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Caveolin 1 Modulates Aldosterone-Mediated Pathways of Glucose and Lipid Homeostasis

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Background—Overactivation of the aldosterone and mineralocorticoid receptor (MR) pathway is associated with hyperglycemia and dyslipidemia. Caveolin 1 (cav-1) is involved in glucose/lipid homeostasis and may modulate MR signaling. We investigated the interplay between cav-1 and aldosterone signaling in modulating insulin resistance and dyslipidemia in cav-1-null mice and humans with a prevalent variant in the CAV1 gene.

Methods and Results—In mouse studies, cav-1 knockout mice exhibited higher levels of homeostatic model assessment of insulin resistance, cholesterol, and resistin and lower ratios of high- to low-density lipoprotein (all P<0.001 versus wild type). Moreover, cav-1 knockout mice displayed hypertriglyceridemia and higher mRNA levels for resistin, retinol binding protein 4, NADPH oxidase 4, and aldose reductase in liver and/or fat tissues. MR blockade with eplerenone significantly decreased glycemia (P<0.01), total cholesterol (P<0.05), resistin (P<0.05), and described enzymes, with no effect on insulin or triglycerides. In the human study, we analyzed the CAV1 gene polymorphism rs926198 in 556 white participants; 58% were minor allele carriers and displayed higher odds of insulin resistance (odds ratio 2.26 [95% CI 1.40–3.64]) and low high-density lipoprotein (odds ratio 1.54 [95% CI 1.01–3.37]). Aldosterone levels correlated with higher homeostatic model assessment of insulin resistance and resistin and lower high-density lipoprotein only in minor allele carriers. CAV1 gene expression quantitative trait loci data revealed lower cav-1 expression in adipose tissues by the rs926198 minor allele.

Conclusions—Our findings in mice and humans suggested that decreased cav-1 expression may activate the effect of aldosterone/MR signaling on several pathways of glycemia, dyslipidemia, and resistin. In contrast, hyperinsulinemia and hypertriglyceridemia are likely mediated by MR-independent mechanisms. Future human studies will elucidate the clinical relevance of MR blockade in patients with genotype-mediated cav-1 deficiency. (J Am Heart Assoc. 2016;5:e003845 doi: 10.1161/JAHA.116.003845)

Key Words: aldosterone • caveolin 1 • dyslipidemia • eplerenone • insulin resistance • mineralocorticoid receptor

Recent studies have demonstrated the important role of aldosterone and mineralocorticoid receptor (MR) activation in modulating cardiometabolic risk factors associated with increased mortality. Inappropriate aldosterone signaling has been associated not only with hypertension but also with impaired glucose metabolism, insulin resistance (IR), and dyslipidemia, especially with a high-sodium (HS) diet. Interestsingly, participants with primary aldosteronism show higher prevalence of diabetes mellitus compared with participants with essential hypertension, despite similar body mass...
Aldosterone Signaling and Caveolin 1  
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index.3 Recent studies have shown, in both humans and rodents, that MR blockade may also reduce hyperglycemia, IR, and mortality.4–7 The proposed mechanisms for the aldosterone-induced abnormalities include adipocyte dysfunction, increased hepatic gluconeogenesis, overproduction of reactive oxygen species, and classic target-organ damage.8–10 It is unclear, however, whether these effects are mediated by genomic or nongenomic MR effects and/or are secondary to alternative crosstalk among aldosterone, plasma membrane proteins, and other receptors.

We previously published findings that caveolin 1 (cav-1) gene variants were associated with IR, dyslipidemia, diabetes mellitus, and metabolic syndrome in two human hypertensive cohorts.11,12 In addition, we and others have shown that cav-1 knockout (KO) mice display several metabolic abnormalities, including hyperglycemia, IR, and dyslipidemia, although they are not obese.13–15 Cav-1 is an important component of the plasma membrane and interacts with multiple signaling pathways, including steroid receptors. We described how cav-1 and MR colocalize and their levels are increased during sodium loading in the heart and the kidney.16,17 Furthermore, we demonstrated that the profound vascular dysfunction observed in the cav-1 KO mice is corrected by MR blockade, suggesting that cav-1 modulates MR function in cardiovascular tissues.16,19 The interplay between cav-1- and MR-mediated mechanisms in other tissues such as the liver and adipose tissue has yet to be determined.

In this study, we tested the hypothesis that cav-1 is a mediator of MR mechanism of action in metabolically active tissues. We characterized cav-1 KO mice and assessed potential downstream mechanisms and further assessed their dependence on MR-mediated transcription following in vivo MR blockade. In addition, in a translational approach, we explored the potential interplay between aldosterone levels and a selected human CAV1 gene variant in modulating IR and dyslipidemia.

Methods

Animal Protocol

Animals

The institutional animal care and use committee at Harvard Medical School approved our studies. We evaluated 16-week-old cav-1 KO and genetically matched wild-type (WT) male mice (stock numbers 004585 and 101045, respectively) from the Jackson Laboratory (Bar Harbor, ME). Animals were housed in a room lighted 12 hours per day at an ambient temperature of 22±1°C and had free access to Purina Lab Chow 5053 (Purina TestDiet) and tap water. All investigators performing the animal procedures were blinded to genotype and treatment.

Study 1 (phenotypic study)

WT mice (n=16) and cav-1 KO mice (n=16) were placed on a HS diet (1.6% Na+) for 11 days to achieve sodium balance, as described previously.18

Study 2 (intervention study)

After acclimation, WT (n=8) and cav-1 KO (n=16) animals were maintained on a HS diet for 14 days. Cav-1 KO mice were then randomized to two treatment groups: placebo (n=8) or eplerenone (100 mg/kg per day) delivered in the HS food (n=8).

Blood and tissue preparation

Animals fasted overnight, and the next morning, body weights were recorded, blood samples were collected via the tail vein, and mice were euthanized under deep isoflurane anesthesia. Retroperitoneal and visceral adipose and liver tissues were excised and immediately placed in liquid nitrogen.18,20

Assays

Blood glucose and serum insulin levels were determined, as described previously,11 using a Freestyle glucometer (Abbott Laboratories) and the Mouse Ultrasensitive Insulin ELISA kit (ALPCO Diagnostics), respectively. Lipid profile was assessed by an enzymatic colorimetric test (Roche Integra). Homeostasis model assessment of IR (HOMA-IR) was calculated, as described previously, and validated in rodents.21 Resistin levels were measured using an enzyme-linked immunosorbent assay (Millipore), with intra- and interassay coefficients of variation of 6.2% and 9.1%, respectively.

Transcript analysis

Quantitative reverse transcriptase polymerase chain reaction was performed. Briefly, total RNA was extracted, and cDNA was synthesized from 1.5 μg RNA with a cDNA kit (GE Healthcare). Polymerase chain reaction amplifications were performed in duplicate using TaqMan gene expression assays with the ABI Prism 7000 (Applied Biosystems). Reactions were analyzed using the ΔΔCt method and normalized to 18S ribosomal RNA levels. Data are presented as fold increase relative to WT.

Glucose-6-phosphate dehydrogenase measurements

Liver glucose-6-phosphate dehydrogenase (G6PD) protein levels were assessed by Western blotting.22 G6PD enzymatic activity in liver tissues was determined by using a plate-reader spectrophotometer (ThermoMax Microplate Reader; Molecular Devices) by measuring absorbance at 340 nm (conversion
of NADP\(^+\) to NADPH). Activity results were standardized to protein concentration.\(^{22}\)

### Human Protocol

The HyperPATH protocol is derived from a multicenter consortium that evaluates hormonal response to a strictly controlled sodium diet intervention. This protocol also includes antihypertensive medication washout and collection of blood samples in an inpatient setting.\(^{11}\) All participants were studied with the same protocol at Brigham and Women’s Hospital (Boston, MA), University of Utah (Salt Lake City, UT), Hôpital Broussais (Paris, France), and San Salvatore Hospital (Rome, Italy). Informed consent was obtained before participant enrollment at all sites. Although other results from HyperPATH have been reported, the present analyses are original.

IR estimation was calculated by the HOMA-IR index; lower high-density lipoprotein (HDL) was defined using World Health Organization criteria,\(^{23}\) and race was obtained by participant self-report, including only white participants. The participants were provided with a HS diet (200 mEq Na\(^+\) per day) for 7 days and then placed on a low-sodium (LS) diet (20 mEq/day) for 7 days. Optimal sodium balance was considered if 24-hour urinary sodium was >150 mEq per 24 hours on HS and <40 mEq per 24 hours on LS.

The measurement of aldosterone was performed for both HS and LS diets to calculate the sodium-modulated aldosterone suppression-to-stimulation index, which associates strongly with individual cardiometabolic risk factors because it reflects abnormal aldosterone physiology and impaired feedback, as published by our group.\(^{23}\) Plasma levels of resistin were measured in a subset of 149 participants by enzyme-linked immunosorbent assay (R&D Systems).

### Genotyping Analysis

The selected polymorphism (rs926198) was recently described as associated with metabolic syndrome\(^{12}\) and was genotyped using a Sequenom platform. Allele and genotype frequencies were in Hardy-Weinberg equilibrium. The selected variant had a completion rate >95%.

We evaluated expression quantitative trait loci using GENEVAR (GENe Expression VARiation), a public database that provides precise gene expression variation. We assessed cav-1 expression levels by rs926198 in adipose tissues from the MuTHER project, a healthy twin study in a white population.\(^{24}\)

### Statistical Analyses

#### Mouse studies

Data are presented as mean±SE. In study 1, the Student \(t\) test for unpaired data was used to compare groups. In study 2, comparisons among 3 groups were made with 1-way ANOVA followed by the Tukey post hoc test using GraphPad Prism 6 (GraphPad Software).

#### Human studies

Baseline analysis comparing by genotype status was performed by unpaired Student \(t\) test for continuous variables and by chi-square for binary variables. A multiple linear model regression analysis was performed to compare continuous outcomes by genotype groups adjusted for age, body mass index, sex, and site of the study. These covariates were chosen for their clinical relevance and were based on univariate analyses. A similar procedure was performed for multiple logistic regression analysis for binary outcomes. Bootstrapping with 1000 iterations was performed if the dependent variable was not normally distributed (ie, resistin). All analyses were performed using Stata 13 (StataCorp).

In all studies, differences were considered statistically significant with a two-tailed \(P<0.05\).

### Results

#### Animal Studies

**Cav-1 deficiency is associated with impaired glucose homeostasis and dyslipidemia**

As reported previously, cav-1 KO mice were leaner than the WT animals, suggesting that their metabolic phenotype was not related to increased adiposity (Table 1).\(^{13}\) Furthermore, fasting glucose levels in cav-1 KO mice were increased by 25% (\(P<0.01\)), whereas insulin and HOMA-IR levels were ≈3-fold higher (\(P<0.001\)) in cav-1 KO compared with WT animals (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>WT ((n=16))</th>
<th>Cav-1 KO ((n=16))</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
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<td>Weight, g</td>
<td>32.2±0.80</td>
<td>28.1±0.83</td>
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<tr>
<td>Glucose, mg/dL</td>
<td>66.1±2.99</td>
<td>83.8±4.78</td>
<td>0.002</td>
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<td>Insulin, (\mu)U/mL</td>
<td>2.99±1.40</td>
<td>7.43±3.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR(^*)</td>
<td>0.48±0.04</td>
<td>1.47±0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>123.5±7.25</td>
<td>180.1±7.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL/LDL ratio</td>
<td>26.9:1</td>
<td>12:1:1</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>95.5±7.25</td>
<td>149.4±16.49</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum resistin, ng/mL</td>
<td>3.4±0.43</td>
<td>6.22±0.47</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^*\)Insulin resistance was determined by homeostatic model assessment.
Cav-1 KO animals on a HS diet displayed dyslipidemia, as shown by higher triglycerides \( (P=0.01) \), total cholesterol, and non-HDL cholesterol (both \( P<0.001 \)), compared with WT mice (Table 1). Because LDL and HDL proportions are different in rodents than in humans, we calculated the HDL/LDL cholesterol ratios and observed that cav-1 KO mice had lower HDL/LDL ratios than WT mice \( (P<0.001) \). These findings confirm and expand previous preliminary findings in animals studied on a normal-sodium diet.11

**Potential pathways altered in the cav-1 KO abnormal metabolic phenotype**

We explored potential downstream pathways in both liver and adipose tissues that could be related to the hyperglycemia, IR, and dyslipidemia observed in the cav-1 KO mice. We initially focused on two well-described markers of IR, inflammation, and dyslipidemia: resistin and retinol binding protein 4 (RBP4).25,26 Serum resistin levels were higher in cav-1 KO mice compared with WT mice (Table 1). Consistently, cav-1 KO animals also had a significant 2- to 3-fold increase in resistin mRNA in both adipose and liver tissues compared with WT controls (Table S1). In addition, we observed that cav-1 KO mice had higher RBP4 mRNA levels in adipose and liver tissues compared with WT mice (Table S1).

We next assessed two factors related to adipose oxidative stress. First, we assessed the mRNA levels of NADPH oxidase 4 (NOX4), an enzyme that is upregulated in animal models of IR and diabetes mellitus27 and that is implicated in aldosterone-mediated reactive oxygen species expression.28 The cav-1 KO mice had significantly higher NOX4 transcript levels in adipose tissues. Second, we determined mRNA levels for aldose reductase (AldoR), an enzyme activated in states of hyperglycemia that has a key role in alternative glucose utilization via the polyol pathway.29 Of note, AldoR mRNA levels in adipose tissue were significantly increased in cav-1 KO compared with WT mice. Interestingly, the transcript levels for both enzymes were similar in liver tissues of cav-1 KO and WT mice (Table S1), consistent with an adipose-specific transcriptional effect of cav-1 deficiency. We then analyzed the levels of G6PD, an enzyme that is associated with diabetic phenotypes30,31 and that has been shown to be modulated by MR activation.32 Compared with WT animals, cav-1 KO mice had significantly higher G6PD mRNA levels in both tissues analyzed, especially in the liver (Table S1). Other factors related to aldosterone/MR activation and adipocyte dysregulation, such as peroxisome proliferator activated receptor \( \gamma \), monocyte chemoattractant protein 1, tumor necrosis factor \( \alpha \), and adiponectin, did not differ in their adipose mRNA levels comparing cav-1 KO and WT (data not shown).

**Effects of eplerenone treatment on the cav-1 KO abnormal phenotype**

The animal data suggested an interplay between cav-1 and aldosterone signaling; therefore, we explored the effect of a two-week treatment with a specific MR antagonist.
Eplerenone significantly decreased glucose levels in cav-1 KO mice by 30% ($P<0.01$) to levels similar to those observed in the control group (Figure 1A); however, insulin levels in cav-1 KO mice were not affected by eplerenone (Figure 1B), and HOMA-IR values did not reach statistical significance (Figure 1C).

Eplerenone treatment in cav-1 KO mice lowered total cholesterol by 20% ($P<0.05$) but did not lower the HDL/LDL ratio (Figure 2). Eplerenone did not modify the abnormal triglyceride profile in the cav-1 KO model, suggesting that MR activation does not play a role in their modulation.

We next explored the effect of MR blockade on resistin and RBP4 expression. Interestingly, eplerenone decreased circulating resistin levels and transcripts in the liver (both $P<0.01$) but did not reduce levels significantly in adipose tissue (Figure 3). In both adipose and liver tissues from cav-1 KO animals treated with eplerenone, RBP4 mRNA levels dropped significantly to levels similar to those observed in WT animals (Figure 4A and 4B). In addition, AldoR and NOX4 transcript levels were significantly decreased by eplerenone treatment in cav-1 KO adipose tissue to levels similar to those observed in WT mice (Figure 4C and 4D).

Consistent with our transcript results, the liver G6PD activity was significantly increased in the cav-1 KO versus WT animals. Furthermore, treatment with eplerenone significantly increased G6PD activity levels in the cav-1 KO model, consistent with the known effect of MR blockade on G6PD activity\(^22\) (Figure 4E). Moreover, G6PD protein levels paralleled the activity results, with significantly higher G6PD levels in the cav-1 KO mice and even higher levels in eplerenone-treated animals (Figure 4F).

**Human Study**

**Study population**

To assess the relevance of cav-1 KO mouse model findings to humans, a total of 556 white adults were analyzed. Our cohort had the following characteristics: age 45.8±10 years (range 18–70 years), body mass index (kg/m\(^2\)) of 27.2±4.2, and 43% female. Using a dominant model for genetic analysis, a homozygous major allele (TT) of the rs926198 CAV1 variant was observed in 42% of participants, and 58% were minor allele carriers (46% had a CT genotype and 12% had a CC genotype).

**Clinical and biochemical characteristics categorized by CAV1 genotype**

Compared with those with the homozygous major allele of rs926198 CAV1 variant, minor allele carriers had no statistical differences in body mass index, HS-related systolic blood pressure, or sex, as described in Table 2.\(^{23}\) Minor allele carriers were slightly older and had higher HOMA-IR values and tended to have higher glycemia and lower HDL levels. Interestingly, the sodium-modulated aldosterone suppression-to-stimulation index, reflecting abnormal aldosterone signaling,\(^{23}\) was consistently higher in the minor allele carrier group (Table 2). In a multiple regression model, sodium-modulated
aldosterone suppression-to-stimulation index levels were significantly higher in CAV1 minor allele carriers compared with noncarriers, even when adjusted by age, sex, body mass index, and site of study (β=0.06, P=0.04). Moreover, in adjusted logistic regression, minor allele carriers had higher odds of having IR (odds ratio 2.36 [95% CI 1.50–3.69]) and low HDL (odds ratio 1.54 [95% CI 1.01–3.37]), consistent with our previous reports on normal sodium diet.\textsuperscript{11,12} There were no differences by genotype in aldosterone or cortisol (Table 2) or in resistin, adiponectin, or 24-hour urinary sodium (data not shown).

**The cav-1 variant modulates the effect of aldosterone on glucose homeostasis and lipid profile**

In agreement with previous studies,\textsuperscript{33} aldosterone levels on a HS diet were associated with higher HOMA-IR values in our cohort (β=0.05, P=0.031), even when adjusted for potential confounders (β=0.06, P=0.009). Interestingly, the association of aldosterone with HOMA-IR was mainly driven by significant changes in glucose (adjusted β=1.13, P<0.001) rather than by insulin (adjusted β=0.05, P=0.459). Because both rs926198 genotype and aldosterone levels correlated with HOMA-IR values, we assessed whether they were independent predictors in an adjusted linear regression model and whether there was an interaction between them. Both rs926198 status and aldosterone levels were significant predictors of HOMA-IR, with a borderline statistical interaction. To further analyze the relationship between these predictors, we stratified the effect of aldosterone on HOMA-IR by genotype. Interestingly, aldosterone levels predicted HOMA-IR only in minor allele carriers in unadjusted models and, notably, in adjusted models, as noted in Table 3.

In addition, lower HDL levels were associated with aldosterone levels in adjusted models in all participants, but again, these results were driven by the effect on minor allele carriers of the CAV1 gene variant (Table 3). There was a consistent significant interaction when analyzing aldosterone levels with rs926198 status predicting HDL levels (P=0.043 for interaction).

We assessed whether changes in aldosterone predicted changes in resistin levels. Indeed, aldosterone predicted higher resistin levels in an adjusted model in CAV1 minor allele carriers but not in major allele homozygotes (Table 3). In contrast, aldosterone levels were not associated with triglycerides, consistent with our cav-1 KO data. Because aldosterone levels (MR ligand) were similar in both CAV1 genotype groups, the differential effect of aldosterone on HOMA-IR, HDL, and resistin levels in minor allele carriers may be attributed to an MR signaling defect predicted by CAV1 genotype status.

**Expression profile by CAV1 genotype**

To determine whether this parallelism between data in humans and cav-1–deficient animals could be explained by lower cav-1 expression in humans, we analyzed available expression quantitative trait loci data for cav-1 expression in human adipose tissues (GENEVAR).\textsuperscript{24} Indeed, cav-1 expression was significantly different by rs926198 genotype (p=0.39, P=10\textsuperscript{-04}), with the lowest expression in minor allele carriers.
homozygotes and identical expression in monozygous twins (Figure S1). These results suggest that the difference in expression likely is not secondary to environmental effects.

Discussion

Our results support the hypothesis that cav-1 is an important mediator of aldosterone’s mechanism of action. In the present study, we provided evidence—in both mice and humans—that impaired aldosterone signaling mediates some of the metabolic characteristics associated with reduced cav-1 levels. MR blockade in cav-1−deficient mice corrected fasting glucose, total cholesterol, and resistin levels but had no effect on insulin and triglyceride levels. In addition, we explored downstream mechanisms in adipose and liver tissues and showed that MR activation in cav-1 KO mice is responsible for the increased transcript levels for resistin, RBP4, and enzymes implicated in the intracellular redox

Figure 4. A two-week treatment with Epl in cav-1 KO mice (gray bars) significantly decreased RBP4 mRNA in liver and fat (A and B) compared with cav-1 KO mice (black bars) to levels observed in control wild-type mice (white bars). In addition, Epl treatment in cav-1 KO mice (gray bars) significantly decreased NOX4 mRNA in fat (C) and aldose reductase mRNA in fat (D) compared with cav-1 KO mice (black bars). Finally, Epl increased both G6PD activity (E) and protein expression (F, top: densitometry analysis; bottom: representative immunoblot) in cav-1 KO mice (gray bars) compared with cav-1 KO mice (black bars). n=4 to 8 per group. Cav-1 indicates caveolin 1; Epl, eplerenone; G6PD, glucose-6-phosphate dehydrogenase; KO, knockout; NOX4, NADPH oxidase 4; ns, not significant; RBP4, retinol binding protein 4.

DOI: 10.1161/JAHA.116.003845
Aldosterone Signaling and Caveolin 1 leading to increased nuclear receptor activity.34 Genomic and rapid MR actions initiated in caveolae and aldosterone levels have direct adverse effects via both to be more complex than initially thought. Inappropriate expression. These results in humans suggest that the gene variant that is associated with lower cav-1 CAV1 HOMA-IR, HDL, and resistin levels in carriers of a prevalent aldosterone suppression-to-stimulation index.

Although aldosterone signaling was proven to be more complex than initially thought. Inappropriate aldosterone levels have direct adverse effects via both genomic and rapid MR actions initiated in caveolae and leading to increased nuclear receptor activity.34–36 Dysfunctional aldosterone signaling in humans has been proposed recently as a potential mechanism for IR and dyslipidemia. This concept is supported by an association of serum aldosterone/MR activation with higher glycemia and lower HDL in participants both with and without primary hyperaldosteronism.3,37,38 Furthermore, HDL maintains the proper lipid environment of caveolae,39 thereby modulating the function of cav-1 and of the various enzymes and receptors in this microenvironment, including those involved in redox and metabolic signaling. In addition, high glucose levels may downregulate caveolae number via NOX-mediated mechanisms.40 Consequently, we hypothesized that the phenotype observed in cav-1 KO mice and human minor allele carriers may be mediated, at least in part, by dysfunctional MR signaling. Further support for the concept that the defect lies in the receptor and not with its ligand is based on the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minor Allele Carrier (n=323)</th>
<th>Major Allele Carrier (n=233)</th>
<th>P Value</th>
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<tbody>
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<td>Sex (female)</td>
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<td>92/233 (40%)</td>
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<td>Age, y</td>
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<td>Glycemia, mg/dL</td>
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<td>2.11±1.4</td>
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<td>Triglycerides, mg/dL</td>
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<td>Aldosterone, ng/dL</td>
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<td>0.41±0.35</td>
<td>0.33±0.27</td>
<td>0.007*</td>
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BP indicates blood pressure; cav-1, Caveolin 1; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; SASSI, sodium-modulated aldosterone suppression-to-stimulation index.

In the past decade, the biology of MR signaling was proven to be more complex than initially thought. Inappropriate aldosterone levels have direct adverse effects via both genomic and rapid MR actions initiated in caveolae and leading to increased nuclear receptor activity.34–36 Dysfunctional aldosterone signaling in humans has been proposed recently as a potential mechanism for IR and dyslipidemia. This concept is supported by an association of serum aldosterone/MR activation with higher glycemia and lower HDL in participants both with and without primary hyperaldosteronism.3,37,38 Furthermore, HDL maintains the proper lipid environment of caveolae,39 thereby modulating the function of cav-1 and of the various enzymes and receptors in this microenvironment, including those involved in redox and metabolic signaling. In addition, high glucose levels may downregulate caveolae number via NOX-mediated mechanisms.40 Consequently, we hypothesized that the phenotype observed in cav-1 KO mice and human minor allele carriers may be mediated, at least in part, by dysfunctional MR signaling. Further support for the concept that the defect lies in the receptor and not with its ligand is based on the abnormal aldosterone feedback by sodium loading, as assessed by the sodium-modulated aldosterone suppression-to-stimulation index in our human cohort, which suggests impaired MR signaling.23 Consistently, aldosterone levels were similar in both carriers and noncarriers of the CAV1 risk allele.

The lipid profile in cav-1–deficient mice shows higher total cholesterol and a lower HDL/LDL ratio compared with the WT mice. Recent evidence supports the importance of the CAV1 gene in human glucose homeostasis; however, the role of cav-1 in human dyslipidemia is poorly understood.13,41–43 Interestingly, a very similar phenotype was observed between our animal model and the one predicted by aldosterone levels in human minor allele carriers. The parallelism between our findings in mice and humans are consistent with the rs926198 minor allele being associated with lower cav-1

<table>
<thead>
<tr>
<th>Cardiometabolic Variable</th>
<th>Category*</th>
<th>Adjusted β (95% CI), P Value</th>
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<tbody>
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<td>HOMA-IR</td>
<td>All participants</td>
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<tr>
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<td>Minor allele carriers</td>
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<tr>
<td></td>
<td>Major allele homozygous</td>
<td>0.04 (–0.03 to 0.11),</td>
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<tr>
<td>HDL cholesterol, mg/dL</td>
<td>All participants</td>
<td>−0.37 (–0.72 to –0.01),</td>
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<td></td>
<td>Minor allele carriers</td>
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<td></td>
<td>Major allele homozygous</td>
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<td>Major allele homozygous</td>
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<td>Resistin, μg/L</td>
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<td>Minor allele carriers</td>
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<td></td>
<td>Major allele homozygous</td>
<td>0.05 (–0.06 to 0.17),</td>
</tr>
</tbody>
</table>

HOMA-IR indicates homeostasis model assessment of insulin resistance.

*Categorized by caveolin 1 genotype rs926198.

†Linear regression adjusted by age, sex, body mass index, and site of study.

‡P<0.05.
expression levels, as described in our exploratory analysis of available human expression quantitative trait loci data.

To support a role for cav-1 in modulating aldosterone-mediated pathways of glucose homeostasis, lipid profile, and resistin, we performed an intervention with eplerenone in the cav-1 KO model. We observed that eplerenone decreased glucose levels but had no effect on insulin levels. The beneficial effects of MR antagonism on glycemia are consistent with increased MR-mediated pathways of glucose modulation in the absence of cav-1. Notably, recent reports propose a detrimental role for liver MR activation on gluconeogenesis and a beneficial effect of MR blockade on hepatic glucose metabolism enzymes.\textsuperscript{4,45} Interventions that reduce aldosterone levels or actions, either by genetic manipulations in mice or by therapeutic interventions in humans with primary aldosteronism, are also associated with lower glycemia, providing convincing evidence of the contribution of aldosterone/MR signaling to glucose modulation.\textsuperscript{4,46,47} Interestingly, the failure of an MR antagonist to correct insulin levels in the cav-1 KO model may be related to different MR pathways regulating glucose and insulin in this model and/or may suggest that some MR downstream pathways are normally inhibited by cav-1. Furthermore, we cannot rule out the possibility that the extreme phenotype of this animal, which includes severely impaired insulin receptor stabilization, mitochondrial dysfunction, and reduced metabolic flexibility, may affect the response to eplerenone.\textsuperscript{14,42} Likewise, treatment with metformin, currently the most commonly used insulin sensitizer, has failed to improve glucose profile in these animals, as we published recently.\textsuperscript{38}

The relationship of aldosterone and MR activation to cardiovascular risks such as IR and low HDL is very well documented.\textsuperscript{37,46,49} In this study, we showed that aldosterone levels significantly predicted changes in HOMA-IR and HDL levels only in minor allele carriers, even when controlling for potential confounders. Treatment with eplerenone in the cav-1 KO mice also decreased total cholesterol, consistent with our data in humans. In contrast, our human and animal data did not support a relation between triglycerides and aldosterone, suggesting that the effects of cav-1 deficiency on triglycerides are MR independent. Moreover, the novel effect of MR activation leading to lower HDL levels in minor allele carriers was not replicated in our animal model, possibly because of the described differences in lipid composition in mice versus humans.\textsuperscript{50}

We also reported new insights into differentially expressed pathways in cav-1 deficiency and MR signaling-related mechanisms when analyzing the response to eplerenone. Interestingly, we observed that cav-1 KO mice expressed higher RBP4 levels in both liver and fat tissues, and eplerenone decreased RBP4 to levels observed in the WT mice. This novel finding is in agreement with evidence that increased RBP4 levels are associated with IR in both human and rodents.\textsuperscript{51,52} One of the described mechanisms by which RBP4 induces IR relates to its direct effects on lowering glucose transporter type 4 levels, a feature that has also been ascribed to cav-1 KO mice.\textsuperscript{53} Because our findings were from measuring gene transcription, the relation of plasma RBP4 and CAV1 genotype in humans warrants future investigation.

Resistin has also been described recently as a biomarker of increased risk of diabetes mellitus, dyslipidemia, and cardiovascular disease.\textsuperscript{25} In the setting of an extended intervention in the presence of a HS diet, we observed that plasma resistin levels and transcripts were increased in the cav-1 KO versus WT mice and were decreased by treatment with eplerenone. This effect is consistent with a recent report in which long-term losartan decreased resistin levels.\textsuperscript{54} Because resistin may modulate glucose and lipids, we cannot rule out the possibility that some metabolic improvement by eplerenone could be mediated indirectly by resistin.\textsuperscript{55} Our data in humans demonstrated that aldosterone levels are positively correlated with circulating resistin levels; these results are consistent with a recent report linking aldosterone and resistin levels in participants with primary aldosteronism.\textsuperscript{25,56} Interestingly, when analyzed by genotype, the relationship between aldosterone and resistin remained significant only in minor allele carriers of the CAV1 gene variant. To date, there are no reports of MR blockade and improvement of resistin levels in humans or any relationship to cav-1 genotype, so this novel finding warrants further confirmation.

Cav-1 actively interplays with two well-known receptors that increase oxidative stress: the angiotensin II receptor and MR.\textsuperscript{36} Consequently, we assessed NOX4 and AldoR, two enzymes related to oxidative stress and NAPDH activation. We observed that cav-1 deficiency is associated with a specific adipose oxidation dysfunction that is reversed by eplerenone to levels observed in the WT mice.\textsuperscript{57,58} Our results are consistent with reports showing that MR blockade decreased NOX4 expression and lowered reactive oxygen species in kidney and heart.\textsuperscript{59,60} To our knowledge, this report is the first showing that AldoR levels are increased in cav-1 KO mice and that eplerenone decreases adipose AldoR transcription. Actually, AldoR inhibitors are used in the treatment of diabetes mellitus and delay the onset of cardiovascular complications\textsuperscript{56,61} while attenuating angiotensin II generation and angiotensin II receptor stimulation.\textsuperscript{62} Nevertheless, given the known crosstalk among cav-1, angiotensin II receptor, and MR,\textsuperscript{37} the increased oxidative stress in the cav-1 KO mice cannot be definitively attributed to overactivation of MR alone. Future studies are needed to unravel this question.

Finally, we explored the expression of G6PD in our cav-1 animal model, given the well-described effects on maintaining
the intracellular redox equilibrium and MR signaling. Our group previously described how aldosterone/MR activation induces a G6PD-deficient state that promotes redox imbalance and vascular dysfunction. Interestingly, G6PD activity in cav-1 deficiency was increased, despite suspected MR signaling dysfunction. Loss of cav-1 has been reported to evoke NOX-mediated oxidant stress and to mimic inflammation and hypoxia, a consequence of which may be aerobic glycolysis and induction of G6PD. This environment of high oxidative stress promoted by cav-1 deficiency may induce atypical behavior for MR function, such as aldosterone-independent transactivation of MR. Alternatively, in the absence of cav-1, the described effect of MR on G6PD levels and function may be overcome by positive regulators of G6PD, such as insulin or nitric oxide synthase, that are increased in the cav-1 KO group previously described how aldosterone/MR activation induces a G6PD-deficient state that promotes redox imbalance and vascular dysfunction. Loss of cav-1 has been reported to evoke NOX-mediated oxidant stress and to mimic inflammation and hypoxia, a consequence of which may be aerobic glycolysis and induction of G6PD. This environment of high oxidative stress promoted by cav-1 deficiency may induce atypical behavior for MR function, such as aldosterone-independent transactivation of MR. Alternatively, in the absence of cav-1, the described effect of MR on G6PD levels and function may be overcome by positive regulators of G6PD, such as insulin or nitric oxide synthase, that are increased in the cav-1 KO model need further assessment in cell culture systems in which the crosstalk between aldosterone and insulin signaling can be further dissected at the molecular level and in future studies with MR blockade in cav-1 heterozygous mice. Furthermore, our human protocol lacks body composition measurements and thus cannot assess whether differences in visceral fat might explain, at least in part, the dissimilar metabolic status by cav-1 genotype. Finally, because the cav-1 variant was studied only in white participants with no long-term follow-up, our human data lack generalizability to other races.

In summary, we explored novel mechanisms such as increased resistin, RBP4, and higher oxidative stress enzymes that could be related to specific enhanced MR pathways associated with cav-1 deficiency. In addition, we corroborated impaired MR signaling by treating the cav-1–deficient mice with eplerenone, with positive effects on glycemia, cholesterol, and resistin. Furthermore, we described an interaction between a highly prevalent CAV1 gene variant and aldosterone levels that may modulate changes in glycemia, lipid profile, and resistin levels in humans. Finally, our data suggest that insulin levels and hypertriglyceridemia associated with cav-1 are not related to cav-1–mediated MR activation. Future preventive and therapeutic interventions targeting the cav-1/MR pathway may provide individualized treatment options for patients that carry this variant.

Acknowledgments

We gratefully recognize the support of the Clinical Research Centers.

Sources of Funding

This work was supported by grants from the NIH: HL104032 (Pojoga), HL103845 (Adler), DK107407 (Vaidya), K23HL111771 (Vaidya), HL105301 (Leopold), HL096518 (Romero), from the American Heart Association 14GRNT20500000 (Pojoga), from the Doris Duke Foundation Grant 2015085 (Vaidya) and from FONDECYT 1150437 (Baudrand), 1150327 (Baudrand), 1160836 (Baudrand), 1160695 (Baudrand) and CORFO 13CTI-21526-P1 (Baudrand).

Disclosures

None.

References


SUPPLEMENTAL MATERIAL

Table S1. Transcript expression profile for potential mechanisms in the cav-1 KO mice (values normalized to 18S mRNA)

<table>
<thead>
<tr>
<th>Variable</th>
<th>WT [n=16]</th>
<th>Cav-1 KO [n=16]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistin (fat)</td>
<td>1.00 ± 0.10</td>
<td>2.09 ± 0.244</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Resistin (liver)</td>
<td>1.00 ± 0.17</td>
<td>3.37 ± 0.92</td>
<td>0.008</td>
</tr>
<tr>
<td>RBP4 (fat)</td>
<td>1.00 ± 0.13</td>
<td>2.10 ± 0.41</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RBP4 (liver)</td>
<td>1.00 ± 0.07</td>
<td>1.74 ± 0.40</td>
<td>0.014</td>
</tr>
<tr>
<td>G6PD (fat)</td>
<td>1.00 ± 0.09</td>
<td>1.84 ± 0.38</td>
<td>0.008</td>
</tr>
<tr>
<td>G6PD (liver)</td>
<td>1.00 ± 0.10</td>
<td>2.45 ± 0.50</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NOX4 (fat)</td>
<td>1.00 ± 0.10</td>
<td>1.80 ± 0.33</td>
<td>0.008</td>
</tr>
<tr>
<td>NOX4 (liver)</td>
<td>1.00 ± 0.21</td>
<td>0.69 ± 0.31</td>
<td>0.395</td>
</tr>
<tr>
<td>AldoR (fat)</td>
<td>1.00 ± 0.09</td>
<td>1.38 ± 0.14</td>
<td>0.026</td>
</tr>
<tr>
<td>AldoR (liver)</td>
<td>1.00 ± 0.11</td>
<td>0.75 ± 0.10</td>
<td>0.149</td>
</tr>
</tbody>
</table>
Figure S1. Adipose tissue cav-1 expression by rs926198 genotype (Genevar database). The minor allele C has a dose-dependent, decreasing effect on cav-1 transcript levels in tissue from the MuTHER Pilot study, p<0.001.

Log2 expression is shown on the y axis.

Rho = -0.39, p < 0.001