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ORIGINAL RESEARCH

Alveolar air and oxidative metabolic demand during exercise in healthy adults: the role of single-nucleotide polymorphisms of the β_2AR gene

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Aerobic exercise, codon 16, exercise capacity, genetic polymorphism, β_2-adrenergic receptor.

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Abstract

The predominating β-adrenergic receptor subtype expressed on human alveolar tissue is the β_2AR. The homozygous arginine (Arg16Arg) single-nucleotide polymorphism (SNP) at codon 16 of the β_2AR gene has been associated with abnormal β_2AR function accompanied by decreased resting alveolar-capillary membrane gas-transfer in certain healthy adults. Although not previously studied in the context of the β_2AR gene, pulmonary gas-transfer is also influenced by alveolar volume (V_A) and with it the availability of alveolar surface area, particularly during exercise. Small V_A implies less alveolar surface area available for O_2 transport. We tested the following hypothesis in healthy adults during exercise: compared with Gly16Gly and Arg16Gly β2AR genotypes, Arg16Arg will demonstrate reduced V_A and ventilation ($\dot{V}_A$) relative to $\dot{V}_E$ and oxidative metabolic demand. Age- BMI- and gender-matched groups of Arg16Arg ($N = 16$), Gly16Gly ($N = 31$), and Arg16Gly ($N = 17$) performed consecutive low (9-min, 40%-peak workload) and moderate (9-min, 75%-peak workload) intensity exercise. We derived V_A and $\dot{V}_A$ using "ideal" alveolar equations via arterialized gases combined with breath-by-breath ventilation and gas-exchange measurements; whereas steady-state $\dot{V}O_2$ was used in metabolic equations to derive exercise economy (EC = workload$\div\dot{V}O_2$). Variables at rest did not differ across β_2AR genotype. Strongest β_2AR genotype effects occurred during moderate exercise. Accordingly, while $\dot{V}_E$ did not differ across genotype ($P > 0.05$), decreased in Arg16Arg versus Arg16Gly and Gly16Gly were $\dot{V}O_2$ (1110 ± 263, 1269 ± 221, 1300 ± 319 mL/(min·m^2), respectively, both $P < 0.05$), $\dot{V}_A$ (59 ± 21, 70 ± 16, 70 ± 21 L/min, respectively, both $P < 0.05$), and V_A (1.43 ± 0.37, 1.95 ± 0.61, 1.93 ± 0.65 L, respectively, both $P < 0.05$). Also reduced was EC in Arg16Arg versus Arg16Gly ($P < 0.05$) and Gly16Gly ($P > 0.05$) (1.81 ± 0.23, 1.99 ± 0.30, and 1.94 ± 0.26 kcal/(L·m^2), respectively). Compared with Gly16Gly and Arg16Gly genotypes, these data suggest the Arg16Arg β_2AR genotype plays a role in the loss of oxidative metabolic efficiency coupled with an inadaptive V_A and, hence, smaller alveolar surface area available for O_2 transport during submaximal exercise in healthy adults.

Introduction

The β_2-adrenergic receptor (β_2AR) is a G-coupled protein expressed on nearly all cell types in the lung (Carstairs et al. 1985; Spina et al. 1989; Green et al. 1994). With receptor distribution and density increasing with each successive airway generation (Carstairs et al. 1985; Spina et al. 1989), β_2ARs play a critical role in helping to maintain total alveolar surface area needed for gas exchange (Sakuma et al. 1994; Kerem et al. 1999; McGraw et al. 2001; Sartori et al. 2002; Mutlu et al. 2004). This is consistent with reports suggesting that while >90% of all

βAR expression in the lung is associated with approximately 300–500 million alveoli, the predominating subtype in this location is the β_2AR (Carstairs et al. 1985; Spina et al. 1989; Ochs et al. 2004).

However, as a consequence of Starling forces (Starling 1896), decreases in alveolar air volume followed by loss of alveolar surface area needed for gas exchange (e.g., convective and diffusive O_2 transport) can occur when fluid accumulates in alveoli. These coupled events may be provoked by exercise and/or stays in extreme environmental conditions (Kerem et al. 1999; Crandall and Matthay 2001; McGraw et al. 2001; Sartori et al. 2002; Snyder et al. 2006d, 2007). In these settings, when hydrostatic pressure of pulmonary capillaries is higher than that of the interstitial space accompanied by interstitial fluid accumulation that exceeds the rate of fluid removal, an influx of fluid into alveoli may occur (Starling 1896; Lauweryns and Baert 1977; Wallin and Leksell 1994; Kerem et al. 1999; Snyder et al. 2006c, 2007). Nevertheless, activation of the β_2AR second messenger pathway including downstream effects on epithelial sodium channels plays an important role in intra-alveolar fluid clearance and maintenance of total alveolar surface area needed for gas exchange (Dumasius et al. 2001; McGraw et al. 2001; Factor et al. 2002; Sartori et al. 2002; Mutlu et al. 2004; Snyder et al. 2007).

In otherwise healthy adults when abnormal lung fluid clearance occurs, this has been attributed to impaired β_2AR function linked to unique single-nucleotide polymorphisms (SNPs) at codon 16 of the β_2AR gene (ADRB2) (Snyder et al. 2007). Snyder et al. (2007) reported that compared with the homozygous glycine (Gly16Gly) β_2AR genotype, healthy adults homozygous for arginine (Arg16Arg) demonstrated reduced alveolar-capillary membrane conductance (D_M) coinciding with decreased lung fluid clearance following rapid intravenous infusions of saline at rest. Whether observations at rest (Snyder et al. 2007) involving pulmonary limitations to gas exchange (e.g., O_2 transfer) and SNPs of the ADRB2 translates to coupling between β_2AR genotype with alveolar respiratory responses and substrate oxidative capacity during exercise remains unclear.

Provided the intrinsic sympathomimetic effect of exercise leads to activated β_2ARs, it could be expected that physiological changes in key components of gas exchange, O_2 transport, and oxidative capacity are not limited to independent effects associated with increased cardiac output ($\dot{Q}$), vasodilation, and so on (Kjaer et al. 1985; Liggett et al. 1988; Large et al. 1997; Snyder et al. 2006a; Wolfarth et al. 2007). Airway factors such as alveolar ventilation ($\dot{V}_A$) and alveolar volume (V_A) with respect to global lung responses of minute ventilation ($\dot{V}_E$) and tidal volume (V_T) also play important roles in gas exchange and O_2 transport (Farhi and Rahn 1955; Hey et al. 1966; Dempsey et al. 1984; Aaron et al. 1992). Thus, it is under these broad assumptions where ours and others' isolated genomics studies involving SNPs of the ADRB2 (Dishy et al. 2001; Garovic et al. 2003; Snyder et al. 2006a, 2007) can be taken to test the following hypothesis in this study: compared with healthy adults demonstrating the Gly16Gly or Arg16-Gly SNP for the ADRB2, there will be a reduced V_A driving an inadequate $\dot{V}_A$ response relative to both $\dot{V}_E$ and metabolic demand (i.e., gross and net substrate oxidation) during submaximal exercise in individuals expressing the Arg16Arg β_2AR genotype. This hypothesis generating study tested in healthy adults involving possible genotype↔phenotype interactions linking SNPs at codon 16 of the ADRB2 to alveolar mechanisms of O_2 transport and oxidative capacity has potential clinical translational implications for patients with advanced cardiopulmonary diseases for whom β_2ARs are targets for pharmacotherapies aimed to improve aerobic capacity (Nelson 1995; Wagoner et al. 2000; Snyder et al. 2006d).

Methods

Participants

Sixty-four Caucasian adults were recruited to participate in this study. All individuals provided written informed consent prior to study participation. All aspects of this study were reviewed and approved by the Mayo Clinic Institutional Review Board and conformed to the Declaration of Helsinki.

Careful review of medical records demonstrated no participant in this study was diagnosed with a cardiovascular, cardiopulmonary, or neuromuscular disease that would confound study interpretations. Participants were also nonsmokers, not pregnant, not on prescribed medications, and not dependent on alcohol or narcotics. Participants in this study were genotyped and stratified into groups according to SNPs at codon 16 of the ADRB2. Although we have previously studied this sample to test the influence of SNPs at codon 16 of the ADRB2 on cardiovascular responses to exercise (Snyder et al. 2006a), aims of this study constitute testing an original hypothesis, presentation of original data, and a logical next step in this research line. We studied 16, 31, and 17 healthy adults who were homozygous for Arg (Arg16Arg), homozygous for Gly (Gly16Gly), or heterozygous for Arg and Gly (Arg16Gly), respectively, at codon 16 of the ADRB2.

Protocol overview

Participants arrived at the General Clinical Research Center (GCRC) for a baseline screening visit where a

pregnancy test was administered to women, blood testing for hemoglobin (Hb) and hematocrit (Hct) levels was given to rule out anemia, and resting flow volume loop spirometry was performed to assess airway function according to the guidelines of the American Thoracic Society (Miller et al. 2005). Participants also performed an incremental cardiopulmonary exercise test (CPET) to assess peak exercise workload, which was confirmed during a mirrored second CPET performed on study visit 2. Test-retest reliability of our CPET from study day 1 to 2 was strong [Intraclass correlation coefficient (Weir 2005; Van Iterson et al. 2017a) across the sample for peak workload between study days 1 and 2 was 0.98 with lower and upper 95% confidence limits (CL): 0.95, 0.99]. Peak workload was used to determine submaximal exercise workloads to be performed for the final visit (study day 3).

Because it is suggested variance in dietary sodium levels can confound the interpretation of β_2AR function (Kotanko et al. 1992), study visit 3 occurred while maintaining a salt-neutral diet as described in detail in Snyder et al. (2006a). With respect to SNPs at codon 16 of the ADRB2, the primary objective of visit 3 for this study was to compare responses pertaining to $\dot{V}_A$ and alveolar and arterial O_2 tensions with respect to metabolic demand. This was accomplished by having participants perform 18 consecutive minutes of submaximal cycle ergometry at two separate blocked workloads set at 40% and 75% of peak workload (determined from CPET).

Data collection

Genotyping

A complete description of the protocol used to genotype codon 16 of the ADRB2 using the polymerase chain reaction (PCR) method is presented in Snyder et al. (2006a) and based on techniques of Bray et al. (2000). Therefore, in brief, the following primer sequences used, forward and backward, were 5'-AGC CAG TGC GCT TAC CTG CCA GAC-3' (at –32) and 3'-CA TGG GTA CGC GGC CTG GTG CTG CAG TGC –5', respectively. This resulted in a PCR product 107 base-pairs in length. As such, the Arg16Arg genotype is represented by a single 107 base-pair band; the Arg16Gly genotype is represented by 25-, 82-, and 107 base-pair bands; and the Gly16Gly genotype is represented by 82- and- 260 base-pair bands.

Pulmonary function

Resting pulmonary function was assessed using flow-volume loop spirometry (CPFS system spirometer, Medical Graphics, St. Paul, MN) in the upright seated position according to guidelines of the American Thoracic Society (Miller et al. 2005). In addition to measuring forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV_1), percent of predicted FVC and FEV_1 were calculated according to equations of Crapo et al. (1981). We calculated maximum voluntary ventilation (MVV) as the product of FEV_1 and 40 (Miller et al. 2005).

Exercise testing

Participants were studied in the postabsorptive state and absence of caffeine ingestion. With continuous rhythm and heart rate monitoring via 12-lead electrocardiogram, participants performed a step-wise CPET to volitional fatigue via upright cycle ergometry (Corival Lode B.V., Netherlands). Testing began with a 3 min rest period followed immediately by a 3 min exercise workload period set at 40 W, increasing thereafter in 40 W increments every 3 min until volitional fatigue (American Thoracic S, and American College of Chest P, 2003; Van Iterson et al. 2017c). Participants were asked to maintain a pedal cadence of 60–65 rpm throughout CPET. An inability of participants to maintain a pedal cadence of 60–65 rpm, a rating of perceived exertion (RPE, Borg 6–20 scale) at the end of an exercise stage ≥17, and/or respiratory exchange ratio (RER) ≥1.10 were closely monitored throughout CPET and were used to assess when peak exercise was achieved (American Thoracic S, and American College of Chest P, 2003; Van Iterson et al. 2017c). Percent of predicted $\dot{V}O_{2peak}$ was calculated using equations of Hansen et al. (1984).

Submaximal cycle ergometry performed on study visit 3 was performed for 18 consecutive min at a pedal cadence of 60–65 rpm and relative workload intensities equivalent to 40% and 75% of peak workload determined from CPET. Following an initial rest period of 3 min, participants transitioned to exercise at 40% of peak workload for 9 consecutive min immediately transitioning thereafter to a workload equivalent of 75% of peak workload for 9 more min. In addition to continuous breath-by-breath measurements of ventilation and gas exchange throughout exercise, arterial draws were performed during steady-state exercise to assess blood gases (described below).

Ventilation and gas exchange measurements

Standard breath-by-breath measurements of ventilation (minute ventilation [$\dot{V}_E$]), volumes (tidal volume [V_T]), and gas exchange ($\dot{V}O_2$ and carbon dioxide output [$\dot{V}CO_2$]) variables occurred continuously throughout all exercise testing in an environmentally controlled human physiological laboratory (FIO_2 = 0.2093 ± 0.0001; room

temperature did not fluctuate more than ± 1°C from 21°C). These variables were acquired using an open circuit indirect calorimetry system (Medical Graphics, St. Paul, MN) customized to sample respired gas fractions in alignment with volume flows via custom software integrated with gas mass spectroscopy (Perkin Elmer MGA-1100, Wesley, MA). Sampling of respired gas fractions using this system has been validated in our laboratory against the Douglas bag technique (Proctor and Beck 1996). Relevant for study visit 3, data from respired gas fractions and arterial gases were used in "ideal" alveolar air equations (Riley and Cournand 1951; Van Iterson et al. 2017b) for the calculation of $\dot{V}_A$ and related variables (see Appendix 1). Calibration of the system using medical grade gases and linearity of the system flowmeter via 3 L syringe across a range of flows was performed using standard routines in the set-up used for testing immediately prior to each exercise test.

Arterial sampling

For calculations relevant to $\dot{V}_A$ [i.e., "ideal" alveolar air equations (Riley and Cournand 1951; Van Iterson et al. 2017b), see Appendix 1] arterial draws were temporally aligned with the 30 sec averaged periods for variables of interest at rest as well as near the end of each 3 min interval throughout submaximal exercise on study visit 3. Temporal alignment of non-invasive and invasive data in this manner is suggested to be accurate during steady-state exercise (Furuike et al. 1982). Accordingly, using standard technique at the left radial artery, percutaneous insertion of a 20-gauge indwelling catheter (Arrow International, Reading, PA) with thermistor was used to draw arterial samples. Arterial samples were drawn into 3 mL heparinized glass syringes and immediately rolled and placed in ice to be transported to the Mayo Clinic institutional Clinical Core Laboratory [meets all routine standards of clinical blood-gas laboratory (Davis et al. 2013)] for measurements of CO_2 tension ($PaCO_2$), O_2 tension (PaO_2), and Hb oxygen saturation (SaO_2). We used the equation, $(0.0134 \times Hb \times SaO_2) + (0.0031 \times PaO_2)$, to calculate CaO_2. There were no between group differences for inspired tension of O_2 (PIO_2) on study visit 3 (143 ± 2, 142 ± 1, and 143 ± 1 mmHg for Arg16Arg, Arg16Gly, and Gly16Gly, respectively, $P > 0.05$).

Metabolic computations

Steady-state mean values for $\dot{V}O_2$ and $\dot{V}CO_2$ representing the final 30 sec of both low (40%) and moderate (75%) intensity exercise periods were used to compute gross metabolic demand as nonprotein substrate oxidation (Brouwer 1957; Coyle et al. 1992; Moseley and Jeukendrup 2001). As such, we quantified exercise economy (EC, i.e., a lower value is worse) as the ratio of work accomplished per L/(min·m^2) of $\dot{V}O_2$ expressed in units of kcal/(L·m^2) as (Moseley and Jeukendrup 2001): workload ÷ $\dot{V}O_2$ where workload is W converted to kcal/min. We also quantified net EC (EC_{NET}, i.e., a higher value is worse) as the absolute difference between energy expended (EE) and work accomplished per L/(min·m^2) of $\dot{V}O_2$ expressed in units of kcal/(L·m^2). We calculated EE in units of kcal/min as in Brouwer (1957):

$$([(\dot{V}O_2 \times 3.869) + (\dot{V}CO_2 \times 1.195) \times (4.186 \div 60) \times 1000 \times 4.2] \div 1000) \times 60$$

Statistical analyses

Data are presented as mean ± SD with 95% confidence limits (CL) where appropriate. All data met assumptions of normality of distribution and homogeneity of variance. The group effect for demographic data was assessed using single-factor ANOVA or Kruskal–Wallis tests with post hoc testing performed using the Tukey–Kramer or Wilcoxon rank sum test, respectively, to identify pairwise differences when the overall group effect was significant.

Data reported and used for statistical analyses with respect to submaximal exercise variables is reflective of steady-state mean values taken from the final 30 s of the low (40%) and moderate (75%) intensity exercise periods. Between group differences were assessed using repeated measures single-factor ANOVA tests. Only when the *F*-test statistic was significant from ANOVA testing did we assess planned pairwise differences using the Tukey–Kramer post hoc test. Where applicable, least squares univariate linear regression models were used to assess the behavior of physiological relationships for β_2AR genotypes [e.g., between $\dot{V}_E$ (independent) and $\dot{V}_A$ (dependent)]. Two-tailed significance was determined using an alpha level set at 0.05. All computations were performed using SAS statistical software (v.9.4., Cary, North Carolina).

Results

Participants

Table 1 illustrates there was no overall group effect for gender, age, height, weight, BMI, BSA, Hb, or Hct. There was also no overall group effect for MVV and resting measurements of absolute or percent of predicted FVC or FEV_1. All participants reached peak exercise during CPET indicated by both RER and RPE (Table 1). Although the

Table 1. Participant characteristics.

	Arg16Arg (N = 16)	Arg16Gly (N = 17)	Gly16Gly (N = 31)	P
% male	44	47	52	0.88
Age, years	29 ± 6 (26, 32)	28 ± 6 (25, 31)	29 ± 6 (27, 32)	0.85
Height, cm	171 ± 9 (167, 176)	176 ± 10 (171, 181)	174 ± 10 (170, 178)	0.41
Weight, kg	67 ± 12 (61, 73)	76 ± 14 (67, 82)	75 ± 13 (70, 80)	0.14
BMI, kg/m^2	23 ± 3 (21, 24)	24 ± 3 (22, 25)	25 ± 4 (23, 26)	0.21
BSA, m^2	1.79 ± 0.05 (1.68, 1.87)	1.91 ± 0.05 (1.78, 2.01)	1.89 ± 0.04 (1.81, 1.97)	0.16
Hemoglobin, g/dL	13.4 ± 1.3 (12.6, 13.9)	13.7 ± 1.1 (13.1, 14.3)	13.7 ± 1.1 (13.2, 14.1)	0.48
Hematocrit, %	39 ± 3 (37, 40)	40 ± 3 (38, 41)	40 ± 3 (38, 42)	0.45
Resting Pulmonary function				
FVC, L	4.5 ± 0.9 (4.0, 5.0)	5.2 ± 1.2 (4.6, 5.9)	5.0 ± 1.1 (4.6, 5.4)	0.16
FVC, %pred.	99 ± 13 (92, 105)	105 ± 9 (100, 110)	102 ± 9 (98, 105)	0.25
FEV_1, L	3.7 ± 0.7 (3.4, 4.1)	4.1 ± 0.8 (3.7, 4.6)	4.2 ± 0.9 (3.8, 4.5)	0.21
FEV_1, %pred.	99 ± 13 (92, 105)	100 ± 9 (95, 105)	103 ± 10 (99, 107)	0.35
MVV, L/min	149 ± 27 (135, 163)	165 ± 34 (148, 183)	166 ± 37 (152, 180)	0.21
Peak exercise				
$\dot{V}O_2$, L/(min·m^2)	1.2 ± 0.3 (1.1, 1.4)	1.4 ± 0.3 (1.3, 1.6)	1.4 ± 0.3 (1.3, 1.5)	0.11
$\dot{V}O_2$, %pred.	86 ± 23 (74, 98)	98 ± 29 (82, 114)	91 ± 22 (82, 100)	0.41
Workload, W	185 ± 56 (156, 214)	244 ± 64 (209, 278)	224 ± 78 (194, 254)	0.06
HR, bpm	189 ± 10 (184, 194)	182 ± 9 (177, 187)	188 ± 10 (184, 192)	0.07
$\dot{V}_E$, L/min	87 ± 31 (71, 103)	100 ± 25 (87, 114)	102 ± 31 (90, 113)	0.24
$\dot{V}_E$, %MVV	57 ± 12 (51, 63)	61 ± 11 (55, 67)	61 ± 12 (57, 66)	0.49
f_B, breaths/min	44 ± 9 (39, 48)	41 ± 7 (37, 45)	40 ± 7 (37, 43)	0.31
V_T, L	1.98 ± 0.56 (1.69, 2.26)	2.50 ± 0.66 (2.15, 2.85)	2.56 ± 0.73 (2.29, 2.84)[1]	0.02
RER	1.17 ± 0.06 (1.13, 1.20)	1.15 ± 0.05 (1.12, 0.17)	1.15 ± 0.06 (1.13, 1.18)	0.44
RPE	19 ± 0 (19, 19)	19 ± 0 (19, 19)	19 ± 0 (19, 19)	0.07
Submaximal exercise workload and energy expended				
Low, W	70 ± 20 (60, 81)	86 ± 22 (74, 98)	84 ± 28 (73, 94)	
EE, kcal/min	5.7 ± 1.7 (4.9, 6.6)	6.5 ± 1.4 (5.8, 7.2)	6.4 ± 1.6 (5.8, 7.1)	
Moderate, W	139 ± 43 (116, 163)	168 ± 45 (144, 192)	169 ± 60 (145, 192)	
EE, kcal/min	11.0 ± 3.5 (9.1, 12.8)	13.0 ± 3.2 (11.3, 14.7)	13.7 ± 4.7 (11.8, 15.5)[1]	

Data are mean ± SD and 95% lower and upper confidence limits (CL), or otherwise noted. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; MVV, maximum voluntary ventilation; $\dot{V}O_2$, pulmonary O_2 uptake; HR, heart rate; $\dot{V}_E$, minute ventilation; f_B, breathing frequency; V_T, tidal volume; RER, respiratory exchange ratio; RPE, rating of perceived exertion (Borg, 6–20 scale); EE, energy expended. Repeated measures ANOVA for group effect on submaximal exercise workload, F[91], $P < 0.001$; there were no pairwise differences at an alpha level of 0.05 after Tukey–Kramer post hoc correction. Test-retest reliability of our CPET was strong [Intraclass correlation coefficient across the sample for peak workload between study 1 and 2 was 0.98 with lower and upper 95% CL: 0.95, 0.99]. P-values in table are overall group effect from ANOVA testing.

[1] $P < 0.05$, Arg16Arg versus Gly16Gly after Tukey–Kramer post hoc correction.

group effect was not significant for peak exercise workload, the Arg16Gly group achieved the highest W. However, except for V_T, there was no overall group effect for $\dot{V}O_2$ (both L/(min·m^2) and percent of predicted), heart rate, $\dot{V}_E$, %MVV, or breathing frequency (f_B) associated with baseline CPET.

Submaximal exercise testing

Although there were no pairwise statistical differences for exercise workload, at both low and moderate intensity Arg16Arg demonstrated the lowest W (Table 1). In contrast, there was a group effect for gross metabolic demand (i.e., nonprotein substrate oxidation during exercise) (F[30], $P < 0.001$), which resulted in significantly lower absolute EE in Arg16Arg compared with Gly16Gly during moderate intensity, but not at low intensity (Table 1). Overall, there were also significant group effects for β_2AR genotype on $\dot{V}_A$, $\dot{V}_A$ as a percentage of $\dot{V}_E$ ($\dot{V}_A/\dot{V}_E$), $\dot{V}_A$ as a percentage of MVV ($\dot{V}_A$/MVV), and physiological dead space to tidal volume ratio (V_D/V_T) in Figure 1; $\dot{V}O_2$, alveolar-to-arterial O_2 tension difference ($PA\text{-}aO_2$) as a quotient with $\dot{V}O_2$ ($PA\text{-}aO_2/\dot{V}O_2$), EC, and EC_{NET} in Figure 2; and $\dot{V}_E$, f_B, V_T, V_A, V_A as percentage of resting FVC (V_A/FVC), alveolar O_2 tension (PAO_2), alveolar CO_2 tension ($PACO_2$),

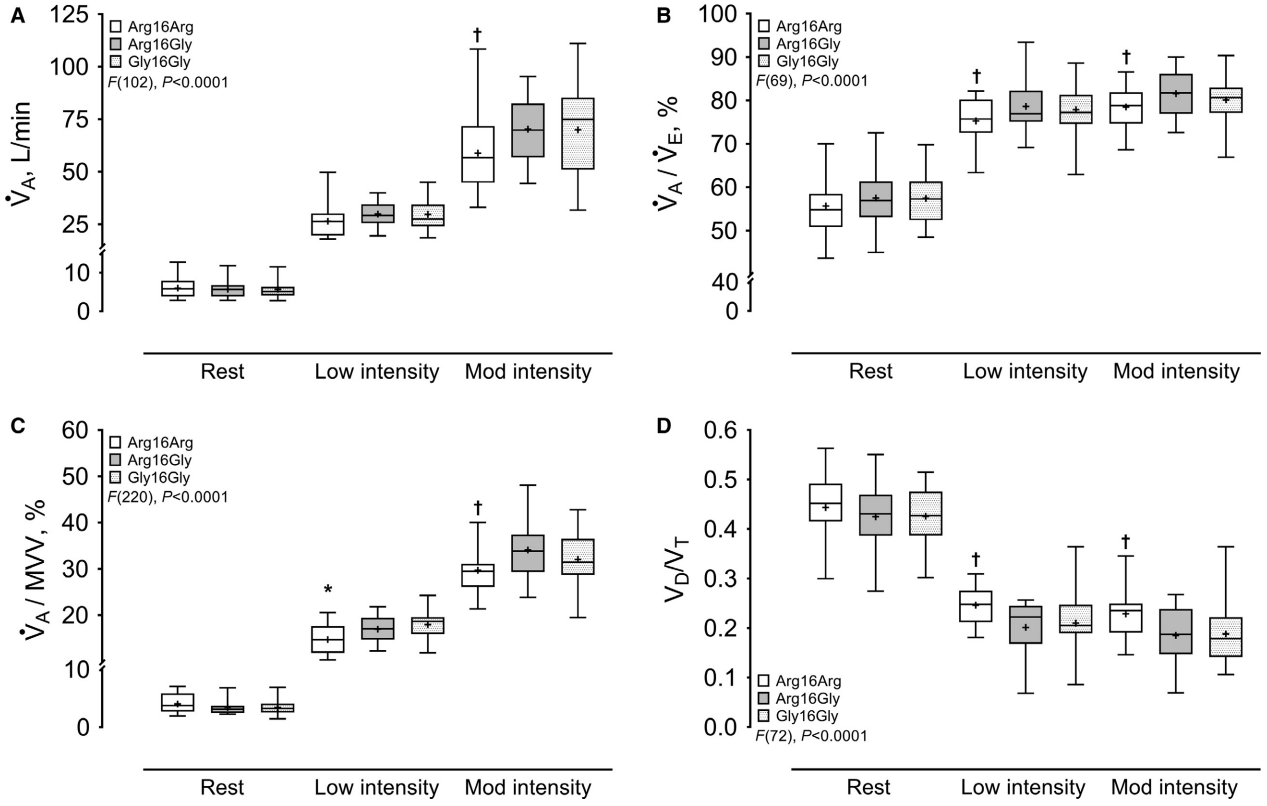

Figure 1. Respiratory responses to low (40% peak workload) and moderate (75% peak workload) intensity exercise in healthy adults stratified by SNPs at codon 16 of ADRB2. N = 16, homozygous for amino acid arginine (Arg16Arg); N = 17, heterozygous for arginine and glycine (Arg16Gly); N = 31, homozygous for glycine (Gly16Gly). Data are interquartile range with the group means indicated by (+). (A) alveolar ventilation, $\dot{V}_A$; (B) $\dot{V}_A$ as a percentage of total minute ventilation, $\dot{V}_A/\dot{V}_E$; (C) $\dot{V}_A$ as a percentage of maximum voluntary ventilation $\dot{V}_A$/MVV; (D) Physiological dead space to tidal volume ratio, V_D/V_T. *P < 0.05, Arg16Arg versus Gly16Gly; †P < 0.05, Arg16Arg versus both Arg16Gly and Gly16Gly. Significance following Tukey–Kramer post hoc correction.

$PaCO_2$, CaO_2, PA-aO_2, and SaO_2 in Table 2 (but not for PaO_2).

Low intensity

There were no pairwise differences for any variable at rest. In contrast, during low intensity exercise at 40% of peak workload, Arg16Arg demonstrated significantly smaller V_A and V_A/FVC compared with Gly16Gly, whereas V_T and CaO_2 trended (P = 0.10 and P = 0.06) lower in Arg16Arg versus Gly16Gly (Table 2). Arg16Arg also demonstrated significantly reduced V_A/MVV in comparison with Gly16Gly (Fig. 1C), whereas V_D/V_T was significantly larger in Arg16Arg compared with both Arg16Gly and Gly16Gly (Fig. 1D). Figure 1B also illustrates V_A/V_E was significantly reduced in Arg16Arg compared with both Arg16Gly and Gly16Gly. Figure 1A shows V_A trended lower in Arg16Arg in comparison with both Arg16Gly and Gly16Gly (P = 0.11 and P = 0.09, respectively). Likewise, though VO_2 trended lower in Arg16Arg versus Arg16Gly in Figure 2A (P = 0.12), EC and EC_{NET} were significantly reduced and increased, respectively, in Arg16Arg compared with Arg16Gly in Figure 2 (panels C and D). Whereas, similar $\dot{V}O_2$ in Arg16Arg and Gly16Gly was accompanied by a pattern of decreased and increased EC and EC_{NET}, respectively, between Arg16Arg (P = 0.10) and Gly16Gly (P = 0.11) (Fig. 2). There were no pairwise group differences for the remaining variables in Table 2 or Figures 1 and 2 at low intensity exercise.

Consistent with rest in Figure 3A, the relationship (coefficient of determination, R^2) between $\dot{V}_E$ (independent) and $\dot{V}_A$ (dependent) during low intensity exercise was significant across the entire sample in Figure 3B. Likewise, individual R^2 for these relationships were equally strong for Arg16Arg (R^2 = 0.96, P < 0.001), Arg16Gly (R^2=0.93, P < 0.001), and Gly16Gly (R^2=0.94, P < 0.001). However, consistent with reduced V_A and V_A/FVC for Arg16Arg in Table 2, the extended response of V_A in driving further increases in $\dot{V}_A$ beyond contributions from f_B was not as strong for Arg16Arg compared with both Arg16Gly and

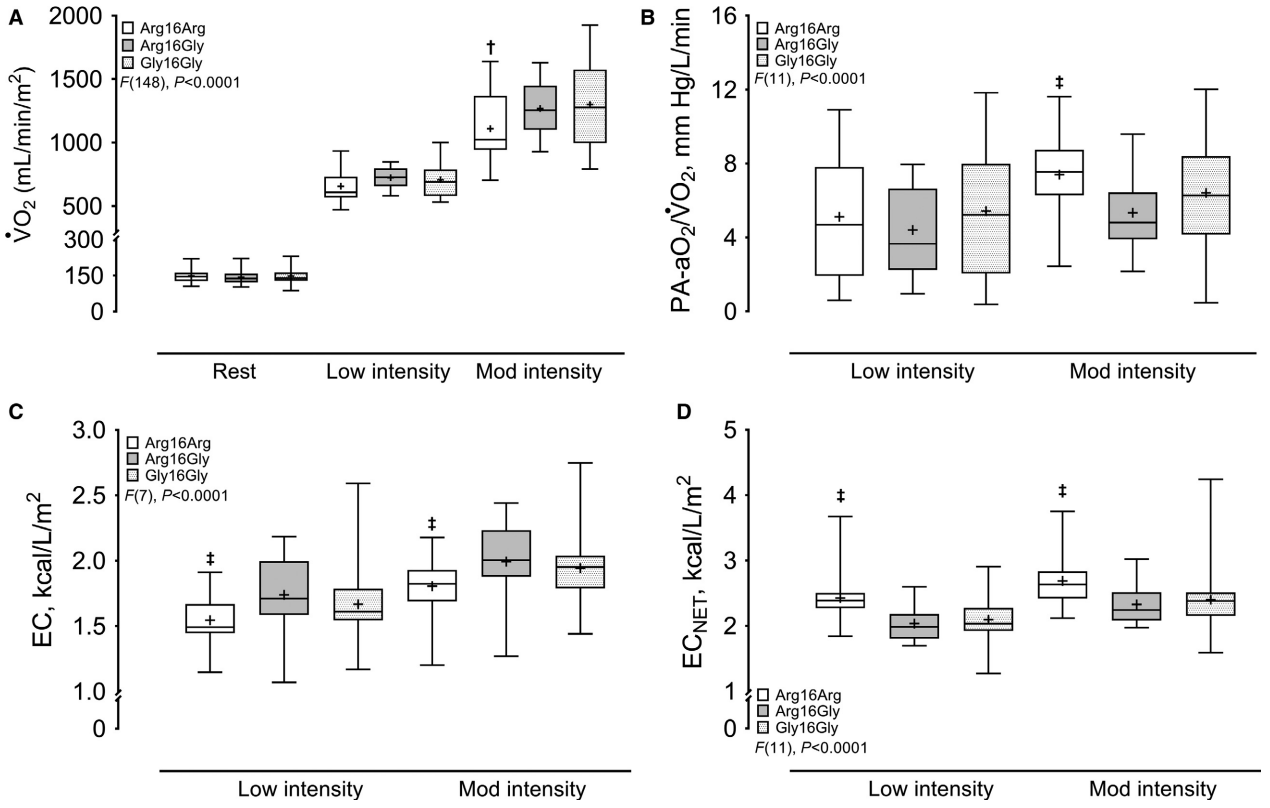

Figure 2. Oxygen uptake and gross metabolic demand during low (40% peak workload) and moderate (75% peak workload) intensity exercise in healthy adults stratified by SNPs at codon 16 of the ADRB2. N = 16, homozygous for amino acid arginine (Arg16Arg); N = 17, heterozygous for arginine and glycine (Arg16Gly); N = 31, homozygous for glycine (Gly16Gly). Data are interquartile range with the group means indicated by (+). A) pulmonary O_2 uptake, $\dot{V}O_2$; (B) quotient of alveolar-to-arterial O_2 tension gradient with $\dot{V}O_2$, PA-aO_2/$\dot{V}O_2$; (C) exercise economy, EC; (D) net exercise economy, EC_{NET}. †$P < 0.05$, Arg16Arg versus both Arg16Gly and Gly16Gly; ‡$P < 0.05$, Arg16Arg versus Arg16Gly. Significance following Tukey–Kramer post hoc correction.

Gly16Gly [standardized β with *95%* CL (i.e., slope) for $V_A \rightarrow \dot{V}_A$ relationships were: 0.57 (0.11, 0.82), 0.74 (0.37, 0.90), and 0.78 (0.58, 0.89), respectively]. This is also illustrated in gray isopleths as progressively steeper slopes for $\dot{V}_E \rightarrow \dot{V}_A$ relationships when we constrained f_B at modest-to-moderate levels (15 and 25 breaths/min) (Fig. 3B).

Moderate intensity

For exercise at 75% of peak workload, Arg16Arg demonstrated significantly lower $\dot{V}_A$ (Fig. 1A), V_T, V_A, and V_A/FVC compared with both Arg16Gly and Gly16Gly (Table 2). Likewise, consistent with significant pairwise differences for $V_A/\dot{V}_E$ and $\dot{V}_A$/MVV (Fig. 1, panels B and C, respectively), Figure 1D illustrates V_D/V_T was significantly larger in Arg16Arg compared with both Arg16Gly and Gly16Gly. This was accompanied by significantly reduced $\dot{V}O_2$ in Arg16Arg compared with both Arg16Gly and Gly16Gly in Figure 2A. In contrast, PA-aO_2/$\dot{V}O_2$ was significantly increased for Arg16Arg compared with Arg16Gly in Figure 2B, but did not differ when Arg16Arg was compared with Gly16Gly ($P = 0.43$). While CaO_2 also did not differ significantly across groups, there was a pattern for lower values in Arg16Arg versus Arg16Gly or Gly16Gly ($P = 0.11$ and $P = 0.15$, respectively; Table 2). However, consistent with differences at low intensity exercise, EC and EC_{NET} were significantly reduced and increased, respectively, for Arg16Arg compared with Arg16Gly in Figure 2, whereas these variables did not differ between Arg16Arg versus Gly16Gly ($P = 0.29$ and $P = 0.24$, respectively). There were no other pairwise group differences for variables presented in Table 2 or Figures 1 and 2 for moderate intensity exercise.

The strength of the relationship between $\dot{V}_E$ (independent) and $\dot{V}_A$ (dependent) across the entire sample at low intensity exercise in Figure 3B persisted to moderate intensity exercise in Figure 3C. Individual R^2 between $\dot{V}_E$ and $\dot{V}_A$ were also strong for Arg16Arg ($R^2 = 0.94$, $P < 0.001$), Arg16Gly ($R^2 = 0.94$, $P < 0.001$), and Gly16Gly ($R^2 = 0.93$, $P < 0.001$). However,

Table 2. Basic ventilation, alveolar air, and arterial blood responses across genotypes for the β_2AR.

	Arg16Arg (N = 16)	Arg16Gly (N = 17)	Gly16Gly (N = 31)
Rest			
$\dot{V}_E$, L/min	11 ± 4 (8, 13)	10 ± 3 (8, 11)	10 ± 4 (8, 11)
f_B, breaths/min	16 ± 4 (13, 18)	15 ± 5 (12, 17)	14 ± 3 (13, 15)
V_T, L	0.74 ± 0.44 (0.51, 0.97)	0.75 ± 0.32 (0.55, 0.95)	0.72 ± 0.39 (0.61, 0.84)
V_A, L	0.43 ± 0.28 (0.28, 0.57)	0.45 ± 0.30 (0.29, 0.61)	0.42 ± 0.20 (0.34, 0.50)
V_A/FVC, %	9.6 ± 6.2 (6.4, 12.8)	8.6 ± 5.0 (6.0, 11.3)	8.4 ± 3.2 (7.2, 9.7)
PAO_2, mmHg	101 ± 11 (95, 106)	98 ± 8 (94, 103)	98 ± 7 (95, 101)
PaO_2, mmHg	96 ± 14 (90, 104)	95 ± 8 (91, 100)	95 ± 11 (91, 99)
$PACO_2$, mmHg	33 ± 5 (31, 35)	34 ± 3 (32, 36)	34 ± 3 (33, 35)
$PaCO_2$, mmHg	34 ± 5 (32, 37)	36 ± 4 (33, 38)	36 ± 4 (34, 37)
CaO_2, mL/dL	18.6 ± 1.9 (17.7, 19.6)	19.4 ± 1.7 (18.5, 20.3)	19.4 ± 1.9 (18.7, 20.1)
PA-aO_2, mmHg	6 ± 3 (4, 8)	4 ± 3 (2, 5)	5 ± 4 (4, 7)
SaO_2, %	98 ± 1 (97, 98)	98 ± 0 (97, 98)	98 ± 1 (97, 98)
Low intensity exercise			
$\dot{V}_E$, L/min	35 ± 9 (30, 39)	37 ± 7 (34 41)	37 ± 8 (35, 40)
f_B, breaths/min	26 ± 6 (23, 29)	26 ± 5 (23, 29)	25 ± 5 (23, 27)
V_T, L	1.37 ± 0.40 (1.17, 1.58)	1.50 ± 0.44 (1.27, 1.73)	1.60 ± 0.49 (1.41, 1.78)
V_A, L	1.04 ± 0.30 (0.88, 1.19)[1]	1.21 ± 0.43 (0.98, 1.44)	1.28 ± 0.46 (1.10, 1.45)
V_A/FVC, %	22.0 ± 5.3 (19.2, 24.7)[1]	23.6 ± 5.9 (20.4, 26.7)	25.3 ± 6.0 (23.0, 27.5)
PAO_2, mmHg	104 ± 5 (101, 106)	103 ± 3 (100, 104)	104 ± 4 (102, 105)
PaO_2, mmHg	99 ± 5 (96, 101)	99 ± 8 (94, 103)	98 ± 6 (96, 100)
$PACO_2$, mmHg	36 ± 3 (35, 38)	36 ± 3 (34, 37)	36 ± 3 (35, 37)
$PaCO_2$, mmHg	37 ± 3 (35, 38)	37 ± 3 (35, 38)	36 ± 3 (35, 37)
CaO_2, mL/dL	19.2 ± 1.8 (18.2, 20.1)	20.0 ± 1.8 (19.0, 21.0)	20.2 ± 1.8 (19.6, 20.9)
PA-aO_2, mmHg	6 ± 4 (4, 8)	6 ± 3 (4, 8)	7 ± 5 (5, 10)
SaO_2, %	98 ± 1 (97, 98)	97 ± 0 (97, 97)	97 ± 1 (97, 98)
Moderate intensity exercise			
$\dot{V}_E$, L/min	75 ± 26 (61, 90)	86 ± 19 (76, 97)	87 ± 27 (76, 97)
f_B, breaths/min	40 ± 7 (36, 44)	38 ± 6 (34, 41)	37 ± 8 (34, 41)
V_T, L	1.85 ± 0.48 (1.59, 2.10)[2]	2.37 ± 0.66 (2.02, 2.72)	2.38 ± 0.75 (2.08, 2.67)
V_A, L	1.43 ± 0.37 (1.24, 1.62)[2]	1.95 ± 0.61 (1.62, 2.28)	1.93 ± 0.65 (1.68, 2.19)
V_A/FVC, %	32.0 ± 4.7 (29.5, 34.5)[2]	36.9 ± 6.4 (33.5, 40.3)	38.0 ± 7.7 (35.0, 41.1)
PAO_2, mmHg	113 ± 4 (111, 115)	112 ± 4 (110, 114)	112 ± 4 (110, 113)
PaO_2, mmHg	101 ± 8 (96, 105)	101 ± 10 (96, 106)	98 ± 9 (95, 102)
$PACO_2$, mmHg	31 ± 4 (30, 33)	31 ± 3 (29, 32)	32 ± 5 (30, 34)
$PaCO_2$, mmHg	31 ± 4 (29, 33)	31 ± 3 (28, 32)	32 ± 3 (30, 33)
CaO_2, mL/dL	19.9 ± 1.9 (18.9, 20.9)	21.0 ± 1.8 (20.0, 22.0)	20.7 ± 1.9 (20.0, 21.4)
PA-aO_2, mmHg	14 ± 5 (11, 17)	12 ± 5 (10, 15)	16 ± 8 (13, 19)
SaO_2, %	97 ± 1 (96, 97)	97 ± 0 (96, 97)	97 ± 1 (96, 97)

Data are mean ± SD with lower and upper 95% confidence limits (CL) in parentheses. Low (40% peak workload) or moderate (75% peak workload) intensity exercise. *F*-statistic from ANOVA for: minute ventilation ($\dot{V}_E$, F[128], $P < 0.0001$); breathing frequency (f_B, F[77], $P < 0.0001$); tidal volume (V_T, F[54], $P < 0.0001$); alveolar volume (V_A, F[58], $P < 0.0001$); V_A as percentage resting forced vital capacity (V_A/FVC, F[80], $P < 0.0001$); alveolar O_2 tension (PAO_2, F[54], $P < 0.0001$); arterial O_2 tension (PaO_2, F[1.3], $P = 0.28$); alveolar CO_2 tension ($PACO_2$, F[12], $P < 0.0001$); arterial CO_2 tension ($PaCO_2$, F[25], $P < 0.0001$); arterial O_2 content (CaO_2, F[48], $P < 0.0001$); alveolar-to-arterial O_2 difference (PA-aO_2, F[8], $P < 0.0001$); and arterial saturation (SaO_2, F[4.5], $P < 0.0001$).

[1] $P < 0.05$, Arg16Arg versus Gly16Gly.

[2] $P < 0.05$, Arg16Arg versus both Arg16Gly and Gly16Gly. Significance following Tukey–Kramer post hoc correction.

consistent with relationships in Figure 3B and absolute values in Table 2, the blunted contribution of V_A to the $\dot{V}_E \rightarrow \dot{V}_A$ relationship when f_B was constrained at moderate-to-high levels (i.e., gray isopleths at 35 and 45 breaths/min, respectively) was indeed more depressed with increasing $\dot{V}_E$ for Arg16Arg in comparison with both Arg16Gly and Gly16Gly (Figure 3C). This was consistent with the standardized β (95% CL) (i.e., slopes) for individual $V_A \rightarrow \dot{V}_A$ relationships for Arg16Arg compared with both Arg16Gly and Gly16Gly [0.53 (0.05, 0.80), 0.83 (0.54, 0.94), and 0.83 (0.65, 0.91), respectively].

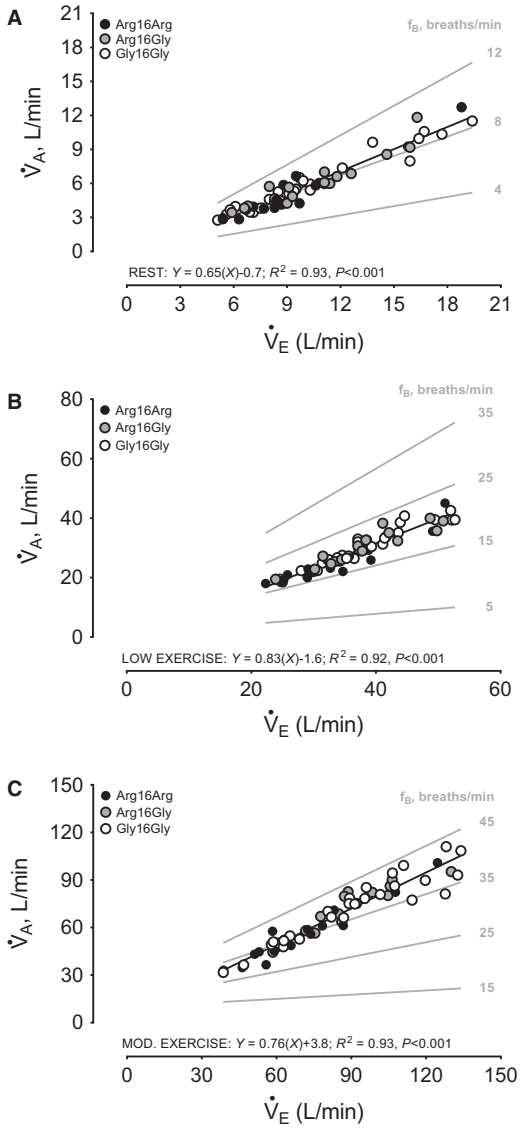

Figure 3. Least squares univariate linear regression between total minute ventilation ($\dot{V}_E$) (independent) and alveolar ventilation ($\dot{V}_A$) (dependent) during low (40% peak workload) and moderate (75% peak workload) intensity exercise in healthy adults stratified by SNPs at codon 16 of the ADRB2. $N = 16$, homozygous for amino acid arginine (Arg16Arg); $N = 17$, heterozygous for arginine and glycine (Arg16Gly); $N = 31$, homozygous for glycine (Gly16Gly). Solid black line is model goodness of fit for the regression across the entire sample. Grey lines are isopleths representing the expected $\dot{V}_A$ response for a given observed $\dot{V}_E$ response when breathing frequency (f_B) is constrained (grey numbers within plots) for a given observed alveolar volume (V_A) response. (A) Rest: Arg16Arg, $Y = 0.67(X)$-1.1, $P < 0.001$; Arg16Gly, $Y = 0.69(X)$-1.0, $P < 0.001$; Gly16Gly, $Y = 0.63(X)$-0.5, $P < 0.001$. (B) 40% peak workload: Arg16Arg, $Y = 0.74(X) + 0.5$, $P < 0.001$; Arg16Gly, $Y = 0.85(X)$-1.7, $P < 0.001$; Gly16Gly, $Y = 0.85(X)$-2.5, $P < 0.001$. (C) 75% peak workload: Arg16Arg, $Y = 0.74(X) + 2.6$, $P < 0.001$; Arg16Gly, $Y = 0.88(X)$-3.9, $P < 0.001$; Gly16Gly, $Y = 0.82(X) + 0.3$, $P < 0.001$.

Discussion

These data suggest that during low-to-moderate intensity aerobic exercise and for a given $\dot{V}_E$, healthy adults demonstrating the Arg16Arg SNP for the ADRB2 display a blunted rise in $\dot{V}_A$ attributable to disproportionately small V_A relative to f_B. Compared with both Arg16Gly and Gly16Gly β_2AR genotypes, Arg16Arg likewise demonstrated consistently larger V_D/V_T throughout exercise, whereas the most prominent rise in PA-aO_2/$\dot{V}O_2$ occurred during the moderate intensity period. While we did not, and were not expecting to observe severe or even moderate exercise induced arterial hypoxemia (SaO_2, <88% or 88–93%, respectively) given the present workload intensities coupled with an absence of cardiopulmonary disease, it is still consistent with these data that relative to each group a modest-to-moderate pattern of decreased CaO_2 occurred for adults demonstrating the Arg16Arg β_2AR genotype. In this context, and as hypothesis generating observations, these data suggest integrated responses of V_A (both absolute and relative to FVC), $\dot{V}_A$ (both absolute and relative to $\dot{V}_E$), V_D/V_T, PA-aO_2/$\dot{V}O_2$ (as a broad surrogate of lung diffusing capacity for O_2) (Morosin et al. 2016), and CaO_2 collectively trended in a direction consistent with supporting our study hypothesis. Though we also acknowledge that it cannot be unequivocally concluded based on our isolated genomics studies that the Arg16Arg SNP of the ADRB2 is fully responsible for the present physiological observations, these results further indicate that given the present study paradigm, compared with Arg16Gly and Gly16Gly β_2AR genotypes, Arg16Arg healthy adults do not demonstrate a similar capacity to drive V_A and $\dot{V}_A$ relative to substrate oxidative capacity and exercise economy.

Independent of our proposed effects of abnormal alveolar respiration, the inability to economically meet metabolic demands of submaximal exercise in Arg16Arg compared with Arg16Gly and Gly16Gly β_2AR genotypes in this study is broadly consistent with reports suggesting ~99% of β-adrenergic receptors in skeletal muscle are β_2ARs (Liggett et al. 1988) and in muscle diseases such as Myasthenia Gravis, there is an increased likelihood for patients demonstrating the Arg16Arg genotype (Xu et al. 2000). Accordingly, while those and other studies of skeletal muscle phenotypes and β_2ARs might be taken to imply limited oxidative capacity associated with the Arg16Arg variant may be directly attributable to skeletal muscle origins (Liggett et al. 1988; Xu et al. 2000; Wolfarth et al. 2007), this SNP for the ADRB2 has also been separately linked to reduced $\dot{Q}$ (attributed to blunted increases in stroke volume), β_2AR desensitization followed by increased vascular resistance, and decreased airway function at rest and/or during exercise in healthy adults

(Dishy et al. 2001; Garovic et al. 2003; Snyder et al. 2006a,b). This suggests that although skeletal muscle factors contribute to changes in oxidative capacity, which perhaps may or may not be underpinned by SNPs of the ADRB2 (Liggett et al. 1988; Xu et al. 2000; Wolfarth et al. 2007), it is also likely that β_2AR expression and function involving the whole body O_2 transport chain including cardiac, smooth, and skeletal muscle is of consequence to exercise capacity (Kjaer et al. 1985; Garovic et al. 2003; Snyder et al. 2006a,b).

Therefore, because in the lung there is a predominating expression of β_2ARs on alveolar tissue coupled with the role these receptors play in helping to maintain the alveolar surface area needed for gas exchange, for the first time, this study sought to assess in what manner might SNPs of the ADRB2 translate to coupled alveolar respiratory and metabolic responses to submaximal exercise in healthy adults. Though it is known β_2ARs are not directly responsible for facilitating the transfer of O_2 across the alveolar-capillary membrane, functional receptors expressed on alveolar tissue are critically needed for proper gas exchange required during exercise and/or stays in extreme environments (e.g., high altitude pulmonary edema) (Kerem et al. 1999; Crandall and Matthay 2001; McGraw et al. 2001; Sartori et al. 2002; Snyder et al. 2006d, 2007).

In this study, the period of moderate exercise performed by participants, despite not being of maximal intensity, has been reported by others as being an adequate stimulus for provoking modest-to-moderate lung fluid accumulation in some, but not all healthy adults (Coates et al. 1984; Koizumi et al. 2001; McKenzie et al. 2005; Snyder et al. 2006c). As such, while we hypothesize that contrasting alveolar respiratory responses during exercise in Arg16Arg compared with Arg16Gly and Gly16Gly variants in this study may have been attributable to abnormal alveolar β_2AR function and reduced total alveolar surface area in the former, we did not directly assess receptor function/density or measure lung fluid changes during exercise and thereby cannot confirm this genotype↔phenotype mechanism as the explanation for our observations. However, because the capacity to recruit V_T as well as V_A as a high proportion of V_T during exercise is preferred for facilitating gas exchange compared with excessive f_B (assuming adequate pulmonary blood volume/distribution in both instances) (Hey et al. 1966; Dempsey et al. 1984; Aaron et al. 1992; Kinker et al. 1992), the disproportionately lower $\dot{V}_A$ relative to $\dot{V}_E$ driven by decreased V_A in the Arg16Arg β_2AR genotype indeed suggests these individuals demonstrated a smaller total alveolar surface area available for O_2 transport compared with Arg16Gly and Gly16Gly variants.

In addition to potential effects of altered alveolar respiration on gas exchange and O_2 transport in adults demonstrating the Arg16Arg β_2AR genotype, we acknowledge that metabolic pathways involving changes to processes of both glycolysis and lipolysis have been separately linked to SNPs of the ADRB2 (Kjaer et al. 1985; Wahrenberg et al. 1987; Large et al. 1997). While not tested in this study, others suggest substitution of Arg for Gly at codon 16 of the ADRB2 (i.e., Gly16Gly or Arg16Gly) leads to increased β_2AR agonist affinity associated with adipocytes (Large et al. 1997). Thus, in theory, it is possible the Arg16Arg β_2AR genotype in this study indeed contributed to low receptor sensitivity to the sympathomimetic effects of exercise. Following could have been lesser than expected lipolytic function in Arg16Arg variants, and thereby a muted ability to preserve glucose for oxidation culminating in reduced peak workload and economy of substrate oxidation compared with Arg16Gly and Gly16Gly β_2AR genotypes (Kjaer et al. 1985; Wahrenberg et al. 1987; Large et al. 1997).

Our initial observations indeed suggest worse aerobic capacity (i.e., both workload and $\dot{V}O_2$) in Arg16Arg compared with Arg16Gly and Gly16Gly β_2AR genotypes, which could have been used to readily explain group differences for $\dot{V}_A$ and $\dot{V}O_2$ during subsequent exercise testing at low and modest relative workload intensities. Nevertheless, we highlight that along with decreased workload and $\dot{V}O_2$ during submaximal exercise, compared with Arg16Gly and Gly16Gly β_2AR genotypes, Arg16Arg variants also demonstrated reduced V_A, $\dot{V}_A/\dot{V}_E$, and V_A/FVC coupled with increased V_D/V_T. These collective respiratory responses in Arg16Arg variants do not resemble changes consistent with individuals performing the lowest workloads at modest or moderate intensity exercise in this study. Therefore, we suggest low external workload and potential effects of SNPs of the ADRB2 on metabolic pathways cannot by themselves explain unique responses of $\dot{V}O_2$, EC, EC_{NET}, and alveolar respiration (i.e., V_A, $\dot{V}_A/\dot{V}_E$, etc.) in adults demonstrating the Arg16Arg β_2AR genotype.

Limitations

In addition to not directly assessing β_2AR expression and function or measuring lung fluid changes during exercise, we acknowledge that we are unable to directly account for intramuscular factors related to microvasculature (e.g., convection, conduction, etc.) and bioenergetics (e.g., mitochondrial function/density, oxidative enzymes, etc.,) in the interpretation of our gross substrate oxidation data. Use of invasive (e.g., skeletal muscle biopsy) and noninvasive (e.g., near-infrared spectroscopy) methods in future work may help to refine the understanding of the

intersecting contributions of skeletal muscle bioenergetic adaptations involved in the O_2 transport chain influential to oxidative capacity as these factors relate with SNPs of the ADRB2. We also recognize that in addition to SNPs at codon 16 of the ADRB2 there are other SNPs at different codons that have been genotyped (e.g., position 27) (Large et al. 1997; Dishy et al. 2001), which may be influential as complex haplotype effects for the hypothesis tested in this study. Nevertheless, compared with the strength of proposed effects of SNPs at codon 16 of the ADRB2 on cardiopulmonary responses to exercise, based on evidence to date, we suggest potential independent influences of SNPs at codon 27 of the ADRB2 would not be expected to explain these data (Large et al. 1997; Dishy et al. 2001; Garovic et al. 2003; Snyder et al. 2006a,b). Our sample sizes respective of each SNP at codon 16 of the ADRB2 were powered to detect physiological differences associated with variability for this single allele (Snyder et al. 2006a). Lastly, we acknowledge that for there to be any possibility for the clinical translation of these data (e.g., heart failure, asthma, etc. (Spina et al. 1989; Van Iterson et al. 2015; Wagoner et al. 2000)), large scale follow-up studies in humans must be performed that include comprehensive genotyping of all allele interactions associated with the ADRB2 as they relate with exercise phenotypes.

Conclusions

These data suggest for the first time that for a given submaximal exercise $\dot{V}_E$, healthy adults expressing the Arg16Arg β_2AR genotype demonstrate blunted elevations in V_A and $\dot{V}_A$ coupled with reduced economy of substrate oxidation compared with Arg16Gly and Gly16Gly variants. Accordingly, because in the lung there is a predominating density and distribution of β_2ARs on alveolar tissue and there is a specific role these receptors play in helping to maintain alveolar surface area needed for proper gas exchange (Carstairs et al. 1985; McGraw et al. 2001; Sartori et al. 2002; Mutlu et al. 2004; Snyder et al. 2006d, 2007), these are hypothesis generating data suggesting the Arg16Arg SNP of the ADRB2 may be associated with decreased total alveolar surface area available for gas exchange during submaximal exercise in some, but not all healthy adults. Upon confirmation of mechanisms proposed in this study following completion of more advanced genomic and exercise phenotype studies in future work, there are potential clinical implications tied to the putative link between SNPs of the ADRB2 and oxidative capacity associated with alveolar respiration in patients with cardiopulmonary diseases (e.g., asthma, heart failure, etc. (Snyder et al. 2006d; Spina et al. 1989; Van Iterson et al. 2015; Wagoner et al. 2000)) for whom pharmacotherapies including β_2AR agonists or blockers are considered part of the routine standard of care.

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Conflict of Interest

The authors of this manuscript have no conflicts of interest to disclose.

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Appendix: 1

Calculation methods involving use of "ideal" alveolar air equations

By using arterial gas measurements and acquired breath-by-breath respiratory gas exchange and volume flow responses, "ideal" alveolar air equations and associated parameters accounting for body temperature [for $PaCO_2$; $PaCO_2 \times (10^{0.021\times(T\text{-}37)}$ (Siggaard-Andersen 1974); and for PaO_2 using equations of Severinghaus (Severinghaus 1979)] could be used to calculate the following variables (as discussed above in methods) (Riley and Cournand 1951; Severinghaus 1966; Van Iterson et al. 2017b):alveolar ventilation,

$$\dot{V}_A(\mathrm{BTPS}) = \frac{[0.760 \times (273 + \mathrm{T}) \div 273] \times \dot{V}O_2(\mathrm{STPD})}{PIO_2 - PaO_2}$$

alveolar volume,

$$V_A = \dot{V}_A(\mathrm{BTPS}) \div f_B$$

alveolar O_2 tension,

$$PAO_2 = PIO_2 + \frac{PACO_2 \times FIO_2 \times (1 - \mathrm{RER})}{100 \times \mathrm{RER}} - \frac{PACO_2}{\mathrm{RER}}$$

alveolar CO_2 tension,

$$PACO_2 = \frac{[0.760 \times (273 + T) \div 273] \times \dot{V}CO_2(STPD)}{\dot{V}_A(BTPS)}$$

respiratory exchange ratio,

$$RER = \frac{PACO_2 \times (1 - FIO_2)}{PIO_2 - PAO_2 - FIO_2 \times PACO_2}$$

inspired O_2 tension,

$$PIO_2 = FIO_2 \times (P_B - 47)$$

physiological dead space to tidal volume ratio,

$$\frac{V_D}{V_T} = (1 - \frac{[0.760 \times (273 + T) \div 273] \times \dot{V}CO_2(STPD)}{\dot{V}_E(BTPS) \times PACO_2})$$

For above parameters related to "ideal" alveolar air equations: $\dot{V}_A$ is alveolar ventilation; V_A is alveolar volume; f_B is breathing frequency; V_D is physiological dead space, V_T is tidal volume; P_B is barometric pressure; 47 is lung water vapor pressure; T is body temperature in °C; RER is respiratory exchange ratio at the lung; $PaCO_2$ is arterial CO_2 tension; $PACO_2$ is alveolar CO_2 tension; PAO_2 is alveolar O_2 tension; FIO_2 is inspired fraction of O_2 equal to room air at sea level; PIO_2 is inspired O_2 tension; $[0.760 \times (273 + T) \div 273]$ is the constant needed when computing partial pressure from fractional concentration involving both volumes/gas (STPD, standard temperature and pressure dry) and volumes/flows (BTPS, body temperature and pressure saturated) standards of measurement.