Lysine-specific demethylase-1 modifies the age effect on blood pressure sensitivity to dietary salt intake

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Abstract How interactions of an individual's genetic background and environmental factors, such as dietary salt intake, result in age-associated blood pressure elevation is largely unknown. Lysine-specific demethylase-1 (LSD1) is a histone demethylase that mediates epigenetic regulation and modification of gene transcription. We have shown previously that hypertensive African-American minor allele carriers

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of the LSD1 single nucleotide polymorphism (rs587168) display blood pressure salt sensitivity. Our goal was to further examine the effects of LSD1 genotype variants on interactions between dietary salt intake, age, and blood pressure. We found that LSD1 single nucleotide polymorphism (rs7548692) predisposes to increasing salt sensitivity during aging in normotensive Caucasian subjects. Using a LSD1 heterozygous knockout mouse model, we compared blood pressure values on low (0.02 % Na⁺) vs. high (1.6 % Na⁺) salt intake. Our results demonstrate significantly increased blood pressure salt sensitivity in LSD1-deficient compared to wild-type animals with age, confirming our findings of salt sensitivity in humans. Elevated blood pressure in LSD1^{+/-} mice is associated with total plasma volume expansion and altered renal Na⁺ excretion. In summary, our human and animal studies demonstrate that LSD1 is a genetic factor that interacts with dietary salt intake modifying age-associated blood pressure increases and salt sensitivity through alteration of renal Na⁺ handling.

Keywords Age-associated blood pressure regulation · Dietary salt · Epigenetic regulation · LSD1

Introduction

Gene–environment interactions determine the onset and progression of cardiovascular diseases, such as

coronary artery disease and hypertension. Exposure to adverse environmental factors cumulates with age, and age is considered the predominant risk factor for the onset of cardiovascular diseases (Lakatta and Levy 2003; Najjar et al. 2005). For example, increased dietary sodium intake has been linked to age-associated vascular dysfunction and arterial hypertension. Daily sodium consumption is estimated to exceed physiological needs by a factor of five in Western societies (Wang and Lakatta 2009). However, the mechanisms of interaction between environmental factors, such as dietary salt intake, and an individual's genetic background resulting in age-associated blood pressure increases remain largely unknown.

Variations in the genes responsible for DNA and histone methylation and demethylation are known to affect an individual's response to internal and environmental factors and, thus, the susceptibility to certain diseases (Thompson et al. 2010a). Lysine-specific demethylase-1 (LSD1), first described in 2004 by Shi et al., was originally shown to demethylate the lysine 4 of histone H3 (H3K4) (Shi et al. 2004). Depending on the site of demethylation and the co-factors involved, LSD1 can either act as a co-repressor or co-activator of transcription (Shi 2007). For example, LSD1 binding to the testosterone–androgen receptor (AR) complex results in demethylation of H3K9, which is crucial for AR-mediated transcription (Metzger et al. 2005; Wissmann et al. 2007). We have reported previously that hypertensive African-American minor allele carriers of the LSD1 single nucleotide polymorphism (SNP) (rs587168) display increased blood pressure sensitivity to dietary salt intake (Williams et al. 2012). Moreover, results from studies in LSD1 heterozygous knockout (LSD1^{+/-}) mice on a high salt diet showed impaired vascular function compared to wild-type animals, as well as suppression of the renin–angiotensin system (RAS) (Pojoga et al. 2011b).

Here, we hypothesized that epigenetic regulator LSD1 interacts with changes in dietary salt intake affecting blood pressure homeostasis with age. Using data from the International Hypertensive Pathotype (HyperPATH) cohort, a multicenter study, which was designed in a rigorous way to minimize modifiable confounders of blood pressure homeostasis, such as body posture, sodium intake, and medications (for details please refer to the “Materials and methods” section), we analyzed the associations between LSD1

genotypes and age-associated blood pressure increases on a high vs. low salt diet in a normotensive Caucasian population. We performed studies in LSD1^{+/-} mice testing the effect of a high vs. low sodium diet on blood pressure regulation during aging, combining results from animal studies with data from humans in a translational approach.

Materials and methods

Human studies

Study population

This current investigation was conducted on data gathered from subjects studied in the HyperPATH Consortium, which was designed to explore genetic and pathophysiological mechanisms of cardiovascular diseases. For this analysis, we used data from 149 subjects. The standardized protocol and inclusion and exclusion criteria for the HyperPATH protocol have been described by us before (Pojoga et al. 2006, 2011a; Vaidya et al. 2011a, b). Briefly, patients with coronary heart disease, heart failure, chronic kidney disease, known causes of secondary hypertension, and active malignancy were excluded from the HyperPATH study. Study participants were considered as normotensive if the average of three consecutive seated blood pressure readings was <140/90 mm Hg, they were not taking antihypertensive medications, and they had no first degree relatives diagnosed with hypertension. Study participants were considered hypertensive if they had one or several of the following conditions: (a) untreated seated diastolic blood pressure (DBP) >100 mmHg, (b) DBP >90 mmHg with one or more antihypertensive medications, and (c) use of two or more antihypertensive medications. To avoid confounding of the study results, all angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and mineralocorticoid receptor antagonists were discontinued 3 months before the study. Beta-blockers were withdrawn 1 month before the study. In case a medication was needed for blood pressure control, subjects were treated with hydrochlorothiazide and/or amlodipine; however, these medications were stopped 3 weeks prior to laboratory evaluation.

Subjects were studied in the Clinical Research Centers of the Brigham and Women's Hospital in

Boston, Massachusetts, USA, and the University of Utah Medical Center in Salt Lake City, Utah, USA. The institutional review board of each institution approved the study, and all study participants gave written informed consent prior to enrollment. Results from the HyperPATH cohort have been reported previously (Underwood et al. 2010; Sun et al. 2011); however, the current analyses are novel and have not been published before.

Study protocol

Briefly, subjects were maintained for 5–7 days on high sodium (HS) (≥ 200 mmolNa⁺/24 h) diet, followed by 5–7 days on low sodium (LS) (≤ 10 mmol Na⁺/24 h) diet. All diets were provided by the Clinical Research Center metabolic kitchen and also included fixed amounts of potassium (100 mmol/24 h) and calcium (1,000 mg/24 h). The sodium amount during the HS phase approximates the average daily sodium intake in Western diets (Chamathi et al. 2010). After each diet phase, participants were admitted to the Institutional Clinical Research Center, and diet compliance and sodium balance were confirmed with a 24-h urine sodium excretion of ≥ 150 mmol for HS and ≤ 30 mmol for LS. Baseline blood pressure was measured while supine between 8:00 AM and 10:00 AM, using the average of five readings from a Dinamap automated device (Critikon, Tampa, FL, USA).

Genotyping

Genotyping of the LSD1 gene was performed using DNA extracted from peripheral leukocytes using the Illumina Bead Station GoldenGate platform. We identified tagging SNPs using the HapMap CEU population, with an R^2 less than 0.9 and a minor allele frequency >10 %. This resulted in seven tagging SNPs, all with a completion rate of >95 %. Concordance with the original genotype call was demonstrated by repeat genotyping for 10 % of the SNPs. Using linkage disequilibrium (LD) constructs from our HyperPath normotensive population, we demonstrated that five of seven tagging SNPs were in LD. Therefore, two tagging SNPs (rs587168 and rs7548692) were analyzed in the HyperPath Caucasian normotensive cohort, with SNP rs758692 showing the most significant association with salt sensitivity. The major and minor alleles for SNP rs7548692, for which we observed the most significant

association with salt sensitivity, are T and A, respectively. In our Caucasian normotensive population, the minor allele frequency is 0.33 with genotype counts of TT 69, AT 75, and AA 16.

Examined phenotype

Basal systolic (SBP) and diastolic (DBP) blood pressure on a liberal salt diet, as well as change in SBP and DBP in response to change in dietary salt intake from low to liberal (HS) salt diet were analyzed as the primary phenotype. As baseline blood pressure was a significant contributor to the change of SBP and DBP in response to dietary salt modification, all the salt sensitivity analyses were corrected for baseline (low salt) blood pressure.

Statistical methods used in the human studies

All tests for association were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA). As it is well known that there can be significant genetic differences between races, we focused on a Caucasian cohort only. We applied a mixed effect linear regression model to test genotype associations with the primary phenotype (i.e., salt sensitivity of BP). All association analyses between LSD1 genotype, age, and the primary endpoints, salt sensitivity of SBP/DBP, were performed assuming an additive model and adjusted for gender, BMI, study site, and baseline BP. To adjust for the relatedness among sibling subjects, the factor “family” was introduced into the models as a repeated measure. The p value threshold for significance was set at $0.05/2=0.025$ for the primary phenotype. The data presented in Supplemental Figs. S1 and S2 as well as in Figs. 1 and 2 all show raw values; all data were adjusted for confounding factors gender, BMI, and baseline blood pressure in the statistical testing.

Animal studies

For all experiments, male lysine-specific demethylase-1 (LSD1) heterozygous knockout (LSD1^{+/-}) animals and age-matched littermate mice (Jackson Laboratories, Bar Harbor, ME, USA) were used. Animal care was in accordance with the Statement of the Institutional Animal Care and Use Committee (IACUC) of the Brigham and Women’s Hospital, Boston, MA, USA. All experimental procedures used aseptic sterile techniques and were approved by the IACUC.

Fig. 1 **a–d** Effect of low (LS) vs. liberal (high, HS) salt intake on **a** SBP and **c** DBP in normotensive Caucasian subjects with age. Age is a significant predictor of blood pressure sensitivity

Blood pressure measurements

Animals were placed on low salt (LS, 0.02 % Na⁺) or high salt (HS, 1.6 % Na⁺) for 7 days prior to blood pressure measurements. Blood pressure was measured using a CODA noninvasive blood pressure system (KENT Scientific) applying the occlusion/volume pressure tail cuff method as described by us previously (Pojoaga et al. 2008, 2010, 2011b). Briefly, during blood pressure measurements, the mice were placed in a mouse restraint holder (KENT Scientific) and set on top of a heating platform to maintain body temperature to 37±1 °C. In order to allow the animals to adapt to the unusual circumstances within the restraint holder, the mice were placed within the holder for a 15-min interval each day starting 3 days prior to the actual

to dietary salt intake, i.e., salt sensitivity ($p=0.002$ for SBP, $p=0.004$ for DBP). **b, d** The distribution patterns of the changes in SBP and DBP from LS to HS

experiment. All readings were performed at 9:00 AM. After ten warm-up cycles, the blood pressure measurements were started and a total of 40 cycles was run for each animal. All results were validated using validation software (KENT Scientific) and a minimum of 15 valid cycles was analyzed. Otherwise, the experiment was repeated. The values were averaged and standard deviations were calculated.

Total plasma volume measurements

After LS or HS diet for a 7-day period, total blood volume of the mice was analyzed using the Evans Blue (EB) method. Briefly, body weight was measured and 100 µl of blood was drawn by submandibular vein puncture; blood was collected in blood collection

Fig. 2 **a, b** Effect of LSD1 genotype on **a** change in SBP and **b** DBP going from LS to HS intake in major allele carriers (*green line*, homozygous, TT; *red line*, heterozygous, AT) and minor

allele homozygous (*blue line*, homozygous, AA) of the LSD1 SNP rs7548692 with age. Age–genotype interaction was significant for SBP ($p=0.02$)

capillaries (Chase Scientific Glass), and the samples were immediately transferred into EDTA-treated microtainers. This was performed to obtain a reference (“blank”) blood sample without the dye prior to the actual experiment. EB was injected into the tail vein at a dosage of 100 μ l of 0.2 mg/ml in 0.9 % NaCl (Sigma). The dye was allowed to distribute in the animals for a period of 3 h before another 100 μ l blood was drawn. All blood samples were centrifuged at 12,000 rpm for 15 min (Hettich Centrifuges), and the supernatant was transferred into a new microcentrifuge tube (Fisherbrand) and diluted with Formamide (Gibco Life Technologies) in a 1:10 ratio in order to obtain a 100- μ l sample. The absorbance of the samples was measured using a spectrophotometer (Beckman Coulter DU 640) at 620 nm, and the concentration in the samples was calculated from a standard curve of EB in order to calculate total plasma volume.

Urinary sodium levels after administration of an acute sodium load

All animals were subjected to LS (0.02 % Na^+) diet for 7 days. On day 7, a bolus of regular drinking water (0.75 % of BW) was administered orally using a

gavage needle (Fine Science Tools). Animals were placed in a metabolic cage atop a flat surface covered in sealing wrap (Borden). Animals were monitored for 1 h, urine samples were collected using a standard pipette, and urine volume was determined gravimetrically. Animals then received a bolus of 2.5 % of their body weight of a 4.6 % NaCl solution (Sigma) using a gavage needle and a syringe, followed by collection of urine samples for the next 4 h in 30-min intervals. Urinary Na^+ concentrations were measured using a commercial kit (Stanbio Sodium Kit). All samples were run in duplicate.

Statistical analysis used in the animal studies

Data are presented as means $\pm$ SD. Normality of the data was assessed using normal probability plots and Shapiro–Wilk test. Assuming normal distribution and equal variance of the data, significance of differences between HS and LS treatment as well as differences between genotypes was tested by two-way or three-way analysis of variance (ANOVA), w/o repeated measures, as applicable. $p<0.05$ was considered significant; all analyses were performed using Sigma Plot software, version 11.

Results

Human studies

Results from epidemiological studies have shown that both blood pressure and salt sensitivity increase with age (Lakatta 2002); please also refer to the Supplement section for data on the relationship between blood pressure and age in our study population. Here, we investigated the effect of low (LS) vs. high (HS) dietary salt intake on DBP and SBP in a normotensive Caucasian population, aged 19–70 years. Panels a and c of Fig. 1 show that age is a highly significant predictor of blood pressure response to dietary salt intake, i.e., salt sensitivity (SBP $p=0.002$, $r=0.27$; DBP $p=0.004$, $r=0.21$), after accounting for baseline blood pressure. Panels b and d of Fig. 1 show the distributions of the changes in

SBP and DBP, respectively, in response to a HS diet in the whole study population. To reduce the influence of spurious outliers, we used the median as the analysis endpoint here. The median value for SBP was 4 mmHg and 2.3 mmHg for DBP, with both distributions being unimodal. Among the majority of study participants, both SBP and DBP levels increased during the HS diet.

We tested SNPs at the LSD1 gene locus for associations with blood pressure response to dietary salt intake. Interestingly, as Fig. 2 demonstrates, the effect of LS vs. HS diet on blood pressure with age depends on the LSD1 genotype. Our analysis of SBP response to dietary salt intake showed a statistically significant interaction between the factors age and rs7548692 ($p=0.02$), indicating that rs7548692 genotype modifies the age effect on salt sensitivity. Figure 3 demonstrates the distributions of salt sensitivity of blood pressure

Fig. 3 a, b Distribution of change in a SBP and b DBP according to LSD1 genotype (AA minor allele homozygous, AT heterozygous, TT major allele homozygous). The distribution in those with the AT and TT genotype is shifted to the right

according to LSD1 genotype. The median values for SBP (a) and DBP (b) are shown in the graphs, with all distributions being unimodal. The distributions for both SBP and DBP are shifted to the right in the subpopulations with the AT (heterozygous) or TT (major allele homozygous) genotype.

Animal studies

The cross-sectional study design of our analysis in human subjects can only provide associations between LSD1 genotypes, salt intake, and blood pressure with age. Therefore, we investigated the relationship of LSD1, salt intake, and blood pressure regulation with age using a LSD1 heterozygous knockout mouse model (LSD1^{+/-}) in more detail. Wild-type and LSD1^{+/-} animals of different ages were switched from regular rat chow (0.3 % Na⁺) to a high salt (HS, 1.6 % Na⁺) diet for 7 days, followed by blood pressure measurements. As demonstrated in Fig. 4, on a HS diet, LSD1^{+/-} mice have significantly higher SBP than their wild-type littermates. Regression analysis shows that the slope of the SBP curve with age is higher in LSD1-deficient mice than in wild-type animals (inset of Fig. 4). Two-way ANOVA shows that the effect of age on SBP depends on the genotype present ($P < 0.024$ for interaction). At every time point analyzed, SBP is significantly higher in heterozygous compared to wild-type animals ($p < 0.05$).

Fig. 4 SBP in LSD1^{+/-} and wild-type animals after a 7-day HS (1.6 % Na⁺) diet. SBP is higher in LSD1-deficient animals compared to wild-type littermates on HS diet. *Inset*: regression analysis indicates more pronounced age-associated rise in SBP in LSD1^{+/-} compared to wild-type mice on HS, $p < 0.024$ for interaction; * $p < 0.05$ LSD1^{+/-} vs. wild type; $n = 5-12$

Using a paired study design, we subjected wild-type and LSD1^{+/-} mice to a low (0.02 % Na⁺) (LS) followed by a high (1.6 % Na⁺) (HS) sodium diet for 7 days each, followed by measurements of SBP and DBP. After 1 week of LS intake, SBP and DBP (Fig. 5a) were similar in wild-type compared to LSD1^{+/-} mice at every time point analyzed. However, high dietary salt intake induced a significant rise in DBP and SBP, indicating increased salt sensitivity in LSD1^{+/-} mice (* $p < 0.05$). This resulted in significantly higher DBP and SBP in LSD1^{+/-} compared to wild-type animals on HS diet (# $p < 0.05$). There is a statistically significant interaction between the factors diet and genotype ($p < 0.001$). Moreover, as we followed these animals over a 2-month period, we observed a significant age-associated increase in DBP and SBP between weeks 44 and 53 in wild-type and LSD1-deficient mice on both diets ($p < 0.05$). Regression analysis indicates increasing age-associated salt sensitivity in LSD1^{+/-} mice (Fig. 5b, c).

Total plasma volume (TPV) is a primary determinant of blood pressure. Figure 6 shows that wild-type and LSD1^{+/-} mice have similar TPV on LS diet at age 54 weeks. However, in contrast to wild-type animals, LSD1-deficient mice responded to HS treatment with significant TPV expansion, resulting in higher SBP and DBP. Two-way ANOVA revealed a significant interaction between the factors

Fig. 5 a–c Effect of LS (0.02 % Na⁺) vs. HS (1.6 % Na⁺) diet and age on SBP and DBP in LSD1^{+/-} and wild-type mice followed over a 2-month period (a). On LS diet, SBP and DBP are similar in both genotypes. HS induces a significant increase in SBP and DBP in LSD1-deficient, but not in wild-type mice. **p* < 0.05 LS vs. HS for LSD1^{+/-}, #*p* < 0.05 wild type vs. LSD1^{+/-} on HS diet. Regression analysis between age and blood pressure—b SBP and c DBP—in wild-type and LSD1^{+/-} animals on LS and HS diets; *n* = 6–8

genotype and salt diet (*p* = 0.01), indicating that the effect of HS diet on TPV depends on the genotype present. Renal Na⁺ handling is a major determinant

Fig. 6 Effect of LS (0.02 % Na⁺) vs. HS (1.6 % Na⁺) treatment on total plasma volume in LSD1^{+/-} and wild-type mice. **p* < 0.05 LS vs. HS in LSD1^{+/-} animals

of TPV homeostasis. To assess Na⁺ sensitivity in LSD1-deficient mice in more detail, we challenged the animals with a Na⁺ bolus after 1 week in LS balance. As Fig. 7 demonstrates, renal Na⁺ excretion was altered in LSD1-deficient mice. The graph shows exaggerated renal Na⁺ excretion in LSD1-deficient compared to wild-type animals during the first 2 h after the salt bolus (*p* < 0.05), indicating a left shift of the Na⁺ excretion curve. After 3 h, natriuresis in wild-type mice reached its peak and was similar in both genotypes 4 h after the bolus. Both genotypes excreted similar cumulative amounts of Na⁺ within the 4-h time period.

Fig. 7 Renal Na⁺ excretion in wild-type and LSD1^{+/-} mice after a Na⁺ bolus following LS balance diet. LSD1-deficient mice show exaggerated Na⁺ excretion after 2 h compared to wild-type animals. **p* < 0.05 vs. 1 h in the respective genotype, #*p* < 0.05 wild type vs. LSD1^{+/-}

Discussion

We have shown that LSD1 SNP (rs7548692) is a genetic factor, which predisposes to increasing salt sensitivity of blood pressure during aging in normotensive Caucasian subjects. Results from our animal studies clearly demonstrate increased salt sensitivity in LSD1-deficient compared to wild-type mice. Elevated blood pressure in LSD1^{+/-} mice on HS diet is associated with TPV expansion and altered renal Na⁺ excretion.

The relationship between genes, epigenetic regulation, and age has been the focus of extensive research during the last several decades (Thompson et al. 2010b). Chromatin is considered one important interface between genes and environment, and epigenetic posttranscriptional modifications of chromatin histone tails are known to affect gene transcription. LSD1 is a flavin-dependent amine oxidase, which removes methyl groups from mono- and dimethylated Lys4 and Lys9 of histone H3 regulating inhibition and activation of gene transcription to environmental stimuli during important physiological processes, such as embryonic development and tumorigenesis (Shi 2007; Adamo et al. 2011; Whyte et al. 2012). Here, we hypothesized that LSD1 is also involved in responses of blood pressure homeostasis to dietary salt intake, i.e., salt sensitivity. The goal of this study was to investigate the relationship between variations of the LSD1 genotype, dietary salt intake, and blood pressure regulation with age.

Our analysis in a normotensive Caucasian population demonstrates age-associated blood pressure increase and increasing salt sensitivity with age, confirming results from earlier studies (Lakatta 2002). Furthermore, our results indicate that the LSD1 genotype is indeed a modifying factor for blood pressure response to dietary salt intake, with major allele T carriers of the LSD1 SNP rs7548692 showing higher salt sensitivity with increasing age than A minor allele homozygotes in response to LS vs. liberal salt diet ($p < 0.05$). We could not observe an effect of the same LSD1 SNP rs7548692 on BP response to salt intake in hypertensive Caucasian subjects (data not shown). A wide variety of genetic and environmental factors are known to contribute to the pathogenesis of hypertension (Agarwal et al. 2005), and even the most rigorous study design will not be able to account for all known confounding factors affecting blood pressure homeostasis. We assume that, in contrast to normotensive subjects, a larger variety of unknown genetic and

environmental confounding factors are likely to contribute to higher blood pressure values in hypertensive patients, masking the potential effect of a single gene or SNP.

As a large variety of uncontrollable factors, such as demographic factors, are likely to confound blood pressure, in particular SBP, there is an ongoing debate about the usefulness of salt sensitivity of blood pressure per se as an intermediate phenotype (Hurwitz et al. 2003). On the other hand, all study subjects underwent a paired intervention design with rigorous control for sodium intake, race, gender, BMI, and blood pressure phenotype, which are all potential confounders of the LSD1 genotype salt sensitivity relationship, representing a major strength of our study. As results from this study are limited to a Caucasian normotensive population, caution is necessary when extrapolating our results to other races. Sample size for other races, such as African Americans and Hispanics, were too small to be included in this analysis. Our analysis in humans consisted of 149 individuals and, thus, may not have been adequately powered to detect some trends that fell short of statistical significance. Using a cross-sectional design, our analysis in humans can only provide associations between LSD1 genotypes, salt intake, and blood pressure regulation. This approach cannot prove causality or directionality of observed associations. Therefore, we also analyzed the relationship between LSD1 and blood pressure regulation in a LSD1 heterozygous knockout model using mice from 24 to 105 weeks of age. As the life span of laboratory mice is approximately 2 years, the animals we used represent the whole life cycle from young to very old. Indeed, results from our animal experiments mirror the findings in humans, strongly supporting the hypothesis that LSD1 affects salt sensitivity with age. On HS diet, SBP is higher and increases with age in LSD1^{+/-} mice.

Here, we also studied the effect of LS vs. HS diet between 44 and 54 weeks of age using a paired study design. In contrast to wild-type mice, LSD1^{+/-} animals responded to high dietary salt intake with elevations in DBP and SBP, indicating increased salt sensitivity. Our animal studies were performed in male mice only. Evidence from the GenSalt study has demonstrated that women are more salt sensitive than men (He et al. 2009). Future investigations in our laboratory will therefore also focus on possible gender differences in salt sensitivity in LSD1 heterozygous mice. Moreover, our regression analysis shows that age-associated salt sensitivity

increased more in LSD1-deficient mice than in wild-type animals, resembling the findings in human major allele carriers of the LSD1 SNP rs7548692. We cannot provide data on LSD1 expression levels in carriers of the LSD1 SNP rs7548692. However, as salt sensitivity increases in rs7548692 T carriers with age, and LSD1^{+/-} mice also show increased salt sensitivity, our results support the view that rs7548692 major allele T is associated with decreased LSD1 expression and/or function.

A variety of factors, such as renal, cardiac, hormonal, and vascular mechanisms, contribute to the complex regulation of blood pressure and salt sensitivity (Blaustein et al. 2006). Long-term increases in blood pressure are suggestive of TPV expansion. Our data confirm the hypothesis that high dietary salt intake increases TPV in LSD1^{+/-} animals, in association with increased blood pressure, indicating that LSD1 is involved in the regulation of whole body sodium homeostasis. We would like to point out that in order to evaluate TPV, we used one single measurement of EB after administration of the dye (Zhang et al. 2005). This approach may overestimate TPV. Previous studies have shown that taking two or more samples within 30 min after EB administration may yield more accurate TPV values of approximately 4 % of total body weight (Morgan et al. 2006; Artunc et al. 2008; Eisner et al. 2012). However, assuming that in our study the clearance rates of EB from the circulation were similar, this should not affect the evaluated differences in TPV between wild-type and LSD1^{+/-} animals after a HS challenge.

Our results suggest that LSD1 deficiency is associated with increased sodium intake and/or impaired sodium excretion in LSD1^{+/-} animals, resulting in volume expansion, thus increasing blood pressure. Various pathophysiological mechanisms, such as increased central salt appetite, impairment of renal vascular reactivity, renal tubular defects, or inappropriate response of the RAS to salt loading (Hall 1986; Guyton 1991; Meneton et al. 2005), are all possible explanations for altered capacity to handle a Na⁺ load in LSD1-deficient animals. Since the state of prior Na⁺ intake affects the Na⁺ excretion rate following an acute load, all animals were placed on a LS diet for 7 days prior to the salt bolus (Tuck et al. 1975). Our results did not demonstrate a blunted capacity to handle an acute salt load in LSD1-deficient mice. In contrast, our experiments demonstrated an enhanced ability to excrete an acute salt load in LSD1-deficient

mice. This result is consistent with previous studies in humans, where low renin hypertensives hyperexcreted a salt load in contrast to normotensives or non-modulating hypertensives (a normal renin form of salt-sensitive hypertension) (Cottier et al. 1958; Krakoff et al. 1970; Luft et al. 1977; Rydstedt et al. 1986; Hollenberg et al. 1986). In this study (data not shown) and previously (Pojoga et al. 2011b), renin and aldosterone levels were suppressed in LSD1-deficient mice on HS diet, indicating that activation of the RAS is not a driving force for elevated blood pressure. As the renal vascular response is a major determinant for the ability to handle a Na⁺ load, and LSD1^{+/-} mice showed enhanced vasoconstriction and impaired vasorelaxation when challenged with HS (Pojoga et al. 2011b), early exaggerated natriuresis is thought to be an indicator of altered renal vascular function, which could contribute to the development of hypertension though the exact mechanisms remain unknown.

Age and high dietary salt intake are predictors of cardiovascular diseases, such as hypertension (Lakatta and Levy 2003; Lakatta et al. 2009). Results from our human and animal studies indicate that LSD1 modulates the interaction of age and salt intake on blood pressure with the animal studies indicating that LSD1 deficiency leads to an increased blood pressure response to dietary salt intake with age. In an approach towards personalized medicine, we identified the LSD1 SNP rs7548692 as genetic factor that predisposes to increasing salt sensitivity with age in a normotensive Caucasian population.

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