Published in final edited form as:
Clin Sci (Lond). 2013 December ; 125(11): 513–520. doi:10.1042/CS20130200.

Mineralocorticoid Receptors Modulate Vascular Endothelial Function in Human Obesity

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Abstract

Obesity increases linearly with age and is associated with impaired vascular endothelial function and increased risk for cardiovascular disease. Mineralocorticoid receptors (MR) contribute to impaired vascular endothelial function in cardiovascular disease; however, their role in uncomplicated human obesity is unknown. Because plasma aldosterone levels are elevated in obesity and adipocytes may be a source of aldosterone, we hypothesized that MR modulate vascular endothelial function in older adults in an adiposity-dependent manner. To test this hypothesis, we administered MR blockade (Eplerenone; 100 mg/day) for 1 month in a balanced, randomized, double-blind, placebo-controlled, crossover study to 22 older adults (10 men, 55–79 years) varying widely in adiposity (body mass index: 20–45 kg/m^2) but who were free from overt cardiovascular disease. We evaluated vascular endothelial function (brachial artery flow-mediated dilation [FMD] via ultrasonography) and oxidative stress (plasma F_2-isoprostanes and vascular endothelial cell protein expression of nitrotyrosine and NADPH oxidase p47^{phox}) during placebo and MR blockade. In the whole group, oxidative stress (P>0.05) and FMD did not change with MR blockade (6.39±0.67 vs. 6.23±0.73 %, P=0.7, placebo vs. Eplerenone). However, individual improvements in FMD in response to Eplerenone were associated with higher total body fat (body mass index: r=0.45, P=0.02 and DXA-derived % body fat: r=0.50, P=0.009) and abdominal fat (total: r=0.61, P=0.005, visceral: r=0.67, P=0.002 and subcutaneous: r=0.48, P=0.03). In addition, greater improvements in FMD with Eplerenone were related with higher baseline fasting glucose

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AUTHOR CONTRIBUTIONS

M.H.H and D.D.C conceived and designed the study; M.H.H., J.K.Y, M.J.L., T.H.M., M.E. and D.D.C. collected the data; M.H.H., J.K.Y., M.J.L. and H.K.K. analyzed the data; T.H.M. and M.E. provide on-site medical supervision for experiments; M.H.H and D.D.C. performed statistical analysis, prepared figures and drafted manuscript; M.H.H, M.S.S. and D.D.C. interpreted results, edited and revised manuscript; J.K.Y, H.K.K., M.J.L., M.S.S., T.H.M. and M.E. provided feedback for manuscript; all authors approved final version of manuscript.

(r=0.53, P=0.01). MR influence vascular endothelial function in an adiposity-dependent manner in healthy older adults.

Keywords

brachial artery; flow-mediated dilation; abdominal visceral and subcutaneous fat

INTRODUCTION

More than one third of adults worldwide is overweight or obese [1] and the prevalence of obesity increases linearly with age [2]. Obesity is associated with increased risk for cardiovascular disease [3], but the underlying mechanisms are not completely understood. Substantial evidence supports an independent role of aldosterone in the development and progression of cardiovascular disease [4–6]. According to the classic view of physiology, aldosterone is secreted by the adrenal gland and is involved in blood pressure regulation by acting on the kidney via activation of epithelial mineralocorticoid receptors (MR) [7]. In the past decade, non-epithelial presence of MR has been demonstrated in cardiac and vascular cells and increasing evidence supports the direct role of MR in modulating vascular function and contributing to cardiovascular disease [8].

Recently, findings from studies *in vitro* and studies performed in rodents demonstrate that adipose tissue is a secondary source of aldosterone [9] and that adipocyte-derived aldosterone contributes to vascular dysfunction in obesity [10]. In humans, several studies have shown that plasma aldosterone levels are positively related with measures of total and abdominal adiposity including body mass index [11], waist circumference [12], abdominal visceral [13] and subcutaneous adipose tissue [14]. In addition, plasma aldosterone concentrations are elevated in the obese compared with lean human subjects [15, 16]. With weight loss, aldosterone levels are significantly decreased [14, 17–19], highlighting the important role of adipose tissue in the obesity-related increases in aldosterone concentration.

Obesity is also associated with impaired endothelial function [20, 21], an independent predictor of future cardiovascular events, disease progression, and long-term outcome [22, 23]. A key component of endothelial dysfunction is decreased nitric oxide bioavailability resulting from either decreased synthesis or increased degradation due to oxidative stress [24]. Activation of vascular NADPH oxidase, eNOS uncoupling and other factors lead to increased production of reactive oxygen species (ROS), which inactivate nitric oxide, thus leading to impaired vascular smooth muscle relaxation and vasodilation [25].

There is strong evidence supporting that aldosterone activation of MR contributes to oxidative stress and decreased nitric oxide activity. Data from experimental models of cardiovascular disease demonstrated that MR activation increases NADPH oxidase expression and activity leading to increased superoxide production, vascular oxidative stress, decreased nitric oxide bioavailability and impaired vascular endothelial function, while MR blockade reverses these effects [26–29]. Human studies in patients with congestive heart failure found that 1 month of MR blockade improves endothelial function and this improvement is associated with increased nitric oxide bioactivity [30, 31].

Taken together these data support a potential role for MR in obesity-related impairments in endothelial function, but this has not been studied in human obesity. Thus, in the current investigation, we hypothesized that MR modulate vascular endothelial function in an adiposity-dependent manner in healthy older adults. To test this hypothesis we administered the selective MR antagonist Eplerenone (100 mg daily for 1 month) in a balanced randomized, double-blind, placebo-controlled, crossover study in healthy older adults varying widely in total and abdominal adiposity. We measured vascular endothelial function and oxidative stress markers during placebo and MR blockade.

METHODS

Subjects

Twenty-two healthy adults (55–79 years), 10 men and 12 women, of a wide range of adiposity (body mass index: 20.0–44.6 kg/m^2; body fat: 25.6–54.1 %) were studied. All subjects were sedentary, non-smokers and were free of overt cardiovascular disease and other clinical disorders (e.g., diabetes, liver and renal disease) as assessed by medical history, physical examination, resting ECG, urinalysis, blood chemistries and hematological evaluation. None of the subjects were taking antihypertensive or vasoactive drugs and subjects who were taking antioxidant supplements completed a 4-week washout prior to study enrolment. All subjects demonstrated normal ECG and blood pressure responses to a graded exercise test on a treadmill. The graded exercise protocol is described below under the aerobic fitness section. Women were all postmenopausal, established by absence of menses for at least 2 years and follicle stimulating hormone >40 IU/L. Postmenopausal women were not on hormone replacement therapy for at least 1 year prior to data collection. The study was carried out in accordance with the Declaration of Helsinki (2008) and was approved by the Institutional Review Boards of the University of Florida, Texas A&M University, and Scott & White Health System. The purpose, nature and risk of all procedures used were explained to the subjects and their written informed consent was obtained prior to participation.

Study design

Subjects were assigned to receive an MR antagonist (Eplerenone; 100 mg per day) for 1 month in a balanced randomized, double-blind, placebo-controlled, crossover study with 1-month washout between treatments (Figure 1). Eplerenone was chosen because it has a higher selectivity for mineralocorticoid receptors and fewer side effects than the other mineralocorticoid receptor antagonist that is currently available (i.e., Spironolactone).

To reduce the risk of hyperkalemia, subjects were not enrolled in the study if their baseline serum potassium was greater than 5.5 mmol/L, serum creatinine was greater than 1.6 mg/dL or creatinine clearance was less than 30 mL/min. Following study enrollment, serum potassium and blood pressure were assessed at baseline, day 3, day 7 and weekly thereafter for each treatment. In response to 1-month treatment with Eplerenone, serum potassium levels did not rise and systolic blood pressure did not decrease excessively requiring subject withdrawal.

General experimental procedures

All measurements were performed in the morning, at the same time each day, in a semi-darkened temperature-controlled room after a 12-hour overnight fast (including abstinence from caffeine and alcohol) and a minimum of 20 minutes of supine rest. Subjects took their morning dose of Eplerenone or placebo exactly one hour prior to data collection.

Vascular endothelial function (flow-mediated dilation; FMD)—Brachial artery FMD was assessed non-invasively following established guidelines [32, 33] by using an ultrasound/Doppler system equipped with a 7.5 MHz vascular transducer (Aplio XV, Toshiba).

Briefly, the subject rested in the supine position with the right arm abducted and fixed in position at heart level by using a Versaform pillow (Sammons Preston Rolyan, Bolingbrook, IL). A pressure cuff connected to a rapid inflator/deflator system (E20 and AG 101, D. E. Hokanson, Bellevue, WA) was placed around the widest part of the subject's forearm. A duplex ultrasound image of the brachial artery (i.e., 2D image and spectral Doppler waveforms) was obtained ~7 cm proximal to the antecubital fossa. The Doppler angle of insonation for assessing blood velocity was set ≤ 60 degrees. Following image optimization the vascular transducer was clamped (Flexbar, Flexbar Machine Corporation, Islandia, NY) in place to prevent movement during data collection. To ensure the same segment of the brachial artery was imaged in the subsequent ultrasound visit, the distance of the transducer relative to the antecubital crease was recorded, a digital photograph of the arm position was stored, and the ultrasound image was printed.

Reactive hyperemia was induced by inflating the forearm cuff to 250 mmHg for 5 minutes followed by rapid deflation. ECG R-gated duplex ultrasound images of the brachial artery were digitally recorded (Vascular Imager, Medical Imaging Applications, LLC, Coralville, IA) for one minute to establish pre-occlusion baseline and for 2 minutes after cuff deflation to assess peak dilatory response (the maximum brachial artery diameter). End-diastolic diameters were analyzed by using a commercially available edge-detection wall-tracking software package (Brachial Analyzer, Medical Imaging Applications, LLC, Coralville, IA). Individual diameters were averaged (bin: 3 R-gated diameters) before identifying the peak diameter. FMD was expressed as absolute change in mm (maximum diameter − baseline diameter) and as % change ([(maximum − baseline diameter)/baseline diameter] × 100). To quantify the hyperemic response, the first 15 post-occlusion spectral Doppler envelopes and at least 15 baseline spectral Doppler envelopes were recorded on super VHS tape and were analyzed with the Toshiba ultrasound system software to obtain blood velocity. Blood flow (ml/min) was calculated as mean blood velocity × [(baseline diameter)^2/4] × μ × 6 × 10^{-1}. Shear stress (dyne/cm^2) was calculated as 8 × μ × mean blood velocity/baseline diameter, where μ was blood viscosity, which was assumed to be 0.035 dyne/cm^2 [34]. Ultrasound images were analyzed by MH and spectral Doppler was analyzed by ML, both of whom were blind to the treatment (i.e., Eplerenone or placebo) and subject identity.

Vascular endothelial cell collection and protein expression—Endothelial cells were collected from an antecubital vein as previously described [35–38]. Briefly, 2 sterile J-

shaped guidewires (Daig, Inc., Minnetonka, MN) were sequentially advanced ~ 10 cm through an 18 gauge intravenous catheter and retracted. Cells were recovered by washing the wires with a dissociation buffer and centrifugation. Cells were fixed with 4% paraformaldehyde (USB corporation, Cleveland, OH), washed thoroughly with PBS, plated on poly-L-lysine coated slides (Sigma Chemical, St. Louis, MO), and stored at −80°C until the immunofluorescence staining was performed.

For immunofluorescence staining, fixed vascular endothelial cells were rehydrated with PBS containing 50 mmol/L glycine and non-specific sites were blocked with 5% donkey serum (Jackson Immunoresearch, West Grove, PA). Slides were incubated with one of the following primary antibodies followed with corresponding secondary antibody with Alexa Fluor 488 (Invitrogen, Carlsbad, CA): nitrotyrosine which is a marker of oxidative stress (Abcam, Inc, Cambridge, MA) and NADPH oxidase $p47^{phox}$ (Millipore, Inc., Billerica, MA) which is one of the major sources of vascular superoxide. Slides were also incubated with a primary antibody for von Willebrand factor (DAKO, Carpinteria, CA) and corresponding secondary antibody with Alexa Fluor 555 (Invitrogen, Carlsbad, CA) to allow identification of endothelial cells. Finally, slides were mounted with Vectashield containing the nuclear stain DAPI (Vector Laboratories, Inc., Burlingame, CA). Because of the large number of slides, staining was performed in several batches, but each subject's slides from the Eplerenone and placebo visits were included in the same batch to avoid the influence of day-to-day variability in staining. To minimize the potential confound of inter-batch variability in staining, 2 slides of human umbilical venous endothelial cells (HUVEC) were stained in each batch and intensity for each protein of interest was expressed relative to the average HUVEC intensity in that batch.

For analysis, cells were examined with a fluorescence microscope (Eclipse 80i, Nikon Instruments, Inc., Melville, NY) at ×100 magnification using the same exposure time. Images of endothelial cells with intact nuclei were digitally captured by a coolSNAP ES2 camera (Photometrics, Tuscon, AR). Endothelial cells were identified by the presence of von Willebrand factor staining and nuclear integrity was confirmed by DAPI staining. Vascular endothelial cell protein expression was measured with NIS Elements software (version 3.2, Nikon Instruments, Inc., Melville, NY) by quantifying Alexa Fluor 488 intensity while correcting for background fluorescence. Vascular endothelial cell protein expression is reported as intensity per HUVEC intensity.

Blood measures—Standard blood chemistries and hematological evaluation were performed at baseline by a clinical laboratory using conventional assays. Insulin resistance was estimated using the homeostasis model of insulin resistance, [HOMA-IR; HOMA-IR = (fasting insulin μU/ml × fasting glucose mg/dl)/405]. Plasma F_2-isoprostanes were measured by the Vanderbilt University Eicosanoid Core Laboratory using gas chromatography-mass spectrometry, as previously described [39].

Height, weight and adiposity measures—Height was measured to the nearest mm using a stadiometer. Body weight was measured to the nearest 0.1 kg with an electronic scale (Tanita, Arlington Heights, IL, USA) while subjects were barefoot and dressed in light clothing. Body mass index (BMI) was calculated as weight divided by height squared

(kg/m^2). Total % body fat was assessed with dual-energy x-ray absorptiometry (DPX-IQ, GE/Lunar, Salt Lake City, UT, USA) as described previously [40]. Abdominal total, visceral and subcutaneous fat were measured at the level of L4-L5 using a single slice computed tomography scan and assessed by a commercially available analysis software (Slice-O-Matic v4.3, Tomovision) [41].

Resting blood pressure—Resting blood pressures were recorded over the brachial artery with a semi-automated device (Dinamap, GE, Salt Lake City, UT, USA).

Aerobic fitness—Aerobic fitness was determined using maximal oxygen consumption (VO_2max) as previously described [40]. Briefly, online computer-assisted open-circuit spirometry was used during incremental treadmill exercise. After subjects walked for 6 to 10 min at a comfortable speed that corresponded to 70 to 80 % of their age-predicted maximal heart rate to warm-up, the treadmill grade was increased 2.5% every two minutes until volitional exhaustion.

Data Analysis

Statistical analyses were performed using SPSS version 21. Statistical significance for all analyses was set at $P< 0.05$. Paired t-tests were used to compare FMD, blood and vascular endothelial cell markers of oxidative stress during MR blockade and placebo treatments. Bivariate relations were determined using Pearson product moment correlation coefficients.

RESULTS

Mean values and ranges for baseline subject characteristics are presented in Table 1. Subjects varied widely in total and abdominal adiposity. At baseline, total and abdominal adiposity were negatively associated with FMD ($r=-0.37$ to 0.49, $P<0.05$) and positively associated with F_2-isoprostanes ($r=0.43$ to 0.68, $P<0.05$).

Vascular responses to MR blockade

In the whole group, mean brachial artery FMD was not different with MR blockade compared to placebo (P=0.7; Table 2). However, individual responses to MR blockade varied from decreased to increased FMD. Subjects whose FMD improved with MR blockade had ~ 40% higher abdominal visceral fat compared with those whose FMD either decreased or did not change with MR blockade (P=0.03). In agreement with these results, greater improvements in FMD in response to MR blockade were related with greater baseline body mass index, total % body fat and total abdominal, visceral and subcutaneous fat ($r=0.45$ to 0.67, $P\leq0.03$; Figures 2 and 3). In addition, greater improvements in FMD were associated with higher baseline fasting glucose ($r=0.53$, $P=0.01$; Figure 4).

Baseline brachial artery diameter was not different between MR blockade and placebo treatment, whereas, baseline shear stress increased in response to MR blockade (P=0.9 and P=0.02, respectively; Table 2). However, hyperemic shear stress and the change in shear stress from baseline did not differ between MR blockade and placebo, indicating that the post-occlusion stimulus to induce vasodilation was similar (P=0.8 and P=0.1, respectively, Table 2). MR blockade resulted in significant reductions in systolic blood pressure

(P<0.0001) and smaller reductions in diastolic blood pressure that did not reach statistical significance (P=0.07; Table 2). However, the change in systolic blood pressure was not related with the change in FMD in response to MR blockade (P>0.05). In addition, accounting for the change in systolic blood pressure in multiple linear regression analysis did not contribute significantly to the model (P>0.05) and did not influence the relation of adiposity with the change in FMD in response to MR blockade.

Plasma oxidative stress and vascular endothelial cell protein expression

MR blockade did not influence plasma F_2-isoprostanes (6.5±1.0 vs. 5.9±0.6 pg/mL, P=0.3; placebo vs. MR blockade). Similarly, vascular endothelial cell protein expression of nitrotyrosine (marker of oxidative stress) and NADPH oxidase (vascular source of superoxide) did not significantly change in response to MR blockade (0.79±0.04 vs. 0.73±0.22 intensity/HUVEC intensity, P=0.2, 0.66±0.04 vs. 0.57±0.04 intensity/HUVEC intensity, P=0.1, respectively). There were no correlations between 1) baseline plasma/endothelial cell oxidative stress measures, and baseline adiposity or change in FMD with MR blockade; and 2) change in plasma/endothelial cell oxidative stress markers and change in FMD with MR blockade.

DISCUSSION

We investigated whether MR modulate vascular endothelial function in an adiposity-dependent manner in healthy older adults with widely varying total and abdominal adiposity. Our study demonstrates for the first time that greater improvement in vascular endothelial function with MR blockade is seen in those who have greater total and abdominal adiposity. Another important finding of our study is that greater enhancements in endothelial function in response to MR blockade are associated with higher baseline fasting glucose.

Findings from two recent studies based on animal and *in vitro* models have shown compelling evidence of aldosterone production in adipocytes and contribution of adipocyte-derived aldosterone to vascular dysfunction in obesity [9, 10]. In humans, several studies have shown elevated plasma aldosterone levels with obesity and some have found that greater BP reduction with MR blockade was associated with higher body mass index [42] and higher waist circumference [43]. Our data extend these findings by demonstrating greater increases in FMD with MR blockade are associated with higher body mass index, total % body fat, total abdominal, visceral and subcutaneous fat.

Aldosterone might be the potential link between adiposity, insulin resistance, and increased risk for cardiovascular disease. A recent review article highlighted data supporting a role for elevated plasma aldosterone levels and MR signaling in the pathophysiology of insulin resistance and vascular dysfunction [44]. Our data demonstrate greater improvements in endothelial function with MR blockade are associated with higher baseline fasting blood glucose. These findings suggest that MR play a larger role in vascular dysfunction in subjects with lower insulin sensitivity.

In our study, systolic blood pressure significantly decreased in response to MR blockade, thus, one might speculate that this could have contributed to the improvements in

endothelial function. However, the change in systolic blood pressure was not related with the change in FMD in response to MR blockade. In addition, accounting for the change in systolic blood pressure in multiple linear regression analysis did not significantly contribute to the model and did not influence the relation of adiposity with the change in FMD in response to MR blockade. Taken together these findings argue against the assumption that reductions in blood pressure might have played a significant role in the beneficial effects of Eplerenone on vascular endothelial function.

Our study has several strengths including: 1) novelty of findings; 2) use of balanced randomized, double-blind, placebo-controlled, cross-over design; 3) exclusion of subjects with overt cardiovascular or other clinical disease and medication use, which could confound the independent relation of MR with obesity; 4) quantification of total % body fat using DEXA and total abdominal, visceral and subcutaneous fat using computed tomography; and 5) rigorous procedures to ensure adherence to intervention.

Our study also has some potential limitations. We did not measure baseline plasma aldosterone to determine if it was elevated in our obese subjects. However, several studies have already established a relation between aldosterone levels and obesity. MR have equal affinity for aldosterone and cortisol, however, the presence of the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD) in tissues (including the vascular wall) converts cortisol to corticone making aldosterone the primary MR agonist [45]. Our current data cannot address whether cortisol might have a role in the observed effects of MR blockade in human obesity. In cardiovascular disease, MR appear to contribute to vascular dysfunction by exacerbating ROS production and oxidative stress, but in our study plasma F_2-isoprostanes and vascular endothelial cell protein expression of nitrotyrosine and NADPH oxidase $p47^{phox}$ did not change in response to MR blockade. Given our limited oxidative stress measures, we cannot rule out that oxidative stress plays a role in the beneficial effects of MR blockade on endothelial function in human obesity. Our protein expression data of oxidative stress markers focused on vascular endothelial cell samples, which does not reflect whether oxidative stress levels changed in vascular smooth muscle cells. In addition, we measured protein expression of a specific subunit of NADPH oxidase, but it is possible that other isoforms/subunits and/or activation of the enzyme are playing a role, which cannot be addressed using our current methodology. Finally, our subjects were older, thus our results might not be applicable to healthy young adults. Additional research is needed to investigate whether MR blockade also improves vascular endothelial function in an obesity-dependent manner in healthy young adults.

Clinical Significance

Aldosterone contributes to vascular dysfunction in cardiovascular disease. Plasma aldosterone is elevated with total and abdominal adiposity in humans, but its influence on vascular function is unknown. We sought to examine the role of MR in vascular endothelial function in human obesity in a balanced randomized, double-blind, placebo-controlled, crossover study using 1 month MR blockade with Eplerenone. We found that Eplerenone-related improvements in FMD were positively associated with total and abdominal adiposity and baseline fasting glucose in healthy older adults. Aldosterone appears to be an important

contributor to vascular endothelial dysfunction in healthy older adults with increased adiposity and fasting blood glucose. These findings have important clinical implications. Therapeutic use of MR blockade to treat hypertension in patients with increased adiposity might confer direct favorable effects on obesity-related vascular alterations and might reduce the risk of developing cardiovascular complications.

CONCLUSIONS

The present findings demonstrate, for the first time, that MR modulate vascular endothelial function in an adiposity-dependent manner in healthy older adults. MR-blockade-related improvements in FMD are positively related with both total and abdominal adiposity. We also demonstrate that changes in vascular endothelial function with MR blockade are related with baseline fasting blood glucose. Our study suggests that MR contribute to the pathophysiology of impaired vascular endothelial function in human obesity.

Acknowledgments

The authors would like to thank Ms. Sharon Greer, R.N., Mr. Creighton Wilson R.N. and the Division of Cardiology and Radiology at the Scott and White Clinic at College Station, Texas, for their contributions. The authors would also like to thank Ms. Molly Cernosek and Ms. Larysa Sautina for technical assistance and the study participants for their time and efforts.

FUNDING

This work was supported by the National Institutes of Health [grant AG 032067] to DDC and American Heart Association [grant 0865117F] to DDC.

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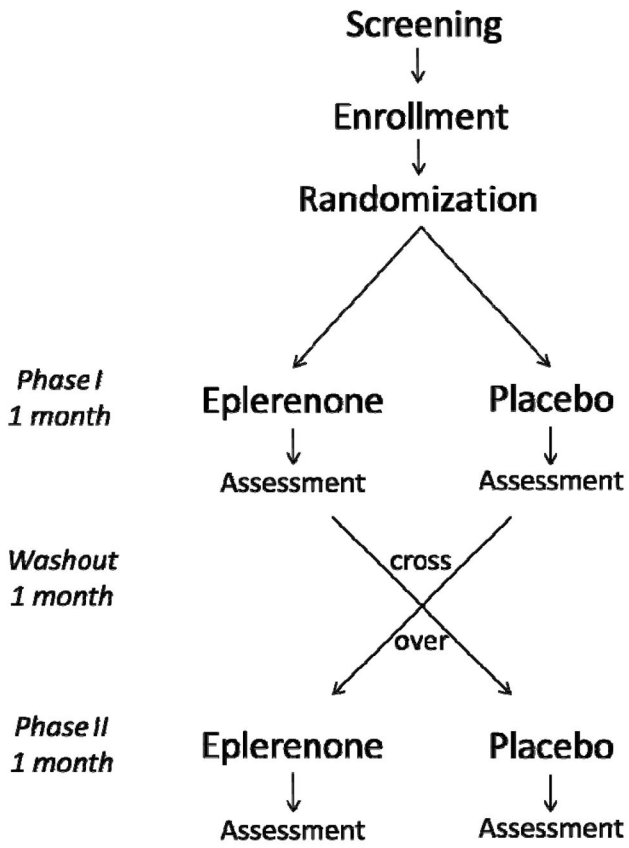

Figure 1.

Study design. Subjects were assigned to receive an MR antagonist (Eplerenone; 100 mg per day) or placebo for 1 month in a balanced randomized, double-blind, crossover study with 1-month washout between treatments.

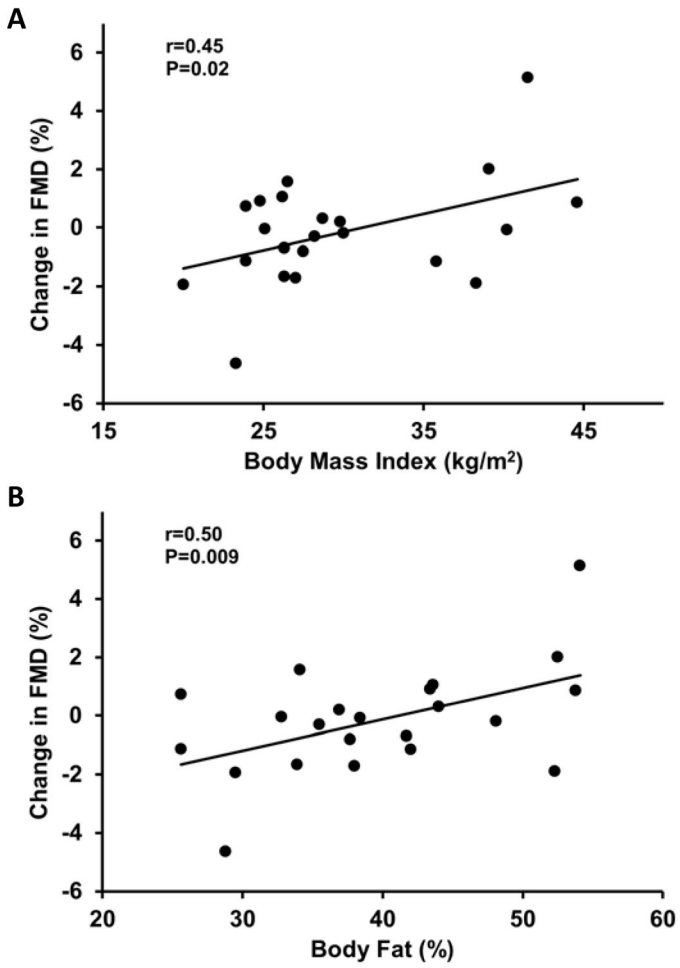

Figure 2.

The relation between body mass index (A) and total % body fat (B) with the change in flow-mediated dilation (FMD) in response to mineralocorticoid receptor blockade.

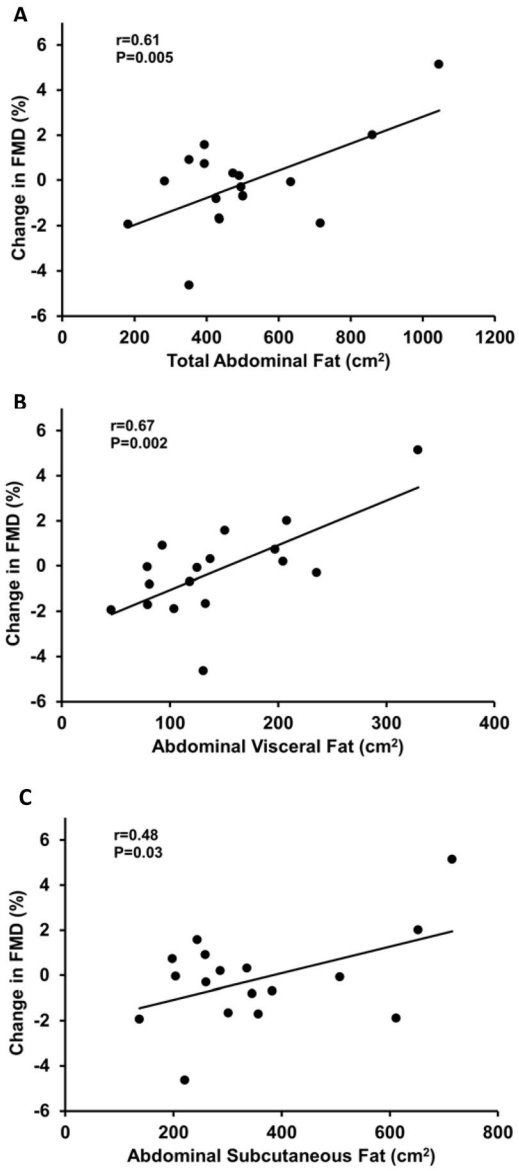

Figure 3.

The relation between total abdominal fat (A), abdominal visceral fat (B) and abdominal subcutaneous fat (C) with the change in flow-mediated dilation (FMD) in response to mineralocorticoid receptor blockade.

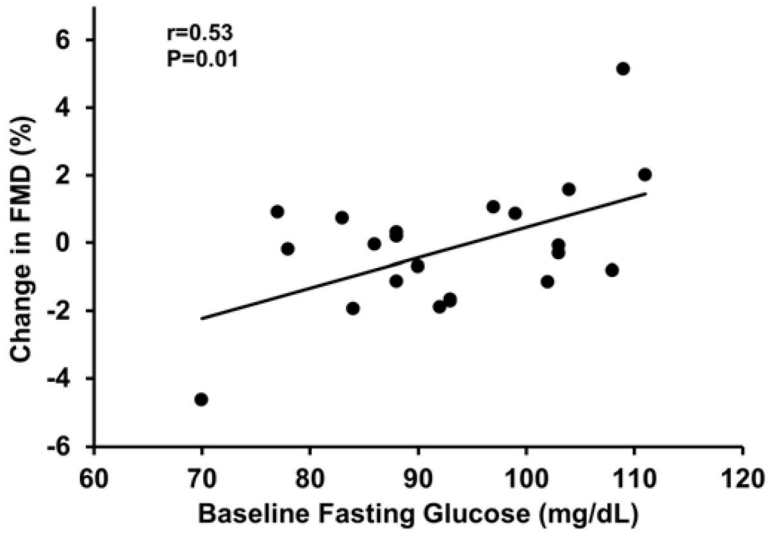

Figure 4.

The relation between baseline fasting glucose and the change in flow-mediated dilation (FMD) in response to mineralocorticoid receptor blockade.

Table 1

Subject Characteristics

	Mean±SE	Min-Max
Sex (male/female)	10/12	
Age, years	63.6±1.5	55–79
Weight, kg	89.6±4.3	54.8–132.7
Body mass index, kg/m^2	29.9±1.4	20.0–44.6
Body fat, %	39.7±1.9	25.6–54.1
Total abdominal fat, cm^2	498.7±51.4	183.1–1045.0
Abdominal visceral fat, cm^2	144.3±17.2	45.8–329.5
Abdominal subcutaneous fat, cm^2	354.4±41.1	137.3–715.5
VO_{2max}, ml/kg/min	24.9±1.4	14.2–37.7
Total cholesterol, mg/dL	185±6	143–225
LDL cholesterol, mg/dL	114±6	69–162
HDL cholesterol, mg/dL	47±3	30–69
Triglycerides, mg/dL	120±18	58–384
Fasting glucose, mg/dL	95±2	84–109
Fasting insulin, μU/mL	3.3±0.5	2–10
HOMA-IR	0.8±0.1	0.4–2.5
F_2-Isoprostanes, pg/mL	69±9	32–198

VO_{2max} = Maximal oxygen consumption; LDL = low density lipoprotein; HDL = high density lipoprotein; HOMA-IR= homeostasis assessment model for insulin resistance.

Table 2

Cardiovascular Responses to Mineralocorticoid Receptor Blockade

	Placebo	MR Blockade	P values
Heart rate, beats/min	60±2	62±2	0.06
Systolic blood pressure, mm Hg	133±3	123±3	<0.0001
Diastolic blood pressure, mm Hg	77±2	72±1	0.07
Baseline diameter, mm	3.77±0.16	3.78±0.15	0.9
Baseline SS, dyne/cm^2	1.75±0.12	2.02±0.18	0.02
Hyperemic SS, dyne/cm^2	7.66±0.44	7.74±0.46	0.8
Change in SS from baseline, %	348±15	314±25	0.1
Flow-mediated dilation, mm	0.23±0.02	0.23±0.02	0.7
Flow-mediated dilation, %	6.39±0.67	6.23±0.73	0.7
Flow-mediated dilation/hyperemic SS	0.03±0.003	0.03±0.003	0.5

Data are mean±SE. SS: shear stress.