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Effect of Selective Mineralocorticoid Receptor Blockade on Flow-Mediated Dilation and Insulin Resistance in Older Adults with Metabolic Syndrome

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Abstract

Background: The prevalence of metabolic syndrome is especially high in older adults. Metabolic syndrome is associated with impaired vascular endothelial function, insulin resistance, and increased risk for cardiovascular disease but the underlying mechanisms are not fully elucidated. Plasma aldosterone is independently associated with metabolic syndrome and is linked to endothelial dysfunction and insulin resistance. Thus, we hypothesized that mineralocorticoid receptor (MR) blockade would improve flow-mediated dilation and insulin resistance in older adults with metabolic syndrome.

Methods: To test this hypothesis, we conducted a balanced, randomized, double-blind, placebo-controlled, crossover study using selective MR blockade (eplerenone; 100 mg/day) for 1 month with 1 month washout in older adults with metabolic syndrome (62.6 ± 3.2 yrs; mean ± standard error). We evaluated brachial artery flow-mediated dilation (ultrasonography), oxidative stress (oxidized low-density lipoproteins and F_2-isoprostanes) and insulin resistance (homeostatic model assessment).

Results: In response to MR blockade, flow-mediated dilation (5.37 ± 0.85 vs. 5.98 ± 1.29%; placebo vs. eplerenone; $P = 0.4$), oxidized low-density lipoproteins (51.6 ± 11.5 vs. 56.1 ± 10.9 U/L; $P = 0.6$), and F_2-isoprostanes (0.07 ± 0.02 vs. 0.06 ± 0.01 pg/mL; $P = 0.3$) did not improve. Insulin resistance also did not change following MR blockade (1.04 ± 0.26 vs. 1.38 ± 0.50; $P = 0.6$). However, MR blockade resulted in a large reduction (10 mmHg) in systolic blood pressure (140 ± 6 vs. 130 ± 6 mmHg; $P = 0.02$), with no significant change in diastolic blood pressure (81 ± 3 vs. 75 ± 2 mmHg; $P = 0.2$).

Conclusions: Our data do not support a contributing role for MRs in endothelial dysfunction and insulin resistance in older adults with metabolic syndrome. However, our findings suggest MR activation is an important contributor to systolic hypertension in this patient group.

Introduction

Aging is associated with an especially high prevalence of metabolic syndrome, occurring in ~44% of men and women over 50 years of age.[1,2] Metabolic syndrome is defined as a clustering of cardiovascular risk factors including hypertension, hyperglycemia, low HDL cholesterol, elevated triglycerides, and abdominal obesity.[3] Individuals with metabolic syndrome have a greater incidence and progression of atherosclerosis and greater incidence of cardiovascular events and death.[4–6] Aldosterone, a mineralocorticoid hormone, is elevated in metabolic syndrome,[7] and there is increasing evidence that both aldosterone and mineralocorticoid receptor activation contribute to insulin resistance and cardiovascular disease (recently reviewed by Briet et al.[8]).

A key early event in the development of atherosclerosis is vascular endothelial dysfunction (*i.e.*, impaired flow-mediated dilation)[9]; however, the mechanisms responsible for this impairment in metabolic syndrome are not yet fully elucidated. Data from experimental models of cardiovascular disease

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provide substantial evidence that mineralocorticoid receptor activation contributes to oxidative stress leading to decreased nitric oxide bioavailability and impaired vascular endothelial function.[10–13] Additionally, these studies demonstrate that mineralocorticoid receptor blockade results in improved vascular endothelial function due to reductions in reactive oxygen species production, restoration of endothelial nitric oxide synthase levels and improvements in nitric oxide bioavailability.[10–13]

In mice with obesity-induced insulin resistance, mineralocorticoid receptor mRNA levels are elevated.[14] In addition, aldosterone inhibits insulin-induced glucose uptake by degrading insulin receptor substrate (IRS) 1 and IRS 2 via oxidative stress based on data from *in vitro* studies.[15] In animal models of obesity-induced insulin resistance[14] and diet-induced diabetes,[16] treatment with mineralocorticoid receptor blockade improves glucose metabolism and insulin resistance.

Recent review articles have proposed that mineralocorticoid receptor activation might contribute to endothelial dysfunction and insulin resistance in metabolic syndrome,[8,17,18] but to our knowledge this has not been directly tested experimentally. Thus, in the current investigation we administered a selective mineralocorticoid receptor antagonist (eplerenone) for 1 month in a randomized, double-blind, placebo-controlled, crossover study to test the hypothesis that flow-mediated dilation and insulin resistance would be improved in response to mineralocorticoid receptor blockade in older adults with metabolic syndrome.

Methods

Subjects

A group of metabolic syndrome patients 55 to 79 years of age ($n = 8$; 4 men and 4 women) were studied. Metabolic syndrome was defined as the presence of three or more of the following five risk factors:[3]

- Elevated blood pressure (systolic: ≥130 mmHg and diastolic: ≥85 mmHg),
- Elevated triglycerides (≥150 mg/dL),
- Elevated fasting glucose (≥100 mg/dL),
- Reduced HDL cholesterol (men: <40 mg/dL and women: <50 mg/dL), or
- Elevated waist circumference (sex, population, and country specific).

All subjects were sedentary, nonsmokers, and were free of overt cardiovascular disease and other clinical disorders (*e.g.*, diabetes, liver and renal disease, sleep apnea) as determined by medical history, physical examination, resting electrocardiogram (ECG), urinalysis, blood chemistries, and hematological evaluation. Subjects demonstrated normal ECG and blood pressure responses to a graded exercise test on a treadmill. For the graded exercise test, subjects walked for 6 min at a comfortable speed that corresponded with 70%–80% of their age-predicted maximal heart rate to warm up, followed by 2.5% increases in the treadmill grade every 2 min until volitional exhaustion. None of the subjects was taking antihypertensive or vasoactive drugs and subjects who were taking antioxidant supplements completed a 4 week washout prior to study enrolment. Women were not on hormone replacement therapy for at least 1 year prior to data collection. All women were postmenopausal, established by absence of menses for at least 2 years and follicle stimulating hormone >40 IU/L.

The study was approved by the Institutional Review Boards of the University of Florida, Texas A&M University, and Scott & White Health System and was carried out in accordance with the Declaration of Helsinki (2008). The purpose, nature, and risk of all study procedures were explained to the subjects and their written informed consent was obtained prior to participation.

Study design

Subjects received a daily dose of the mineralocorticoid receptor antagonist eplerenone (100 mg per day) or placebo for 1 month, following a balanced randomized crossover, double-blind design. Data collection occurred at the end of each treatment arm. A washout period of 1 month was allowed between treatments. Subjects were not enrolled in the study if serum potassium was >5.5 mmol/L, serum creatinine was >1.6 mg/dL, or creatinine clearance was <30 mL/min to reduce the risk of hyperkalaemia. Serum potassium and blood pressure were assessed at baseline, day 3, day 7, and day 14 after each treatment. Serum potassium levels following eplerenone administration did not significantly increase (4.5 ± 0.1, 4.5 ± 0.2, 4.7 ± 0.1 and 4.7 ± 0.1 mmol/L for baseline, day 3, day 7, and day 14, respectively; $P = 0.4$) and no subjects were withdrawn from the study due to elevated serum potassium. In addition, there were no symptoms of orthostatic hypotension reported while subjects received eplerenone.

General experimental procedures

Measurements were performed at the same time in the morning after a 12-hour overnight fast (including abstinence from caffeine and alcohol). Subjects rested supine for a minimum of 20 min in a semidarkened temperature-controlled room prior to data collection.

Brachial artery flow-mediated dilation

Flow-mediated dilation was assessed at the brachial artery noninvasively using an ultrasound/Doppler system equipped with a 7.5 MHz linear vascular transducer (Aplio XV, Toshiba) following established guidelines.[19,20]

The subject's right arm was abducted and fixed in position at heart level. A rapid inflation/deflation pressure cuff (E20 and AG 101, D. E. Hokanson, Bellevue, WA) was placed around the widest part of the subject's forearm and was used to induce reactive hyperemia by inflating the cuff to 250 mmHg for 5 min followed by rapid deflation. A duplex ultrasound image of the brachial artery (*i.e.*, 2D image and spectral Doppler waveforms) was obtained approximately midway between the antecubital fossa and the shoulder. Blood velocity was assessed with the Doppler angle of insonation set at ≤60 degrees. To ensure an image of the same segment of the brachial artery was obtained in both ultrasound visits, 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. To prevent movement during data collection, the vascular transducer was clamped in place (Flexbar, Flexbar Machine Corporation, Islandia, NY). ECG R-gated duplex ultrasound images of the brachial artery were digitally recorded for 1 min (Vascular Imager, Medical Imaging Applications, LLC, Coralville, IA) to establish end-diastolic pre-

occlusion baseline diameter and for 2 min post-occlusion to determine the maximum brachial artery diameter.

A commercially available edge-detection wall-tracking software (Brachial Analyzer, Medical Imaging Applications, LLC) was used to analyze the diameters. A moving average using a 3-R-gated image bin was applied prior to identifying the peak diameter. Flow-mediated dilation was expressed as absolute change in mm (maximum diameter − baseline diameter), as % change {[(maximum − baseline diameter)/baseline diameter] × 100} and as flow-mediated dilation normalized for hyperemic shear stress. To quantify the hyperemic response, blood velocity was analyzed using the first 15 post-occlusion spectral Doppler envelopes and 15 baseline spectral Doppler envelopes using the Toshiba ultrasound system software. Blood flow (mL/min) was calculated as [mean blood velocity × (baseline diameter/2)^2 × π × 0.6].[21] Shear stress (dyn/cm^2) was calculated as (8 × μ × mean blood velocity/baseline diameter), where μ was blood viscosity, which was assumed to be 0.035 dyn/cm^2.[22] Ultrasound images were analyzed by the same investigator who was blind to the treatment (*i.e.*, mineralocorticoid receptor blockade or placebo).

Blood measures

Standard blood chemistries and hematological evaluation were performed by a clinical laboratory using conventional assays. Plasma oxidized low-density lipoprotein, an indirect measure of oxidative stress, was measured using a commercially available ELISA kit (Mercodia). Plasma F_2-isoprostanes, another marker of oxidative stress, were measured by the Vanderbilt University Eicosanoid Core Laboratory using gas chromatography–mass spectrometry, as previously described.[23] Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) as [fasting insulin (μU/mL) × fasting glucose (mg/dL)/405].[24]

Body weight

Body weight was measured to the nearest 0.1 kg with an electronic scale (Tanita, Arlington Heights, IL) while subjects were barefoot and dressed in light clothing.

Resting blood pressure

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

Statistical analyses

Statistical analyses were performed using SPSS version 21. Statistical significance for all analyses was set at $P < 0.05$. Data are presented as means ± standard error. To test the study hypothesis, paired *t*-tests were performed. To test whether potassium levels changed during eplerenone administration, repeated measures analysis of variance was performed.

Results

The average age of our metabolic syndrome subjects was 62.6 ± 3.2 years. Mean values and ranges for other baseline subject characteristics are presented in Table 1.

Mineralocorticoid receptor blockade with eplerenone resulted in 10 mmHg reduction in systolic blood pressure (140 ± 6 vs. 130 ± 6 mmHg; $P = 0.02$). Diastolic blood pressure and heart rate were unaffected (81 ± 3 vs. 75 ± 2 mmHg and 59 ± 2 vs. 61 ± 2 bpm, respectively, $P > 0.05$).

Table 1. Baseline Subject Characteristics

	Mean ± SE	*Range*
Weight, kg	100.8 ± 7.0	75.8–122.7
Waist circumference, cm	107.0 ± 5.1	84.0–128.8
Triglycerides, mg/dL	155 ± 26	78–280
LDL cholesterol, mg/dL	102 ± 8	79–140
HDL cholesterol, mg/dL	40 ± 2	31–49
Fasting glucose, mg/dL	100 ± 4	78–111
Systolic blood pressure, mmHg	136 ± 5	110–169
Diastolic blood pressure, mmHg	82 ± 3	67–92

LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Baseline brachial artery diameter and shear stress did not change ($P > 0.05$; Table 2) in response to eplerenone. In addition, the post-occlusion stimulus for inducing vasodilation was not different between the eplerenone and placebo treatment as evidenced by the similar hyperemic shear stress and the similar change in shear stress from baseline ($P = 0.6$ and $P = 0.7$, respectively; Table 2).

In response to eplerenone, flow-mediated dilation did not improve ($P = 0.4$ for flow-mediated dilation in %, $P = 0.5$ for flow-mediated dilation in mm and $P = 0.8$ for flow-mediated dilation normalized for hyperemic shear stress; Table 2). In addition, plasma oxidized low-density lipoprotein (51.6 ± 11.5 vs. 56.1 ± 10.9 U/L, $P = 0.6$; placebo vs. eplerenone) and plasma F_2-isoprostanes (0.07 ± 0.02 vs. 0.06 ± 0.01 pg/mL, $P = 0.3$) did not change following treatment with eplerenone. Insulin resistance also did not improve in response to eplerenone (HOMA-IR: 1.04 ± 0.26 vs. 1.38 ± 0.50, $P = 0.6$).

Discussion

Our study is the first in older adults with metabolic syndrome to test the hypothesis that brachial artery flow-mediated dilation and insulin resistance would improve in response to mineralocorticoid receptor blockade. Contrary to our hypothesis, mineralocorticoid receptor blockade did not influence flow-mediated dilation or insulin resistance. However, our findings support that use of 100 mg oral daily dose of eplerenone for 1 month is safe and dramatically improves systolic blood pressure (−10 mmHg) in older adults with metabolic syndrome.

Table 2. Cardiovascular Responses to Mineralocorticoid Receptor

	Placebo	*Eplerenone*
Baseline diameter, mm	4.10 ± 0.31	4.07 ± 0.31
Baseline SS, dyn/cm^2	1.62 ± 0.20	1.91 ± 0.33
Hyperemic SS, dyn/cm^2	7.05 ± 0.77	7.41 ± 0.85
Change in SS, %	351 ± 27	333 ± 47
FMD, %	5.37 ± 0.85	5.98 ± 1.29
FMD, mm	0.21 ± 0.03	0.23 ± 0.04
FMD/hyperemic SS	0.03 ± 0.005	0.03 ± 0.004

Data are mean ± SE.
SS, shear stress; FMD, flow-mediated dilation.

Endothelial dysfunction

Although recent reviews have proposed that mineralocorticoid receptor activation might possibly be involved in endothelial dysfunction in metabolic syndrome,[8,17] we are not aware of any studies directly investigating the effect of selective mineralocorticoid receptor blockade on endothelial function in metabolic syndrome. In the present study, we provide data for the first time showing that mineralocorticoid receptor blockade does not lead to improved flow-mediated dilation, suggesting that mineralocorticoid receptors do not appear to contribute to vascular endothelial dysfunction in older adults with metabolic syndrome. Our results are consistent with a prior study in type 1 diabetic men (21 to 64 years of age) which demonstrated that 6 week eplerenone administration did not improve brachial artery flow-mediated dilation.[25] Contrary to these findings, a study in type 2 diabetic patients (43 to 79 years of age) found that 1-month mineralocorticoid receptor blockade resulted in impairments in endothelial function.[26]

Insulin resistance

Data from animal studies link aldosterone to insulin resistance and altered insulin signaling pathways, whereas mineralocorticoid receptor blockade has been reported to improve insulin resistance (reviewed in Underwood et al.[18]), but this has not been previously investigated in individuals with metabolic syndrome. Our data do not support that mineralocorticoid receptor blockade influences insulin resistance in older adults with metabolic syndrome; however, our measure of insulin resistance cannot address whether insulin-mediated glucose uptake by the muscle was affected. Our findings, are in agreement with Garg et al.,[27] who recently reported in human obesity no changes in HOMA-IR, area under the curve for insulin and glucose, or insulin sensitivity index with 6 weeks of mineralocorticoid receptor blockade using spironolactone.

Blood pressure

The efficacy of mineralocorticoid receptor blockade in arterial hypertension has recently been reviewed by Pellicia et al.[28] Eleven randomized clinical trials ranging in dose from 25 to 100 mg of eplerenone resulted in a dose-dependent reduction in blood pressure. However, none of these randomized trials focused on patients with metabolic syndrome. Two nonrandomized open label studies[29,30] have examined the add-on effects of mineralocorticoid receptor blockade on blood pressure in patients with metabolic syndrome who were already using other antihypertensive medications. These studies reported significant reductions in blood pressure ($P < 0.05$), but their findings have not been confirmed by well-controlled trials.

Our randomized double-blind placebo-controlled study demonstrated that 100 mg of daily dose of eplerenone for 1 month results in a rapid and dramatic decrease in systolic blood pressure compared to placebo in older adults with metabolic syndrome. Our findings suggest that in older adults with metabolic syndrome activation of mineralocorticoid receptors plays a significant role in arterial hypertension. These findings may have important clinical implications because apart from the antihypertensive effects of eplerenone, reducing blood pressure may be beneficial in limiting end organ damage (*e.g.*, cardiac and renal dysfunction) in older adults with metabolic syndrome.

Study strengths

One of the strengths of the current study is the use of a randomized, double-blind, placebo-controlled, crossover design. Our data significantly contribute to scientific progress in several ways: (1) they address an important gap in knowledge, (2) they extend our current understanding related to mineralocorticoid receptors; (3) they guide the design of future experiments in the area of metabolic syndrome; and (4) they provide a balanced perspective to the proposed important pathophysiological role of mineralocorticoid receptor activation in endothelial dysfunction and insulin resistance in metabolic syndrome as described in recent review articles.[8,17,18]

Study limitations

First, we have studied a small number of older adults with metabolic syndrome. However, based on power analysis using the very small effect size found in this study, we estimated that a very large number of subjects (109 to 1081) would be required to demonstrate a statistically significant but slight increase in endothelial function (0.06% and 0.015 mm change in flow-mediated dilation and 0.0007 change in flow-mediated dilation normalized for hyperemic shear stress). We also estimated that more than 100 subjects would be required to show a significant change in insulin resistance (0.34 units increase in HOMA-IR). Such minor changes in endothelial function and insulin resistance, even if statistically significant, would likely not be important in influencing future cardiovascular outcomes or clinical management of older metabolic syndrome patients.

Second, the present investigation included both men and women. The mechanisms of endothelial dysfunction and the influence of mineralocorticoid receptors on endothelial function and insulin resistance may differ between sexes, which could have influenced our findings. Future investigations should focus on these potential sex differences.

Third, our conclusions are restricted to older adults with metabolic syndrome. The interaction between aging and metabolic syndrome could have contributed to our results. Future studies should examine the effect of mineralocorticoid receptor blockade in young patients with metabolic syndrome to directly determine if it is effective in improving endothelial function and insulin resistance.

Finally, our results could have been influenced by administering eplerenone, the selective mineralocorticoid receptor antagonist, instead of spironolactone, the nonselective mineralocorticoid receptor antagonist, and/or by the length of eplerenone administration and washout period. Although both eplerenone and spironolactone effectively block the mineralocorticoid receptors, we chose to use eplerenone in this investigation because it has very low binding affinity for androgen, glucocorticoid and progesterone receptors (100- to 1000-fold lower compared with spironolactone).[31] Eplerenone is also better tolerated and is associated with lower risk for hyperkalemia and with no or very low sexual side effects compared to spironolactone.[31] In our study, we administered eplerenone for 1 month, which should be sufficiently long to result in changes in flow-mediated dilation and insulin resistance, but we cannot rule out that longer treatment might have resulted in different findings. However, other studies that

blocked the mineralocorticoid receptors with eplerenone or spironolactone for similar or longer period also showed no improvement in endothelial function or insulin resistance.[25–27] The 1 month washout in the current study should be adequate given the relatively short elimination half-life of eplerenone (4–6 hours) and absence of active metabolites. Although the biological effect of eplerenone could be longer than the elimination half-life, there is no evidence suggesting that biological effects would have persisted longer than the 1 month washout period. Studies investigating the effect of 1 month treatment with spironolactone on endothelial function have used a 2 week washout[26,32]; spironolactone elimination half-life is 1.3 hours and 2.8–11.2 hours for the active metabolites.

Conclusion

It is clinically important to understand the mechanisms that contribute to impairments in flow-mediated dilation and insulin resistance in order to develop therapeutic strategies to prevent or reverse them. Our data demonstrate, for the first time using a placebo-controlled randomized trial, that selective mineralocorticoid receptor blockade using a daily dose of 100 mg of eplerenone is safe, well tolerated, and dramatically improves systolic blood pressure in older metabolic syndrome patients. These findings suggest that in older adults with metabolic syndrome, mineralocorticoid receptor activation is an important contributor to systolic hypertension. However, our data do not provide support to the proposed underlying role of the mineralocorticoid receptors in vascular endothelial dysfunction and insulin resistance in metabolic syndrome.

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Author contributions were as follows: Moon-Hyon Hwang and Demetra Christou conceived and designed the study; Moon-Hyon Hwang, Jeung-Ki Yoo, Meredith Luttrell, Thomas Meade, Mark English, and Demetra Christou collected the data; Moon-Hyon Hwang, Jeung-Ki Yoo, and Meredith Luttrell analyzed the data; Thomas Meade and Mark English provided on-site medical supervision for experiments; Moon-Hyon Hwang and Demetra Christou performed statistical analysis, interpreted results, and wrote the manuscript; Jeung-Ki Yoo, Meredith Luttrell, Thomas Meade, and Mark English reviewed the manuscript and provided feedback.

Author Disclosure Statement

No competing financial interests exist.

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