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Interactions between beta-2 adrenoceptor gene variation, cardiovascular control and dietary sodium in healthy young adults

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Key points

- Common single nucleotide polymorphisms in the beta-2 adrenoceptor (ADRB2) gene influence cardiovascular function, but they exist in combinations (haplotypes), so it is crucial to characterize the haplotypes to improve the functional predictive power of ADRB2 gene variation.
- Dietary sodium affects ADRB2 function.
- In a large randomized cross-over phenotyping trial that administered low, normal and high dietary sodium, we determined the interactions between ADRB2 haplotype, receptor density on lymphocytes, cardiovascular haemodynamics during stress manoeuvres, forearm blood flow vasodilator responsiveness and dietary sodium.
- Healthy young adults were recruited based on the homozygous haplotypes of the ADRB2 gene: Arg16+Gln27, the rare Gly16+Gln27 and Gly16+Glu27.
- Independent of dietary sodium, the Gly16+Glu27 haplotype had the greatest ADRB2 density and Arg16+Gln27 had the least, suggesting that ADRB2 haplotype influences ADRB2 protein expression, yet the haemodynamic consequences appear modest in healthy humans, necessitating larger trials that explore variation in multiple candidate genes.

Abstract Dietary sodium affects function of the beta-2 adrenoceptor (ADRB2). We tested the hypothesis that haplotype variation in the ADRB2 gene would influence the cardio-vascular and regional vasodilator responses to sympathoexcitatory manoeuvres following low, normal and high sodium diets, and ADRB2-mediated forearm vasodilation in the high sodium condition. Seventy-one healthy young adults were grouped by double homozygous haplotypes: Arg16+Gln27 ($n = 31$), the rare Gly16+Gln27 ($n = 10$) and Gly16+Glu27 ($n = 30$). Using a randomized cross-over design, subjects were studied following 5 days of controlled low, normal and high sodium with 1 month or longer between diets (and low hormone phase of the menstrual cycle). All three visits utilized ECG and finger plethysmography for haemodynamic measures, and the high sodium visit included a brachial arterial catheter for forearm vasodilator responses to isoprenaline with plethysmography. Lymphocytes were sampled for *ex vivo* analysis of ADRB2 density and binding conformation. We found a main effect of haplotype on ADRB2 density ($P = 0.03$) with the Gly16+Glu27 haplotype having the greatest density (low, normal, high sodium: 12.9 ± 0.9, 13.5 ± 0.9 and 13.6 ± 0.8 fmol mg^{-1} protein, respectively) and Arg16+Gln27 having the least (9.3 ± 0.6, 10.1 ± 0.5 and 10.3 ± 0.6 fmol mg^{-1} protein, respectively), but there were no sodium or haplotype effects on receptor binding conformation. In the mental stress

trial, there was a main effect of haplotype on cardiac output ($P = 0.04$), as Arg16+Gln27 had the lowest responses. Handgrip and forearm vasodilation yielded no haplotype differences, and no correlations were present for ADRB2 density and haemodynamics. Our findings support cell-based evidence that ADRB2 haplotype influences ADRB2 protein expression independent of dietary sodium, yet the haemodynamic consequences appear modest in healthy humans.

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Abbreviations　　ACh, acetylcholine; ADRB2, beta-2 adrenoceptor; Arg, arginine; BP, blood pressure; CO, cardiac output; DBP, diastolic blood pressure; FBF/FVC, forearm blood flow/conductance; Gln, glutamine; Glu, glutamate; Gly, glycine; L-NMMA, *NG*-monomethyl-L-arginine; MAP, mean arterial pressure; NTP, sodium nitroprusside; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; SV, stroke volume; SVR, systemic vascular resistance

Introduction

In the circulatory system, the beta-2 adrenoceptor (ADRB2) is a major contributor to heart rate, myocardial contractility and peripheral vasodilation (Eisenach & Wittwer, 2010). ADRB2 function is also modulated by dietary sodium intake. It has long been established that in hypertension, ADRB2-mediated vasodilation is reduced, which is corrected by a low sodium diet (Feldman *et al.* 1984, 1987; Feldman, 1990; Naslund *et al.* 1990). Parallel to these findings, the functional relevance of variation in the ADRB gene has been characterized over the last 20 years (Green *et al.* 1993). Single nucleotide polymorphisms (SNPs) in the ADRB2 gene that have been suggested to affect physiological function include amino acid position 16, which contains either glycine or arginine (major/minor allele: Gly16/Arg), and amino acid position 27, which contains either glutamine or glutamic acid (Gln27/Glu).

Over the last decade our laboratory has conducted a series of experiments examining the cardiovascular pleiotropic effects of ADRB2 gene variation in relation to dietary sodium. First, we established that individuals homozygous for Gly16 possess a greater forearm vasodilator response to intra-arterial graded infusions of the beta-agonist isoprenaline – when compared to Arg 16 homozygotes – after 5 days of a controlled normal sodium diet (Garovic *et al.* 2003). Subsequently, we demonstrated that the greater forearm vasodilator response to isoprenaline in Gly16 homozygotes was no longer present following 5 days of a controlled low sodium diet (Eisenach *et al.* 2006). Next, in support of the regional blood flow studies, we infused the beta-2 agonist terbutaline intravenously during baroreflex inhibition with trimethaphan to 'isolate' the systemic vasodilator response, and found that terbutaline evoked greater systemic vasodilation (lower systemic vascular resistance, SVR) in Gly16 *vs.* Arg16 homozygotes; this study was conducted after a normal sodium diet (Hesse *et al.* 2010). We also found that Gly16 was associated with a greater density of ADRB2s on lymphocytes, whereas the ADRB2 high and low affinity binding conformation was similar between genotype groups (Hesse *et al.* 2010).

A separate series of studies examining the ADRB2 SNPs in response to sympathoexcitatory manoeuvres demonstrated that Gly16 homozygotes have a lower resting heart rate (HR) and greater HR and cardiac output (CO) response to isometric handgrip (Eisenach *et al.* 2004, 2005). During head-up tilt, Arg16 homozygotes have a greater HR, greater SVR and greater arterial plasma noradrenaline response than Gly16 homozygotes (Wittwer *et al.* 2011). Haemodynamic and forearm vasodilator responses to mental stress were not dependent on Gly16/Arg genotype (Liu *et al.* 2006). Importantly, these latter studies did not control for dietary sodium intake prior to the sympathoexcitation protocols.

Summarizing these findings, evidence suggests that the Gly16 and Glu27 SNPs may be associated with augmented peripheral vasodilator function and myocardial function (Tang *et al.* 2003; Snyder *et al.* 2006*b*). Clinically, these phenotypes may confer favourable outcomes in patients with acute coronary syndrome (Lanfear *et al.* 2005) and heart failure (Kaye *et al.* 2003; de Groote *et al.* 2005; Metra *et al.* 2010). Conversely, the Arg16 and Gln27 alleles appear to harbour unfavourable characteristics in cardiovascular and pulmonary health, owing to blunted indices of cardiac function, vasodilatation and perhaps post-exercise bronchodilatation, as recently reviewed (Eisenach & Wittwer, 2010). The two commonly tested SNPs in the ADRB2 gene are in linkage disequilibrium, such that Glu27 homozygotes are homozygous for Gly16, and Arg16 homozygotes are homozygous for Gln27 (Drysdale *et al.* 2000; Hawkins *et al.* 2006). Therefore, if an individual is homozygous for both positions 16 and 27, that individual will contain one of three homozygous haplotype combinations: Arg16+Gln27, Gly16+Gln27 or Gly16+Glu27. Thus, it is reasonable to postulate that if the individual SNPs functionally impact ADRB2 function and exist in linkage disequilibrium, then individuals with homozygous forms of the haplotypes should demonstrate the most contrasting physiological function.

Hypertension as a modifiable risk factor for cardiovascular disease has reached global epidemic proportions (Sliwa *et al.* 2011). High dietary sodium increases blood pressure (BP), cardiovascular morbidity and mortality (Aaron & Sanders, 2013). Therefore, dietary sodium has emerged as a major focus of cardiovascular disease prevention, with ongoing evidence to support widespread recommendations calling for reduced sodium intake (Cook *et al.* 2014). However, the intermediate physiological effects of dietary sodium on cardiovascular control with respect to genetic variation in key regulatory pathways are indeterminate. The functional relevance of individual SNPs in the ADRB2 gene have been characterized with mixed results. Even less is known on how these SNPs interact in haplotype combinations.

With this information as background, in the present study we tested the overall hypothesis that dietary sodium affects cardiovascular control in an ADRB2 haplotype-dependent manner. In a randomized cross-over design, we administered 5 days of controlled low, normal and high dietary sodium with an interval of 1 month or greater between diets. Our aims were: (1) to determine the effect of dietary sodium and ADRB2 genotype on lymphocyte ADRB2 density and binding conformation; (2) to determine the effect of dietary sodium on the haemodynamic and forearm vasodilator responses to mental stress; (3) to determine the effect of dietary sodium on the haemodynamic responses to isometric handgrip; (4) to determine the influence of ADRB2 gene variation on these cardiovascular responses, and whether genotype-dependent responses would no longer be present after beta-blockade with propranolol. As a follow-up to our prior forearm blood flow (FBF) protocols during normal and low sodium diets, (5) our final aim was to determine whether ADRB2 genotype influenced the forearm vasodilator response to isoprenaline in the high dietary sodium condition.

Methods

Ethical approval

This study was approved by the Mayo Clinic IRB and conformed to the standards set by the latest revision of the Declaration of Helsinki. Written, informed consent was obtained from each participant at the screening visit prior to study participation.

Subjects

This study was conducted between 2009 and 2013. The duration of study completion was dependent on recruitment of individuals who were identified as homozygous for both ADRB positions 16 and 27. Of the 71 participants, approximately half of the subjects were recruited from community-wide genotyping efforts performed by our laboratory from 2000 to 2010 ($n = 2226$ samples). The remainder were recruited from the Mayo Clinic Centre for Individualized Medicine's Biobank repository in 2009–2012 ($n = 1000$ samples). To control for race and ethnicity, enrolment was limited to white Caucasians. The subjects were non-obese (body mass index ≤28), non-smokers, non-diabetic and not currently taking anti-hypertensive or other medications except for oral contraceptives. Candidates were considered ineligible if they were men over age 40, women over age 50 (or post-menopausal), used tobacco products, or had any acute or chronic disorders associated with alterations in cardiovascular structure or function (such as hypertension or diabetes). Female volunteers had a negative pregnancy test within 48 h of being studied and on each visit, and were studied during the low hormone phase of the menstrual cycle or the placebo phase of oral contraceptives. Prior to the study protocol, each subject was evaluated by one of the investigators who reviewed the subject's medical history and performed a physical examination. Subjects were considered ineligible if they participated in strenuous regular physical activity such as training for competitive distance events.

Diets

In randomized order separated by 1 month or longer, subjects were placed on one of three diets: the low sodium diet contained 10 mmol (0.23 g Na$^+$; 0.6 g salt) of sodium daily; normal sodium diet contained 150 mmol (3.45 g Na$^+$; 8.6 g salt) of sodium daily; and high sodium diet contained 400 mmol (9.2 g Na$^+$; 23 g salt) of sodium daily. The diets provided constant daily amounts of protein (1.4 g (kg body weight)$^{-1}$ day^{-1}), potassium (100 mmol day^{-1}) and calcium (1100 mg day^{-1}). The caloric content of the diet was adjusted using the Harris Benedict equation to maintain constant body weight, and no more than 35% of calories were provided by fat. All meals were prepared in the dietary kitchen of the Mayo Clinical Research Unit (CRU). On day 5, urine was collected for 24 h for measurement of sodium, potassium and creatinine excretion. All study visits were conducted between 07:00 and 16:00 h. Subjects remained fasting on the morning of each study except for water until the study measurements were completed. If an individual was studied in the afternoon, a light breakfast administered by the research kitchen consistent with the dietary condition was allowed to be consumed more than 4 h before data collection.

Protocol

From this trial, two recent interim analyses on the effects of dietary sodium on HR variability and sex differences in sodium sensitivity describe the details of the study

visit protocols (Eisenach *et al.* 2012; Allen *et al.* 2014). The data included in this report have not been reported on all 71 participants with respect to the interactions between ADRB2 genotype, ADRB2 lymphocyte density, cardiovascular haemodynamics during stress manoeuvres, FBF and dietary sodium. Briefly, the study visit days after the low and normal sodium diets were identical. Subjects were placed in a semi-recumbent chair and an intravenous (I.V.) catheter was placed in the non-dominant arm and used for blood sampling and systemic propranolol infusion. On the study day following the high sodium diet, subjects were initially placed in the supine position. A 20-gauge brachial arterial catheter was placed under local anaesthesia in the non-dominant arm for BP measurement and the forearm drug infusion protocol as detailed previously (Eisenach *et al.* 2012). FBF was measured with venous occlusion plethysmography. Upon completion of the forearm protocol, subjects were allowed a toilet break and *ad libitum* intake of water, and were transferred to the semi-recumbent chair. An I.V. catheter and finger plethysmography cuff were placed in the non-dominant arm. The remainder of the protocol on the high sodium day was identical to the other visits.

Measurements

HR measured with a three-lead ECG. BP was measured non-invasively with finger plethysmography (NexFin, Edwards Lifesciences, Irvine, CA, USA) and verified by brachial cuff oscillometric sphygmomanometry prior to each data collection period. Stroke volume (SV), CO and SVR were derived from pulse contour analysis of the finger plethysmography waveform according to NexFin algorithms (Critoph *et al.* 2013). NexFin has shown low numbers for within-patient variability or tracking errors and reliable tracking of rapid BP changes compared to intra-arterial data (Martina *et al.* 2012; Truijen *et al.* 2012). On the high sodium visit, the brachial arterial catheter provided BP measures, and the NexFin was calibrated to the arterial pressure and recorded to allow for SV, CO and SVR comparisons across dietary conditions. Following each sodium condition, data were recorded at 1000 Hz and analysed on LabChart (AD Instruments, Colorado Springs, CO, USA). Haemodynamic variables were averaged during pre-stress baseline and during the entire period of stress for mental stress and isometric handgrip. FBF was averaged during the 2 min baseline (pre-drug infusion) and final 1 min of each vasodilator dose.

Forearm drug infusion protocol

The forearm vasodilation protocol was conducted after the high sodium diet and conducted in identical fashion to our previous report in the low sodium condition (Eisenach *et al.* 2006). To measure endothelium-independent vasodilation, sodium nitroprusside (NTP) was infused for 2 min at 1.0 μg (100 ml limb volume)$^{-1}$ min^{-1}. To measure ADRB2-mediated vasodilation, isoprenaline was infused for 2 min at 1.0, 3.0, 6.0 and 12.0 ng (100 ml limb volume)$^{-1}$ min^{-1}. To measure endothelium-dependent vasodilation, acetylcholine (ACh) was infused for 2 min at 4.0 μg (100 ml limb volume)$^{-1}$ min^{-1}. Following a 20 min washout period, the NO synthase inhibitor *NG*-monomethyl-L-arginine (L-NMMA; 50 mg) was infused over 10 min, followed by a maintenance dose of 1 mg min^{-1} for the remainder of the forearm protocol. The vasodilator drugs were repeated in reverse order, to allow determination that the NO component of endothelium-dependent dilation was markedly inhibited in response to ACh.

Laboratory stressors

After baseline recording for 2 min, instructions were narrated into participant headphones followed by automated subtraction problems for a total time of 5 min with 81 problems in random order and difficulty (Allen *et al.* 2014). The audio track verbalized random extraneous numbers and was interrupted with a narration urging subjects to improve their performance. A study team member stood behind the subject and recorded the number of incorrect responses. The test concluded with a 2 min recovery period. A mental stress survey developed by Reims *et al.* (2004) was administered. To prevent familiarity with the tests across diets, three maths files were created and randomly selected on each study visit. After 15 min quiet rest and 2 min of baseline recording, subjects performed isometric handgrip at 40% of maximal voluntary contraction (determined on screening visit) until exhaustion. To ensure maximal effort, subjects were encouraged to continue squeezing until fatigue was reached, defined as the inability to maintain force within 10% of the target. After a 10 min rest, an I.V. loading dose of propranolol (0.15 mg kg^{-1}) was infused at a rate of 1 mg min^{-1}, followed by a maintenance infusion of 0.004 mg kg^{-1} min^{-1} for systemic beta-adrenoceptor blockade (Hjemdahl *et al.* 1983; Freyschuss *et al.* 1988). Ten minutes later, the mental stress and handgrip protocols were completed, and the subjects were de-instrumented and discharged.

Blood samples for lymphocyte ADRB2 density and binding affinity

At the beginning of low and normal sodium visits, 80 ml of venous blood was withdrawn from the I.V. catheter. At the beginning of the high sodium visit, 80 ml of arterial blood was withdrawn from the arterial catheter. Lymphocyte ADRB2 density and binding conformation assays were conducted as previously described (Hesse *et al.*

Table 1. Subject characteristics by genotype and dietary sodium condition (low, normal, high)

Characteristic	Arg16 ± Gln27 (n = 31: 20F, 11M)			Gly16 ± Gln27 (n = 10: 4F, 6M)			Gly16 ± Glu27 (n = 30: 21F, 9M)			P values		
	Low	Normal	High	Low	Normal	High	Low	Normal	High	P_{sodium}	$P_{haplotype}$	$P_{interaction}$
Weight (kg)	70 ± 2	71 ± 2	72 ± 2	80 ± 5	79 ± 5	80 ± 5	66 ± 2	69 ± 2	69 ± 2	<0.001	0.26	0.08
HR (b.p.m.)	76 ± 2	73 ± 2	70 ± 2	70 ± 3	71 ± 5	64 ± 4	78 ± 2	71 ± 2	71 ± 2	<0.001	0.71	0.55
SBP (mmHg)	118 ± 2	117 ± 2	118 ± 2	114 ± 4	119 ± 3	115 ± 4	115 ± 2	117 ± 2	116 ± 3	0.37	0.66	0.63
DBP (mmHg)	72 ± 2	69 ± 1	69 ± 1	67 ± 4	64 ± 3	62 ± 4	69 ± 2	67 ± 2	66 ± 1	0.06	0.06	0.86
MAP (mmHg)	87 ± 2	85 ± 1	85 ± 1	83 ± 4	83 ± 2	79 ± 3	84 ± 2	84 ± 1	83 ± 1	0.23	0.13	0.79
24 h urine volume (ml)	1646 ± 127	1695 ± 126	2450 ± 160	2255 ± 252	2605 ± 435	3385 ± 901	1504 ± 122	1589 ± 168	2025 ± 157	<0.001	<0.01	0.45
24 h Na$^+$ excretion (mmol)	20 ± 2	116 ± 6	343 ± 18	22 ± 3	117 ± 7	323 ± 32	21 ± 3	101 ± 6	314 ± 21	<0.001	0.34	0.40
ADRB2 receptor density on lymphocytes	9.3 ± 0.6	10.1 ± 0.5	10.3 ± 0.6	10.6 ± 1.2	13.5 ± 1.6	11.8 ± 1.9	12.9 ± 0.9	13.5 ± 0.9	13.6 ± 0.8	<0.01	0.03	0.11

Values are means ± SEM. Haemodynamic values were obtained on arrival for each study visit after 5 days of each sodium diet. Urinary sodium (Na$^+$) was obtained from a 24 h urine collection on the final day of the diets. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

2010). These assays were performed in yearly batches, and each participant's samples collected from the three sodium conditions were assayed together.

Statistical analysis

Subject characteristics in each sodium condition were compared between haplotype groups using ANOVA. Haemodynamic variables' changes with stress (stress – baseline) were analysed using mixed-effect linear models (repeated measures analysis) taking into account the longitudinal study design. The mixed-effect modelling was performed using PROC MIXED (SAS version 9.3, SAS Institute, Cary, NC, USA). For these models, the change in the given haemodynamic variable with stress was the dependent variable, and the explanatory variables included haplotype group as a between-subject effect, and diet and propranolol as within-subject effects. The diet-by-haplotype interaction terms were included in the models to assess whether dietary differences in the haemodynamic response to stress were dependent on haplotype. FBF responses to isoprenaline, NTP and ACh were analysed using mixed linear models with FBF as the dependent variable. For these models, haplotype was a between-subject effect, drug dose was a within-subject effect and the analysis was repeated using the data collected before and after the administration of L-NMMA.

The comparison between the common haplotypes Arg16+Gln27 and Gly16+Glu27 was of primary interest, with an approximate sample size of 30 individuals per group based on previous studies comparing the CO response to isometric exercise, and FBF responses to isoprenaline between groups that were homozygous for the Arg16 or Gly16 variant (Garovic *et al.* 2003; Eisenach *et al.* 2006). Given our final sample size, the statistical power (two-sided, $\alpha = 0.05$) to detect differences between Arg16+Gln27 and Gly16+Glu27 was 81% for a difference

of 0.75 SD, 97% for a difference of 1.0 SD and 99% for 1.25 SD. Individuals with Gly16+Gln27 haplotype were recruited with a sample size goal of 15 to provide a statistical power of 63, 86 and 97% for a difference of 0.75, 1.0 and 1.25 SD, respectively, in comparison with the other two groups. However, only 10 individuals were enrolled due to the rare occurrence of the homozygous form of this haplotype. All data are presented means±SEM. *P* values of<0.05 were considered statistically significant.

Results

Demographics, lymphocyte ADRB2 density and binding conformation

Subject characteristics based on haplotype and dietary condition are shown in Table 1. Because there was a greater proportion of males in the Gly16+Gln27 group, the *P* values generated in this table included sex as a covariate. There was a main effect of dietary sodium on weight for all subjects irrespective of genotype, as the mean ± SEM weight in low, normal and high sodium conditions was 70.1 ± 1.4, 71.5 ± 1.5 and 72.2 ± 1.4 kg, respectively. There was a main effect of dietary sodium on HR, as low sodium was associated with a greater HR, but this was not dependent on haplotype. For BP, there was a trend toward main effects of sodium and haplotype on diastolic BP (DBP). Within haplotype groups DBP was greater in the low sodium condition, but there was no interaction effect, suggesting that DBP did not respond differently to dietary sodium based on haplotype. Urine volume was dependent on both sodium and haplotype, with the Gly16+Gln27 haplotype displaying greater urine output than the other haplotypes. As expected, urine sodium excretion was dependent on dietary sodium condition. Potassium and creatinine excretion were not affected by diet or haplotype (data not shown). Finally, there was a

main effect of sodium and haplotype on ADRB2 density on lymphocytes, with the Arg16+Gln27 haplotype displaying a lower density than the other groups (Fig. 1). There was no sodium-by-haplotype interaction, suggesting that ADRB2 density did not react differently from the sodium conditions based on haplotype. Because the ADRB2 density assays were performed in yearly batches over the 4 years of the study, we repeated the analysis with time as a covariate and the effects remained significant (Table 1). Finally, there was no evidence to suggest that ADRB2 high and low affinity binding conformation was influenced by dietary sodium or haplotype (data not shown).

Dietary sodium and the haemodynamic and forearm vasodilator responses to mental stress

Table 2 gives the cardiovascular haemodynamics in each dietary condition, at rest (immediately before mental stress) and during mental stress, before and after propranolol. There was a main effect of dietary sodium on the change in HR (stress–baseline), such that the incremental sodium restriction (from high to low sodium) evoked a decrease in the HR response. A similar pattern was generally present for CO. For BP, there was no main effect of sodium on systolic BP (SBP), DBP or mean arterial pressure (MAP), but there was a sodium-by-propranolol interaction for MAP, suggesting that dietary sodium altered the MAP response to beta-blockade. There was also a main effect of sodium on SVR, as SVR was greatest after high sodium, followed by low sodium and least after normal sodium. Dietary sodium did not affect the
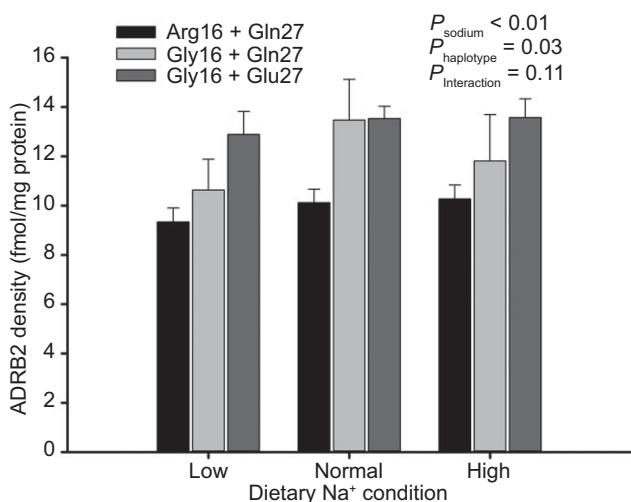
change in forced vital capacity (FVC), but similar to MAP, there was a significant sodium-by-propranolol interaction, indicating that dietary sodium altered the FVC response to beta-blockade. The Reims perceived stress scores that were obtained immediately after the mental stressor were not affected by dietary sodium (between sodium visits). However, within each study visit (under similar sodium conditions), the Reims stress scores were reduced after propranolol, probably as a consequence of test familiarity on a given study day.

ADRB2 haplotype and mental stress responses

Also shown in Table 2, there were modest effects of haplotype on the study variables. For CO, there was a main effect of haplotype and a haplotype-by-propranolol interaction, indicating that the difference between haplotypes in CO response to mental stress was attenuated with propranolol. Interestingly, SV was significant for a haplotype-by-propranolol interaction and a tendency for a sodium-by-haplotype interaction, indicating that propranolol and sodium independently affected SV based on haplotype. However, the three-way interaction was not significant. For regional vasodilator responses to mental stress, there was no evidence to suggest FVC differed based on haplotype.

Dietary sodium and the haemodynamic responses to isometric handgrip

Table 3 displays the cardiovascular haemodynamics in each dietary condition, at rest (immediately before handgrip) and during handgrip, before and after propranolol. Consistent with the mental stress trial, there was a main effect of dietary sodium on HR, CO and SVR. Dietary sodium did not affect blood pressure (SBP, DBP, MAP) or SV. For ADRB2 haplotype during handgrip, there was no evidence to suggest that the haemodynamic and forearm variables were influenced by haplotype.

Figure 1. Lymphocyte density of beta-2 adrenoceptor (ADRB2) based on haplotype and dietary sodium condition
There was a main effect of sodium and haplotype on receptor density, as the Gly16+Glu27 haplotype was associated with greater density than the other groups. Error bars denote SEM.

Forearm vasodilation in the high sodium condition

The forearm vasodilator responses in the high sodium condition are displayed in Table 4. As shown in Fig. 2, there was no effect of haplotype on the FBF response to isoprenaline. Moreover, there was no effect of haplotype on the FBF responses to NTP and ACh. As expected, L-NMMA evoked a reduction in baseline FBF and the FBF responses to isoprenaline and ACh.

Table 2. Mental stress variables before and after propranolol by genotype and dietary sodium condition

	Arg16+Gln27			Gly16+Gln27			Gly16+Glu27			P values	
	Low	Normal	High	Low	Normal	High	Low	Normal	High		
HR (b.p.m.)										P_{sodium}	<0.01
Pre-propranolol										$P_{haplotype}$	0.15
Rest	67 ± 2	64 ± 2	64 ± 2	65 ± 3	65 ± 4	61 ± 4	68 ± 2	65 ± 2	62 ± 3	$P_{prop.}$	<0.001
Mental stress	81 ± 2	80 ± 2	79 ± 2	81 ± 6	87 ± 7	82 ± 7	84 ± 3	82 ± 3	82 ± 3	$P_{sodium*prop.}$	0.12
Post-propranolol										$P_{haplotype*prop.}$	0.27
Rest	57 ± 1	55 ± 1	55 ± 1	55 ± 2	55 ± 3	52 ± 3	58 ± 1	55 ± 1	55 ± 1	$P_{sodium*haplotype}$	0.65
Mental stress	62 ± 1	61 ± 1	60 ± 1	59 ± 2	61 ± 3	60 ± 4	63 ± 2	61 ± 2	61 ± 2	$P_{sodium*haplotype*prop.}$	0.59
SBP (mmHg)										P_{sodium}	0.96
Pre-propranolol										$P_{haplotype}$	0.34
Rest	121 ± 2	120 ± 2	124 ± 2	121 ± 3	124 ± 5	126 ± 5	115 ± 4	121 ± 3	121 ± 3	$P_{prop.}$	<0.001
Mental stress	128 ± 12	128 ± 2	134 ± 2	130 ± 4	135 ± 6	139 ± 8	128 ± 2	128 ± 2	134 ± 3	$P_{sodium*prop.}$	0.07
Post-propranolol										$P_{haplotype*prop.}$	0.57
Rest	118 ± 2	117 ± 2	123 ± 2	118 ± 2	117 ± 2	128 ± 5	116 ± 3	120 ± 3	125 ± 3	$P_{sodium*haplotype}$	0.054
Mental stress	124 ± 2	124 ± 3	127 ± 2	123 ± 3	126 ± 3	134 ± 4	124 ± 2	126 ± 3	131 ± 3	$P_{sodium*haplotype*prop.}$	0.89
DBP (mmHg)										P_{sodium}	0.99
Pre-propranolol										$P_{haplotype}$	0.39
Rest	73 ± 1	72 ± 1	75 ± 1	75 ± 2	73 ± 3	76 ± 3	70 ± 2	72 ± 1	74 ± 2	$P_{prop.}$	<0.01
Mental stress	79 ± 1	77 ± 1	82 ± 1	80 ± 2	80 ± 3	83 ± 4	78 ± 2	77 ± 2	82 ± 2	$P_{sodium*prop.}$	0.14
Post-propranolol										$P_{haplotype*prop.}$	0.34
Rest	74 ± 1	71 ± 2	76 ± 1	73 ± 3	74 ± 2	79 ± 2	71 ± 2	73 ± 2	77 ± 2	$P_{sodium*haplotype}$	0.62
Mental stress	78 ± 1	75 ± 1	79 ± 1	78 ± 3	81 ± 2	84 ± 3	76 ± 2	77 ± 2	80 ± 2	$P_{sodium*haplotype*prop.}$	0.91
MAP (mmHg)										P_{sodium}	0.79
Pre-propranolol										$P_{haplotype}$	0.39
Rest	91 ± 1	90 ± 1	94 ± 2	92 ± 2	92 ± 3	96 ± 4	90 ± 2	91 ± 2	94 ± 2	$P_{prop.}$	<0.001
Mental stress	98 ± 1	97 ± 2	102 ± 2	99 ± 2	102 ± 4	105 ± 5	98 ± 2	98 ± 2	102 ± 2	$P_{sodium*prop.}$	0.050
Post-propranolol										$P_{haplotype*prop.}$	0.90
Rest	91 ± 1	89 ± 2	94 ± 1	90 ± 2	91 ± 2	98 ± 3	89 ± 2	91 ± 2	96 ± 2	$P_{sodium*haplotype}$	0.48
Mental stress	96 ± 1	94 ± 2	98 ± 1	96 ± 3	99 ± 2	104 ± 3	96 ± 2	97 ± 2	100 ± 3	$P_{sodium*haplotype*prop.}$	0.95
CO (l min⁻¹)										P_{sodium}	<0.01
Pre-propranolol										$P_{haplotype}$	0.04
Rest	6.4 ± 0.2	6.3 ± 0.2	6.4 ± 0.2	6.2 ± 0.4	6.8 ± 0.4	6.4 ± 0.4	6.4 ± 0.2	6.6 ± 0.2	6.2 ± 0.3	$P_{prop.}$	<0.001
Mental stress	7.4 ± 0.3	7.8 ± 0.2	7.8 ± 0.2	7.9 ± 0.6	9.0 ± 0.8	8.6 ± 0.6	8.1 ± 0.3	8.3 ± 0.3	8.0 ± 0.3	$P_{sodium*prop.}$	0.58
Post-propranolol										$P_{haplotype*prop.}$	<0.01
Rest	5.2 ± 0.2	5.4 ± 0.2	5.3 ± 0.2	5.4 ± 0.3	5.3 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	5.3 ± 0.2	$P_{sodium*haplotype}$	0.34
Mental stress	5.5 ± 0.2	5.9 ± 0.1	5.8 ± 0.2	5.6 ± 0.3	5.7 ± 0.2	5.9 ± 0.3	5.9 ± 0.2	6.0 ± 0.2	5.8 ± 0.2	$P_{sodium*haplotype*prop.}$	0.65
SV (ml)										P_{sodium}	0.27
Pre-propranolol										$P_{haplotype}$	0.11
Rest	100 ± 4	104 ± 4	102 ± 3	97 ± 4	107 ± 4	107 ± 4	96 ± 4	104 ± 4	100 ± 3	$P_{prop.}$	<0.01
Mental stress	98 ± 4	108 ± 7	101 ± 3	99 ± 4	107 ± 4	108 ± 5	99 ± 4	103 ± 4	100 ± 3	$P_{sodium*prop.}$	0.16
Post-propranolol										$P_{haplotype*prop.}$	0.03
Rest	97 ± 3	99 ± 2	99 ± 3	100 ± 6	98 ± 4	104 ± 2	96 ± 3	101 ± 3	98 ± 3	$P_{sodium*haplotype}$	0.06
Mental stress	94 ± 3	99 ± 2	98 ± 3	86 ± 11	95 ± 3	100 ± 3	96 ± 3	100 ± 3	98 ± 3	$P_{sodium*haplotype*prop.}$	0.13
SVR (units)										P_{sodium}	<0.01
Pre-propranolol										$P_{haplotype}$	0.38
Rest	1196 ± 48	1155 ± 32	1216 ± 46	1209 ± 73	1105 ± 63	1212 ± 32	1157 ± 39	1130 ± 39	1250 ± 44	$P_{prop.}$	<0.001
Mental stress	1105 ± 47	1003 ± 29	1077 ± 36	1050 ± 72	942 ± 66	998 ± 48	1005 ± 40	986 ± 40	1061 ± 43	$P_{sodium*prop.}$	0.62
Post-propranolol										$P_{haplotype*prop.}$	0.10
Rest	1491 ± 84	1343 ± 44	1450 ± 41	1360 ± 90	1402 ± 70	1474 ± 58	1346 ± 47	1378 ± 46	1513 ± 92	$P_{sodium*haplotype}$	0.59
Mental stress	1508 ± 96	1292 ± 35	1375 ± 39	1379 ± 79	1394 ± 62	1424 ± 69	1322 ± 51	1317 ± 44	1441 ± 91	$P_{sodium*haplotype*prop.}$	0.80
FVC (units)										P_{sodium}	0.26
Pre-propranolol										$P_{haplotype}$	0.29
Rest	1.9 ± 0.1	2.1 ± 0.1	2.9 ± 0.2	2.0 ± 0.3	2.7 ± 0.4	2.3 ± 0.3	2.3 ± 0.2	2.2 ± 0.2	3.1 ± 0.2	$P_{prop.}$	<0.001
Mental stress	3.3 ± 0.2	3.5 ± 0.3	5.3 ± 0.5	3.5 ± 0.8	5.2 ± 0.9	4.5 ± 0.7	3.8 ± 0.4	4.4 ± 0.5	5.6 ± 0.4	$P_{sodium*prop.}$	0.02
Post-propranolol										$P_{haplotype*prop.}$	0.93
Rest	2.0 ± 0.1	2.1 ± 0.1	3.4 ± 0.8	1.7 ± 0.2	2.5 ± 0.4	2.4 ± 0.3	1.9 ± 0.1	2.2 ± 0.2	2.6 ± 0.2	$P_{sodium*haplotype}$	0.60
Mental stress	3.0 ± 0.2	2.8 ± 0.2	3.7 ± 0.3	2.3 ± 0.2	3.3 ± 0.5	3.4 ± 0.4	3.2 ± 0.5	3.0 ± 0.3	3.6 ± 0.3	$P_{sodium*haplotype*prop.}$	0.21
Perceived stress scores										P_{sodium}	0.49
Pre-propranolol	20.4 ± 0.8	19.8 ± 0.8	19.9 ± 0.8	18.9 ± 0.9	19.6 ± 1.4	18.9 ± 1.6	21.4 ± 1.1	21.1 ± 0.9	21.9 ± 0.8	$P_{haplotype}$	0.47
Post-propranolol	18.5 ± 0.9	18.3 ± 0.8	18.2 ± 1.0	17.3 ± 1.5	17.5 ± 1.8	18.7 ± 1.4	19.3 ± 1.1	19.4 ± 1.0	21.0 ± 0.7	$P_{prop.}$	<0.001

Values are means ± SEM. Values at rest were recorded immediately prior to beginning handgrip. HR, heart rate; MAP, mean arterial pressure; CO, cardiac output; SV, stroke volume; SVR, systemic vascular resistance; prop., propranolol.

Table 3. Handgrip variables before and after propranolol by haplotype and dietary sodium condition

	Arg16 ± Gln27			Gly16 ± Gln27			Gly16 ± Glu27			*P* values	
	Low	Normal	High	Low	Normal	High	Low	Normal	High		
HR (b.p.m.)										P_{sodium}	<0.001
Pre-propranolol										$P_{haplotype}$	0.54
Rest	67 ± 1	64 ± 1	64 ± 1	65 ± 3	63 ± 3	59 ± 4	67 ± 2	66 ± 2	64 ± 2	$P_{prop.}$	<0.001
Handgrip	85 ± 2	87 ± 4	85 ± 2	83 ± 5	81 ± 5	82 ± 5	83 ± 2	81 ± 2	83 ± 2	$P_{sodium*prop.}$	0.91
Post-propranolol										$P_{haplotype*prop.}$	0.13
Rest	58 ± 1	56 ± 1	55 ± 1	55 ± 2	55 ± 2	52 ± 3	58 ± 1	55 ± 1	56 ± 1	$P_{sodium*haplotype}$	0.44
Handgrip	69 ± 1	68 ± 1	70 ± 1	66 ± 3	67 ± 3	68 ± 4	69 ± 2	67 ± 2	70 ± 2	$P_{sodium*haplotype*prop.}$	0.49
SBP (mmHg)										P_{sodium}	0.58
Pre-propranolol										$P_{haplotype}$	0.95
Rest	119 ± 2	120 ± 2	123 ± 2	121 ± 3	117 ± 2	121 ± 7	119 ± 2	121 ± 3	126 ± 3	$P_{prop.}$	0.01
Handgrip	135 ± 4	138 ± 3	140 ± 3	140 ± 5	132 ± 2	137 ± 7	136 ± 3	138 ± 4	142 ± 3	$P_{sodium*prop.}$	0.69
Post-propranolol										$P_{haplotype*prop.}$	0.79
Rest	115 ± 2	117 ± 2	122 ± 2	115 ± 3	116 ± 3	120 ± 2	116 ± 3	119 ± 3	124 ± 2	$P_{sodium*haplotype}$	0.58
Handgrip	134 ± 3	136 ± 2	142 ± 3	133 ± 5	134 ± 3	137 ± 4	136 ± 3	137 ± 3	142 ± 3	$P_{sodium*haplotype*prop.}$	0.61
DBP (mmHg)										P_{sodium}	0.29
Pre-propranolol										$P_{haplotype}$	0.68
Rest	74 ± 1	73 ± 1	75 ± 1	75 ± 2	73 ± 2	76 ± 3	73 ± 1	73 ± 2	76 ± 1	$P_{prop.}$	<0.001
Handgrip	87 ± 2	88 ± 2	88 ± 1	90 ± 3	87 ± 2	88 ± 3	86 ± 2	86 ± 2	89 ± 2	$P_{sodium*prop.}$	0.26
Post-propranolol										$P_{haplotype*prop.}$	0.94
Rest	72 ± 1	72 ± 2	76 ± 1	70 ± 4	74 ± 2	76 ± 2	72 ± 2	73 ± 2	75 ± 2	$P_{sodium*haplotype}$	0.74
Handgrip	89 ± 1	87 ± 2	92 ± 2	87 ± 4	89 ± 2	92 ± 2	87 ± 2	87 ± 2	90 ± 2	$P_{sodium*haplotype*prop.}$	0.65
MAP (mmHg)										P_{sodium}	0.10
Pre-propranolol										$P_{haplotype}$	0.83
Rest	92 ± 1	91 ± 2	94 ± 1	93 ± 1	91 ± 2	93 ± 4	91 ± 1	92 ± 2	96 ± 2	$P_{propranolol}$	0.03
Handgrip	108 ± 2	109 ± 2	110 ± 2	111 ± 3	106 ± 2	108 ± 4	107 ± 2	108 ± 3	111 ± 2	$P_{sodium*prop.}$	0.54
Post-propranolol										$P_{haplotype*prop.}$	0.99
Rest	89 ± 1	89 ± 2	94 ± 1	87 ± 3	90 ± 2	94 ± 2	89 ± 2	91 ± 2	94 ± 2	$P_{sodium*haplotype}$	0.54
Handgrip	107 ± 2	106 ± 2	112 ± 2	105 ± 4	107 ± 2	110 ± 2	106 ± 2	107 ± 2	111 ± 2	$P_{sodium*haplotype*prop.}$	0.65
CO (l min^{-1})										P_{sodium}	<0.001
Pre-propranolol										$P_{haplotype}$	0.99
Rest	6.1 ± 0.2	6.3 ± 0.2	6.4 ± 0.2	6.4 ± 0.4	6.2 ± 0.3	5.8 ± 0.4	6.4 ± 0.2	6.3 ± 0.3	6.3 ± 0.2	$P_{prop.}$	<0.001
Handgrip	7.3 ± 0.3	7.8 ± 0.2	8.1 ± 0.3	7.7 ± 0.5	7.6 ± 0.5	7.8 ± 0.5	7.7 ± 0.4	7.7 ± 0.3	7.9 ± 0.3	$P_{sodium*prop.}$	0.65
Post-propranolol										$P_{haplotype*prop.}$	0.39
Rest	5.3 ± 0.2	5.3 ± 0.2	5.2 ± 0.2	5.5 ± 0.3	5.1 ± 0.2	5.1 ± 0.2	5.3 ± 0.2	5.4 ± 0.1	5.4 ± 0.2	$P_{sodium*haplotype}$	0.17
Handgrip	5.7 ± 0.2	6.1 ± 0.2	6.0 ± 0.2	5.8 ± 0.3	5.7 ± 0.3	5.9 ± 0.3	6.0 ± 0.2	5.9 ± 0.2	6.3 ± 0.3	$P_{sodium*haplotype*prop.}$	0.54
SV (ml)										P_{sodium}	0.67
Pre-propranolol										$P_{haplotype}$	0.31
Rest	98 ± 4	100 ± 2	102 ± 3	100 ± 6	102 ± 4	100 ± 5	97 ± 4	102 ± 4	101 ± 3	$P_{prop.}$	<0.001
Handgrip	92 ± 4	96 ± 2	97 ± 3	95 ± 5	96 ± 3	98 ± 3	94 ± 3	98 ± 3	97 ± 3	$P_{sodium*prop.}$	0.053
Post-propranolol										$P_{haplotype*prop.}$	0.19
Rest	96 ± 3	98 ± 2	97 ± 3	102 ± 6	93 ± 4	100 ± 2	94 ± 3	99 ± 3	99 ± 3	$P_{sodium*haplotype}$	0.71
Handgrip	87 ± 3	91 ± 2	88 ± 2	90 ± 6	87 ± 4	88 ± 2	88 ± 3	93 ± 3	92 ± 3	$P_{sodium*haplotype*prop.}$	0.18
SVR (units)										P_{sodium}	<0.001
Pre-propranolol										$P_{haplotype}$	0.90
Rest	1252 ± 54	1181 ± 35	1204 ± 40	1202 ± 77	1192 ± 69	1316 ± 59	1175 ± 45	1257 ± 97	1242 ± 42	$P_{propranolol}$	<0.001
Handgrip	1266 ± 66	1135 ± 38	1132 ± 42	1191 ± 70	1168 ± 85	1144 ± 68	1168 ± 47	1174 ± 52	1169 ± 47	$P_{sodium*prop.}$	0.95
Post-propranolol										$P_{haplotype*prop.}$	0.76
Rest	1389 ± 55	1361 ± 41	1473 ± 43	1313 ± 114	1473 ± 109	1492 ± 55	1368 ± 47	1378 ± 44	1458 ± 84	$P_{sodium*haplotype}$	0.36
Handgrip	1563 ± 60	1425 ± 41	1520 ± 45	1495 ± 125	1550 ± 110	1539 ± 79	1468 ± 54	1499 ± 74	1492 ± 94	$P_{sodium*haplotype*prop.}$	0.47

Values are means ± SEM. Values at rest were recorded immediately prior to beginning handgrip. HR, heart rate; MAP, mean arterial pressure; CO, cardiac output; SV, stroke volume; SVR, systemic vascular resistance; prop., propranolol.

Polymorphic variation based on position 16

To determine the effect of SNP position 16 alone on the study variables, we repeated all analyses with individuals grouped as either Arg16 ($n = 31$) *vs.* Gly16 ($n = 40$). For ADRB2 density on lymphocytes, the Gly16 group demonstrated a significantly greater density across all sodium conditions. Additionally, the low sodium condition was associated with the least ADRB2 density within groups. In the mental stress trial, similar to the haplotype analysis, there was a sodium-by-propranolol interaction effect for SBP, DBP and MAP, but these were not influenced by position 16. Also for mental stress, there was a main effect of position 16 genotype on CO,

Table 4. Forearm blood flow following high dietary sodium

	Arg16 ± Gln27		Gly16 ± Gln27		Gly16 ± Glu27			P values	
	Pre-L-NMMA	Post-L-NMMA	Pre-L-NMMA	Post-L-NMMA	Pre-L-NMMA	Post-L-NMMA		Pre-L-NMMA	Post-L-NMMA
Isoprenaline									
Baseline	2.3 ± 0.3	1.8 ± 0.1	2.3 ± 0.3	1.8 ± 0.3	2.4 ± 0.2	1.6 ± 0.1	P_{dose}	<0.001	<0.001
1.0	6.3 ± 0.4	3.2 ± 0.2	7.0 ± 1.1	3.6 ± 0.7	6.7 ± 0.4	3.1 ± 0.3	$P_{haplotype}$	0.88	0.89
3.0	7.5 ± 0.6	4.3 ± 0.3	7.7 ± 0.7	4.6 ± 1.0	7.5 ± 0.4	4.2 ± 0.4	$P_{dose*haplotype}$	0.98	0.42
6.0	9.7 ± 0.7	5.7 ± 0.4	10.1 ± 1.7	7.1 ± 1.8	10.3 ± 0.7	6.0 ± 0.7			
12.0	12.3 ± 0.9	8.0 ± 0.7	13.0 ± 2.1	7.6 ± 1.1	13.1 ± 0.8	7.9 ± 0.7			
Nitroprusside							P_{dose}	<0.001	<0.001
Baseline	2.3 ± 0.1	1.8 ± 0.1	2.3 ± 0.3	1.8 ± 0.3	2.2 ± 0.1	1.7 ± 0.1	$P_{haplotype}$	0.77	0.63
1.0	15.1 ± 0.8	15.8 ± 0.9	14.4 ± 1.5	14.2 ± 1.7	15.9 ± 1.0	16.4 ± 1.1	$P_{dose*haplotype}$	0.58	0.50
Acetylcholine							P_{dose}	<0.001	<0.001
Baseline	2.9 ± 0.3	1.8 ± 0.1	3.1 ± 0.5	1.8 ± 0.3	2.9 ± 0.2	1.6 ± 0.1	$P_{haplotype}$	0.80	0.40
4.0	19.8 ± 1.4	16.1 ± 1.3	17.2 ± 3.0	12.1 ± 2.2	19.5 ± 1.9	16.3 ± 1.7	$P_{dose*haplotype}$	0.65	0.31

Forearm blood flow is expressed as mean ± SE, in ml (100 ml limb volume)$^{-1}$ min^{-1}. Doses for isoprenaline are in ng (100 ml limb volume)$^{-1}$ min^{-1}, acetylcholine are in μg (100 ml limb volume)$^{-1}$ min^{-1} and nitroprusside are in μg (100 ml limb volume)$^{-1}$ min^{-1}.

and a significant genotype-by-propranolol interaction, consistent with the haplotype analysis. For handgrip, no variables were significant based on SNP position 16.

Discussion

The purpose of this investigation was to determine the interaction between three distinct levels of dietary sodium intake and functional ADRB2 gene haplotypes. Our first finding was that ADRB2 density on circulating lymphocytes was dependent on ADRB2 haplotype and SNP position 16 alone. Analysis of lymphocyte ADRB2 density and function has been correlated with receptor properties in less accessible tissues such as the heart and vasculature (Fraser *et al.* 1981; Aarons & Molinoff, 1982; Feldman *et al.* 1984). For example, the density of the ADRB2s on lymphocytes by radioligand binding has been shown to correlate with the density of ADRB2s in cardiac tissue using positron emission tomography (Qing *et al.* 1997). In this context, lymphocyte ADRB2 density or agonist binding affinity may predict the influence of ADRB2 haplotype variation on receptor function *in vivo*. Furthermore, *ex vivo* analysis of ADRB2 function and polymorphic variation using lymphocytes has been described but the influence of the common genetic variations in the ADRB2 gene has been inconsistent among studies (Aziz *et al.* 1999; Lipworth *et al.* 2002; Bao *et al.* 2005; Oostendorp *et al.* 2005; Snyder *et al.* 2006*b*). The present study is the third in a series of studies at our institution to demonstrate that ADRB2 density is greater in Gly16 homozygotes (Snyder *et al.* 2006*b*; Hesse *et al.* 2010), which appears to be the dominant effect when comparing position 16 + 27 haplotypes. Importantly, 64 of the current 71 subjects had not participated in previous trials. Our findings are consistent with formative work by Drysdale *et al.* (2000), who first reported the importance of ADRB2 haplotype and transfected the corresponding haplotypes into HEK293 cells, resulting in approximately 50% greater mRNA and ADRB2 density in Gly16+Glu27 than Arg16+Gln27. Taken together, these reports lend further evidence that ADRB2 gene variation affects ADRB2 cell membrane density.

Our finding that dietary sodium restriction decreased lymphocyte ADRB2 density and sodium loading increased ADRB2 density in all subjects, regardless of genotype, is consistent with a similarly designed trial in healthy young adult normotensive men over three decades ago (Fraser *et al.* 1981). Our rationale to determine the percentage of ADRB2 receptor in high and low affinity binding conformation was based on seminal work showing that the high affinity conformation is altered by dietary sodium restriction and loading in a small study of hypertensive and normotensive subjects (Naslund *et al.* 1990). It is unclear why we were unable to demonstrate either a sodium or a haplotype effect on ADRB2 binding conformation. However, this may explain the statistically non-significant interactions between sodium and haplotype on the haemodynamic results during mental stress and handgrip.

We recently reported an interim analysis of HR variability on the present cohort, showing the effects of dietary sodium on HR and cardiac autonomic modulation (Allen *et al.* 2014), but in the present analysis these effects were independent of haplotype (data not shown). Sodium loading increases total blood volume and reduces resting HR (McNeely *et al.* 2008), while sodium restriction increases sympathetic activity and circulating catecholamines (Graudal *et al.* 2012). In the present study, resting HR was inversely associated with sodium intake, but this was not dependent on haplotype. Dietary sodium did not change resting BP in our cohort, which is consistent with other studies of short-term dietary
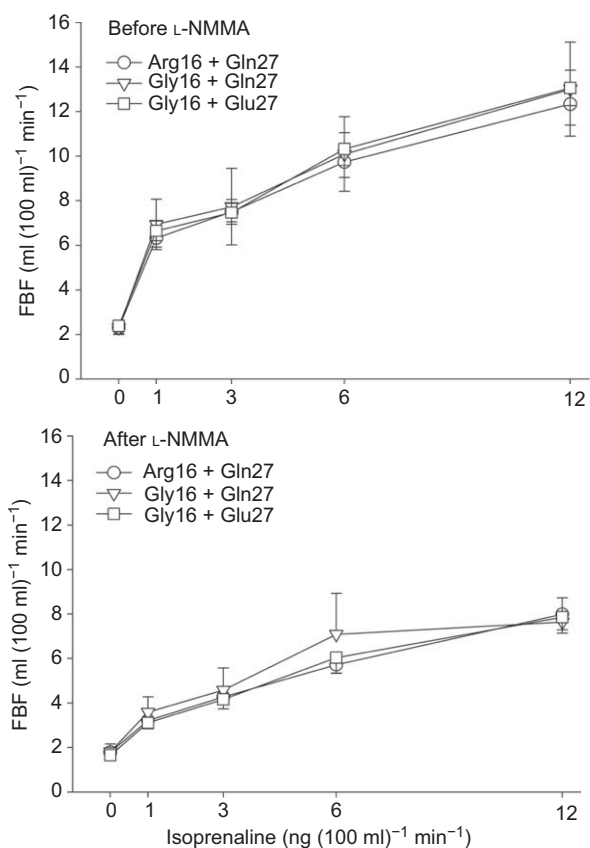
sodium manipulation in healthy normotensives (Wedler *et al.* 1992; DuPont *et al.* 2013). Furthermore, there was no effect of haplotype on BP in any of the sodium conditions, consistent with a recent meta-analysis that failed to associate positions 16 and 27 with essential hypertension (Lou *et al.* 2010). It is unclear why 24 h urine volume was dependent on haplotype, even after sex was included as a covariate due to the greater proportion of men in the Gly16+Gln27 group. The haplotype variants may influence renal sodium handling (Snyder *et al.* 2006*c*), although in the present study this is purely speculative as 24 h sodium excretion was unaffected by haplotype.

The next major finding was a main effect of dietary sodium on HR and CO for the mental stress trial, probably related to circulating blood volume and total body water, as evidenced by sodium-related weight changes. It is of note that resting SVR was greatest after

Figure 2. During the high sodium condition, forearm blood flow (FBF) response to administration of isoprenaline via the brachial artery in individuals homozygous for both amino acid positions 16 and 27 in the beta-2 adrenoceptor
Baseline FBF did not differ between groups. Isoprenaline increased FBF and this was not different between haplotypes. In the lower panel, NO synthase inhibition with L-NMMA decreased FBF and significantly blunted the FBF response to isoprenaline, but this was not dependent on haplotype.

high sodium, followed by low sodium and least after normal sodium, which remained in this general pattern during mental stress before and after propranolol. The mechanism for elevated SVR during sodium loading is postulated to be a result of elevated cerebral spinal fluid sodium concentration, which increases hypothalamic ouabain release and promotes a cascade of events to increase sympathetic nerve activity (Blaustein *et al.* 2012). In acute sodium restriction, blood volume decreases, which activates baroreceptors and sympathetic activity, concomitant with renin–angiotensin–aldosterone system activation (Graudal *et al.* 2012). Thus, both extremes of sodium intake evoke diverse mechanisms to increase SVR, while adaptive mechanisms during sodium restriction promote a reduction in SVR and BP over time (Makela *et al.* 2008). Therefore, our findings support the previous mechanistic work, in that dietary sodium extremes evoked perturbations in cardiovascular control (HR, CO and SVR) to a greater extent than in normal sodium conditions.

A major hypothesis in this study was that the sympathoexcitatory manoeuvres would reveal haplotype-dependent differences in haemodynamic responsiveness. In the mental stress trial, the main effects of haplotype were present for CO but not HR or stroke volume. The haplotype-by-propranolol interaction for CO reflects that beta blockade is altering CO in a haplotype-dependent manner. This finding was also present for position 16 alone. The interesting distinction with SV is that the haplotype effect did not reach significance ($P = 0.11$) but displayed interactions between haplotype and propranolol ($P = 0.03$), and a trend toward significance between haplotype and sodium ($P = 0.06$). Other investigators have shown that Gly16 homozygotes have greater fractional shortening, ejection fraction, mid-wall shortening and stress-corrected mid-wall shortening compared to both heterozygotes and Arg16 homozygotes using echocardiography (Tang *et al.* 2003). In a separate cohort from our institution, Gly16 homozygotes demonstrated greater CO and SV at rest compared to Arg16 homozygotes, as measured by the open-circuit acetylene wash-in method (Snyder *et al.* 2006*b*). During low and high intensity exercise, the Gly16 homozygotes also had greater CO and SV compared to Arg16 homozygotes (Snyder *et al.* 2006*a*). Together with our findings, this suggests that haplotype variation may mediate SV that is influenced by dietary sodium and is affected differentially by beta blockade.

While few haplotype effects were present in the mental stress trial, there were no variables influenced by haplotype in the handgrip trial. These statistically non-significant findings were in contrast to our hypothesis which was based on experimental evidence that the Arg16/Gly SNP influences the HR and CO response to handgrip

(Eisenach *et al.* 2004, 2005) in separate study cohorts from nearly a decade ago. We also reasoned that forearm ADRB2-mediated vasodilation would be greater in the individuals homozygous for Gly16 and/or Glu27, as these variants have been associated with augmented regional vasodilator responsiveness (Cockcroft *et al.* 2000; Dishy *et al.* 2001; Garovic *et al.* 2003) and systemic vasodilator responsiveness during ganglionic blockade (Hesse *et al.* 2010). While it may remain that Gly16 and/or Glu27 is associated with augmented ADRB2 vasodilation during the normal sodium condition, we are confident that these effects are not apparent in the low sodium and high sodium dietary conditions. Furthermore, while ADRB2 lymphocyte density was different based on haplotype, linear regression analysis did not demonstrate correlations between density and the haemodynamic variables.

The strength of this investigation was our strategy to improve the predictive power of gene variation by studying the interaction between SNP positions 16 and 27 by recruiting only subjects who were double homozygous at both sites. This in turn served to classify our subjects according to the common haplotypes originally described by Drysdale *et al.* (2000). Despite our efforts to recruit these genetically specific individuals from a large pool of samples from the general population, the limitations are centred on the possibility that there are functionally relevant variants in the ADRB2 gene beyond ADRB2 positions 16 and 27. Along these lines, a more recent study constructed eight common ADRB2 haplotypes derived from 26 polymorphisms in the coding and non-coding regions of the gene, and performed whole-gene transfection in COS-7 cells to reveal haplotype differences in ADRB2 protein expression (Panebra *et al.* 2010). Of those eight haplotypes, only one contains Gly16+Glu27, and this was the haplotype with the greatest ADRB2 protein expression, which is in accord with the Gly16+Glu27 subjects in our study. Unfortunately, in Panebra's study the haplotype with the second-highest ADRB2 expression was one of the five haplotypes that included Arg16+Gln27, which underlines the authors' conclusions that extended additional variations in extended haplotypes may provide greater discrimination of phenotype (Panebra *et al.* 2010). In this context, we acknowledge that phenotyping based on individual SNPs is probably inadequate to characterize functional gene variation, but alternative strategies must be discovered to allow for practical and comprehensive phenotyping that can be replicated in human populations. Finally, despite the large genotyping recruitment effort, we were unable to complete enrolment of the Gly16+Gln27 haplotype; therefore, we acknowledge that the lack of difference in haemodynamics between this rare haplotype group and the others could be due the low sample size (type II error). In addition, beyond the primary aims of this study, there were analyses of secondary outcomes that were performed without adjustment for multiple comparisons. These secondary analyses should be considered exploratory given the increased possibility of type I error.

In conclusion, we have explored the interactions among sympathoexcitatory manoeuvres, adrenergic agonists and antagonist infusions, and dietary sodium intake to generate a detailed picture of how ADRB2 gene variation influences physiological responses pertinent to the development of hypertension and cardiovascular disease. Most notably the Gly16+Glu27 haplotype and Gly16 alone is associated with greater ADRB2 receptor density on lymphocytes than Arg16 and Gln27. Moreover, we have shown that CO is affected by ADRB2 haplotype and position 16 alone during the mental stress trial but was no longer apparent in the handgrip trial. Finally, we found no evidence to suggest that the interaction between ADRB2 gene variation and the physiological responses to sympathoexcitatory stress are influenced by dietary sodium. In the overall context of ADRB2 gene variation, dietary sodium and cardiovascular control, this high-resolution phenotyping trial suggests that common ADRB2 polymorphisms influence ADRB2 density on circulating lymphocytes, but only modest effects on haemodynamics.

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Additional information

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None declared.

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