Activation of the Renin-Angiotensin System Mediates the Effects of Dietary Salt Intake on Atherogenesis in the Apolipoprotein E Knockout Mouse

Chris Tikellis, Raelene J. Pickering, Despina Tsorotes, Olivier Huet, Jaye Chin-Dusting, Mark E. Cooper, Merlin C. Thomas

Abstract—Dietary salt intake is a major determinant of the activation state of renin-angiotensin-aldosterone system. Given the important role of the renin-angiotensin-aldosterone system in plaque accumulation, we investigated its role in the development of atherogenesis associated with sodium intake in apolipoprotein E knockout mice. Six-weeks of a low-salt diet (containing 0.03% sodium) resulted in a 4-fold increase in plaque accumulation in apolipoprotein E knockout mice when compared with mice receiving normal chow (containing 0.30% sodium). This was associated with activation of the renin-angiotensin-aldosterone system, increased vascular expression of adhesion molecules and inflammatory cytokines, and increased adhesion of labeled leukocytes across the whole aorta on a dynamic flow assay. These changes were blocked with the angiotensin-converting enzyme inhibitor perindopril (2 mg/kg per day). A high-salt diet (containing 3% sodium) attenuated vascular inflammation and atherogenesis, associated with suppression of the renin-angiotensin-aldosterone system, although systolic blood pressure levels were modestly increased (5±1 mmHg). Constitutive activation of the renin-angiotensin-aldosterone system in angiotensin-converting enzyme 2 apolipoprotein E knockout mice was also associated with increased atherosclerosis and vascular adhesion, and this was attenuated by a high-salt diet associated with suppression of the renin-angiotensin-aldosterone system. By contrast, a low-salt diet failed to further activate the renin-angiotensin-aldosterone system or to increase atherosclerosis in angiotensin-converting enzyme 2 apolipoprotein E knockout mice. Together, these data validate a relationship between salt-mediated renin-angiotensin-aldosterone system activation and atherogenesis, which may partly explain the inconclusive or paradoxical findings of recent observational studies, despite clear effects on blood pressure. (Hypertension. 2012;60:98-105.)

Key Words: aldosterone ■ angiotensin ■ atherosclerosis ■ inflammation ■ renin angiotensin system ■ salt ■ sodium

Hypertension is an important modifiable risk factor for cardiovascular disease (CVD). Nutritional guidelines advocate that adults should restrict their dietary intake of salt to <100 mmol/d to reduce blood pressure and the consequent risk of CVD. This logic has been used to project that lowering the amount of salt taken by Americans each day by 3 g would reduce the annual number of new cases of coronary heart disease by >60,000. Although such logic is compelling, the actions of salt on vascular physiology are potentially more complicated, contextual, and probably extend beyond effects on blood pressure. For example, even modest salt restriction is associated with failure, and treated hypertension in young adults and most recently in patients at high risk of CVD. These observational studies have been criticized for failing to adjust for unmeasured influences. Because of potential confounding, experimental studies must be performed to test the hypothesis that sodium intake has pleiotropic effects that may counterbalance its actions on blood pressure. In this study, we specifically examined the role of the RAAS in determining the effects on plaque accumulation arising from modifying the dietary intake of salt in the apolipoprotein E knockout (apoE KO) mouse. In addition, we examined the effect of dietary salt in the angiotensin-converting enzyme 2 (Ace2) apoE KO mouse, a model associated with a constitutively active RAAS.
Materials and Methods

Animal Models
Apoe KO mice and Ace2/ApoE KO mice bred on a c57b/6J background were sourced and generated in house, as described previously. All strains are inbred and litter mates assigned to different study groups. In these studies, male mice aged 10-weeks and weighing between 20 to 25 g were allocated to receive an isocaloric diet with low-salt content (0.03%), normal salt content (0.3%) or a high-salt content (3%) containing 6% fat (Specialty Feeds, Perth, Australia). Apoe KO on a low-salt diet were further randomized to receive treatment with the angiotensin-converting enzyme (ACE) inhibitor perindopril (Servier, Neulilly, France) at a dose of 2 mg/kg per day in drinking water. Each group contained ≥20 animals.

After 6 weeks of study, all of the mice were placed individual metabolic cages (Iifa Credo, L’Arbresele, France) for 24 hours, and their weight, food, and water (sodium) intake and urine (sodium) output were documented. Systolic blood pressure was measured by tail-cuff plethysmography in conscious, prewarmed mice. Animals were then culled using an IP injection of Euthal (10 mg/kg; Delvet Limited, Seven Hills, New South Wales, Australia) followed by exsanguination via cardiac puncture. Total cholesterol and triglycerides were measured in fasting plasma samples using a COBAS INTEGRA 400 auto-analyzer (Roche Diagnostics, Indianapolis, IN). The sodium concentration was estimated in diluted urine on the same machine using an ion-sensitive electrode and the result adjusted for urinary output (in micromoles per day).

Plasma aldosterone was measured using a commercial radioimmunoassay (proSearch, Malvern, Victoria, Australia). Aortas were collected and placed in 10% neutral buffered formalin and quantitated for lesion area before being processed for subsequent immunohistochemical analysis or snap frozen and stored at −70°C for subsequent RNA extraction. All of the experiments were approved by the animal ethics committee of the Alfred Medical Research Precinct and conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (publication No. 85-23, revised 1996).

Plaque Area Quantitation
Plaque area was quantitated as described previously. In brief, aortas were removed from mice, cleaned of excess fat, and stained with Sudan IV-Hersheimer solution (0.5% w/v; Gurr, BDH Limited, Poole, United Kingdom). Aortas were then dissected longitudinally and pinned flat onto wax. Plaque accumulation across the aortic arch and total aortic surface was quantitated as the percentage of area stained red.

Quantitative Real-Time PCR
Gene expression of the adhesion molecules and proinflammatory cytokines were assessed in aortic and liver homogenates by quantitative real-time RT-PCR. This was performed using the TaqMan system based on real-time detection of accumulated fluorescence (ABI Prism 7700, Perkin-Elmer Inc, PE Biosystems, Foster City, California). The sodium concentration was estimated in diluted urine on the same machine using an ion-sensitive electrode and the result adjusted for urinary output (in micromoles per day).

Circulating Inflammatory Markers
To measure various adhesion molecules, serum-soluble vascular cell adhesion molecule (VCAM-1), soluble intercellular adhesion molecule (ICAM-1), macrophage chemotactic factor (MCP-1), and interleukin 6 (IL-6) were measured using commercial ELISA kits (Rand D Biosystems and Quantakine, respectively) performed according to the manufacturer’s instructions.

Dynamic Flow Adhesion Studies
To examine the earliest changes that contribute to atherogenesis in this model, apoe KO mice (6–8 weeks of age, n=6 per group) were randomized to receive a diet low in sodium (0.03% salt), normal chow (0.3% salt), or a high-salt diet (3.00%) for 1 week. At this time, animals were culled and aortas were isolated and mounted in a vessel chamber primed with Krebs buffer and maintained at physiological pH by infusing carbogen gas (95% O2; 5% CO2) through the buffer at 37°C, as described previously. As a positive control, vessels (n=4–5 per group) were pretreated with tumor necrosis factor α–α (10 ng/mL; 4 hours at 37°C) before being perfused through the aorta at 0.12 mL/min. Images of vessel wall-cell interactions were observed using a fluorescence microscope (Zeiss Discovery.V20) and analyzed with AxioVision software. Two to 3 frames were taken at each time point and the number of adherent cells per frame recorded.

Statistics
Continuous data are expressed as mean±SEM. Differences in the mean among groups were compared using 2-way ANOVA. Pairwise multiple comparisons were made with the Student-Newman-Keuls post hoc analysis to detect significant differences between groups. P<0.05 was considered statistically significant.

Results
Effect of Dietary Salt Intake on Metabolic, Blood Pressure, and Aldosterone Levels in Apoe KO Mice
Circulating lipids levels were elevated in all of the groups of apoe KO mice. Changes in the intake of dietary sodium had no effect on lipid or glucose levels, weight gain, or feeding behavior (Table). Systolic blood pressure was modestly elevated by a high-sodium diet (Table). Daily urinary sodium excretion was correlated with the dietary sodium content in apoe KO mice, with the lowest sodium excretion observed in mice on a low-salt diet containing 0.03% sodium and the highest excretion observed in mice on a high-salt diet containing 3.00% sodium. The intake of dietary sodium was also correlated with circulating aldosterone levels, a marker of systemic RAAS activation, such that circulating aldosterone levels were increased in mice on a low-salt diet and undetectable (<20 pg/mL) in mice on a high-salt diet when compared with those receiving normal chow (Table).

Effect of Low Dietary Salt Intake on Plaque Accumulation and Vascular Inflammation in Apoe KO Mice
Plaque accumulation, quantitated as a percentage area of the aorta stained red with Sudan IV, was significantly increased in apoe KO mice fed a low-salt (0.03% sodium) diet compared with apoe KO mice receiving normal chow containing 0.30% sodium (P<0.001; Figure 1). This change was particularly marked in the aortic arch, although it was significant across all of the aortic segments.

A low-salt diet was also associated with increased gene expression of a number of vascular adhesion molecules and inflammatory cytokines in the aortas of apoe KO mice, including tumor necrosis factor α, IL-6, MCP-1, VCAM-1, and the proinflammatory mediator junctional adhesion molecule A. In addition, expression of the leukocyte marker cluster of differentiation molecule 11b and the T-cell markers, cluster of differentiation molecule 3 and major histocompatibility factor class II were also increased, denoting the presence of vascular inflammation (Figure 2A). The expression of leukocyte markers was also increased in apoe KO mice, receiving a low-salt diet, consistent with the induction of vascular inflammation (Figure 2B). Tissue levels of
MCP-1, VCAM-1, and ICAM-1 protein were also increased in the aortas of apoE KO mice receiving a low-salt diet (Figure 2C), and circulating levels of soluble ICAM-1, VCAM-1, MCP-1, and IL-6 were also elevated (Figure 2D). In addition, the increase in circulating levels of IL-6 in apoE KO mice receiving a low-salt diet was associated with increased gene expression of IL-6 in the liver, the major source of circulating IL-6, compared with mice receiving normal chow (low-salt, 1.6±0.2; normal chow, 1.0±0.2; \( P<0.01 \)).

To further explore the early induction of vascular inflammation associated with a low-salt diet, aortas were taken from preatherosclerotic apoE KO mice exposed to 1 week or 6 weeks of a low-salt diet or normal chow and then subjected to dynamic flow adhesion studies. Consistent with the increased expression of adhesion molecules and subsequent plaque accumulation, mice fed a low-salt diet showed increased adhesion of labeled human leukocytes (Figure 3), comparable to that observed in the positive control after pretreatment of aortas with tumor necrosis factor-\( \alpha \) (10 ng/mL; 4 hours at 37°C).

Figure 1. Atherosclerotic plaque accumulation in apolipoprotein E (apoE) knockout (KO) mice. A, Representative Sudan IV staining in the aorta of mice fed a low-salt (LS), low-salt with perindopril (LS+P), normal chow (NS), or a high-salt (HS) diet. B and C, Quantitated percentage area stained red with Sudan IV across the total aorta (B) and aortic arch (C); \( n=8 \) per group. Data show mean±SEM; \( * \) vs normal chow apoE KO, \( P<0.01 \).
Effect of ACE Inhibition With Perindopril on Plaque Accumulation and Vascular Inflammation Associated With a Low-Salt Diet

To explore the potential role of the RAAS in increased atherogenesis observed in apoE KO mice fed a low-salt diet, these mice were further randomized to receive treatment with the ACE inhibitor perindopril. Treatment with the ACE inhibitor perindopril attenuated sodium retention and reduced aldosterone levels in apoE KO mice fed a low-salt diet, consistent with its suppression of the RAAS. This treatment also prevented plaque accumulation associated with a low-salt diet (Figure 1). Treatment with perindopril also attenuated increases in inflammatory mediators in the aorta and in the circulation associated with a low-salt diet in apoE KO mice (Figure 2). In addition, treatment with perindopril also prevented any increase in dynamic leukocyte adhesion observed in apoE KO mice receiving a low-salt diet (Figure 3).

Effect of High Dietary Salt Intake in ApoE KO Mice

Consistent with suppression of the RAAS (Table), intake of a diet high in sodium (3%) for 6 weeks resulted in a reduction in plaque accumulation in apoE KO mice when compared with apoE KO mice on normal chow (Figure 1). Although significant, the effect was small, because apoE KO mice on normal chow have accumulated only limited plaque by 14 weeks of age. Nonetheless, a high-salt intake was also associated with reduced gene expression of proinflammatory cytokines and adhesion molecules in the aorta when compared with apoE KO mice receiving normal chow (Figure 2A). In addition, the tissue and circulating levels of MCP-1 and circulating IL-6 were also reduced in mice fed a high-salt diet, similar in magnitude to that observed in mice receiving the ACE inhibitor perindopril (Figure 2B and 2C).

Effect of Dietary Salt in Ace2/ApoE KO Mice

We have shown previously that Ace2/apoE KO mice have a constitutionally active RAAS, with increased circulating and vascular levels of angiotensin II (Ang II), leading to a modest increase in systolic blood pressure and accelerated plaque accumulation when bred onto a susceptible mouse phenotype. In the present study, basal aldosterone levels were also elevated in Ace2/apoE KO mice on normal chow when compared with apoE KO mice (P<0.01), consistent with constitutional RAAS activation resulting from ACE2 deficiency. However, sodium excretion was significantly higher in Ace2/apoE KO mice fed a low-salt diet, when compared with apoE KO mice (P<0.01), consistent with reduced suppression of aldosterone after feeding with a low-salt diet in Ace2/apoE KO mice, when compared with apoE KO mice.

Figure 2. The expression of adhesion molecules, proinflammatory cytokines, and leukocyte markers in the aortas of apolipoprotein E (apoE) knockout (KO) mice fed a low-salt (LS; 0.03% sodium, □), low-salt with perindopril (P; □□), normal chow (NS; 0.30% sodium, □□), or high-salt diet (HS; 3.00% sodium, ■); n=8 per group. A, Expression profile of adhesion mediators and proinflammatory markers in the aortas and (B) the expression of leukocyte markers in the aortas, as measured by real-time RT-PCR. C, Protein expression of proinflammatory and adhesion molecules vascular cell adhesion molecule (VCAM-1), soluble intercellular adhesion molecule (iCAM-1), and macrophage chemotactic factor (MCP-1) as measured by ELISA. D, Concentration of circulating cytokines in the plasma; n=8 per group. Data show mean±SEM; *vs normal chow, P<0.01.

Tikellis et al Dietary Salt and Atherosclerosis 101
By contrast, a high-salt diet suppressed aldosterone to a significant extent in both Ace2/apoE KO mice and apoE KO mice (Table).

We have shown previously that RAAS blockade prevents atherosclerosis in Ace2/apoE KO mice. We have shown previously that RAAS blockade prevents atherosclerosis in Ace2/apoE KO mice.11 Consistent with this observation, in the present study a high-salt diet, which also suppressed the RAAS, also attenuated the induction of proinflammatory genes (Figure 4) and prevented plaque accumulation (Figure 5). By contrast, a low-salt diet did not further increase plaque accumulation beyond that observed in Ace2/apoE KO mice receiving normal chow (Figure 2), possibly because the activation state of the RAAS was not further increased by a low-salt diet (Table). In addition, the increase in the expression of VCAM-1, IL-6, and tumor necrosis factor-α observed in apoE KO mice on a low-salt diet was not observed in Ace2/apoE KO mice. However, the gene expressions of ICAM-1, MCP, and junctional adhesion molecule A were further induced by a low-salt diet when compared with Ace2/apoE KO mice animals receiving normal chow (Figure 4).

**Discussion**

Atherogenesis is a complex process in which a combination of pathogenic factors activate common molecular pathways that lead to the development of atherosclerotic plaques. Atherogenesis is a complex process in which a combination of pathogenic factors activate common molecular pathways that lead to the development of atherosclerotic plaques.15–17 One of the most important is activation of the RAAS.18,19 In this study, we showed that dietary salt intake, a key regulator of the circulating and local RAAS, modifies early plaque development in atherosclerosis-prone apoE KO mice (Figure 2). A low-salt diet increased atherogenesis and vascular inflammation in this model, in association with compensatory renal sodium retention and increased RAAS activity. Importantly, these changes could be attenuated after pharmacological RAAS blockade and were not observed in the absence of additional RAAS activation in the Ace2/apoE KO mouse. Together these data validate a relationship between salt-

**Figure 3.** A low-salt diet increases the dynamic adhesion of labeled human leukocytes to the aortas of apolipoprotein E (apoE) knock-out (KO) mice mounted in a vessel chamber at 37°C. Data show mice fed a low-salt (LS; 0.03% sodium, □ solid line), low-salt with perindopril (LS + P; ■ dashed line), normal chow (NS; 0.30% sodium, ▪), and aortas pretreated with tumor necrosis factor (TNFα; 10 ng/mL for 4 hours) as a positive control; n=6 to 8 per group. A, Quantitated leukocyte-binding per field. Data show mean±SEM; *P<0.05 vs normal chow. B, Representative image showing leukocyte binding to the aortic surface.

**Figure 4.** The gene expression profile expression of adhesion molecules and proinflammatory cytokines in the aortas of angiotensin-converting enzyme 2 (Ace2)/apolipoprotein E (apoE) knockout (KO) mice fed for 6 weeks a diet low in sodium (0.03%; □) vs normal chow (0.30% sodium; ▪) or a high-salt diet vs normal chow (3.00% sodium; ▪), as measured by real-time RT-PCR; n=8 per group. Expression is normalized to that observed in an apoE KO mouse on normal chow. Data show mean±SEM; *vs normal chow, P<0.01.
mediated RAAS activation and atherosclerosis. By contrast, a diet high in salt significantly reduced plaque accumulation in apoE KO mice and Ace2/apoE KO mice with an efficacy similar to that achieved after blockade of the RAAS with an ACE inhibitor.11

Although such data demonstrate the effects of dietary salt in early atherosclerosis, in no way should these findings be taken to imply that salt restriction is detrimental to human cardiovascular health or that a high-salt intake is beneficial. On the contrary, sodium intake is clearly correlated with blood pressure levels, both in our study (Table) and in humans.20 There are overwhelming data that hypertension is a key risk factor in the development and progression of CVD. However, there is also strong epidemiological and experimental evidence that RAAS activation is also a risk factor for CVD. Consequently, the net effects of dietary sodium on atherosclerosis are complicated and contextually. Many observational studies have examined the association between salt intake and the risk of CVD in humans.20 There are studies showing that high-salt intake is associated with poor cardiovascular outcomes21–25 and some have found no effect, whereas others have suggested that individuals with a low-salt intake have worse clinical outcomes.5,8,9 The possibility of a J-shaped relationship between sodium intake and cardiovascular outcomes has also emerged in patients with diabetes mellitus4 and more recently in patients at high risk for CVD.10 This is despite trials clearly showing that salt restriction lowers blood pressure.20 This inconsistency forms the basis of the so-called salt wars in public health. We would argue that there is potentially a middle ground, in which positive and negative effects of sodium intake on vascular physiology both exist and may be observed to a greater or lesser extent in specific contexts. For example, we have shown here that a low-salt diet accelerates atherosclerosis in apoE KO mice, the same model in which blood pressure lowering with anldopine fails to prevent plaque accumulation.27 However, if the activation state of the RAAS is “fixed-on,” as in the Ace2/apoE KO mouse, or “fixed-off,” as in mice receiving RAAS blockade, then any pleiotropic effects of a low-salt diet on atherogenesis are not observed. Consistent with this hypothesis, a high-salt diet combined with Ang II infusion to “fix” the RAAS has been reported previously to accelerate atherosclerosis in the apoE KO mouse,28 although a high-salt diet appears to suppress atherogenesis when RAAS activity is suppressible (Figure 1).

In the vasculature, ACE2 is the major enzyme that metabolizes Ang II. We have shown previously that deficiency of ACE2 results in constitutive RAAS activation is associated with increased vascular inflammation and accelerated atherosclerosis, in the absence of systemic hypertension.11 Notably, atherosclerosis in this model can be prevented by blockade of the RAAS with perindopril.11 In the present study, we showed that a high-salt diet is also able to prevent atherosclerosis in Ace2/apoE KO mice, consistent with its actions to suppress the RAAS in this model, although this strain was modestly more salt sensitive. However, a low-salt diet did not increase plaque accumulation in Ace2/apoE KO mice, beyond that observed in mice receiving normal chow. It is possible to speculate that this relates to the failure of a low-salt diet to further activate the RAAS beyond that observed in Ace2/apoE KO mice on normal chow that already have an overactive RAAS. However, even in the absence of RAAS activation, a low-salt diet was still able to increase the expression of some vascular markers of inflammation in Ace2/apoE KO mice, suggesting that a low-salt diet also activates other proatherogenic pathways (eg, the sympathetic nervous system).

Activation of the RAAS may contribute to atherogenesis in a number of different ways. Most research has focused on the pro-oxidant and proinflammatory actions of Ang II, which promotes monocyte and endothelial cell activation29 and ultimately plaque accumulation.30 Increasing levels of aldosterone associated with renin-angiotensin system activation may also have direct atherogenic effects in apoE KO mice.31 Finally, it is also possible that reduced levels of the antiatherosclerotic peptide angiotensin 1-7 contribute to the proinflammatory and atherogenic phenotypes associated with a low-sodium diet, as reported previously in ACE2 deficiency.11 Indeed, the effects of angiotensin 1-7 on Ang II–induced constriction are attenuated when mice are fed a low-salt diet.32 However, studies in healthy humans suggest that low-sodium intake elicits a rise in angiotensin 1-7 that parallels the rise in other components of the RAAS.33

The key limitation of this research is its reliance on the apoE KO mouse model. Because of very efficient lipoprotein metabolism, a proatherogenic phenotype is required for the development of any atherosclerotic plaque in mice. Although the apoE KO mouse is the most widely used experimental model for the study of atherosclerosis and the sequential events involved in initial fatty streak formation are similar to those in humans, it remains contentious to what extent this model fully reflects atherogenesis in a human context. Features of advanced human atherosclerosis, such as plaque rupture, thrombosis, and coronary lesions, are only infrequently observed in apoE KO mice. The model is also dominated by markedly elevated very–low-density lipoprotein cholesterol levels, similar to combined dyslipidemia.
found in humans, which can be genetic or acquired, usually as part of the metabolic syndrome or diabetes mellitus. Vascular lesions in this model are also clearly dependent on activation of the RAAS, because blockade of the type 1 angiotensin receptor antagonists or inhibition of ACE prevents plaque accumulation. Similarly, increased RAAS activation in apoe KO mice accelerates plaque formation, such as after an infusion of Ang II or in the Ace2/apoE KO mouse. Previous studies have suggested that a low-sodium intake may accelerate atherosclerosis in high-fat–fed apoE KO mice, although the actions of a high-salt diet are less clear. Notwithstanding these limitations, there appears to be sufficient commonality with early human disease, both histologically and in the response to risk factors and interventions, to suggest that our findings may also be clinically relevant.

It also possible that the genetic background of our mice has influenced the phenotypic response to dietary sodium. Mice bred on a c57BL/6j background are not especially salt sensitive, as demonstrated by the modest changes in blood pressure in response to changes in sodium intake (Table). It is possible to speculate that, had these experiments been conducted in a salt-sensitive context, then the adverse vascular effects of salt-induced hypertension may have offset its beneficial actions to inhibit RAAS activation. Equally, the impact of blood pressure reduction may have offset the proatherosclerotic actions of RAAS activation associated with a low-sodium diet. Nonetheless, by specifically performing our experiments in the absence of salt sensitivity, we have been able to uncover the off-target actions of dietary sodium. It is also worth noting the majority of humans are not salt sensitive (outside of elderly or black patients).

Another potential limitation of this work is the range of salt intake used in this study. Animals receiving a low-salt diet ate ∼10 times less salt that those on a normal diet, whereas those on a high-salt diet received ∼10 times more. By contrast, the difference between the fifth and 95th percentile of sodium intake in humans is only 7- to 10-fold. Nonetheless, the degree of RAAS activation observed with a low-salt diet was no more than that achieved by diabetes mellitus (data not shown). Similarly, on plasma aldosterone levels, the achieved degree of RAAS activation observed with a low-salt diet was comparable to that achieved when using an ACE inhibitor. Because the relationship between dietary salt intake and RAAS activation is continuous in both humans and mice, we speculate that lesser changes in salt intake would likely also influence chronic atherogenesis, albeit more slowly. Certainly, the same pathways that were modified by salt intake in our 6-week experiments are also clearly implicated in long-term human atherogenesis.

PERSPECTIVES

In the apoE KO mouse model of atherosclerosis, any reduction in dietary salt intake (from high to normal or low) is proatherogenic because of activation of the RAAS. In contrast, increasing salt in the diet suppresses RAAS-dependent atherogenesis. Although hypertension is a leading cause of atherosclerosis, the balance of benefits and risks that may arise out of encouraging adults to reduce their (usually high) salt intake is likely to be variable, contextual, and to extend beyond the known beneficial effects on blood pressure. We postulate that there may be some clinical settings in which neurohormonal activation in response to a reduction in sodium intake may lead to less favorable or even paradoxical cardiovascular outcomes, despite clear beneficial effects on blood pressure. Although this has been suggested previously in observational clinical studies, our study demonstrates that this hypothesis is physiologically plausible.

SOURCES OF FUNDING

M.C.T. is supported by a National Health and Medical Research Council Senior Research Fellowship. C.T. is supported by a Juvenile Diabetes Research Foundation Career Development Award. Research and infrastructure at the Baker IDI Heart & Diabetes Institute is supported by the Victorian State Government.

DISCLOSURES

None.

REFERENCES


**Novelty and Significance**

**What Is New?**
- Although reducing sodium intake improves blood pressure levels, it is associated with activation of the renin-angiotensin system and augmented atherogenesis in apoE KO mice.
- Although increasing sodium intake increases blood pressure levels, it is associated with suppression of the renin-angiotensin system and reduced atherogenesis in apoE KO mice.

**What Is Relevant?**
- Universal recommendations for sodium restriction are based on the effects of sodium on blood pressure.

**What Is New?**
- We demonstrate that there are other “off-target effects” of sodium restriction, which have the potential to offset the cardiovascular benefits achieved through blood pressure lowering.

**Summary**

Together these data validate a relationship between salt-mediated RAAS activation and atherogenesis, which may partly explain the inconclusive or paradoxical findings of recent observational studies despite clear effects on blood pressure.
Activation of the Renin-Angiotensin System Mediates the Effects of Dietary Salt Intake on Atherogenesis in the Apolipoprotein E Knockout Mouse

Chris Tikellis, Raelene J. Pickering, Despina Tsrortes, Olivier Huet, Jaye Chin-Dusting, Mark E. Cooper and Merlin C. Thomas

_Hypertension_. 2012;60:98-105; originally published online May 29, 2012; doi: 10.1161/HYPERTENSIONAHA.112.191767

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2012 American Heart Association, Inc. All rights reserved.

Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://hyper.ahajournals.org/content/60/1/98

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:

http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:

http://hyper.ahajournals.org//subscriptions/