammal. The method mammal can be a human. The sGC agonist can be ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliciguat, olinciguat, bis-

heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.

In another aspect, this document features a method for treating a mammal, where the method includes (a) identifying the mammal as having, or as being at risk
5 for, hypertension when in a supine position, and (b) administering to a sGC agonist to the mammal, thereby reducing blood pressure in the supine position. The mammal can be a human. The sGC agonist can be ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550,
10 IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770. The method can further include administering a PDE5A inhibitor to the mammal. The method can further include identifying the mammal as being a male mammal prior to the administering. The administering may not reduce blood pressure when the mammal is in an upright position.

In still another aspect, this document features a method for treating a
15 population of mammals identified as having, or as being at risk for developing hypertension in a supine position, wherein the population includes at least one male mammal and at least one female mammal, and where the method includes administering a sGC agonist to the at least one male mammal, thereby reducing blood
20 pressure and preventing deleterious side effects of hypertension while in the supine position in the at least one male mammal, and not administering the sGC agonist to the at least one female mammal. The mammals can be human. The sGC agonist can be ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole
25 IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770. The method can further include administering a PDE5A inhibitor to the at least one male mammal. The method can include administering an anti-fibrotic agent to the at least one female mammal. The administering may not reduce blood pressure when the mammal is in an upright
30 position.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent

to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including
5 definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

10 DESCRIPTION OF DRAWINGS

FIGS. 1A and 1B are graphs plotting levels of phospho-VASP²³⁹ in aortic valve tissue of male (**FIG. 1A**) and female (**FIG. 1B**) mice with established aortic valve disease after treatment with ataciguat/HMR1766 (WD+ATA) vs. control (WD). These studies demonstrated that ataciguat effectively activated soluble guanylyl
15 cyclase in treated mice. Phospho-VASP²³⁹ levels were assessed using immunohistochemical techniques and confocal microscopy.

FIGS. 2A and 2B are graphs plotting levels of phospho-SMAD1/5/8, a key mediator of bone morphogenetic protein (BMP) signaling, in male (**FIG. 2A**) and female (**FIG. 2B**) mice with established aortic valve disease after treatment with
20 ataciguat/HMR1766 (WD+ATA) or control (WD). These studies showed that ataciguat effectively reduced canonical bone morphogenetic signaling in both treated mice. Phospho-SMAD1/5/8 levels were assessed using immunohistochemical techniques and confocal microscopy.

FIGS. 3A-3D are a series of graphs plotting levels of molecular markers of
25 tissue calcification in both male and female mice with established aortic valve disease after treatment with ataciguat/HMR1766 (WD+ATA) or control (WD). Ataciguat effectively reduced both osteopontin (SPP1; **FIGS. 3A and 3B**) and Runx2 (a master regulator of osteogenesis; **FIGS. 3C and 3D**) in male (**FIGS. 3A and 3C**) and female (**FIGS. 3B and 3D**) mice, consistent with reduced tissue calcification observed *in vivo* (see, **FIGS. 4A and 4B**). mRNA levels were assessed using quantitative real-
30 time RT-PCR.

FIGS. 4A and 4B are graphs plotting levels of calcification of aortic valve tissue in male (**FIG. 4A**) and female (**FIG. 4B**) mice with established aortic valve

disease after treatment with ataciguat/HMR1766 (WD+ATA) or control (WD). Calcification was assessed histologically using Alizarin red staining followed by brightfield microscopic imaging.

FIGS. 5A and 5B are graphs plotting levels of canonical transforming factor beta (TGF β) signaling in aortic valve tissue of male (**FIG. 5A**) and female (**FIG. 5B**) mice with established aortic valve disease, after treatment with ataciguat/HMR1766 (WD+ATA) or control (WD). TGF β signaling was assessed based on levels of SMAD2/3 phosphorylation, which was measured using immunohistochemical techniques and confocal microscopy.

FIGS. 6A-6D are graphs plotting levels of collagen isoform expression (a major determinant of tissue fibrosis) in male (**FIGS. 6A and 6C**) and female (**FIGS. 6B and 6D**) mice with established aortic valve disease, after treatment with ataciguat/HMR1766 (WD+ATA) or control (WD). HMR1766 did not significantly affect expression of collagen 1a1 (a major structural collagen isoform) in male mice (**FIG. 6A**), but significantly reduced Colla1 expression in female mice (**FIG. 6B**). Further, expression of Coll5a1 (a significant basement membrane structural isoform) was increased in male mice (**FIG. 6C**) but tended to be reduced in female mice (**FIG. 6D**). Collectively, these studies showed that ataciguat did not alter or increased collagen isoform expression in males, but reduced collagen isoform expression in females. mRNA levels were assessed using quantitative real-time RT-PCR.

FIGS. 7A and 7B are graphs plotting the level of matrix metalloproteinase 2 (MMP2, a major determinant of collagen fiber degradation/remodeling) expression in male (**FIG. 7A**) and female (**FIG. 7B**) mice with established aortic valve disease, after treatment with ataciguat/HMR1766 (WD+ATA) or control (WD). HMR1766 did not significantly affect MMP2 mRNA levels in either male or female mice; it was noted that the female mice tended to have higher levels of MMP2 mRNA than littermate-matched males. Collectively, these data suggested that although HMR1766 did not appear to impact matrix remodeling enzyme expression, female mice might be more prone to excess matrix remodeling over time. mRNA levels were assessed using quantitative real-time RT-PCR.

FIGS. 8A-8D are graphs plotting collagen fiber architecture in male (**FIGS. 8A and 8B**) and female (**FIGS. 8C and 8D**) mice with established aortic valve disease, after treatment with ataciguat/HMR1766 (WD+ATA) or control (WD).

Ataciguat did not impact relative collagen fiber thicknesses in male mice, but ataciguat did increase the fraction of thinner fibers (and reduced the fraction of thicker fibers) in female mice. Importantly, these changes are consistent with the molecular changes in collagen isoform and MMP2 expression in each gender. Collagen fiber thickness was assessed histologically using picosirius red staining followed by microscopic imaging under circularly polarized light (which leverages the birefringent properties of collagen).

FIGS. 9A and 9B are graphs plotting aortic valve function in male (**FIG. 9A**) and female (**FIG. 9B**) mice with established aortic valve disease, after treatment with ataciguat/HMR1766 (WD+ATA) or control (WD). Data were obtained using a previously validated approach of measuring cusp separation distance by high resolution echocardiography, where a larger separation distance indicates better function. The data suggested that only male mice received a therapeutic benefit from long-term treatment with ataciguat.

FIGS. 10A-10D are graphs plotting measures of ventricular function in male (**FIGS. 10A and 10C**) and female (**FIGS. 10B and 10D**) mice with established aortic valve disease, after treatment with ataciguat/HMR1766 (WD+ATA) or control (WD). Consistent with prior reports showing resistance to systolic dysfunction in the face of moderate pressure overload in mice, ejection fraction was well-preserved in WD-fed mice and was not improved further by ataciguat (**FIGS. 10A and 10B**). Diastolic function (measured using E/e' , which is clinically known to also predict poor outcomes in numerous patient populations) was impaired in WD-fed mice of both genders (the normal value approximately 20-25 in mice), and was significantly improved in male mice after ataciguat treatment (**FIG. 10C**) but not in female mice (**FIG. 10D**). Ventricular function was assessed using high resolution echocardiography.

FIGS. 11A-11D are graphs plotting seated systolic (**FIGS. 11A and 11B**) and diastolic (**FIGS. 11C and 11D**) blood pressure in humans with mild-to-moderate aortic valve stenosis before and after treatment with placebo (**FIGS. 11A and 11C**) or ataciguat/HMR1766 (**FIGS. 11B and 11D**) for 14 days (randomized, double-blind study design, doses as indicated). As indicated in **FIGS. 11B and 11D**, 100 mg/day of ataciguat significantly reduced blood pressure while in a seated position, but 200 mg/day did not have a significant effect on blood pressure. This suggested that the

higher dose of ataciguat would not put patients at risk for side effects of hypotension (light headedness, etc.).

FIGS. 12A-12D are graphs plotting changes in systolic (**FIGS. 12A and 12B**) and diastolic (**FIGS. 12C and 12D**) blood pressure upon standing in humans with
5 mild-to-moderate aortic valve stenosis, before and after treatment with placebo (**FIGS. 12A and 12C**) or ataciguat (**FIGS. 12B and 12D**) for 14 days (randomized, double-blind study design, doses as indicated). Treatment with ataciguat did not significantly or consistently alter blood pressure responses to the orthostatic stress of transitioning from a seated to a standing position. This again suggested that ataciguat
10 would not put patients at risk for side effects of hypotension.

FIGS. 13A and 13B are graphs plotting changes in heart rate upon standing in humans with mild-to-moderate aortic valve stenosis, before and after treatment with placebo (**FIG. 13A**) or ataciguat/HMR1766 (**FIG. 13B**) for 14 days (randomized, double-blind study design, doses as indicated). Consistent with the observed changes
15 in blood pressure, treatment with ataciguat did not significantly or consistently alter heart rate responses to the orthostatic stress of transitioning from a seated to a standing position. This again suggested that ataciguat would not put patients at risk for side effects of hypotension, and also that excessive tachycardia is not a compensatory mechanism masking unwanted effects of ataciguat on vascular
20 tone/vasomotor regulation.

FIGS. 14A-14D are graphs plotting supine systolic (**FIGS. 14A and 14B**) and diastolic (**FIGS. 14C and 14D**) blood pressure in humans with mild-to-moderate aortic valve stenosis, before and after treatment with placebo (**FIGS. 14A and 14C**) or ataciguat/HMR1766 (**FIGS. 14B and 14D**) for 14 days (randomized, double-blind
25 study design, doses as indicated). Treatment with ataciguat significantly reduced both systolic and diastolic blood pressure, suggesting that ataciguat is a viable strategy to mitigate vascular stiffness and reduce nocturnal hypertension without putting patients at risk for symptoms related to reduced blood pressure while upright.

FIGS. 15A-15D are graphs plotting changes in systolic (**FIGS. 15A and 15B**)
30 and diastolic (**FIGS. 15C and 15D**) blood pressure upon progressive head-up tilting in humans with mild-to-moderate aortic valve stenosis, before and after treatment with placebo (**FIGS. 15A and 15C**) or ataciguat/HMR1766 (**FIGS. 15B and 15D**) for 14 days (randomized, double-blind study design, doses as indicated). Treatment

with ataciguat did not consistently or significantly alter blood pressure responses to head-up tilt compared to pre-treatment or placebo-treated subjects. These observations were consistent with the more functional “standing test” data (**FIGS. 12A-12D and 13A-13B**), and were conducted as a more sensitive and controlled
5 measure of orthostatic tolerance.

FIGS. 16A and 16B are graphs plotting changes in heart rate upon progressive head-up tilting in humans with mild-to-moderate aortic valve stenosis, before and after treatment with placebo (**FIG. 16A**) or ataciguat/HMR1766 (**FIG. 16B**) for 14 days (randomized, double-blind study design, doses as indicated).
10 Treatment with ataciguat did not consistently or significantly alter heart rate responses to head-up tilt compared to pre-treatment or placebo-treated subjects, again suggesting that ataciguat would not put patients at risk for side effects of hypotension, and that excessive tachycardia is not compensatory mechanism masking unwanted effects of ataciguat on vascular tone/vasomotor regulation.

FIGS. 17A-17C are graphs plotting changes in aortic valve calcification in humans with mild-to-moderate aortic valve stenosis, before and after treatment with placebo or ataciguat/HMR1766 (200 mg/day, once daily) for six months (randomized, double-blind study design). Treatment with ataciguat significantly attenuated
15 progression of valve calcification when data from subjects of both sexes are combined (**FIG. 17A**). When data were split by gender, however, it became evident that male patients (**FIG. 17B**) received a much greater therapeutic benefit from treatment with ataciguat, compared to females (**FIG. 17C**).

FIGS. 18A-18F are graphs plotting changes in aortic valve function in humans with mild-to-moderate aortic valve stenosis, before and after treatment with
25 placebo or ataciguat/HMR1766 (200 mg/day, once daily) for six months (randomized, double-blind study design). Using changes in aortic valve area (**FIGS. 18A-18C**) as a measure of valve function, it was concluded that treatment with ataciguat can attenuate progression of aortic valve dysfunction when subjects of both sexes are combined (**FIG. 18A**). Similar to changes in valve calcium, a therapeutic benefit was
30 predominantly conferred to male patients (**FIG. 18B**) as compared to female patients (**FIG. 18C**). While the changes in peak transvalvular velocity (**FIGS. 18D-18F**) as a measure of valve dysfunction could be considered to indicate a negligible impact of ataciguat on progression of valve dysfunction, this measure is critically influenced by

changes in left ventricular systolic function. As depicted in **FIGS. 19A-19F**, improvements in left ventricular systolic function did explain the artifactual “masking” of therapeutic benefit when velocimetric measures were used.

FIGS. 19A-19F are graphs plotting changes in left ventricular systolic function in humans with mild-to-moderate aortic valve stenosis, before and after treatment with placebo or ataciguat/HMR1766 (200 mg/day, once daily) for six months (randomized, double-blind study design). Using either left ventricular ejection fraction (**FIGS. 19A-19C**) or left ventricular stroke volume (**FIGS. 19D-19F**) as a measure of valve function, it was concluded that treatment with ataciguat could prevent the decline in left ventricular function that was observed in the placebo group when all subjects were combined (**FIGS. 19A and 19D**). When only male subjects were evaluated, however, the data suggested that ataciguat not only prevented declines in left ventricular function, but also improved left ventricular systolic function (**FIGS. 19B and 19E**), which would serve to drive “artifactual” elevations in peak transvalvular velocity as observed in **FIG. 18** and mask preserved valvular function. Female subjects appeared to receive no consistent benefit from ataciguat on left ventricular systolic function (**FIG. 19C**), and even demonstrated a potential worsening of stroke volume (**FIG. 19F**).

FIGS. 20A-20F are graphs plotting changes in left ventricular diastolic function in humans with mild-to-moderate aortic valve stenosis before and after treatment with placebo or ataciguat/HMR1766 (200 mg/day, once daily) for six months (randomized, double-blind study design). Using either changes in E/e' (**FIGS. 20A-20C**) or the E/A ratio (**FIGS. 20D-20F**), the data suggested that ataciguat can attenuate further progression of diastolic dysfunction associated with aortic valve stenosis when all subjects were combined (**FIGS. 20A and 20D**). When only male subjects were examined, however, it was evident that males are the predominant recipients of any therapeutic benefit conferred by long-term treatment with ataciguat (**FIGS. 20B and 20E**). In contrast, females received negligible therapeutic benefit with regard to left ventricular diastolic function from long-term treatment with ataciguat compared to placebo-treated subjects (**FIGS. 20C and 20F**).

FIGS. 21A-21C are graphs plotting changes in aortic pulse pressure in seated humans with mild-to-moderate aortic valve stenosis, before and after treatment with placebo or ataciguat/HMR1766 (200 mg/day, once daily) for six months (randomized,

double-blind study design). The data suggested that ataciguat attenuated pulse pressure (an index of arterial stiffness) when all subjects were combined (**FIG. 21A**). When data from only male subjects were examined, however, it was evident that males are the predominant recipients of any therapeutic benefit with regard to pulse pressure or arterial stiffness conferred by long-term treatment with ataciguat (**FIG. 21B**). In contrast, females received no apparent therapeutic benefit from long-term treatment with ataciguat with regard to changes in pulse pressure function, as compared to placebo-treated female subjects (**FIG. 21C**).

FIG. 22 is a graph resulting from the use of a non-biased, machine-learning/neural network algorithm to determine if baseline phenotypic variables can predict progression of aortic valve dysfunction and response to treatment in patients. Patient gender, age, treatment group (ataciguat or placebo), and measurements from blood tests and echocardiograms, were used as features to train a neural network regression model for predicting change in aortic valve area. Model performance was evaluated through 10-fold cross-validation. The bottom right inset indicates the top eight variables that were the most informative to the model's decision-making process. Critically, treatment group (placebo vs. ataciguat) and gender (male vs. female) were the two strongest predictive components identified from this non-biased approach.

FIG. 23 is a graph plotting expression of the bone morphogenetic protein (BMP) target gene, Osterix (also known as transcription factor Sp7), in aortic valve interstitial cells treated with nelociguat. Nelociguat concentrations of 1 nM to 1 μ M reduced BMP signaling (arrows denote the efficacious range).

FIG. 24 is a graph plotting expression of Osterix in aortic valve interstitial cells treated with vericiguat. Only relatively high doses of vericiguat were effective to reduce BMP target gene expression (denoted by the arrow).

FIG. 25 is a graph plotting expression of Osterix in aortic valve interstitial cells treated with lificiguat. Similar to nelociguat, lificiguat appeared to lose its efficacy at higher concentrations *in vitro*.

FIG. 26 is a graph plotting expression of Osterix in aortic valve interstitial cells treated with BAY41-8543. BAY41-8543 tended to drive dose-dependent reductions in BMP target gene expression, and was highly efficacious at 10 μ M.

FIG. 27 is a graph plotting expression of Osterix in aortic valve interstitial cells treated with lysophosphatidic acid (LPA; specifically, 18:0 lyso-PA), with or without ataciguat (ATA), to assess LPA-induced BMP signaling and BMP target gene expression. Treatment with 18:0 LPA increased expression of the BMP target gene
5 (center bar), while ataciguat dramatically suppressed this effect (right bar).

FIG. 28 is a graph plotting the relationship between plasma LPA levels and baseline levels of valvular calcium (measured using CT scanning) in patients with mild to moderate aortic valve stenosis. Higher plasma LPA levels were associated with greater calcium levels in this patient cohort.

10 **FIG. 29** is a graph plotting the relationship between baseline plasma LPA levels and changes in valvular calcium (measured using CT scanning) in patients with mild to moderate aortic valve stenosis who were treated with ataciguat for 6 months. Patients with higher levels of LPA tended to show a greater attenuation of excess BMP signaling when treated with ataciguat.

15

DETAILED DESCRIPTION

This document provides methods and materials involved in treating cardiovascular conditions such as calcific aortic valve stenosis based, at least in part, on gender. For example, this document provides methods and materials for using one
20 or more sGC agonists or a combination of one or more sGC agonists and one or more PDE5A inhibitors to reduce calcification of heart valves and/or vessels or to slow progression of aortic sclerosis to calcific aortic valve stenosis in male patients. As described herein, sGC agonists appear to be less effective in females than in males. Methods and materials for excluding females from such treatment also are provided
25 herein, as are alternative methods for treating females.

In some cases, this document provides methods for treating mammals on the basis of gender, where the methods include identifying a mammal as having, or as being at risk of developing, a cardiovascular condition (e.g., heart valve calcification, vessel calcification, aortic sclerosis, arterial stiffness, or left ventricular systolic or
30 diastolic dysfunction), and identifying the gender of the mammal. If the mammal is a male, the method can include administering a sGC agonist in order to slow progression of the cardiovascular condition (e.g., calcification of a heart valve or vessel, or progression of aortic sclerosis to calcific aortic valve stenosis) in the male

mammal. If the mammal is a female, the method can exclude administering a sGC agonist to the mammal. In some cases, if the mammal is a female, the method can include administering a treatment that is not a sGC agonist, in order to slow progression of the cardiovascular condition. In some cases, if the mammal is a female, the method can include administering an anti-fibrotic agent, in order to slow progression of the cardiovascular condition. In some cases, if calcification develops in the female mammal after administration of the anti-fibrotic agent, a sGC agonist can subsequently be administered to the female mammal.

In some cases, the methods provided herein can include treating a population of mammals that includes at least one male and at least one female that have been identified as having, or as being at risk for developing, a cardiovascular condition (e.g., heart valve calcification, vessel calcification, aortic sclerosis, or left ventricular systolic or diastolic dysfunction). Such methods can include administering a sGC agonist to the at least one male mammal, thereby slowing progression of the cardiovascular condition within the at least one male mammal, and not administering the sGC agonist to the at least one female mammal. The methods provided herein therefore can avoid the unnecessary use of sGC agonists to treat female mammals identified as having, or at risk of developing, a cardiovascular condition such as heart valve calcification, vessel calcification, aortic sclerosis, or left ventricular systolic or diastolic dysfunction. In some cases, the methods can include administering an anti-fibrotic agent to the at least one female mammal. If calcification develops in the at least one female mammal after treatment with the anti-fibrotic agent, the mammal can be treated with a sGC agonist.

The methods disclosed herein also can be used to reduce blood pressure in mammals who have, or who are at risk of having, hypertension (e.g., hypertension while in a supine position). The methods also can be used to treat diastolic dysfunction and mitigate elevated pulse pressure. In some cases, these methods also can be carried out on a gender-dependent basis, such that male mammals are treated with a sGC agonist (with or without a PDE5A inhibitor), while females are excluded from treatment. In some cases, the methods can be carried out on a gender-dependent basis, such that female mammals are treated with an anti-fibrotic agent.

Any type of mammal having a cardiovascular condition such as calcific aortic valve stenosis can be treated as described herein. For example, male humans and

other primates such as monkeys having a cardiovascular condition such as calcific aortic valve stenosis can be treated with one or more sGC agonists, or with one or more sGC agonists and one or more PDE5A inhibitors. In some cases, male dogs, cats, horses, cows, pigs, sheep, mice, and rats can be treated with one or more sGC agonists or a combination of one or more sGC agonists and one or more PDE5A inhibitors as described herein. In some cases, female humans or other female mammals (e.g., non-human primates, dogs, cats, horses, cows, pigs, sheep, mice, and rats) having a cardiovascular condition such as calcific aortic valve stenosis can be treated with one or more anti-fibrotic agents as described herein.

10 Any appropriate method can be used to identify a mammal having a cardiovascular condition such as calcific aortic valve stenosis or aortic sclerosis. For example, echocardiography or computed tomography scanning can be used to identify a human having aortic sclerosis that is at risk of progressing into calcific aortic valve stenosis.

15 In some cases, a mammal can be identified for treatment based on an elevated level of LPA, a phospholipid derivative that can act as a signaling molecule and a mitogen. Increased LPA levels can indicate an increased risk of aortic valve calcification and stenosis. Moreover, as described in the Examples herein, elevated LPA levels may also be a primary indicator of response to sGC agonists. The term “elevated level” as used herein with respect to blood levels of LPA refers to any level that is above a threshold level (e.g., a threshold plasma level) for a control population of healthy mammals (e.g., an age-matched random population of 10, 20, 30, 40, 50, 100, or 500 healthy mammals) that do not have a cardiovascular condition as described herein. In some cases, a threshold LPA level can be, without limitation, 5 mg/dL or greater (e.g., 10 mg/dL, 20 mg/dL, 25 mg/dL, 30 mg/dL, 40 mg/dL, or 50 mg/dL). Any appropriate method can be used to determine a blood level of LPA. For example, polypeptide detection methods such as immunoassays (e.g., ELISAs or radioimmunoassays) and mass spectrometry (e.g., LC-MS/MS) can be used to determine the level of LPA or specific LPA species (e.g., 18:0 LPA) in a plasma or whole blood sample. In some cases, radioimmunoassays can be used to determine the blood or plasma level of LPA.

Once identified as having a cardiovascular condition such as calcific aortic valve stenosis or aortic sclerosis with the potential to progress into calcific aortic

valve stenosis and identified as being a male mammal, the male mammal can be administered or instructed to self-administer one or more sGC agonists or a combination of one or more sGC agonists and one or more PDE5A inhibitors.

Examples of sGC agonists include, without limitation, ataciguat (5-chloro-2-
 5 [[(5-chloro-2-thienyl)sulfonyl]amino]-N-[4-(4-morpholinylsulfonyl)phenyl]-
 benzamide; HMR1766), YC-I (5-[1-(phenylmethyl)-1H-indazol-3-yl]-2-
 furanmethanol), BAY 58-2667 (4-[[[(4-carboxybutyl)(2-[(4-phenethylbenzyl)oxy]
 phenethyl)amino)methyl]benzoic]acid hydrochloride; cinaciguat), BAY 41-2272 (3-
 (4-amino-5-cyclopropylpyrimidin-2-yl)-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-
 10 b]pyridine), BAY-41-8543 (2-[1-[(2-fluorophenyl)methyl]-1H-pyrazolo[3,4-
 b]pyridin-3-yl]-5-(4-morpholinyl)-4,6-pyrimidinediamine), BAY 63-2521 (methyl
 (4,6-diamino-2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-
 yl)(methyl)carbamate; riociguat), CFM-1571 (3-[3-(dimethylamino)propoxy]-N-(4-
 methoxyphenyl)-1-(phenylmethyl)-1H-pyrazole-5-carboxamide hydrochloride), A-
 15 350619 (3-[2-(4-chlorophenylthio)phenyl]-N-(4-dimethylaminobutyl)acrylamide),
 vericiguat (BAY 1021189, also referred to as MK-1242; methyl (4,6-diamino-2-(5-
 fluoro-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-yl)carbamate),
 praliguat (IW-1973, IWP-121; 1,1,1,3,3,3-hexafluoro-2-[(5-fluoro-2-[1-(2-
 fluorobenzyl)-5-(1,2-oxazol-3-yl)-1H-pyrazol-3-yl]pyrimidin-4-
 20 yl)amino)methyl]propan-2-ol), olinciguat (IW-1701; (2R)-3,3,3-trifluoro-2-[(5-
 fluoro-2-{1-[(2-fluorophenyl)methyl]-5-(1,2-oxazol-3-yl)-1H-pyrazol-3-
 yl}pyrimidin-4-yl)amino]methyl}-2-hydroxypropanamide), bis-heteroaryl pyrazole
 IWP-051 (5-fluoro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-
 yl)pyrimidin-4(3H)-one), IW-6463, GSK2181236A (1-(6-{2-[(3-methyl-4'-
 25 [(trifluoromethyl)oxy]-4-biphenyl)methyl]oxy]phenyl}-2-pyridinyl)-5-
 (trifluoromethyl)-1H-pyrazole-4-carboxylic acid), IWP-550, IWP-854 (4-(5-fluoro-2-
 (1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-yl)-1-(3-methyl-
 3H-diazirin-3-yl)-N-(37-oxo-41-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-
 d]imidazol-4-yl)-3,6,9,12,15,18,21,24,27,30,33-undecaoxa-36-azahentetracontyl)-
 30 7,10,13,16-tetraoxa-4-azanonadecan-19-amide), IWP-953, nelociguat (methyl (4,6-
 diamino-2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-
 yl)carbamate; BAY 60-4552), MG354 ((S)-1-(6-(3-((4-(1-
 (cyclopropanecarbonyl)piperidin-4-yl)-2-methylphenyl)amino)-2,3-dihydro-1H-

inden-4-yl)pyridin-2-yl)-5-methyl-1H-pyrazole-4-carboxylic acid), BI 703704 ((1R,5S,8s)-3-(4-(5-methyl-2-((2-methyl-4-(piperidine-1-carbonyl)benzyl)oxy)phenyl)thiazol-2-yl)-3-azabicyclo[3.2.1]octane-8-carboxylic acid), S3448 (2-[[[(4-chlorophenyl)sulfonyl]amino]-4,5-dimethoxy-N-[4-(4-thiomorpholinylsulfonyl)phenyl]benzamide), and BAY 60-2770 (4-[[[(4-carboxybutyl)[2-[5-fluoro-2-[[4'-(trifluoromethyl)[1,1'-biphenyl]-4-yl]methoxy]phenyl]ethyl]amino]methyl]benzoic acid). See, Buys et al., *Nitric Oxide* 78:72-78, 2018; Sandner et al., “Soluble Guanylate Cyclase Stimulators and Activators,” In: Handbook of Experimental Pharmacology, Springer, Berlin, Heidelberg, pp. 1-40, 2018; and Friebe et al., *Naunyn-Schmiedeberg's Arch Pharmacol* 390, 1177-1188, 2017).

A sGC agonist can be a sGC “stimulator” or a sGC “activator.” sGC stimulators bind to sGC and potentiate nitric oxide- (NO-) mediated cGMP signaling. sGC stimulators can increase sGC activity in the absence of NO, but in the presence of NO, sGC stimulators can act synergistically with the NO to amplify NO signaling (Buys et al., *supra*). In contrast, sGC activators bind to the heme pocket of the sGC enzyme, and therefore can only activate heme-free sGC, independent of NO (Buys et al., *supra*).

Thus, in some cases, a sGC agonist can be a stimulator that synergistically increases sGC enzyme activity with NO. Examples of sGC stimulators that can be used as described herein include, without limitation, riociguat (BAY 63-2521), vericiguat (BAY 1021189), praliguat (IW-1973, IWP-121), olinciguat (IW-1701), nelociguat (BAY 60-4552), IW-6463, A-350619, BAY 41-2272, BAY 41-8543, CFM-1571, GSK2181236 A, IWP-051, IWP-550, IWP-854, IWP-953, and YC-1. In some cases, a sGC agonist can be a sGC activator. Examples of sGC activators that can be used as described herein include, without limitation, cinaciguat (BAY 58-2667), ataciguat (HMR1766), MGV354, BI 703704, S3448, BAY 60-2770.

Examples of PDE5A inhibitors include, without limitation, sildenafil, vardenafil, tadalafil, EMD 360527, DA 8159, UK-343-664 (Walker *et al.*, *Xenobiotica*, 31:651-664 (2001)), UK-427-387, UK-357903 ([1-ethyl-4-{3-[3-ethyl-6,7-dihydro-7-oxo-2-(2-pyridylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-5-yl]-2-(2-methoxyethoxy)-5-pyridylsulphonyl}piperazine]) (Gardiner *et al.*, *J. Pharmacol. Exp. Ther.*, 312:265-271 (2005)), UK-371800 (Pfizer), UK-313794 (Pfizer), UK-343664

(Abel *et al.*, *Xenobiotica*, 31:665-76 (2001)), TA-1790 (Tanabe Seiyaku), CP-248 (Osi Pharmaceuticals), CP-461 (Osi Pharmaceuticals), exisulind (Deguchi *et al.*, *Molecular Cancer Therapeutics*, 803-809 (2002); (Osi Pharmaceuticals)), pyrazolinone, EMD82639 (Merck KgaA, Darmstadt, DE; (4-(4-[2-ethyl-
5 phenylamino)-methylene]-3-methyl-5-oxo-4,5-di-hydro-pyrazol-1-yl)-benzoic acid; Senzaki *et al.*, *FASEB J.*, 15:1718-1726 (2001), and Scutt *et al.*, *BMC Pharmacol.*, 4:10 (2004)), EMD360527 (Merck KgaA, Darmstadt, DE; [7-(3-Chloro-4-methoxy-benzylamino)-l-methyl-3-propyl-lH-pyrazolo[4,3-d]pyrimidin-5-ylmethoxy]-acetic acid; Scutt *et al.*, *BMC Pharmacol.*, 4:10 (2004)), EMD221829 (Merck KgaA,
10 Darmstadt, DE; 4-[4-(3-Chloro-4-methoxy-benzylamino)-benzo[4,5]thieno[2,3-d]-pyrimidin-2-yl]-cyclohexanecarboxylic acid, ethanolamin salt; Scutt *et al.*, *BMC Pharmacol.*, 4:10 (2004)), EMDI 71827 (Merck KgaA, Darmstadt, DE; 5-[4-(3-Chloro-4-methoxy-benzylamino)-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-2-yl]-pentanoic acid; Scutt *et al.*, *BMC Pharmacol.*, 4:10 (2004)), DA-
15 8259 (3-(l-Methyl-7-oxo-3-propyl-6,7-dihydro-lH-pyrazolo-[4,3-d]pyrimidin-5-yl)-N-[2-(l-methylpyrrolidin-2-yl)ethyl]-4-propoxybenzenesulfonamide), E-4021 (Dukarm *et al.*, *Am. J. Respir. Crit. Care Med.*, 160:858-865 (1999)), pentoxifylline, and FR22934 (Fujisawa). Additional examples of PDE5A inhibitors can be set forth in U.S. Patent Nos: 6,916,927, 6,911,542, 6,903,099, 6,878,711, 6,872,721, 6,858,620,
20 6,825,197, 6,774,128, 6,723,719, 6,699,870, 6,670,366, 5,859,006, and 5,250,534 and International Patent Application Publication No. WO 03/063875, WO 03/1012761, WO 2004/037183, and WO 98/38168. In some cases, a sGC agonist used as described herein can be an activator of an oxidized or non-oxidized form of sGC.

In some cases, one or more sGC agonists (e.g., one, two, three, four, five, or
25 more sGC agonists) or a combination of one or more sGC agonists (e.g., one, two, three, four, five, or more sGC agonists) and one or more PDE5A inhibitors (e.g., one, two, three, four, five, or more PDE5A inhibitors) can be administered to a male mammal to reduce calcification of heart valves and/or vessels or to slow progression of aortic sclerosis to calcific aortic valve stenosis, and/or to slow progression of
30 established mild-to-moderate valvular stenosis to more severe stenosis.

Administration of such agents at early or moderate disease stages also can be used to prevent or reduce organ dysfunction stemming from long term increases in cardiovascular/valvular stiffness (e.g., left ventricular maladaptation or renal

dysfunction from aortic stiffness). The one or more sGC agonists and the one or more PDE5A inhibitors can be administered simultaneously (e.g., in separate compositions administered at substantially the same time, or in the same composition), or can be administered at separate times (e.g., 10 to 30 minutes apart, 30 to 60 minutes apart, 5 one to two hours apart, two to four hours apart, four to six hours apart, six to 12 hours part, 12 to 24 hours apart, or more than 24 hours apart). In some cases, one or more sGC agonists or a combination of one or more sGC agonists and one or more PDE5A inhibitors can be formulated into a pharmaceutically acceptable composition. For example, a therapeutically effective amount of ataciguat can be formulated together 10 with one or more pharmaceutically acceptable carriers (additives) and/or diluents. A pharmaceutical composition can be formulated for administration in solid or liquid form including, without limitation, sterile solutions, suspensions, sustained-release formulations, tablets, capsules, pills, powders, and granules.

After being identified as having a cardiovascular condition such as calcific 15 aortic valve stenosis or aortic sclerosis with the potential to progress into calcific aortic valve stenosis and identified as being a female mammal, the female mammal can be administered or instructed to self-administer one or more anti-fibrotic agents. In some cases, if the female mammal develops calcification after administration of the anti-fibrotic agent(s), the female mammal can be administered or instructed to self- 20 administer one or more sGC agonists as described above.

Examples of anti-fibrotic agents include, without limitation, TGF-beta neutralizing antibodies, angiotension II inhibitors, inhibitors of fibrogenic cytokine signaling (e.g., TNF-alpha neutralizing antibodies, and inhibitors or neutralizing antibodies against IL6 signaling), integrin inhibitors, and other small molecules or 25 inhibitors or neutralizing antibodies that may directly target fibrogenic signaling, indirectly target fibrogenic signaling (e.g., upstream or downstream modulators), or suppress fibrogenic signaling. Specific examples of anti-fibrotic agents include, without limitation, SHP-627, hydronidone PXS-25, disitertide, fresolimumab, LY2382770, STX-100, CWHM-12, SB-431542, THR-184, PF-06473871, RXI-109, 30 FG-3019, imatinib, BOT-191, nilotinib, dasatinib, nintedanib, sorafenib, thalidomide, pomalidomide, etanercept, belimumab, refanalin (BB-3), dectrekumab (QAX-576), tralokinumab, anakinra, rilonacept, SAR156597, carlumab, bindarit, maraviroc, RS-504393, actimmune, IFN- α , batimastat (BB-49), marimastat, macitentan, bosentan,

ambrisentan, sparsentan (RE-021), atrasentan, losartan, BMS-986020, SAR-100842, PAR1 antagonists, curcumin, silymarin, β -caryophyllene, beraprost, iloprost, treprostinil, aviptadil, sivelestat, UK-396082, serelaxin, PRM-151, dioscin, NTU281, rapamycin, palomid-529 (RES-529), ruxolitinib, baricitinib, omipalisib
5 (GSK2126458), PF-562271, tanzisertib (CC-930), MMI-0100, IMD-1041, bardoxolone methyl (CDDO-Me), antisense NF- κ B, baicalein, sulfasalazine, Y-27632, bortezomib, emricasan, VX-166, Z-VAD-fmk, CTP-499, VBY-376, CA-074Me, paquinimod, HOE-077, rosiglitazone, elafibranor (GFT-505), saroglitazar, pioglitazone, docosahexaenoic acid, INT-767, PX-102, obeticholic acid, turofexorate
10 isopropyl (WAY-362450), GW4064, triamcinolone, genistein, pirfenidone, pentoxifylline, SIS-3, glycyrrhizin, anti-miR-21, ademetionine (SAM), β -aminopropionitrile (BAPN), simtuzumab (GS-6624), GM-CT-01, GR-MD-02, GCS-100, GKT137831, N-acetylcysteine, mitoquinone, salvianolic acid B, resveratrol, pyridoxamine, α -tocopherol, and IW001.

15 Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions described herein include, without limitation, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or
20 electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. If required, the
25 solubility and bioavailability of a sGC agonist and/or a PDE5A inhibitor in a pharmaceutical composition can be enhanced using lipid excipients and/or block copolymers of ethylene oxide and propylene oxide. See, e.g., U.S. Patent No. 7,014,866 and U.S. Patent Publication Nos. 20060094744 and 20060079502.

A pharmaceutical composition described herein can be designed for oral or
30 parenteral (including subcutaneous, intramuscular, intravenous, and intradermal) administration. Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that can contain anti-oxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the

intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations can be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

5 Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.

Such injection solutions can be in the form, for example, of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation can be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1, 3-butanediol. Among the acceptable vehicles and solvents that can be used are mannitol, water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be used including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives can be used in the preparation of injectables, as can natural pharmaceutically-acceptable oils, such as olive oil or castor oil, including those in their polyoxyethylated versions. These oil solutions or suspensions can contain a long-chain alcohol diluent or dispersant.

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In some cases, a pharmaceutically acceptable composition including one or more sGC agonists and/or one or more PDE5A inhibitors can be administered locally or systemically. For example, a composition containing a sGC agonist can be administered systemically by injection to a mammal (e.g., a human). When two or more sGC agonists are to be administered, each sGC agonist can be administered by the same or different routes. For example, ataciguat can be administered orally, and YC-I can be administered by injection. In some cases, one or more sGC agonists can be administered via one route, and one or more PDE5A inhibitors can be administered via the same or a different route.

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Compositions containing one or more sGC agonists or a combination of one or more sGC agonists and one or more PDE5A inhibitors can be administered to a male mammal in any amount, at any frequency, and for any duration effective to achieve a

desired outcome (e.g., to reduce calcification of heart valves and/or vessels or to slow progression of aortic sclerosis to calcific aortic valve stenosis). Compositions containing one or more anti-fibrotic agents can be administered to a female mammal in any amount, at any frequency, and for any duration effective to achieve a desired
5 outcome (e.g., to reduce calcification of heart valves and/or vessels or to slow progression of aortic sclerosis to calcific aortic valve stenosis).

Effective doses can vary, as recognized by those skilled in the art, depending on the severity of the condition (e.g., calcific aortic valve stenosis), the route of administration, age and general health condition of the subject, excipient usage, the
10 possibility of co-usage with other therapeutic treatments such as use of other agents and the judgment of the treating physician.

An effective amount of a composition containing one or more sGC agonists or a combination of one or more sGC agonists and one or more PDE5A inhibitors can be any amount that reduces the severity of a symptom of a condition being treated (e.g.,
15 calcific aortic valve stenosis) without producing significant toxicity to the male mammal. For example, an effective amount of a sGC agonist (e.g., YC-I) can be from about 0.5 mg/kg to about 80 mg/kg (e.g., from about 0.5 mg/kg to about 70 mg/kg, from about 0.5 mg/kg to about 60 mg/kg, from about 0.5 mg/kg to about 50 mg/kg, from about 0.5 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg,
20 from about 0.5 mg/kg to about 20 mg/kg, from about 0.5 mg/kg to about 10 mg/kg, from about 0.5 mg/kg to about 5 mg/kg, from about 0.5 mg/kg to about 1 mg/kg, from about 0.75 mg/kg to about 10 mg/kg, from about 1 mg/kg to about 10 mg/kg, or from about 2 mg/kg to about 10 mg/kg). In some cases, between about 0.1 mg and about 500 mg (e.g., between about 0.1 mg and about 0.5 mg, between about 0.5 mg and
25 about 1 mg, between about 1 mg and about 5 mg, between about 5 mg and about 10 mg, between about 10 mg and about 25 mg, between about 25 mg and about 50 mg, between about 50 mg and 200 mg, between about 50 mg and 180 mg, between about 50 mg and 150 mg, between about 50 mg and 125 mg, between about 60 mg and 200 mg, between about 75 mg and 200 mg, between about 100 mg and 200 mg, between
30 about 75 mg and 150 mg, between about 100 mg and 150 mg, between about 100 mg and about 200 mg, between about 200 mg and about 300 mg, between about 300 mg and about 400 mg, or between about 400 mg and about 500 mg) of a sGC agonist such as ataciguat can be administered to a mammal (e.g., a male human) per day for a

suitable length of time. If a particular mammal fails to respond to a particular amount, then the amount of sGC agonist or PDE5A inhibitor can be increased by, for example, two fold. After receiving this higher amount, the mammal can be monitored for both responsiveness to the treatment and toxicity symptoms, and adjustments
5 made accordingly.

An effective amount of a composition containing one or more anti-fibrotic agents can be any amount that reduces the severity of a symptom of a condition being treated (e.g., calcific aortic valve stenosis) without producing significant toxicity to the female mammal. For example, an effective amount of an anti-fibrotic agent can
10 be from about 0.01 mg/kg to about 80 mg/kg (e.g., from about 0.01 mg/kg to about 0.1 mg/kg, from about 0.1 mg/kg to about 0.5 mg/kg, from about 0.5 mg/kg to about 70 mg/kg, from about 0.5 mg/kg to about 60 mg/kg, from about 0.5 mg/kg to about 50 mg/kg, from about 0.5 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.5 mg/kg to about 20 mg/kg, from about 0.5 mg/kg to about 10
15 mg/kg, from about 0.5 mg/kg to about 5 mg/kg, from about 0.5 mg/kg to about 1 mg/kg, from about 0.75 mg/kg to about 10 mg/kg, from about 1 mg/kg to about 10 mg/kg, or from about 2 mg/kg to about 10 mg/kg). In some cases, between about 0.1 mg and about 500 mg (e.g., between about 0.1 mg and about 0.5 mg, between about 0.5 mg and about 1 mg, between about 1 mg and about 5 mg, between about 5 mg and
20 about 10 mg, between about 10 mg and about 25 mg, between about 25 mg and about 50 mg, between about 50 mg and 200 mg, between about 50 mg and 180 mg, between about 50 mg and 150 mg, between about 50 mg and 125 mg, between about 60 mg and 200 mg, between about 75 mg and 200 mg, between about 100 mg and 200 mg, between about 75 mg and 150 mg, between about 100 mg and 150 mg, between about
25 100 mg and about 200 mg, between about 200 mg and about 300 mg, between about 300 mg and about 400 mg, or between about 400 mg and about 500 mg) of an anti-fibrotic agent can be administered to a mammal (e.g., a female human) per day for a suitable length of time. If a particular mammal fails to respond to a particular amount, then the amount of anti-fibrotic agent can be increased by, for example, two
30 fold. After receiving this higher amount, the mammal can be monitored for both responsiveness to the treatment and toxicity symptoms, and adjustments made accordingly.

The effective amount can remain constant or can be adjusted as a sliding scale or variable dose depending on the mammal's response to treatment. Various factors can influence the actual effective amount used for a particular application. For example, the frequency of administration, duration of treatment, use of multiple
5 treatment agents, route of administration, and severity of the condition (e.g., calcific aortic valve stenosis) may require an increase or decrease in the actual effective amount administered.

The frequency of administration can be any frequency that reduces the severity of a symptom of a condition to be treated (e.g., calcific aortic valve stenosis)
10 without producing significant toxicity to the male mammal. For example, the frequency of administration can be from about once a week to about three times a day, or from about twice a month to about six times a day, or from about twice a week to about once a day. The frequency of administration can remain constant or can be variable during the duration of treatment. In some cases, a course of treatment of a
15 male mammal with a composition containing one or more sGC agonists or a combination of one or more sGC agonists and one or more PDE5A inhibitors, or a course of treatment of a female mammal with a composition containing one or more anti-fibrotic agents, can include rest periods. For example, a composition containing one or more sGC agonists or a combination of one or more sGC agonists and one or
20 more PDE5A inhibitors can be administered daily over a two week period followed by a two week rest period, and such a regimen can be repeated multiple times. As with the effective amount, various factors can influence the actual frequency of administration used for a particular application. For example, the effective amount, duration of treatment, use of multiple treatment agents, route of administration, and
25 severity of the condition (e.g., calcific aortic valve stenosis) may require an increase or decrease in administration frequency. An effective duration for administering a composition containing one or more sGC agonists or a combination of one or more sGC agonists and one or more PDE5A inhibitors can be any duration that reduces the severity of a symptom of the condition to be treated (e.g., calcific aortic valve
30 stenosis) without producing significant toxicity to the male mammal. Thus, the effective duration can vary from several days to several weeks, months, or years. In general, the effective duration for the treatment of calcific aortic valve stenosis can range in duration from several months to several years. In some cases, an effective

duration can be for as long as an individual mammal is alive. Multiple factors can influence the actual effective duration used for a particular treatment. For example, an effective duration can vary with the frequency of administration, effective amount, use of multiple treatment agents, route of administration, and severity of the condition
5 being treated.

In certain instances, a course of treatment and the severity of one or more symptoms related to the condition being treated can be monitored. Any appropriate method can be used to determine whether or not the severity of a symptom is reduced. For example, the severity of a symptom of calcific aortic valve stenosis can be
10 assessed using imaging techniques at different time points.

This document also provides methods and materials to assist medical or research professionals in determining whether or not a mammal identified as having, or as being likely to have, a cardiovascular condition (e.g., heart valve calcification, vessel calcification, aortic sclerosis, arterial stiffness, or left ventricular systolic or
15 diastolic dysfunction) is likely to benefit from treatment with a sGC agonist or a sGC agonist and a PDE5A inhibitor. Medical professionals can be, for example, doctors, nurses, medical laboratory technologists, and pharmacists. Research professionals can be, for example, principal investigators, research technicians, postdoctoral trainees, and graduate students. A professional can be assisted in determining
20 whether or not a mammal has, or is likely to have, a cardiovascular condition, and/or whether a mammal having a cardiovascular condition is likely to respond to a sGC agonist (e.g., ataciguat) by (1) determining a blood LPA level, and (2) communicating information about that level to that professional.

Any method can be used to communicate information to another person (e.g.,
25 a professional). For example, information can be given directly or indirectly to a professional. In addition, any type of communication can be used to communicate the information. For example, mail, e-mail, telephone, and face-to-face interactions can be used. The information also can be communicated to a professional by making that information electronically available to the professional. For example, the information
30 can be communicated to a professional by placing the information on a computer database such that the professional can access the information. In addition, the information can be communicated to a hospital, clinic, or research facility serving as an agent for the professional.

participation in a randomized, double-blind, placebo-controlled trial. After screening, patients were randomized into two groups:

- (1) placebo treatment for a minimum of 6 months, or
- (2) ataciguat treatment for a minimum of 6 months

5 Patients underwent computed tomography measurements of aortic valve calcification, echocardiographic assessments of ventricular and aortic valve function, and DEXA scanning for assessment of systemic bone mineral density at baseline and at six month intervals after initiation of treatment.

In vitro cell culture studies: Aortic valve interstitial cells were isolated using
10 an outgrowth method, and were expanded in DMEM/F-10 medium with 10% FBS and studied in passages 2-10. Cells were treated for 24 hours under each experimental condition, at which point mRNA was harvested using lysis buffer. After mRNA isolation and reverse transcription, gene expression was measured using
15 quantitative real-time RT-PCR and Taqman primers. Changes in gene expression were assessed by the ddCt method and expressed as fold-change normalized to control conditions.

LPA studies in humans with aortic valve calcification: Human plasma LPA levels were measured using a colorimetric, sandwich/quantitative ELISA assay (AB212165) after samples were diluted in diluent buffer. Computed tomography
20 scanning was performed using clinical grade, high-resolution scanning methods that are well-established for assessment of aortic valve calcification. The calcium burden was measured from 3-dimensional, multi-slice images and expressed as arbitrary units (AU).

25 Example 2 - Ataciguat increases sGC signaling and attenuates BMP signaling *in vitro*

To confirm that ataciguat effectively activated sGC in cells, mouse aortic valve interstitial cells (mVICs) were isolated from C57BL6/J mice treated with ataciguat or other sGC agonists as internal controls. In otherwise naïve cells, treatment with ataciguat significantly increased sGC-dependent signaling (indicated
30 by phospho-VASP²³⁹ levels) over vehicle-treated cells. Importantly, both sodium nitroprusside and DEA-NONOATE increased phospho-VASP²³⁹ levels to a similar extent, suggesting that sGC resides in both reduced and heme-free states in mVICs.

To determine whether ataciguat could attenuate signaling cascades central to the initiation and progression of valve calcification *in vitro*, cells were treated with BMP2 with or without ataciguat. In these studies, ataciguat reduced phospho-SMAD1/5/8 signaling (e.g., canonical BMP signaling) as well as expression of
5 osteogenic genes induced by BMP2 (e.g., osterix).

Example 3 – Ataciguat slows progression of established FCAVS *in vivo*

A therapeutically-relevant treatment strategy in which *ldlr*-deficient, apoB100-only mice were fed a western diet for six months (to develop mild/moderate FCAVS)
10 followed by western diet plus ataciguat (150 ppm mixed in food). To confirm that ataciguat effectively binds oxidized sGC in aortic valve tissue *in vivo*, immunohistochemistry was used to demonstrate that pVASP²³⁹ was significantly increased in mice of both genders (**FIGS. 1A and 1B**). Consistent with other reports in mouse models of hyperlipidemia, treatment with ataciguat did not alter blood
15 pressure in mice of either gender.

To subsequently evaluate the effects of ataciguat on signaling pathways that drive progression of FCAVS *in vivo*, immunohistochemistry and quantitative real-time RT-PCR were used to assess changes in canonical BMP and TGF β signaling in aortic valve tissue. Similar to observations in valve interstitial cells *in vitro*, treatment
20 with ataciguat for three months significantly attenuated SMAD1/5/8 phosphorylation in valve tissue from male and female mice (**FIGS. 2A and 2B**), and reduced markers of osteogenesis such as Runx2 and SPP1 (**FIGS. 3A-3D**). Greater reductions in valvular calcium also were observed in male mice (**FIG. 4**). Interestingly, treatment with ataciguat increased canonical TGF β signaling (SMAD2/3 phosphorylation) in
25 valve tissue from both male and female mice (**FIGS. 5A and 5B**), but paradoxically reduced the expression of the SMAD2/3 target genes CTGF and COL1A1 only in female mice (**FIGS. 6A-6D**). MMP2 expression was not altered by ataciguat in either male or female mice, but was markedly higher in female mice than in male mice (**FIGS. 7A and 7B**). Consistent with these molecular changes in matrix
30 metalloproteinase and collagen isoform expression, changes in relative collagen fiber thickness were only evident in female mice (**FIGS. 8A and 8B**). In addition, consistent with observed changes in valvular calcification, long-term treatment with ataciguat significantly attenuated progression of valvular dysfunction and associated

reductions in left ventricular diastolic function in male mice only (**FIGS. 9A-9B and 10A-10D**).

Example 4 – Ataciguat is well-tolerated in patients with mild/moderate FCAVS *in*

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vivo

Combining ataciguat's role as an activator of heme-free sGC with reports of increased systemic oxidative stress in patients with FCAVS (Mourino-Alvarez et al., *Int. J. Cardiol.*, 225:99-106 (2016)), it was conceivable that administration of ataciguat could elicit acute hypotension or increase propensity to experience orthostatic hypotension. To investigate this possibility, a randomized, double-blind, placebo-controlled, dose-escalation study was conducted to assess the effects of ataciguat on orthostatic tolerance using both a standing test (highly functional) and a tilt table test (highly sensitive). These studies revealed that 14 days of treatment with placebo or either dose of ataciguat did not significantly alter resting blood pressure (**FIGS. 11A-11D**) or augment any reductions in blood pressure or heart rate in response to standing (**FIGS. 12A-12D and 13A-13B**) or head-up tilt (**FIGS. 14A-14D, 15A-15D, and 16A-16D**).

Example 5 – Ataciguat slows progression of valve disease in patients with
20 mild/moderate FCAVS

To determine whether ataciguat could slow progression of FCAVS, a randomized, double-blind, placebo-controlled study was conducted in patients with mild/moderate valvular disease. Key eligibility criteria for the study included valvular calcium levels above 300 AU, a valve area between 1.0 and 2.0 cm², an ejection fraction greater than 50%, and absence of congenital heart valve disease (full inclusion/exclusion criteria is shown in **TABLE 1**). Patients were randomized to placebo or ataciguat treatment for six months, and a subset of patients received treatment for 12 months. Subject characteristics at baseline are shown in **TABLE 2**. Importantly, there was no difference in measured hemodynamic, blood biochemistry, drug treatment, or comorbidity indices between treatment groups at baseline. Given the relatively short duration of follow-up, the primary outcome variable was the change in valvular calcium over time. Secondary and tertiary outcomes were aortic valve and left ventricular function, respectively.

After six months of treatment, it was found that treatment with ataciguat attenuated progression of aortic valve calcification by ~70% compared to placebo-treated patients (**FIGS. 17A-17C**). Interestingly, and consistent with the observations in mice, subgroup analyses suggested that ataciguat conferred a greater benefit to men with FCAVS than women with FCAVS. While placebo-treated patients displayed progressive worsening of aortic valve function during the six month follow-up period ($p < 0.01$ vs baseline), aortic valve function did not worsen significantly in patients treated with ataciguat ($p = 0.24$ vs baseline) (**FIGS. 18A-18F**). Similar to observed changes in aortic valve calcium levels, treatment with ataciguat tended to confer a greater therapeutic benefit to men with FCAVS compared to women.

Studies were then conducted to assess whether ataciguat had any impact on cardiac function and adaptations to chronic ventricular overload presented by aortic valve dysfunction. After six months of treatment, it was observed that treatment with ataciguat could prevent decline in left ventricular systolic function (assessed by either ejection fraction or stroke volume), compared to placebo-treated subjects (**FIGS. 19A-19F**). Interestingly, ataciguat also tended to improve left ventricular diastolic function (assessed by either E/e' or the E/A ratio) compared to placebo-treated patients (**FIGS. 20A-20F**). A reduction in pulse pressure also was observed in males treated with ataciguat as compared to placebo; such a reduction was not apparent in females (**FIGS. 21A-21C**). Strikingly, and similar to the observed changes in aortic valve function, these data suggested that men received a greater and more consistent therapeutic benefit from ataciguat in both systolic and diastolic function when compared to women.

TABLE 1: Inclusion/Exclusion Criteria
<i>Criteria for Inclusion</i>
Age > 50 years
Male or female gender
Aortic valve area greater than 1.0 cm ² but less than 2.0 cm ²
Aortic valve calcium levels greater than 150 AU from chest CT
Ejection fraction >50%
<i>Criteria for Exclusion</i>
Orthostatic intolerance or symptomatic hypotension prior to study or during study visits
Positive pregnancy test during screening visit
Nitrate use within 24 hours
Systolic blood pressure <110 mm Hg
Mean systemic arterial pressure <75 mm Hg
Severe mitral or aortic regurgitation
Retinal or optic nerve problems
Recent (≤30 days) acute coronary syndrome
Oxygen saturation <90% on room air
Congenital valve disease
Hepatic dysfunction/elevated liver enzymes
Prescription of drugs known to alter NO-sGC-cGMP signaling (sildenafil, nitrates, etc.)
Prescription of Warfarin (Coumadin) for chronic anticoagulation
Concomitant participation in other trials at Mayo Clinic or elsewhere
Use of phenytoin or related compounds for any indication
Chronic midazolam treatment for any indication
Use of monoamine oxidase inhibitors for any indication
Use of anti-diabetic drugs in the sulfonylurea family
Use of fluvastatin (predominantly degraded by CYP2C9)

TABLE 2: Patient Characteristics at Baseline (mean \pm SD).			
Characteristic	PLACEBO (n = 11)	ATACIGUAT (n = 12)	p-value
Median age (yr)	72 \pm 8	74 \pm 4	
Male sex (no.)	8	7	
Body habitus			
Height (cm)	172 \pm 10	168 \pm 8	
Weight (kg)	99 \pm 18	88 \pm 11	
Body mass index (AU)	34 \pm 7	31 \pm 3	
Blood pressure (mm Hg)			
Systolic	129 \pm 12	131 \pm 14	
Diastolic	74 \pm 9	68 \pm 12	
Cardiac function			
Left ventricular ejection fraction (%)	65 \pm 5	65 \pm 6	0.99
Heart rate	62 \pm 14	62 \pm 15	0.52
Aortic valve function/disease severity			
Valve calcium levels (AU)	1208 \pm 776	1055 \pm 594	0.85
Valve area (cm ²)	1.33 \pm 0.15	1.36 \pm 0.24	0.51
Peak transvalvular velocity (m/sec)	3.3 \pm 0.4	3.2 \pm 0.4	0.64
Peak transvalvular gradient (mm Hg)	26 \pm 7	26 \pm 7	0.86
Biochemistry			
Total cholesterol (mg/dl)	164 \pm 28	197 \pm 53	0.12
LDL cholesterol (mg/dl)	82 \pm 24	110 \pm 50	0.15
HDL Cholesterol (mg/dl)	52 \pm 18	65 \pm 15	0.07
Triglycerides (mg/dl)	151 \pm 70	118 \pm 50	0.11
Glucose (mg/dl)	115 \pm 35	105 \pm 17	0.37
Creatinine	1.0 \pm 0.2	0.9 \pm 0.2	0.73
Alanine aminotransferase (U/L)	25 \pm 13	25 \pm 11	0.94
Aspartate aminotransferase (U/L)	25 \pm 11	30 \pm 25	0.59
APTT (sec)	33 \pm 3	33 \pm 3	0.98
INR (Units)	1.0 \pm 0.1	1.0 \pm 0.1	0.59
Hematocrit (%)	43 \pm 3	41 \pm 5	0.12
Hemoglobin (g/dl)	14.3 \pm 1.4	13.7 \pm 1.5	0.15
Coexisting conditions (no.)			
Hypercholesterolemia	8	8	
Hypertension	7	7	
Diabetes	2	1	
Atrial fibrillation	0	0	
Current smoker			
Coronary heart disease	4	1	
Cerebrovascular disease	0	0	

Peripheral vascular disease	0	0	
Charlson Comorbidity Index			
Not age-adjusted	2.6 ± 3.1	0.8 ± 0.8	0.07
Age-adjusted	5.1 ± 2.8	3.8 ± 0.9	0.11
Medications			
Statins	10	10	
ACE inhibitor or ARB	6	6	
Beta blocker	7	4	
Other			

Example 6 – Utility of baseline variables in predicting progression of aortic valve dysfunction

Further analyses used a neural network algorithm trained on baseline variables to determine whether there were readily available phenotypic characteristics that would predict changes in aortic valve area over time. Following 10-fold cross validation, the algorithm performed reasonably well at predicting the change in aortic valve function in data from all subjects. Critically – and consistent with the data presented above, the model identified both treatment group (placebo vs. ataciguat) and sex (male vs. female) as the most informative variables predicting the change in valve area over time (**FIG. 22**).

In summary, the studies described above demonstrated the following:

In mice, ataciguat activated sGC in valve tissue, reduced osteogenic signaling and calcification in valve tissue from male and female animals, and did not consistently impact collagen expression in males but reduced collagen expression in females which, combined with higher MMP2 expression, caused a shift toward a phenotype of thinner collagen fibers in females. This work suggested that ataciguat preferentially slows valvular and cardiac diastolic dysfunction in male mice.

In humans, ataciguat was well tolerated with regard to side effects and blood pressure regulation. Ataciguat effectively reduced blood pressure at low doses and was more effective when subjects were supine. It also tended to slow progression of valve calcification by about 70%, slow progression of valve dysfunction, and preserve or prevent decline in left ventricular systolic and diastolic function, although these benefits were predominantly observed in men.

Finally, a non-biased neural network model revealed that treatment group (ataciguat vs. placebo) and gender (male vs. female) were the strongest predictors of therapeutic effectiveness when predicting changes in aortic valve area.

Example 7 – Effects of sGC agonists on expression of Osterix

In vitro studies were conducted in aortic valve interstitial cells to determine the effects of several sGC agonists on mRNA levels of Osterix, a BMP target gene.

5 Cells were treated with nelociguat, vericiguat, lificiguat, or BAY41-8543, and Osterix mRNA levels were determined. Nelociguat and BAY41-8543 were used at concentrations ranging from 1 nM to 10 μ M, while vericiguat and lificiguat were used at concentrations ranging from 1 nM to 1 μ M.

Nelociguat effectively reduced BMP signaling at concentrations from 1 nM to 10 1 μ M (**FIG. 23**, arrows). This finding suggested that a concentration 10 μ M or higher may be cytotoxic, as has been observed with some other compounds. Vericiguat was effective to reduce Osterix expression only at the highest dose tested (**FIG. 24**, arrow). Similar to nelociguat, lificiguat appeared to lose efficacy at higher concentrations *in vitro* (**FIG. 25**; effective concentrations indicated by arrows). 15 BAY41-8543 tended to drive dose-dependent reductions in Osterix expression, and was highly efficacious at 10 μ M (**FIG. 26**, arrow).

Example 8 – Effects of ataciguat on LPA-induced BMP signaling

Additional studies were conducted in aortic valve interstitial cells to examine 20 the effects of ataciguat on LPA-induced BMP signaling and BMP target gene expression. Cells were treated with 18:0 lyso-PA, with or without ataciguat, and Osterix mRNA levels were determined. These studies showed that treatment with 18:0 lyso-PA increased expression of Osterix and that ataciguat dramatically suppressed the LPA effect (**FIG. 27**, “LPA” vs. “LPA+ATA”), suggesting that 25 ataciguat would be efficacious in the treatment of valve disease in patients with high LPA levels.

The relationship between plasma LPA levels and levels of valvular calcium was evaluated in patients with mild to moderate aortic valve stenosis. Plasma LPA was measured at baseline, and valvular calcium levels were assessed by CT scanning 30 at baseline or after sGC agonist treatment. At baseline, higher plasma LPA levels were associated with greater valvular calcium levels in this patient cohort (**FIG. 28**). After treatment with ataciguat for six months, patients who had higher baseline levels

of plasma LPA tended to receive greater therapeutic benefit (greater attenuation of excess BMP signaling) than patients with lower levels of plasma LPA (**FIG. 29**).

OTHER EMBODIMENTS

- 5 It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for treating a mammal on the basis of the mammal's gender, said method comprising:
 - (a) identifying a mammal as having, or as being at risk of developing, heart valve calcification or vessel calcification,
 - (b) identifying said mammal as a male mammal or a female mammal, and
 - (c) when said mammal is a male mammal, administering a sGC agonist to said male mammal, thereby slowing progression of calcification of a heart valve or vessel within said male mammal, and when said mammal is a female mammal, not administering a sGC agonist to said female mammal and, optionally, administering a treatment that is not a sGC agonist to said female mammal to slow progression of calcification of a heart valve or vessel within said female mammal.
2. The method of claim 1, wherein said mammal is a human.
3. The method of claim 1 or claim 2, wherein said sGC agonist is:
 - ataciguat (5-chloro-2-[[[(5-chloro-2-thienyl)sulfonyl]amino]-N-[4-(4-morpholinylsulfonyl)phenyl]-benzamide; HMR1766),
 - YC-I (5-[1-(phenylmethyl)-1H-indazol-3-yl]-2-furanmethanol),
 - BAY 58-2667 (4-[[[(4-carboxybutyl)(2-[(4-phenethylbenzyl)oxy]phenethyl)amino)methyl]benzoic]acid hydrochloride),
 - BAY 41-2272 (3-(4-amino-5-cyclopropylpyrimidin-2-yl)-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridine),
 - BAY-41-8543 (2-[1-[(2-fluorophenyl)methyl]-1H-pyrazolo[3,4-b]pyridin-3-yl]-5-(4-morpholinyl)-4,6-pyrimidinediamine),
 - BAY 63-2521 (methyl (4,6-diamino-2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-yl)(methyl)carbamate),
 - CFM-1571 (3-[3-(dimethylamino)propoxy]-N-(4-methoxyphenyl)-1-(phenylmethyl)-1H-pyrazole-5-carboxamide hydrochloride),
 - A-350619 (3-[2-(4-chlorophenylthio)phenyl]-N-(4-dimethylaminobutyl)acrylamide),
 - vericiguat (methyl (4,6-diamino-2-(5-fluoro-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-yl)carbamate),

praliciguat (1,1,1,3,3,3-hexafluoro-2-[(5-fluoro-2-[1-(2-fluorobenzyl)-5-(1,2-oxazol-3-yl)-1H-pyrazol-3-yl]pyrimidin-4-yl}amino)methyl]propan-2-ol),

olinciguat ((2R)-3,3,3-trifluoro-2-[(5-fluoro-2-{1-[(2-fluorophenyl)methyl]-5-(1,2-oxazol-3-yl)-1H-pyrazol-3-yl}pyrimidin-4-yl)amino]methyl}-2-hydroxypropanamide),

bis-heteroaryl pyrazole IWP-051 (5-fluoro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4(3H)-one),

IW-6463,

GSK2181236A (1-(6-{2-[(3-methyl-4'-(trifluoromethyl)oxy]-4-biphenyl)methyl]oxy}phenyl)-2-pyridinyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid),

IWP-550,

IWP-854 (4-(5-fluoro-2-(1-(2-fluorobenzyl)-5-(isoxazol-3-yl)-1H-pyrazol-3-yl)pyrimidin-4-yl)-1-(3-methyl-3H-diazirin-3-yl)-N-(37-oxo-41-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-3,6,9,12,15,18,21,24,27,30,33-undecaaza-36-azahentetracontyl)-7,10,13,16-tetraoxa-4-azanonadecan-19-amide),

IWP-953,

nelociguat (methyl (4,6-diamino-2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)pyrimidin-5-yl)carbamate),

MGV354 ((S)-1-(6-(3-((4-(1-(cyclopropanecarbonyl)piperidin-4-yl)-2-methylphenyl)amino)-2,3-dihydro-1H-inden-4-yl)pyridin-2-yl)-5-methyl-1H-pyrazole-4-carboxylic acid),

BI 703704 ((1R,5S,8s)-3-(4-(5-methyl-2-((2-methyl-4-(piperidine-1-carbonyl)benzyl)oxy)phenyl)thiazol-2-yl)-3-azabicyclo[3.2.1]octane-8-carboxylic acid),

S3448 (2-[(4-chlorophenyl)sulfonyl]amino]-4,5-dimethoxy-N-[4-(4-thiomorpholinylsulfonyl)phenyl]benzamide), or

BAY 60-2770 (4-[(4-carboxybutyl)[2-[5-fluoro-2-[4'-(trifluoromethyl)[1,1'-biphenyl]-4-yl]methoxy]phenyl]ethyl]amino]methyl]benzoic acid).

4. The method of any one of claims 1-3, wherein said method further comprises administering a PDE5A inhibitor to said male mammal.

5. The method of any one of claims 1-4, wherein said method further comprises identifying said male mammal as having an elevated plasma level of lysophosphatidic acid (LPA).
6. The method of claim 1 or claim 2, wherein said mammal is a female and said method comprises administering an anti-fibrotic agent to said female mammal to slow progression of calcification of a heart valve or vessel within said female mammal.
7. A method for treating a population of mammals identified as having, or as being at risk for developing, heart valve calcification or vessel calcification, wherein said population comprises at least one male mammal and at least one female mammal, said method comprising administering a sGC agonist to said at least one male mammal, thereby slowing progression of calcification of a heart valve or vessel within said at least one male mammal, and not administering said sGC agonist to said at least one female mammal.
8. The method of claim 7, wherein said mammals are human.
9. The method of claim 7 or claim 8, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliciguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.
10. The method of any one of claims 7-9, wherein said method further comprises administering a PDE5A inhibitor to said at least one male mammal.
11. The method of any one of claims 7-10, wherein said at least one male mammal also was identified as having an elevated plasma level of LPA.
12. A method for treating a population of mammals based on gender, said method comprising:
 - (a) identifying the gender of mammals in a population determined to have, or to be at risk for developing, heart valve calcification or vessel calcification, and
 - (b) administering a sGC agonist to mammals identified as being male mammals and not administering a sGC agonist to mammals identified as being female

mammals and, optionally, administering a treatment that is not a sGC agonist to said female mammals.

13. The method of claim 12, wherein said mammals are human.

14. The method of claim 12 or claim 13, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.

15. The method of any one of claims 12-14, wherein said method further comprises administering a PDE5A inhibitor to said mammals identified as being male mammals.

16. The method of any one of claims 12-15, wherein said mammals identified as being male mammals also were determined to have an elevated plasma level of LPA.

17. The method of claim 12 or claim 13, comprising administering an anti-fibrotic agent to said mammals identified as being female mammals.

18. A method for avoiding the unnecessary use of a sGC agonist to treat a mammal having, or at risk of developing, heart valve calcification or vessel calcification, said method comprising:

identifying said mammal as being a female mammal and as having, or at risk of developing, said heart valve calcification or vessel calcification, and

excluding said female mammal from treatment with said sGC agonist based, at least in part, on the female gender of said female mammal.

19. The method of claim 18, wherein said mammal is a human.

20. The method of claim 18 or claim 19, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.

21. A method for treating a mammal on the basis of the mammal's gender, said method comprising:
- (a) identifying a mammal as having, or as being at risk of developing, aortic sclerosis,
 - (b) identifying said mammal as a male mammal or a female mammal, and
 - (c) when said mammal is a male mammal, administering a sGC agonist to said male mammal, thereby slowing progression of aortic sclerosis to calcific aortic valve stenosis within said male mammal, and when said mammal is a female mammal, not administering a sGC agonist to said female mammal and, optionally, administering a treatment that is not a sGC agonist to said female mammal to slow progression of aortic sclerosis to calcific aortic valve stenosis within said female mammal.
22. The method of claim 21, wherein said mammal is a human.
23. The method of claim 21 or claim 22, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliciguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.
24. The method of any one of claims 21-23, wherein said method further comprises administering a PDE5A inhibitor to said male mammal.
25. The method of any one of claims 21-24, wherein said method further comprises identifying said male mammal as having an elevated plasma level of LPA.
26. The method of claim 21 or claim 22, comprising administering an anti-fibrotic agent to said female mammal.
27. A method for treating a population of mammals identified as having, or as being at risk for developing, aortic sclerosis, wherein said population comprises at least one male mammal and at least one female mammal, said method comprising administering a sGC agonist to said at least one male mammal, thereby slowing progression of aortic sclerosis to calcific aortic valve stenosis within said at least one male mammal, and not administering said sGC agonist to said at least one female mammal.

28. The method of claim 27, wherein said mammals are human.
29. The method of claim 27 or claim 28, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.
30. The method of any one of claims 27-29, wherein said method further comprises administering a PDE5A inhibitor to said at least one male mammal.
31. The method of any one of claims 27-30, wherein said method further comprises identifying said at least one male mammal as having an elevated plasma level of LPA.
32. A method for treating a population of mammals based on gender, said method comprising:
- (a) identifying the gender of mammals in a population determined to have, or to be at risk for developing, aortic sclerosis, and
 - (b) administering a sGC agonist to mammals identified as being male mammals and not administering a sGC agonist to mammals identified as being female mammals and, optionally, administering a treatment that is not a sGC agonist to said female mammals.
33. The method of claim 32, wherein said mammals are human.
34. The method of claim 32 or claim 33, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.
35. The method of any one of claims 32-34, wherein said method further comprises administering a PDE5A inhibitor to said mammals identified as being male mammals.

36. The method of any one of claims 32-35, wherein said mammals identified as being male mammals were further determined to have an elevated plasma level of LPA.
37. The method of claim 32 or claim 33, comprising administering an anti-fibrotic agent to said mammals identified as being female mammals.
38. A method for avoiding the unnecessary use of a sGC agonist to treat a mammal having, or at risk of developing, aortic sclerosis, said method comprising:
identifying said mammal as being a female mammal and as having, or at risk of developing, said aortic sclerosis, and
excluding said female mammal from treatment with said sGC agonist based, at least in part, on the female gender of said female mammal.
39. The method of claim 38, wherein said mammal is a human.
40. The method of claim 38 or claim 39, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.
41. A method for treating a mammal, comprising
(a) identifying said mammal as having, or as being at risk for, hypertension when in a supine position, and
(b) administering to a sGC agonist to said mammal, thereby reducing blood pressure in the supine position.
42. The method of claim 41, wherein said mammal is a human.
43. The method of claim 41 or claim 42, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MGV354, BI 703704, S3448, or BAY 60-2770.

44. The method of any one of claims 41-43, wherein said method further comprises administering a PDE5A inhibitor to said mammal.
45. The method of any one of claims 41-44, wherein said method further comprises identifying said mammal as being a male mammal prior to said administering.
46. The method of any one of claims 41-45, wherein said administering does not reduce blood pressure when the mammal is in an upright position.
47. A method for treating a population of mammals identified as having, or as being at risk for developing hypertension in a supine position, wherein said population comprises at least one male mammal and at least one female mammal, said method comprising administering a sGC agonist to said at least one male mammal, thereby reducing blood pressure and preventing deleterious side effects of hypertension while in the supine position in said at least one male mammal, and not administering said sGC agonist to said at least one female mammal.
48. The method of claim 47, wherein said mammals are human.
49. The method of claim 47 or claim 48, wherein said sGC agonist is ataciguat, YC-I, BAY 58-2667, BAY 41-2272, BAY-41-8543, BAY 63-2521, CFM-1571, A-350619, vericiguat, praliguat, olinciguat, bis-heteroaryl pyrazole IWP-051, IW-6463, GSK2181236A, IWP-550, IWP-854, IWP-953, nelociguat, MG3354, BI 703704, S3448, or BAY 60-2770.
50. The method of any one of claims 47-49, wherein said method further comprises administering a PDE5A inhibitor to said at least one male mammal.
51. The method of claim 47 or claim 48, comprising administering an anti-fibrotic agent to said at least one female mammal.
52. The method of any one of claims 47-50, wherein said administering does not reduce blood pressure when the mammal is in an upright position.

FIG. 1B

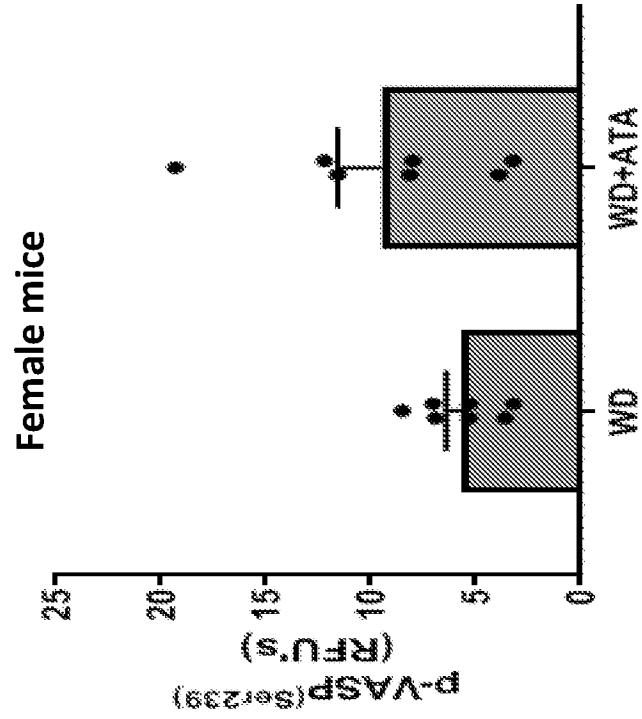


FIG. 1A

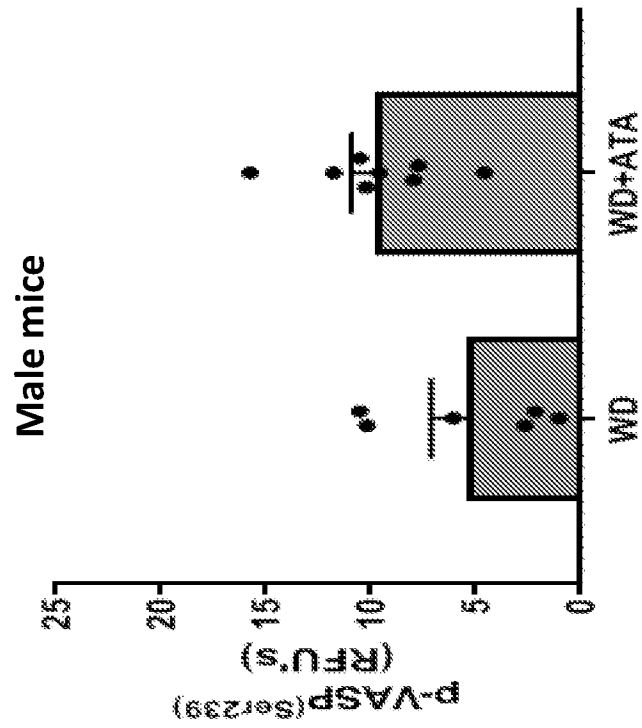


FIG. 2A

Male mice

$p < 0.01$

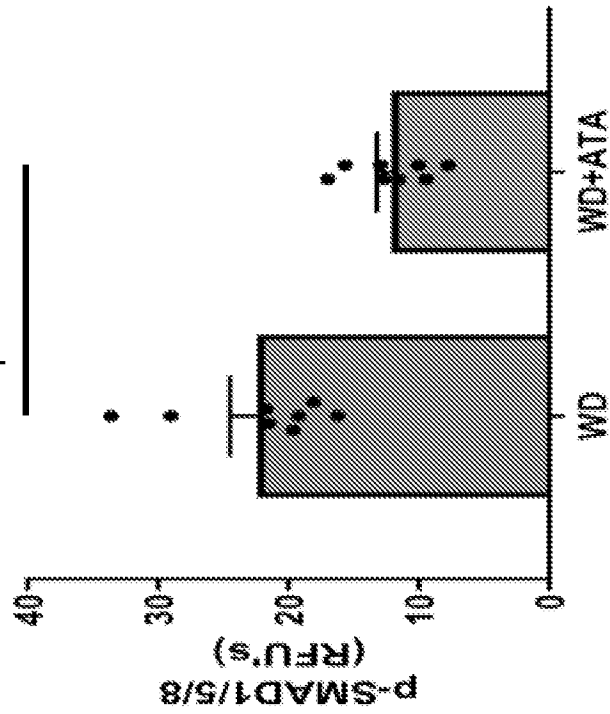


FIG. 2B

Female mice

$p < 0.05$

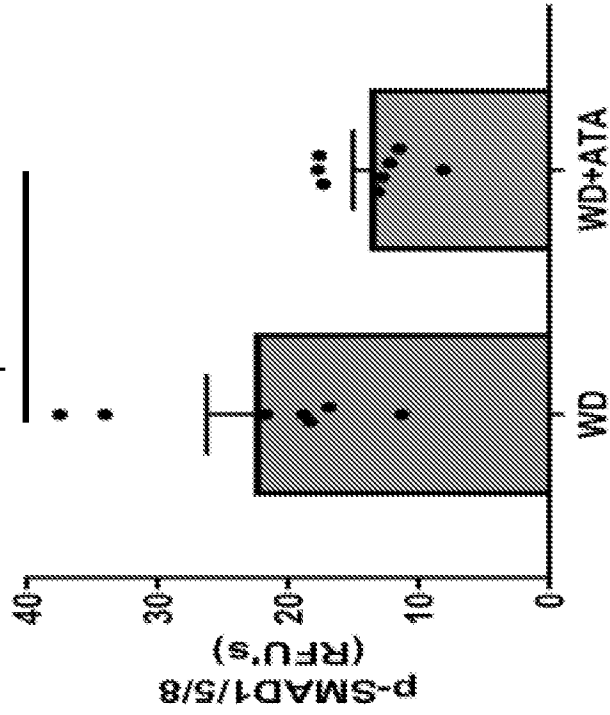


FIG. 3B
Female mice

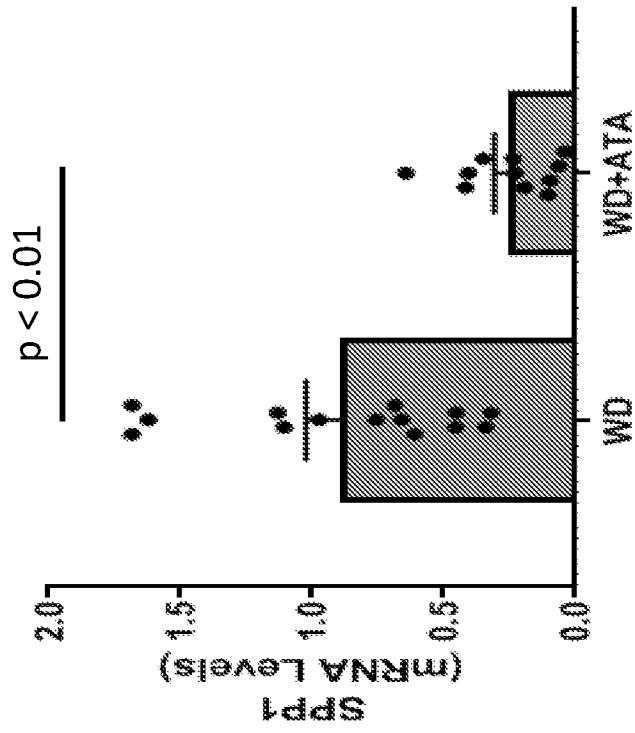


FIG. 3A
Male mice

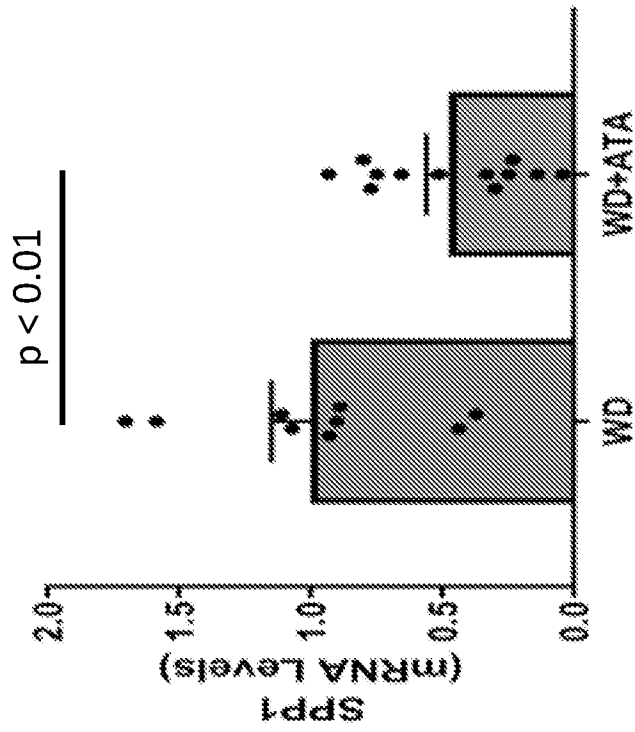


FIG. 3D

Female mice

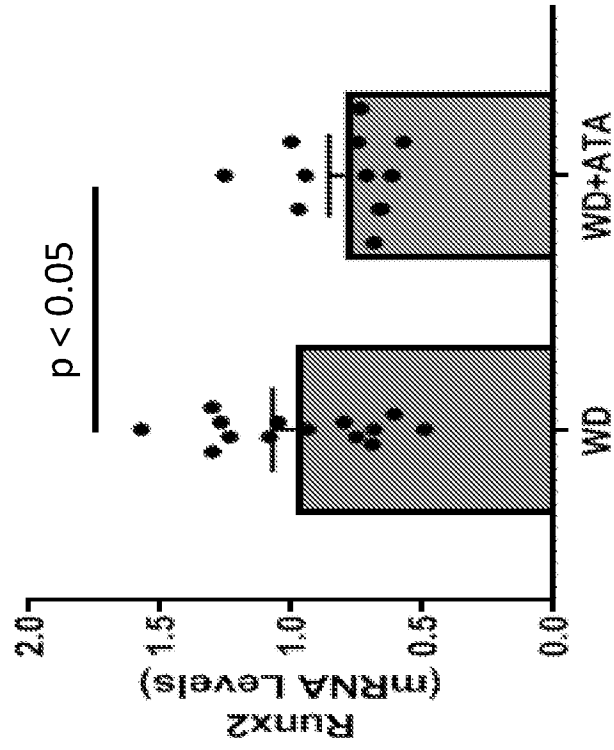


FIG. 3C

Male mice

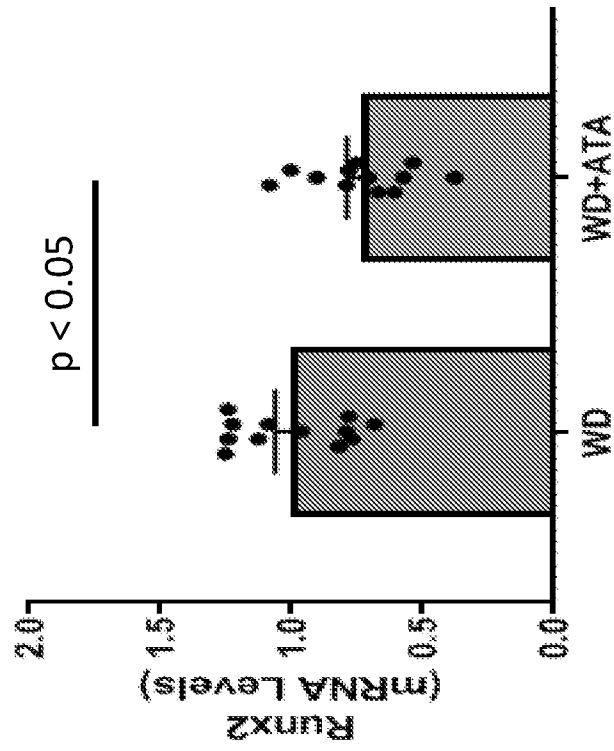


FIG. 4A

Male mice

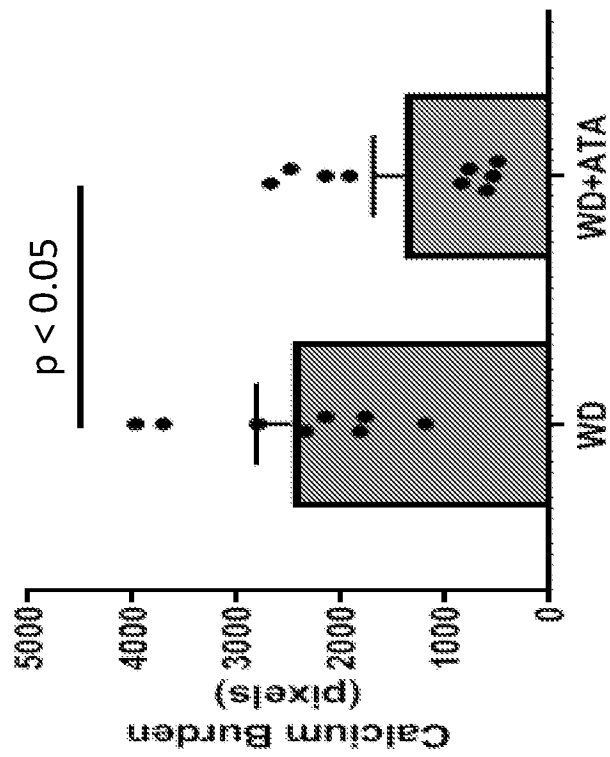


FIG. 4B

Female mice

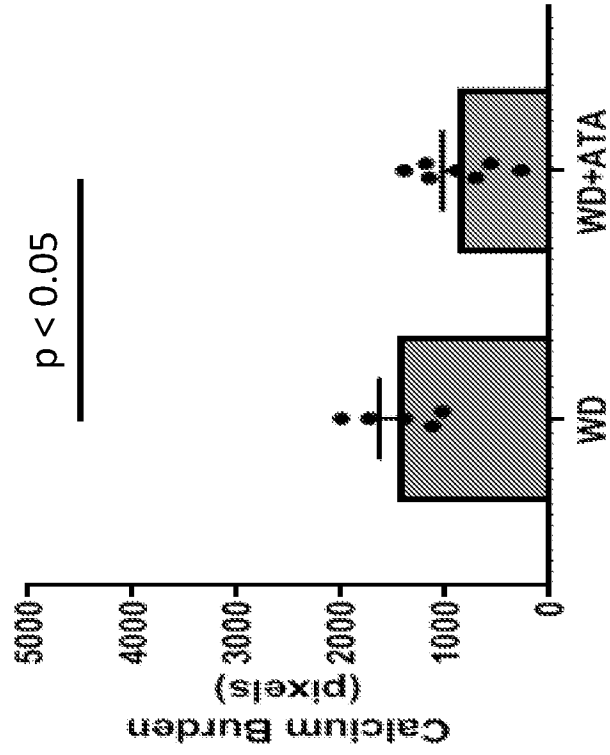


FIG. 5B

Female mice

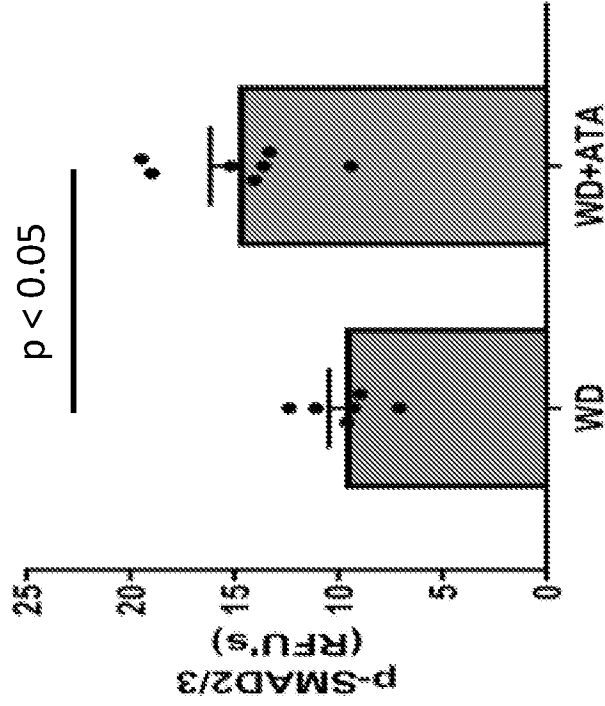


FIG. 5A

Male mice

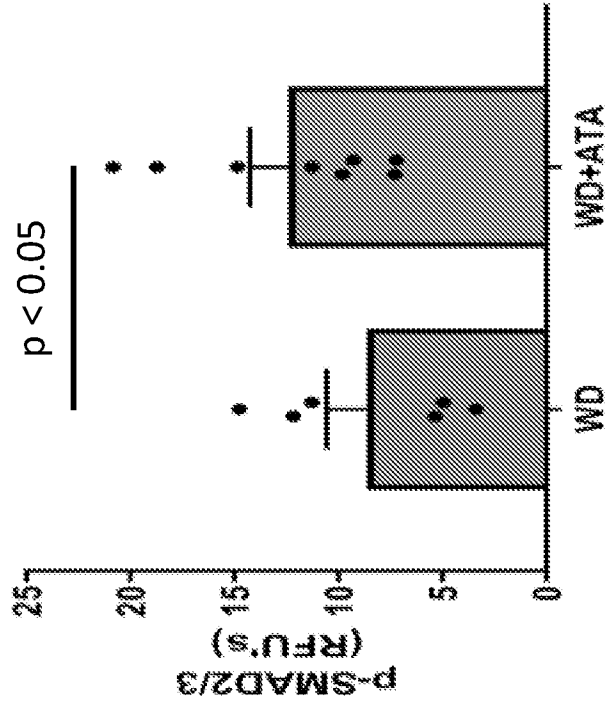


FIG. 6B

Female mice

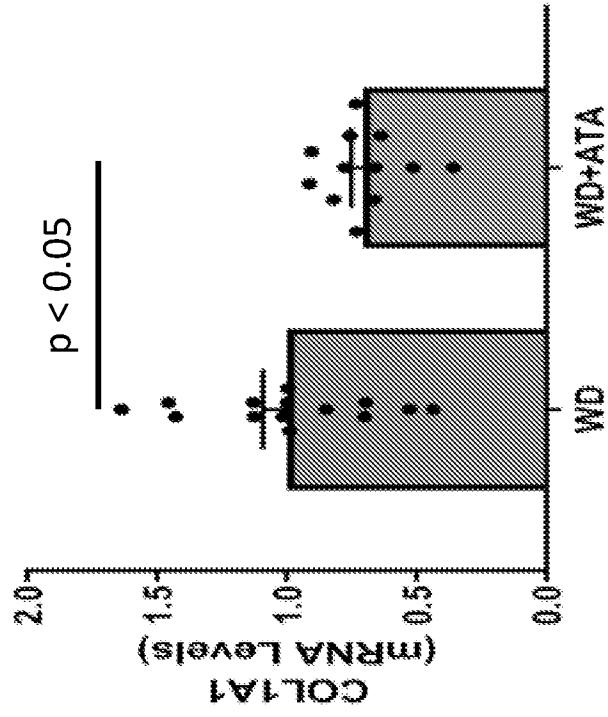


FIG. 6A

Male mice

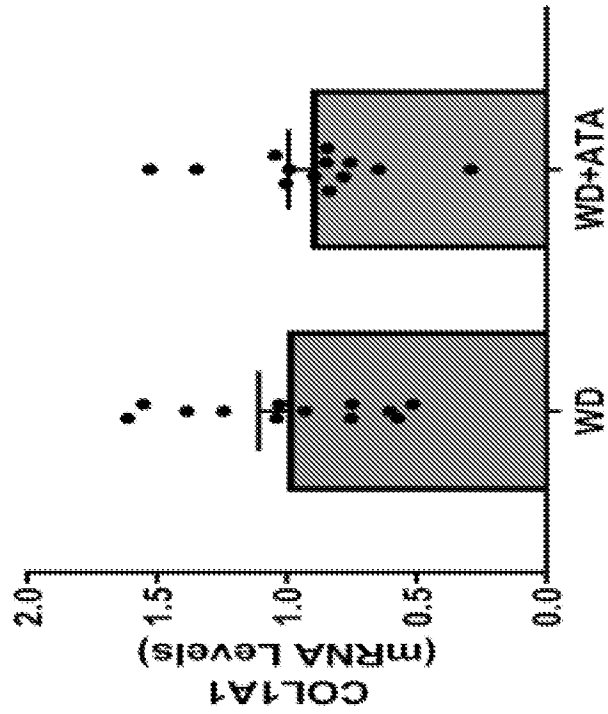


FIG. 6D

Female mice

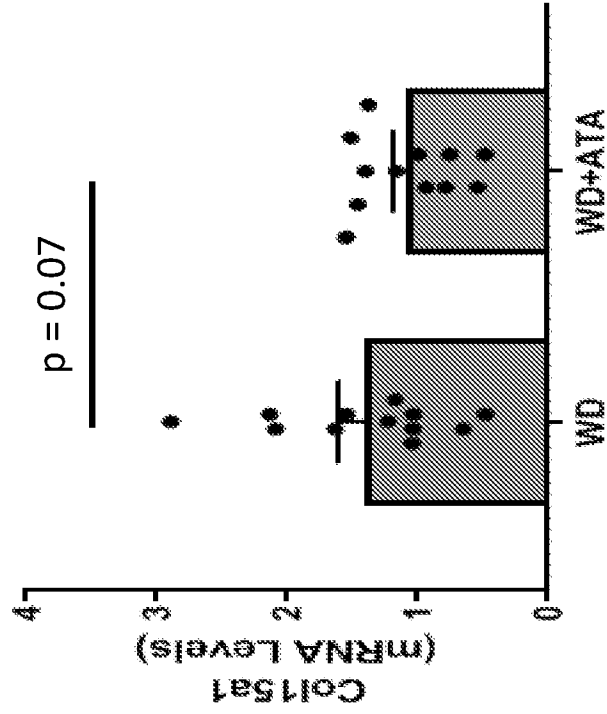


FIG. 6C

Male mice

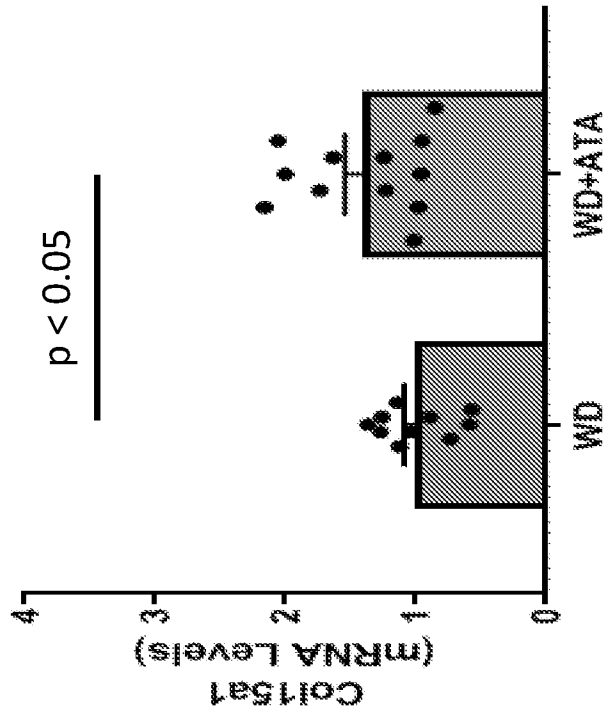


FIG. 7B

Female mice

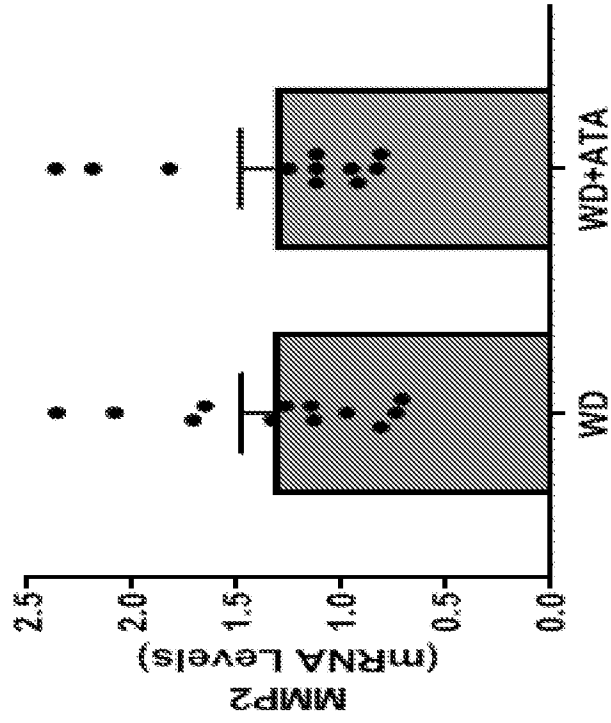


FIG. 7A

Male mice

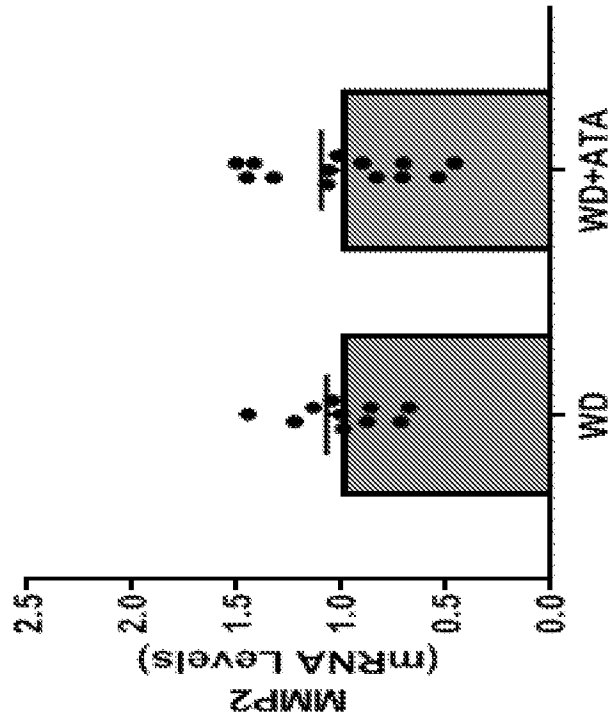


FIG. 8B

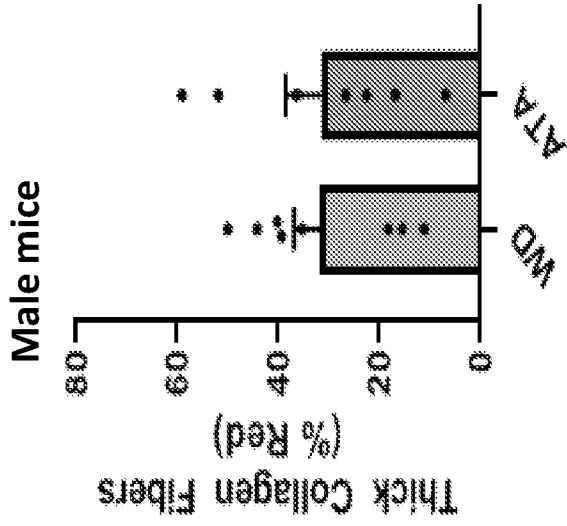


FIG. 8D

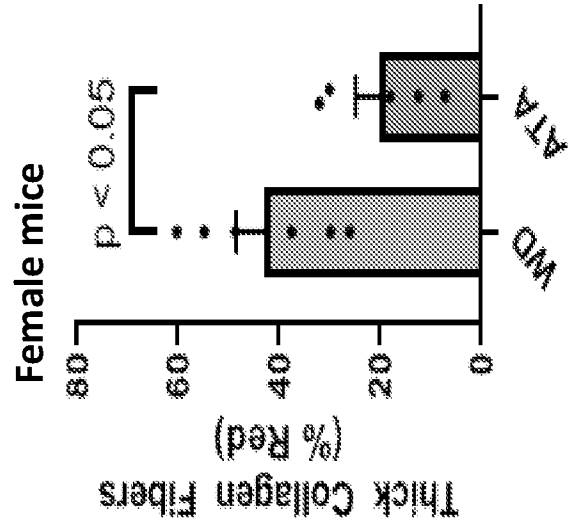


FIG. 8A

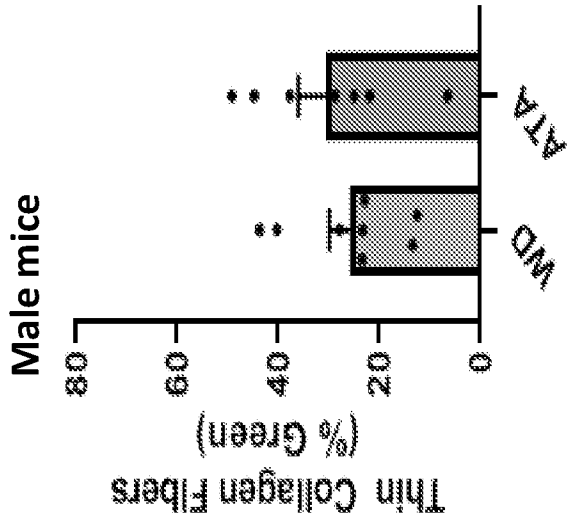


FIG. 8C

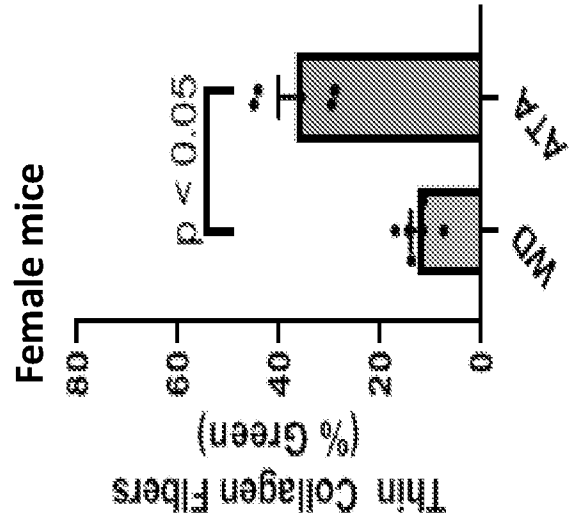


FIG. 9B
Female mice

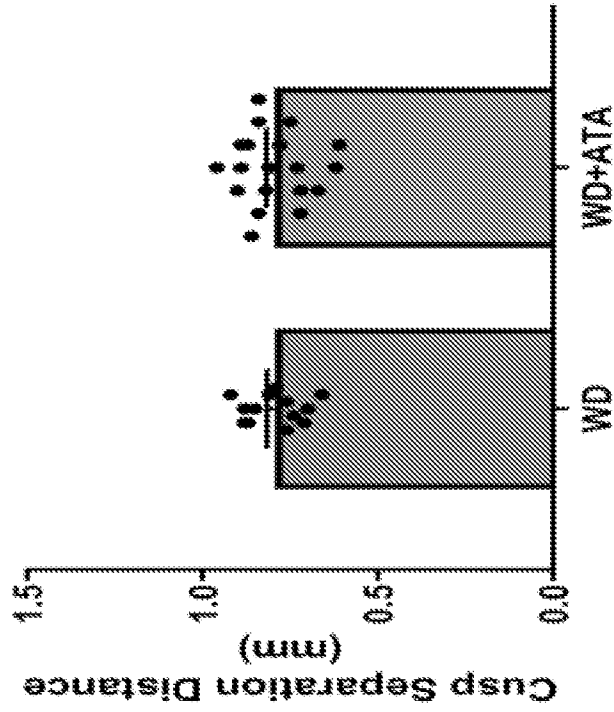


FIG. 9A
Male mice

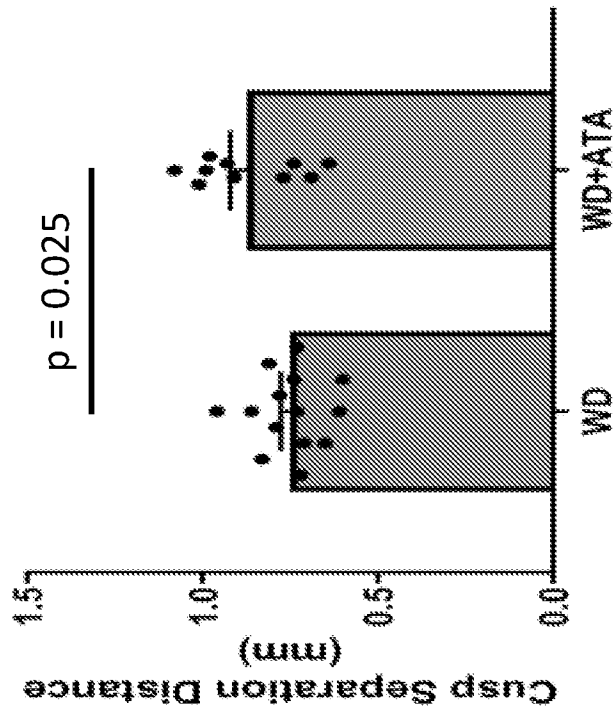


FIG. 10B

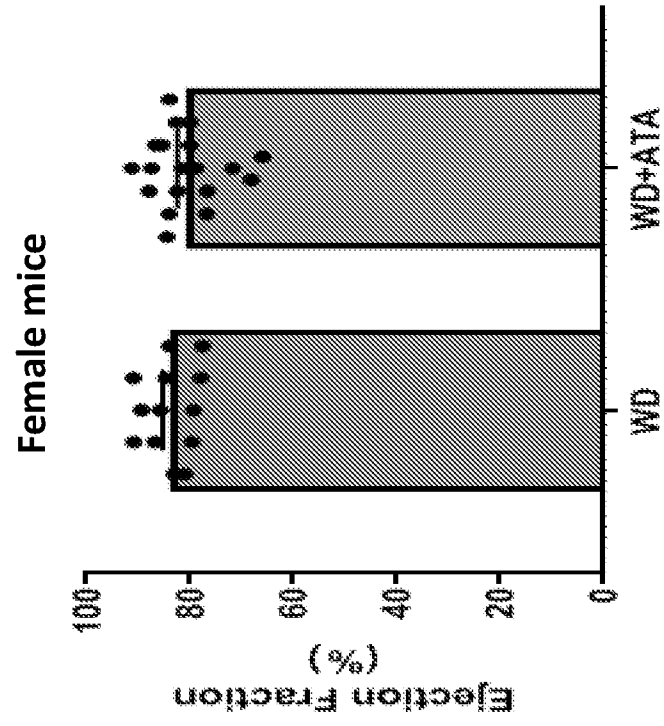


FIG. 10A

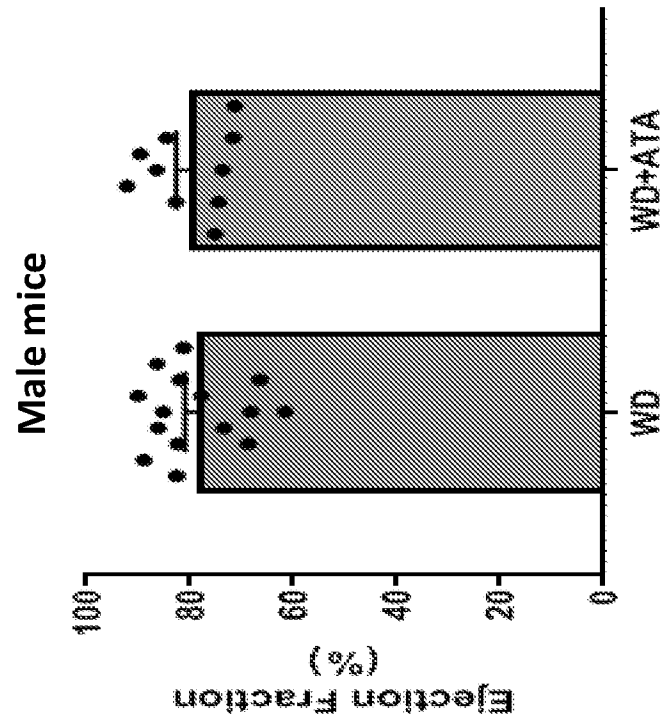


FIG. 10D

Female mice

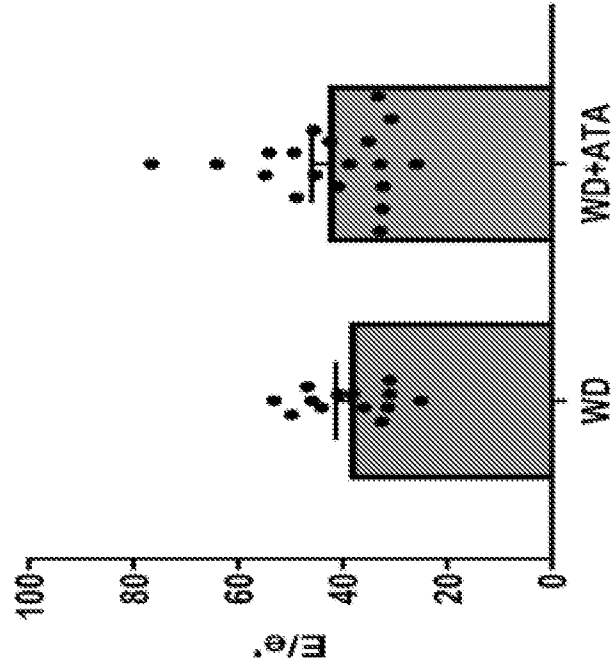


FIG. 10C

Male mice

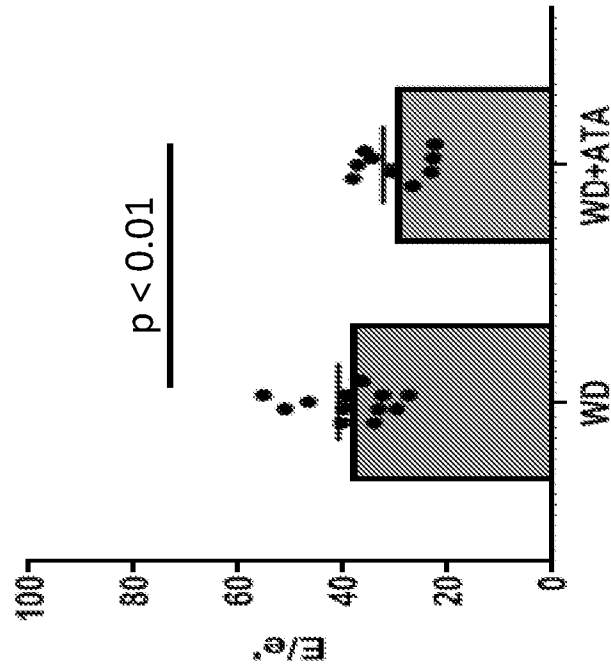


FIG. 11B

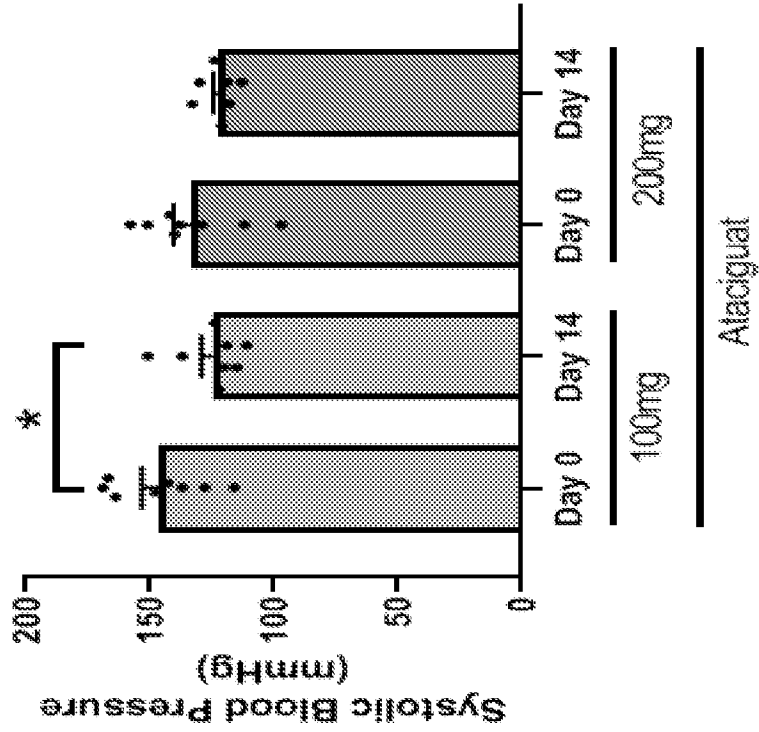


FIG. 11A

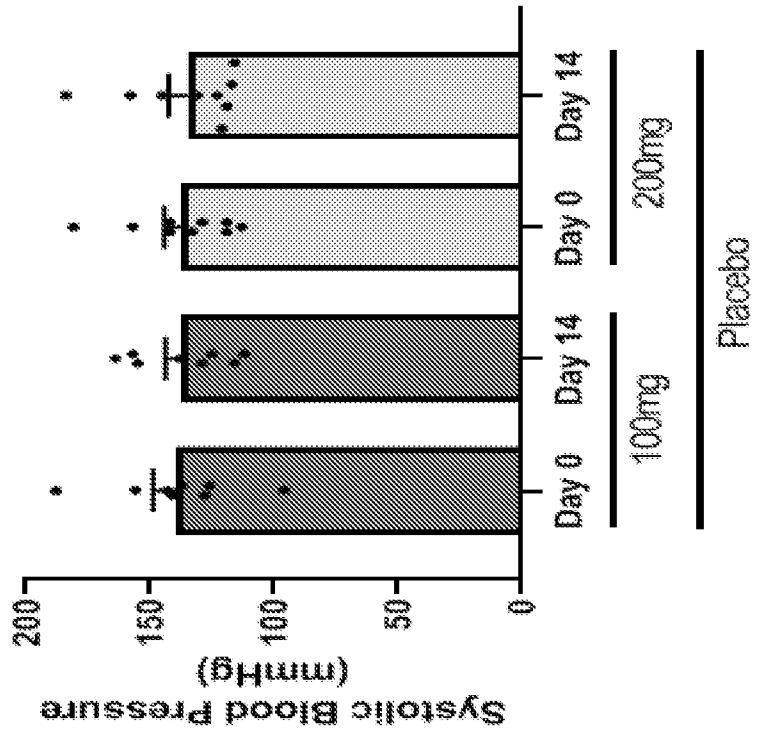


FIG. 11D

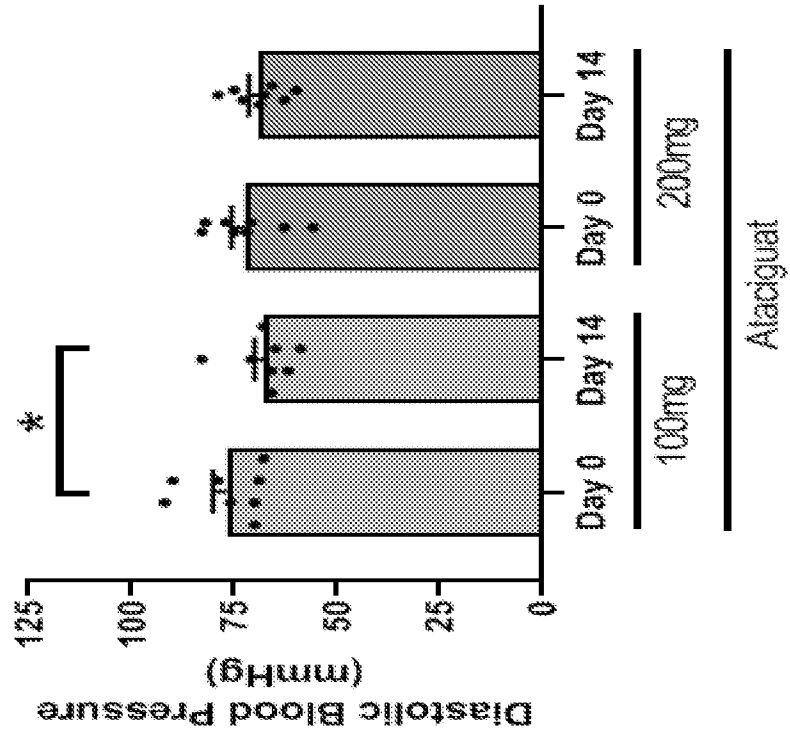


FIG. 11C

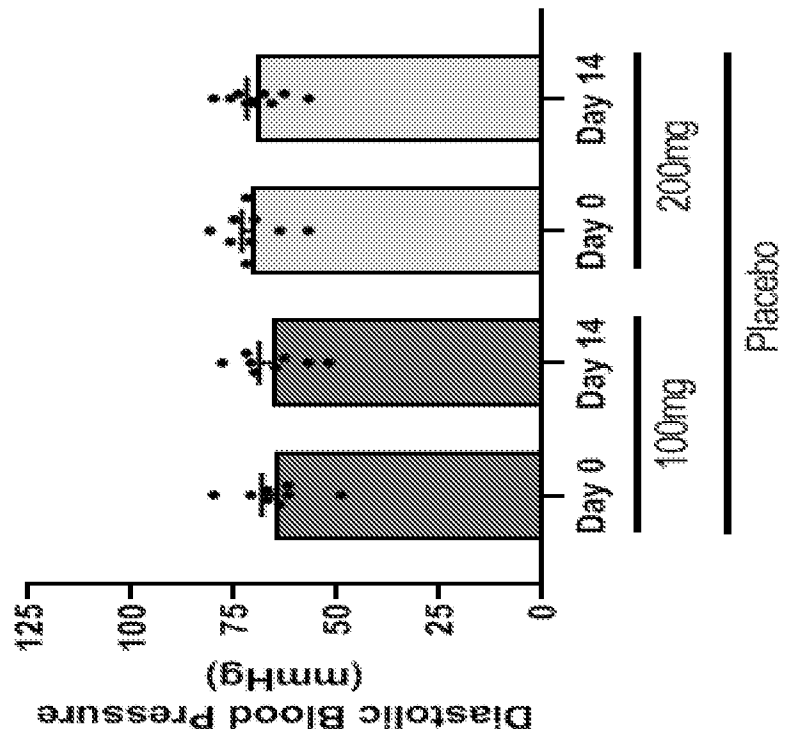


FIG. 12B

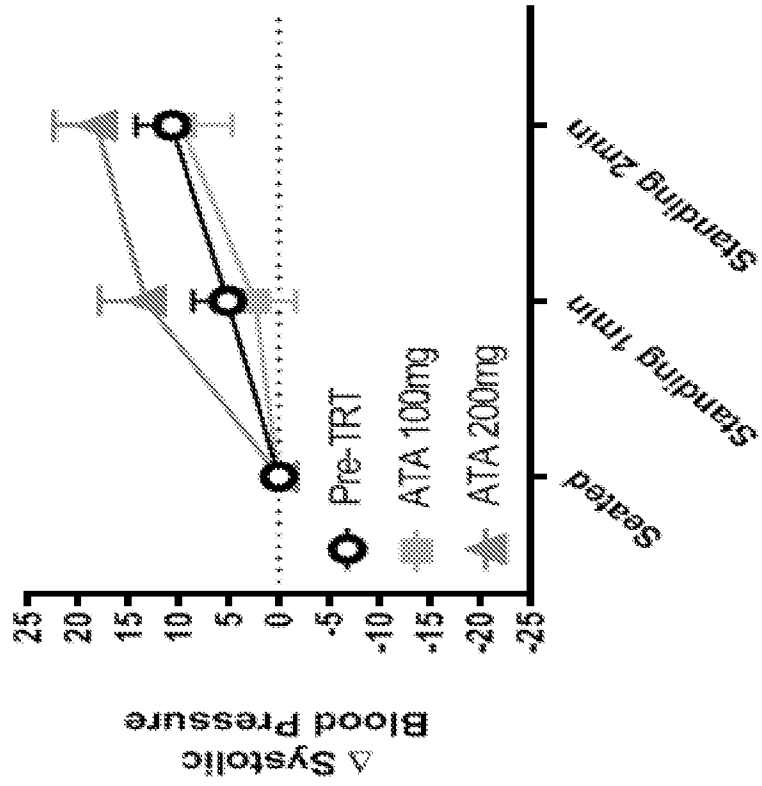


FIG. 12A

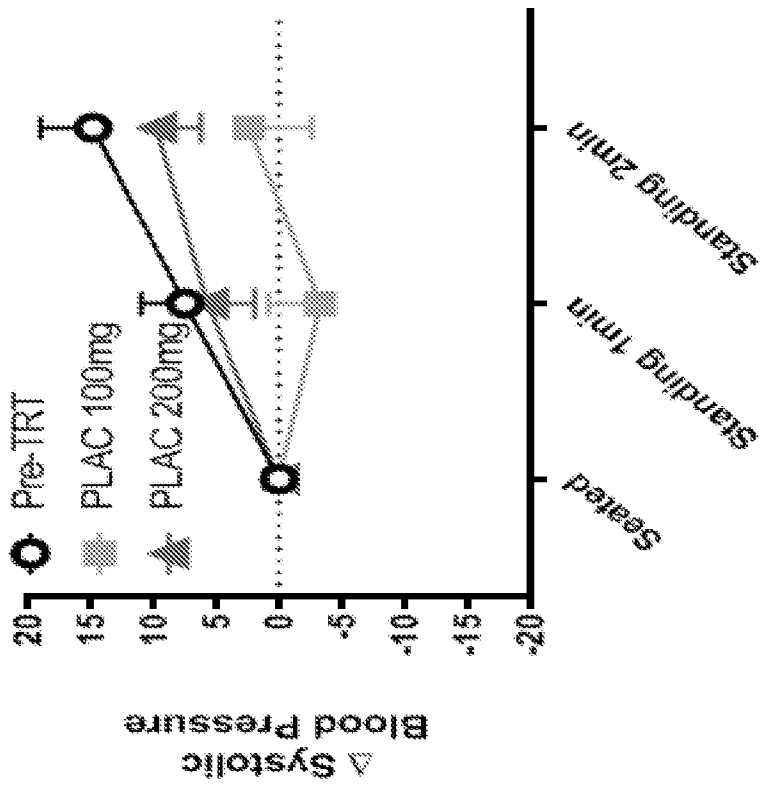


FIG. 12D

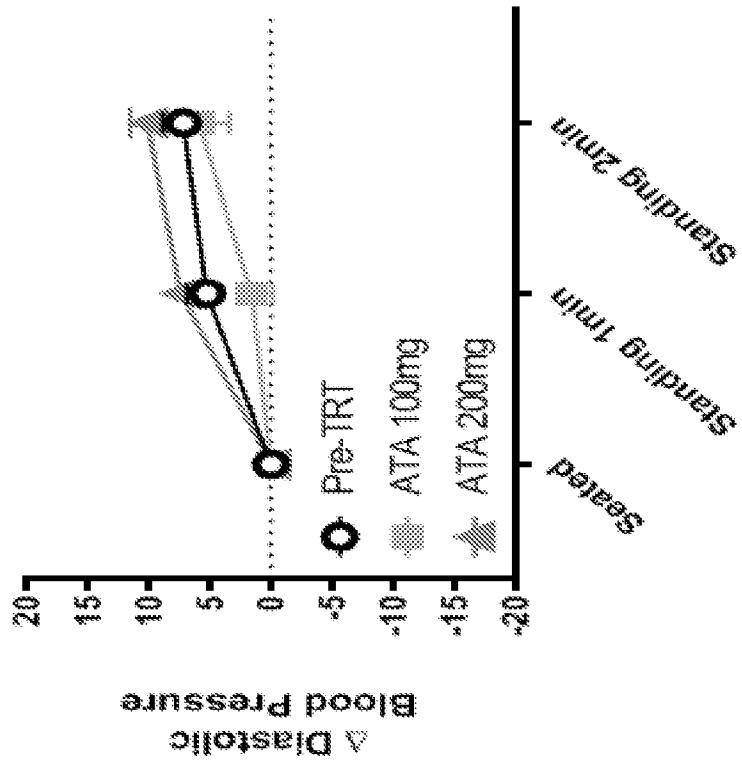


FIG. 12C

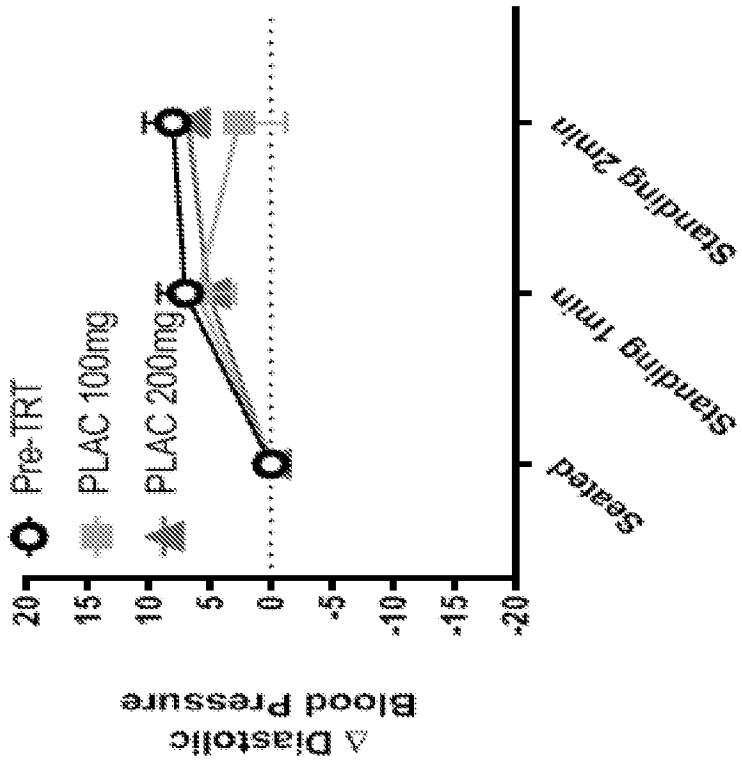


FIG. 13B

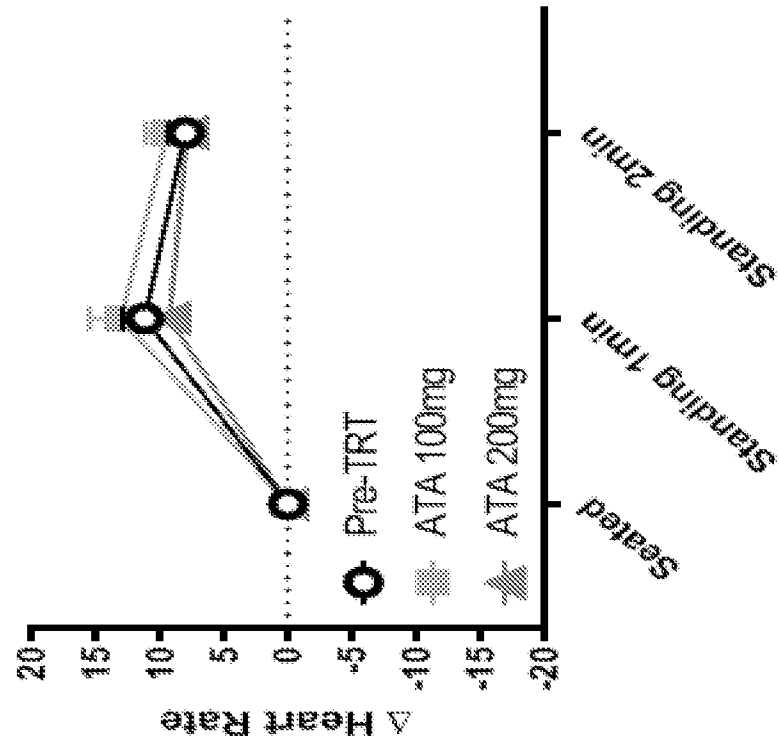


FIG. 13A

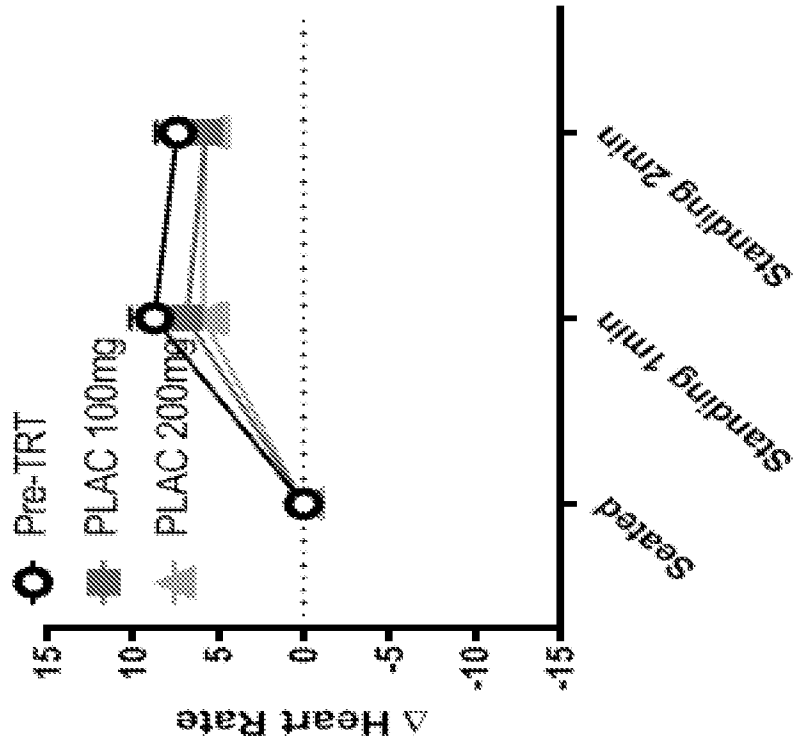


FIG. 14B

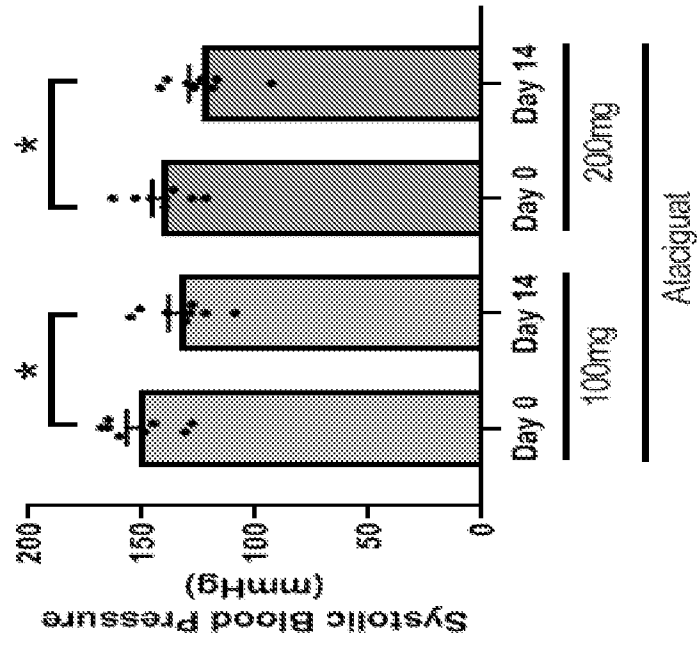


FIG. 14A

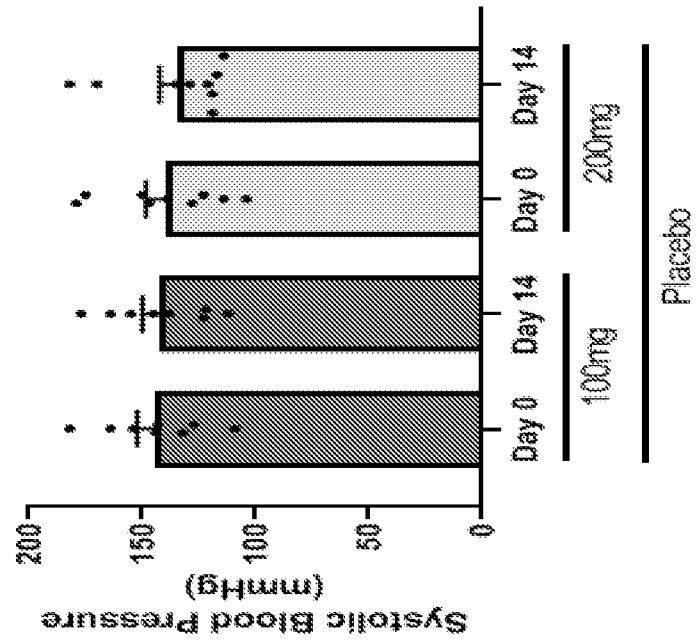


FIG. 14D

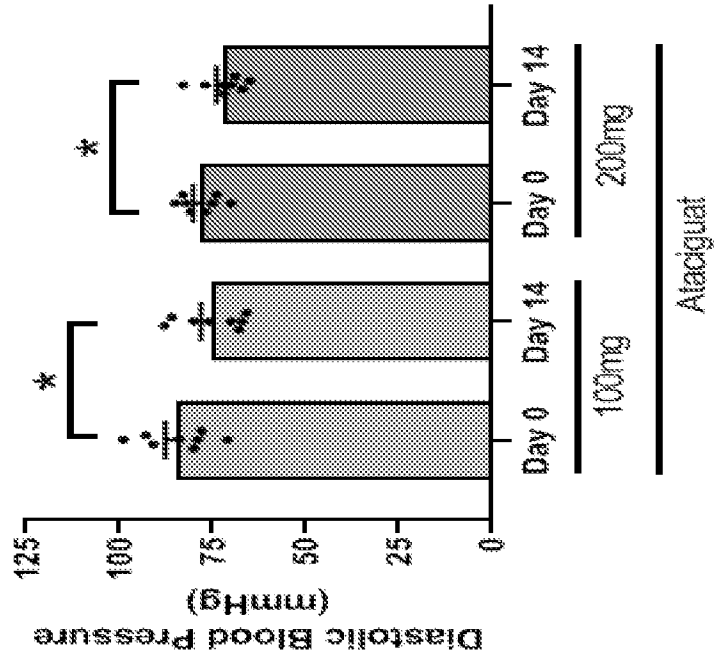


FIG. 14C

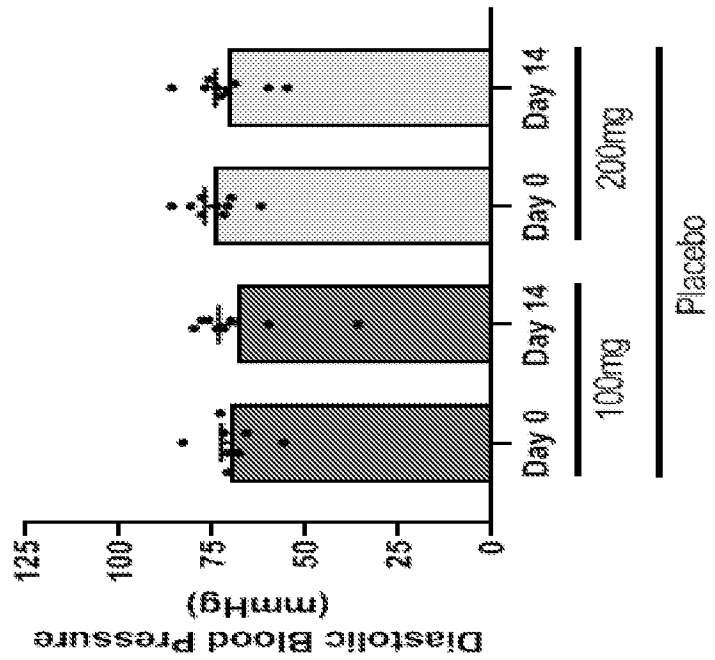


FIG. 15B

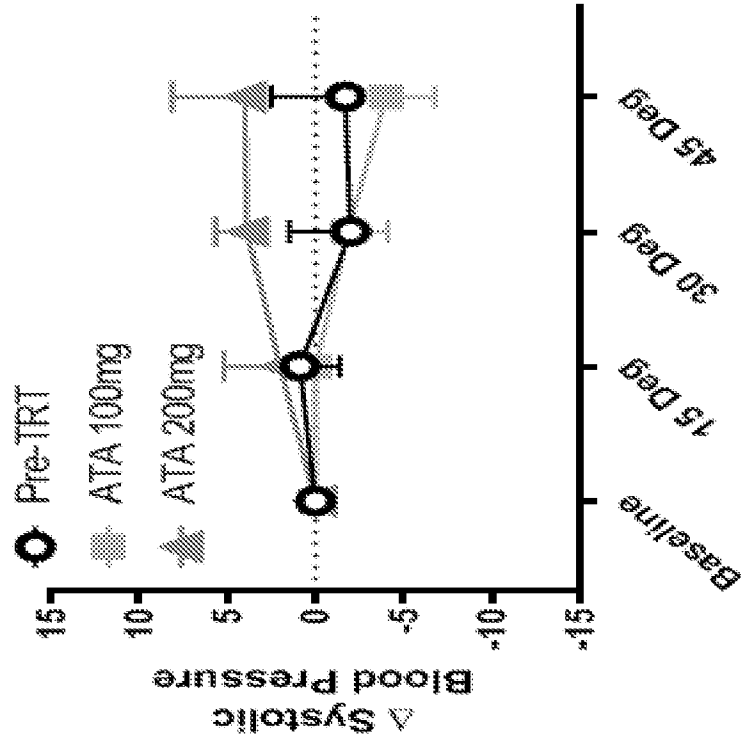


FIG. 15A

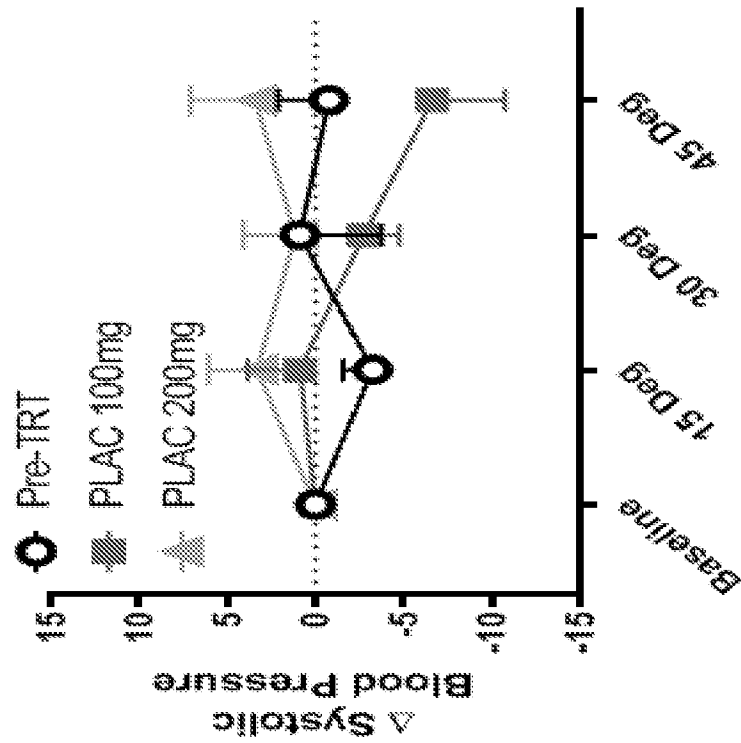


FIG. 15D

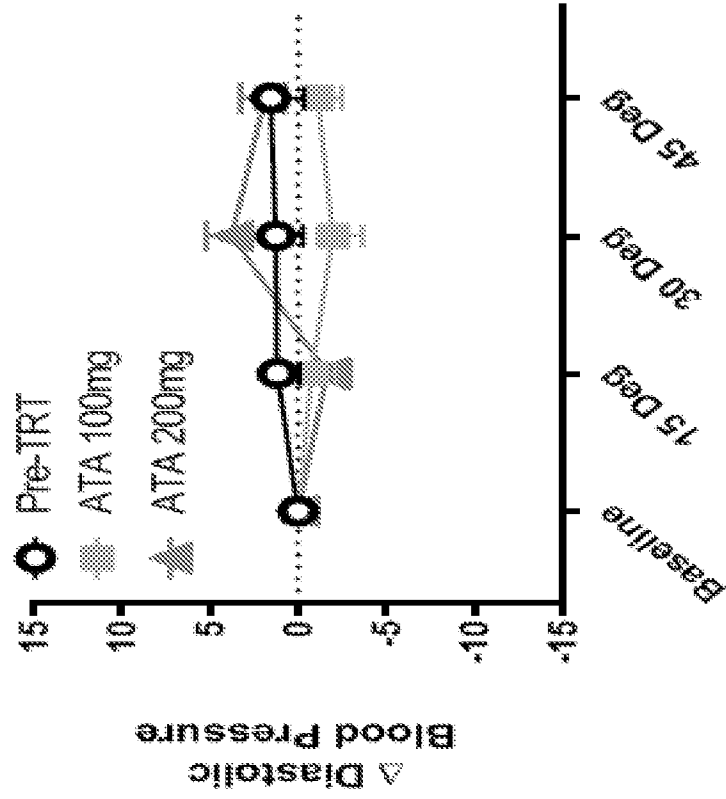


FIG. 15C

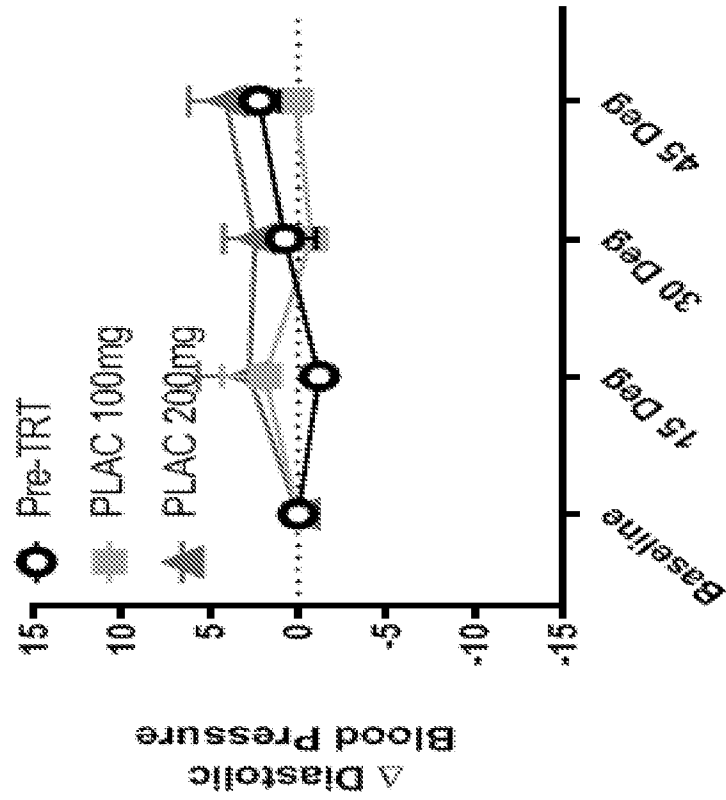


FIG. 16B

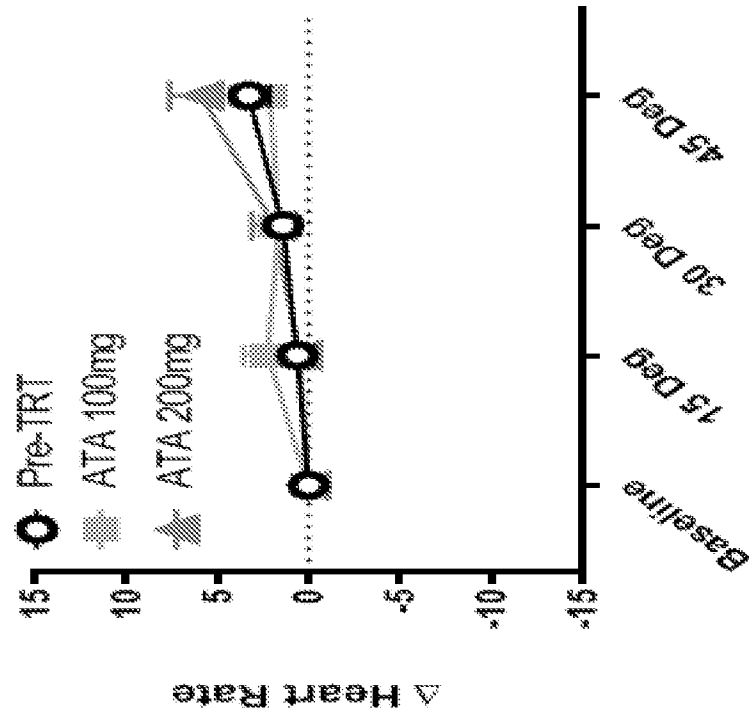


FIG. 16A

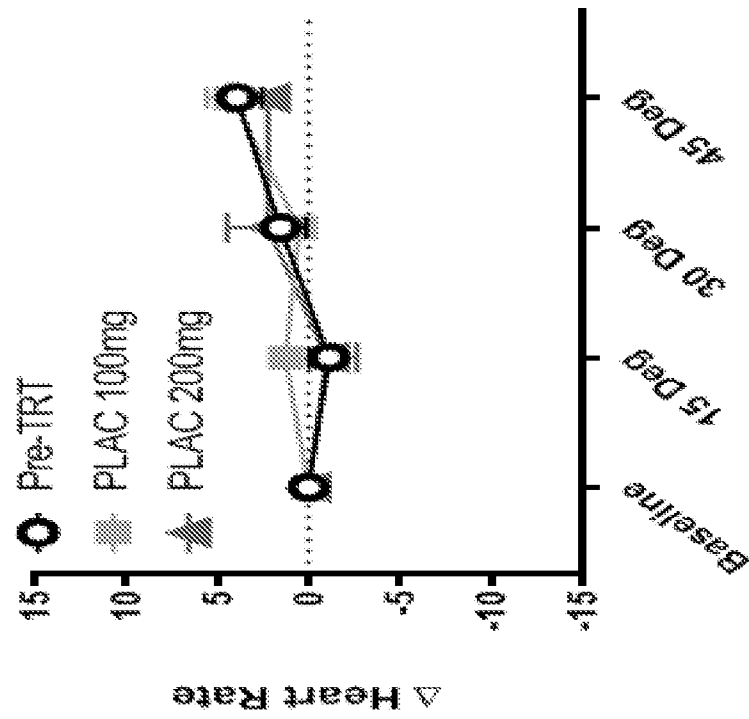


FIG. 17A

Both sexes

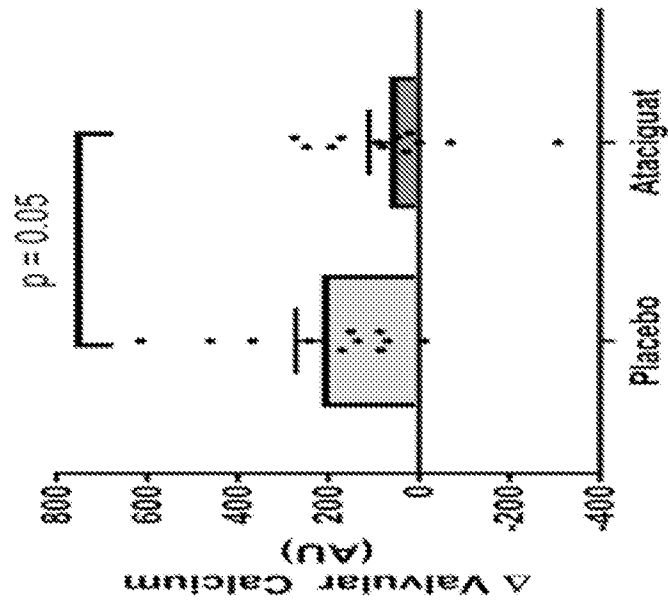


FIG. 17B

Men only

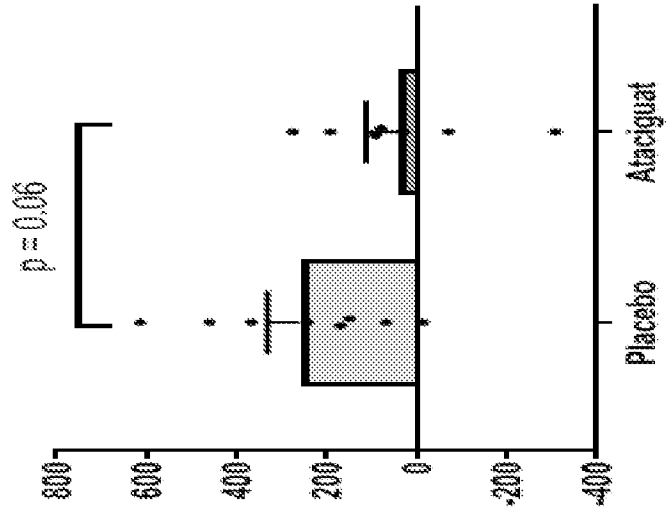


FIG. 17C

Women only

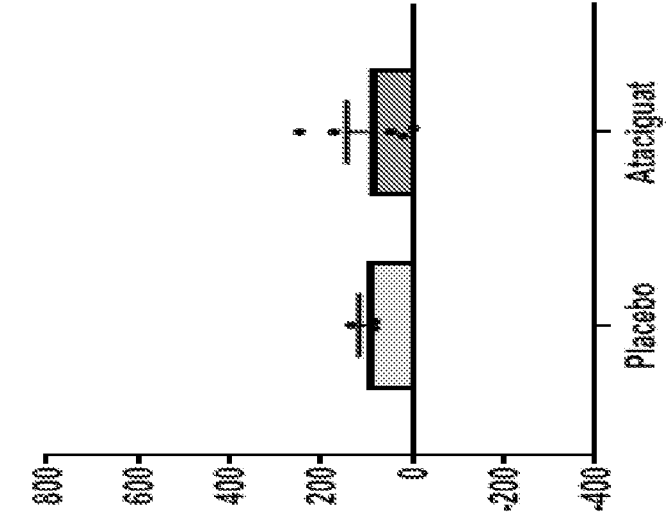


FIG. 18A

Both sexes

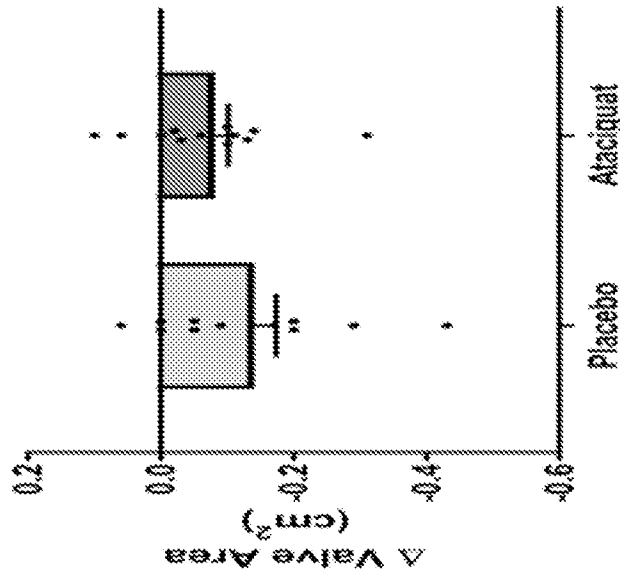


FIG. 18B

Men only

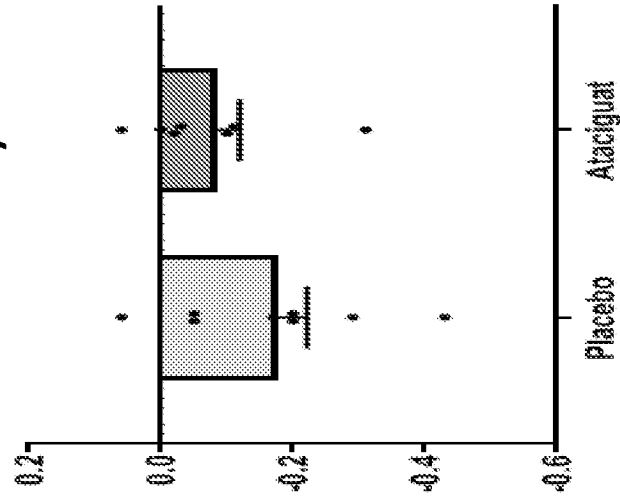
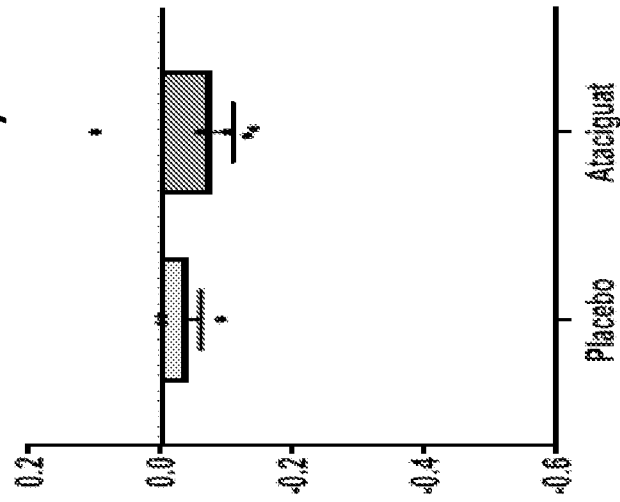


FIG. 18C

Women only



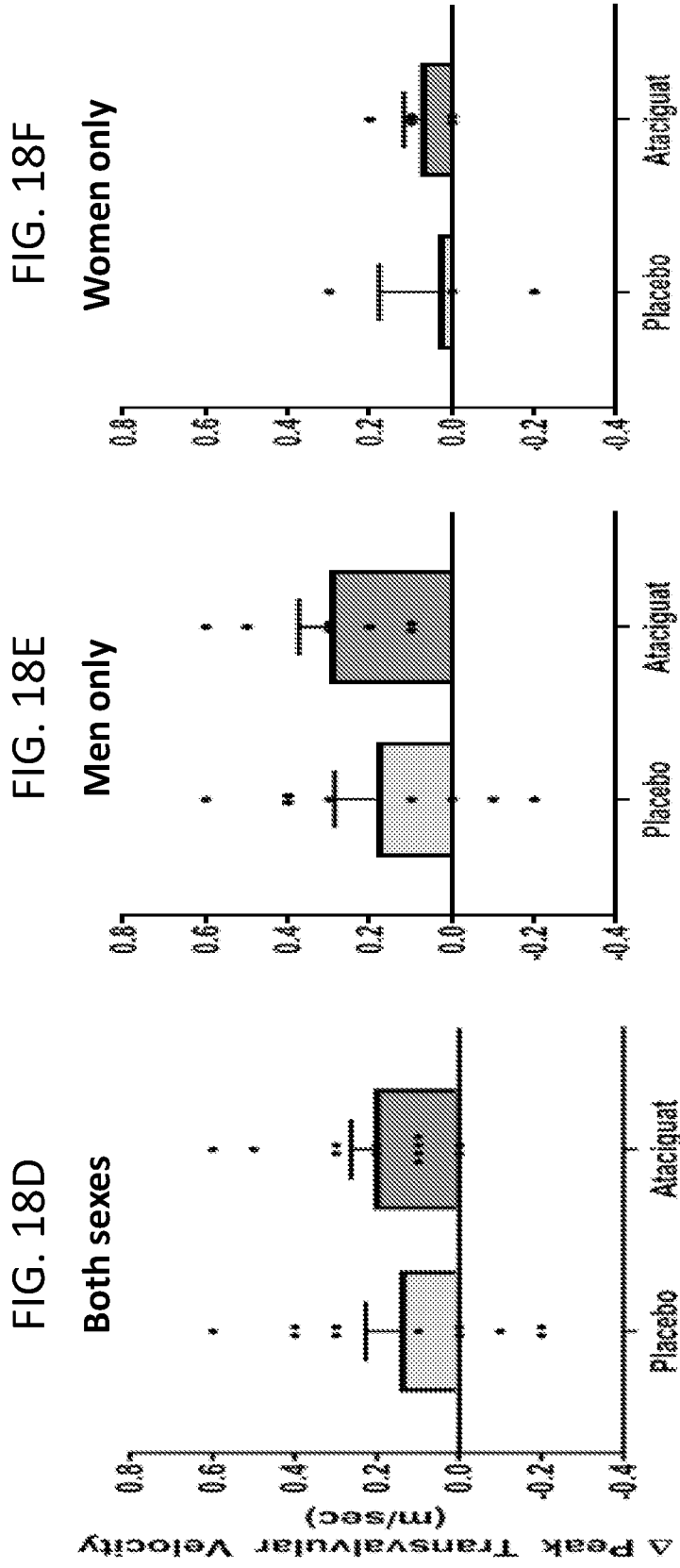


FIG. 18D

FIG. 18E

FIG. 18F

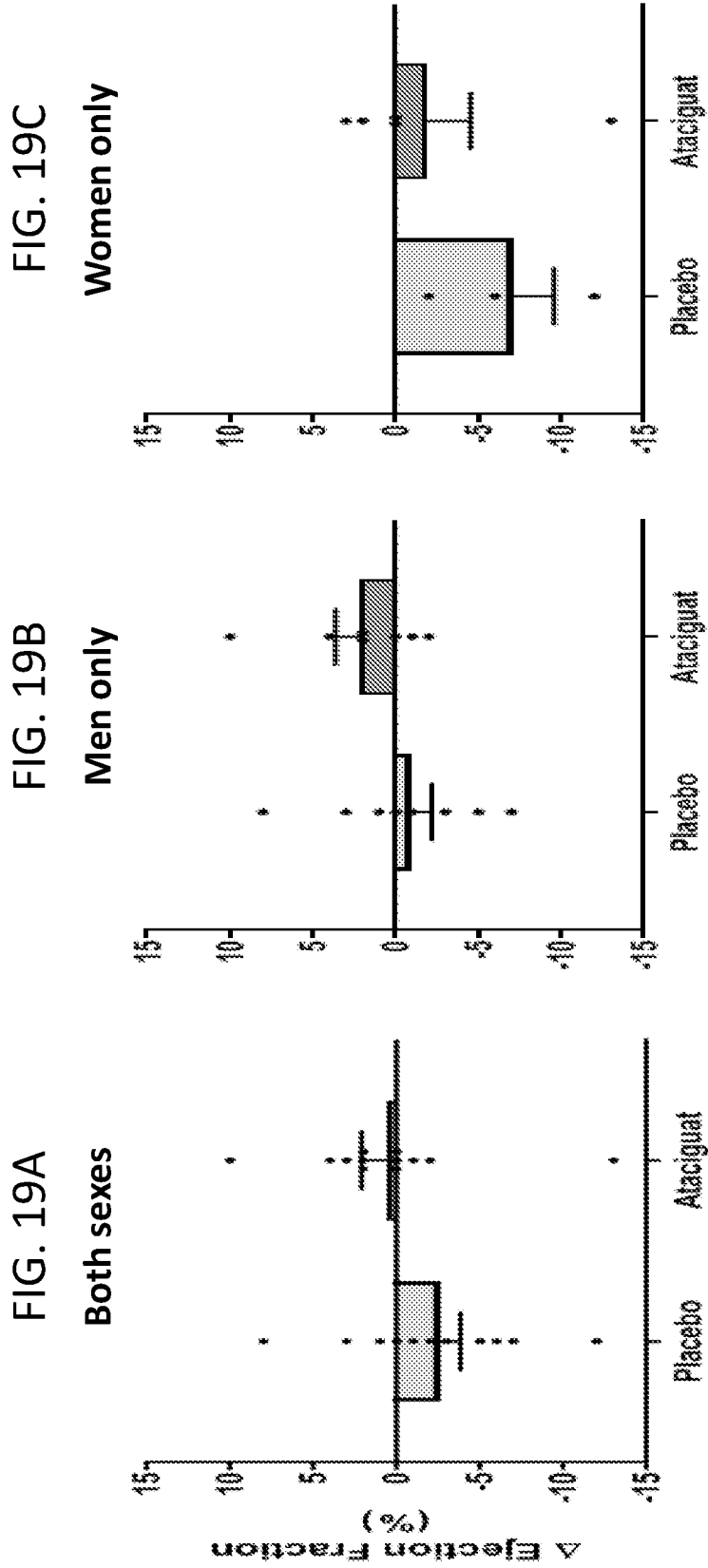


FIG. 19D

Both sexes

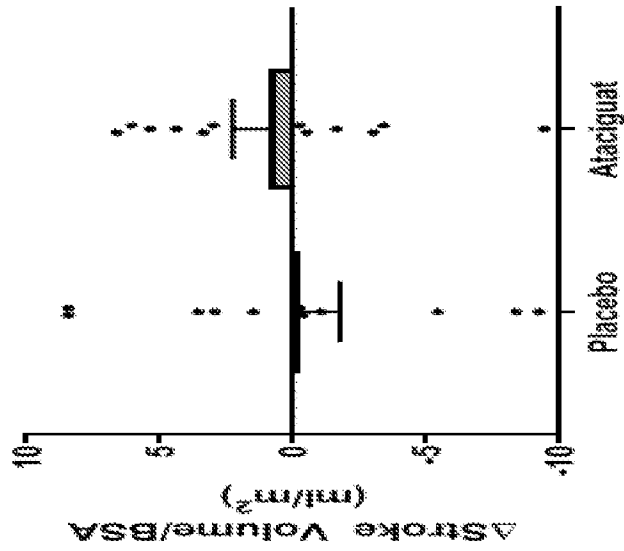


FIG. 19E

Men only

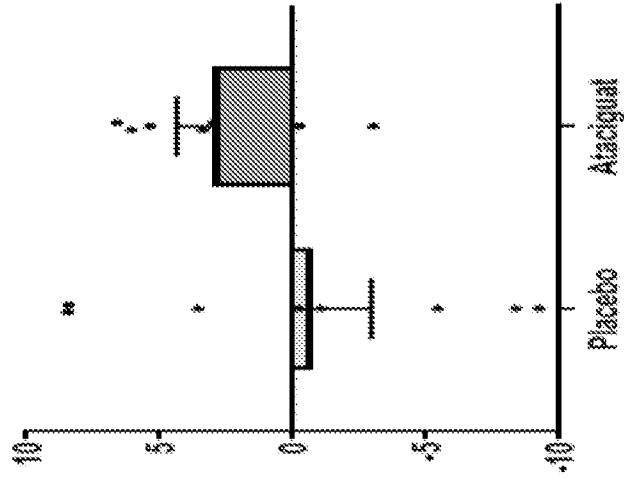


FIG. 19F

Women only

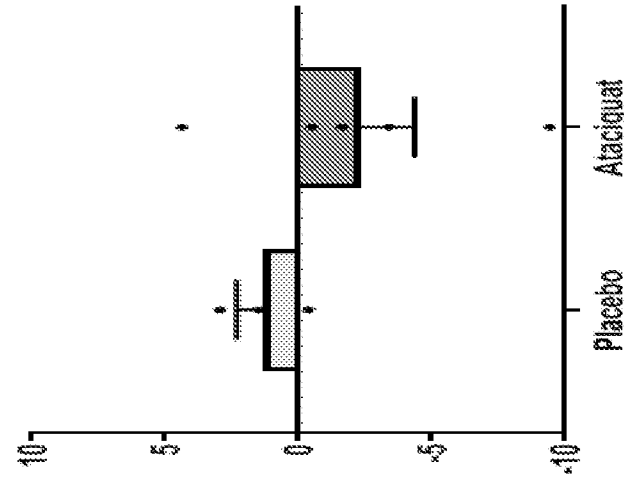


FIG. 20A

Both sexes

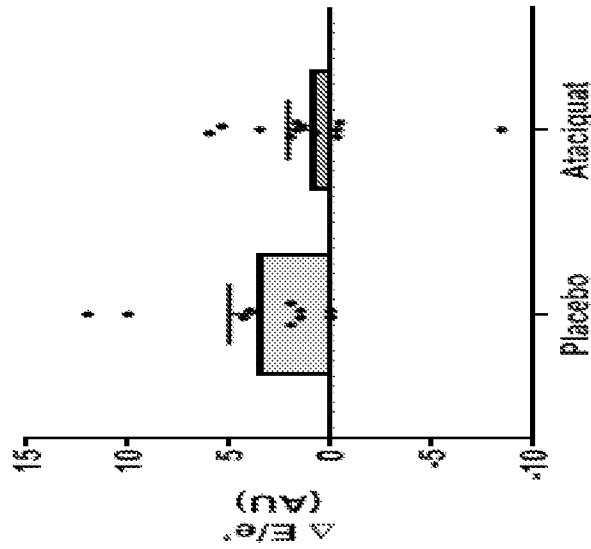


FIG. 20B

Men only

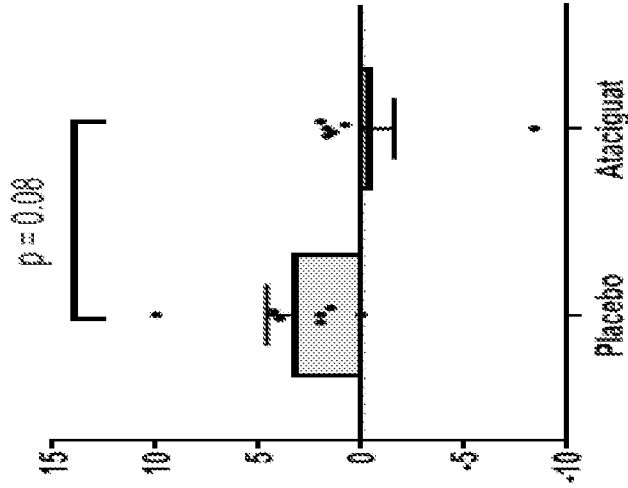


FIG. 20C

Women only

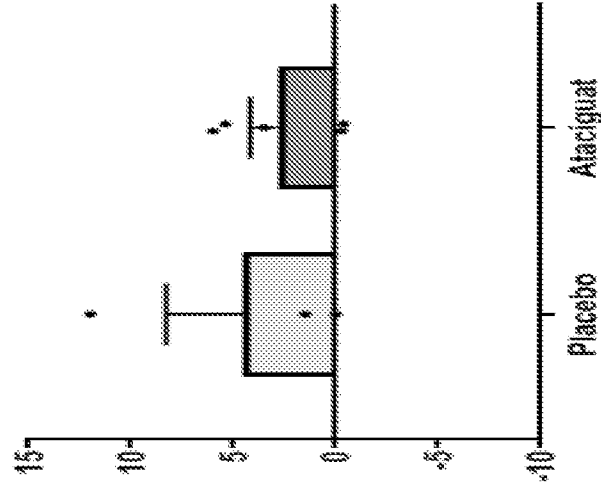


FIG. 20D
Both sexes

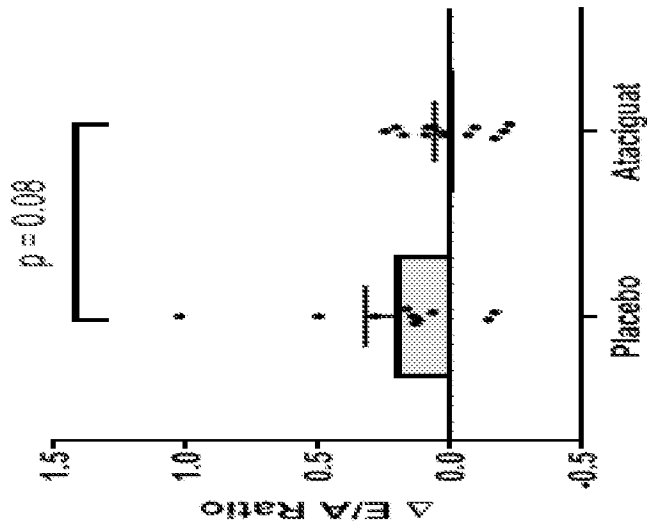


FIG. 20E
Men only

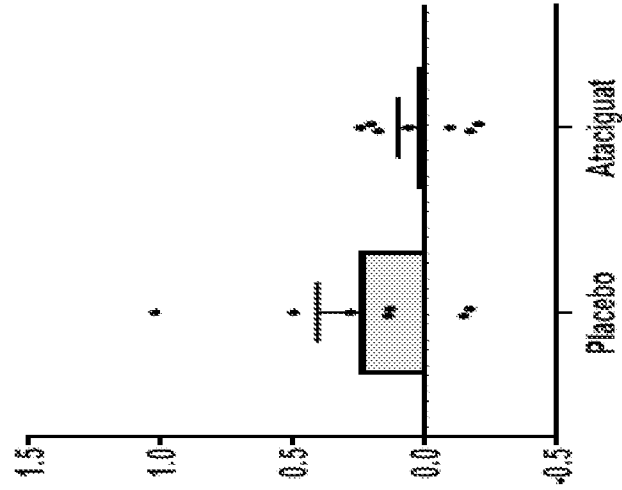
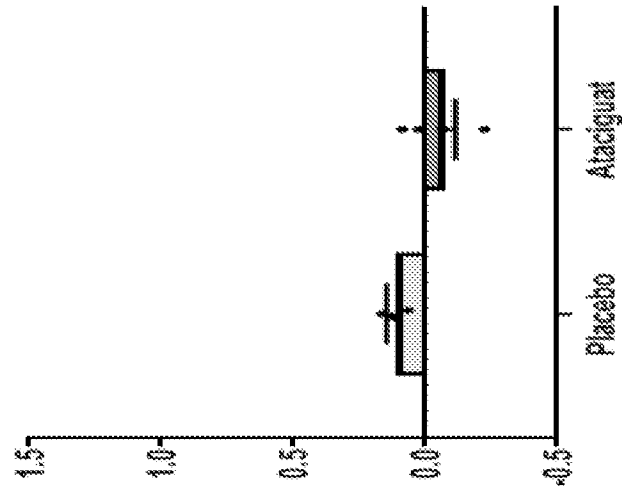


FIG. 20F
Women only



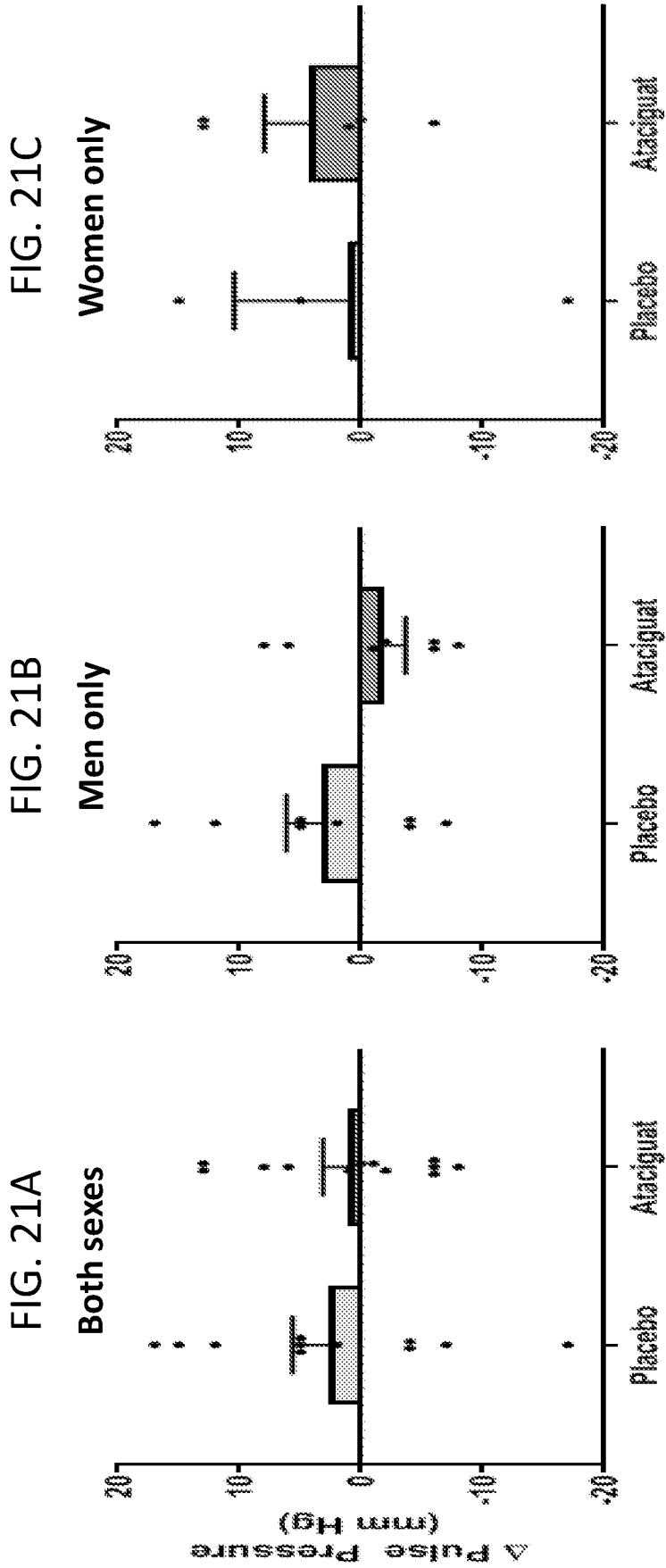


FIG. 22

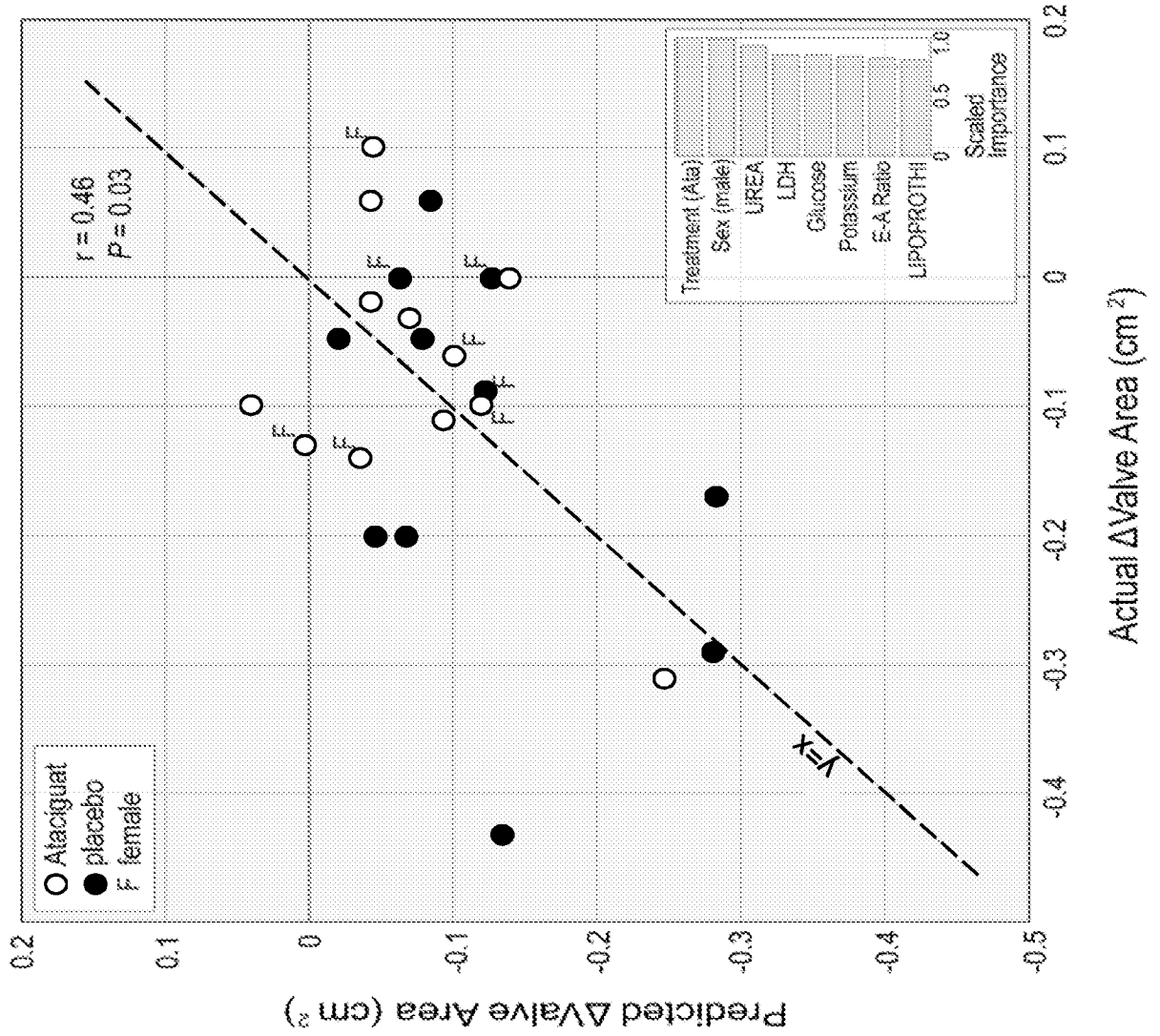


FIG. 23

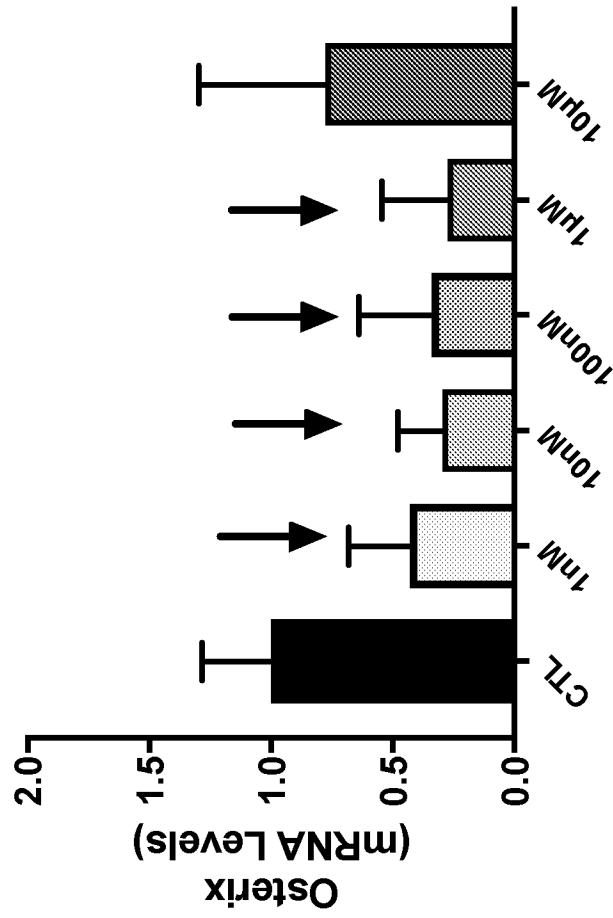


FIG. 24

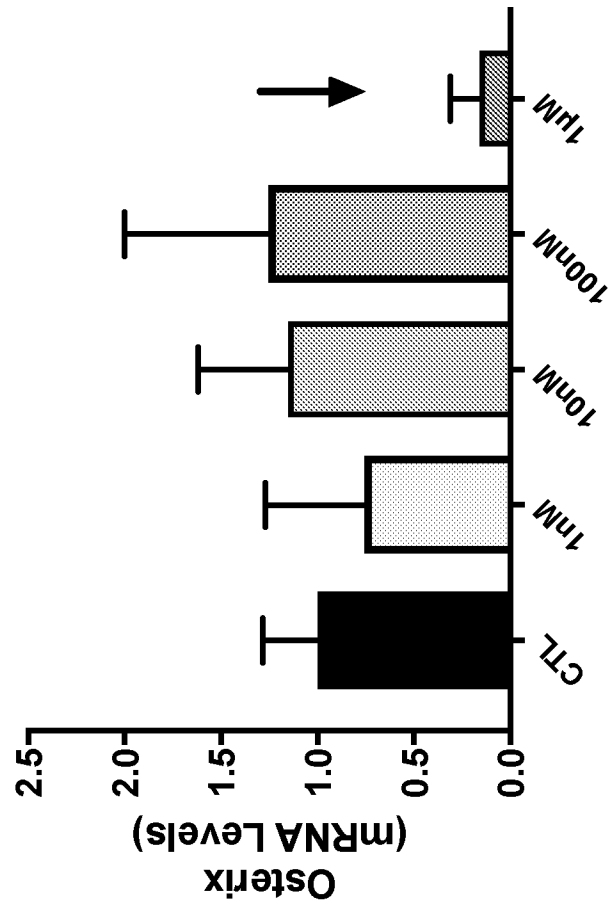


FIG. 25

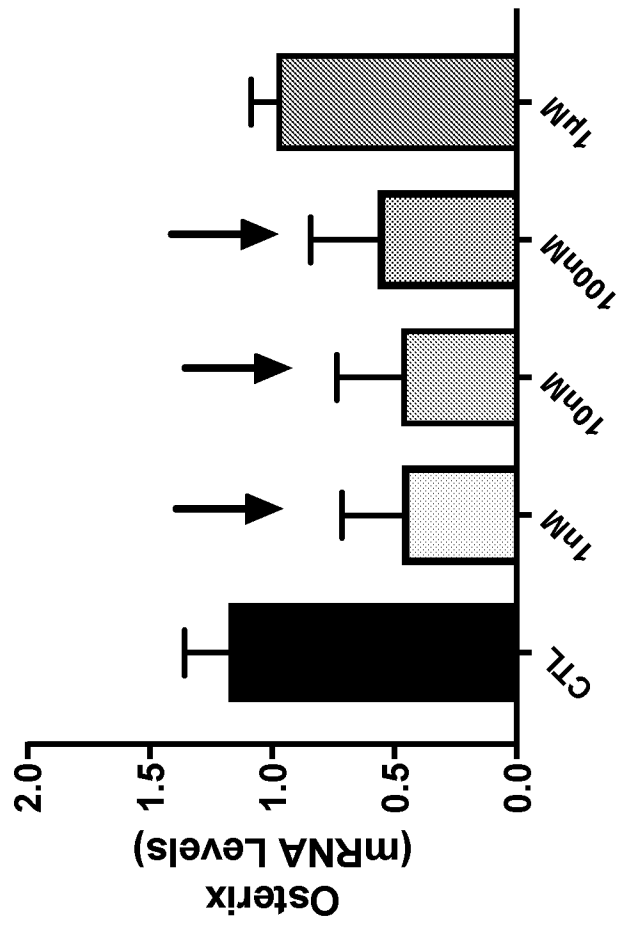


FIG. 26

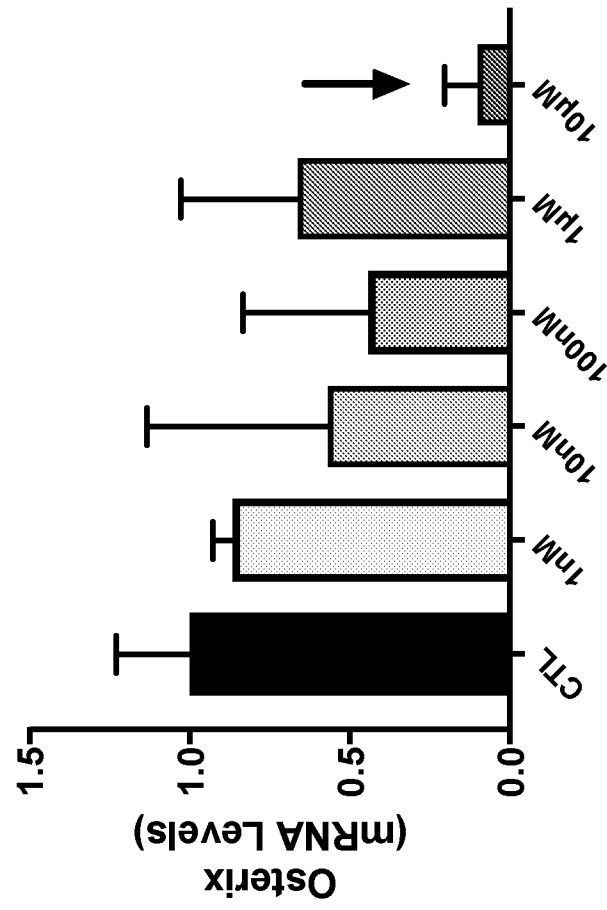


FIG. 27



FIG. 28

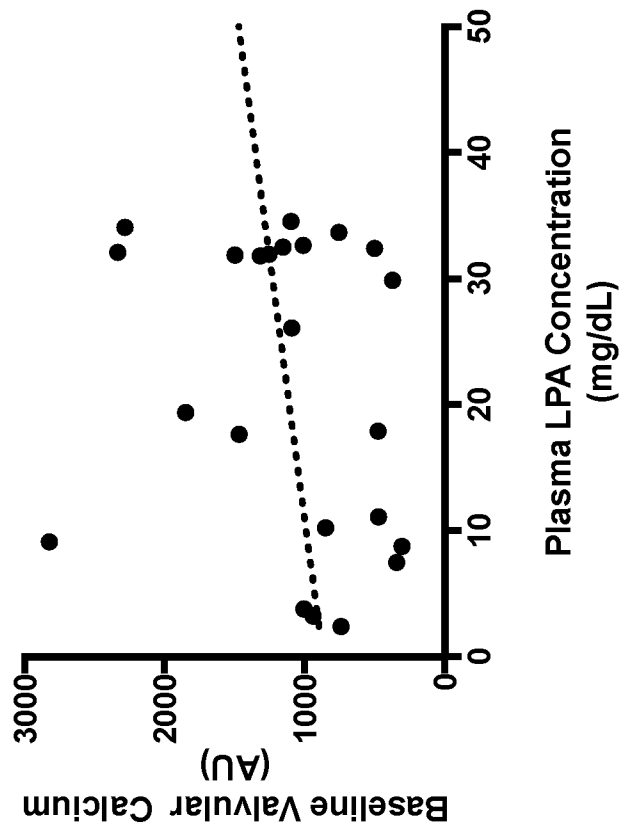


FIG. 29

