# Genetic Variation Of The β$_2$ Adrenergic Receptor Is Associated With Differences In Lung Fluid Accumulation In Humans.

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## Abstract

The beta-2 adrenergic receptors ($\beta_2$AR)play an important role in lung fluid regulation. Previous research has suggested that subjects homozygous for Arginine at amino acid 16 of the $\beta_2$AR (Arg16) may have attenuated receptor function relative to subjects homozygous for Glycine at the same amino acid (Gly16). We sought to determine if the Arg16Gly polymorphism of the $\beta_2$AR influenced lung fluid balance in response to rapid saline infusion. We hypothesized that subjects homozygous for Arg at amino acid 16 (n=14) would have greater lung fluid accumulation compared to those homozygous for Gly (n=15) following a rapid intravenous infusion of isotonic saline (30mls/kg over 17 minutes). Changes in lung fluid were determined using measures of lung density and tissue volume (CT imaging) and measures of pulmonary capillary blood volume (Vc) and alveolar-capillary conductance ($D_M$, determined from the simultaneous assessment of the diffusing capacities of the lungs for carbon monoxide and nitric oxide). The saline infusion resulted in elevated catecholamines in both genotype groups (Arg16= 283±117% vs. Gly16= 252±118%, p>0.05). The Arg16 group had a larger decrease in $D_M$ and increase in lung tissue volume and lung water post saline infusion relative to the Gly16 group ($D_M$ -13±14 vs 0±26%, p<0.05, lung tissue volume=13±11 vs 3±11%, lung water +90±66 vs +48±144ml, p=0.10, p<0.05, for Arg vs. Gly16, respectively, mean±SD). These data suggest that subjects homozygous for Arg at amino acid 16 of the $\beta_2$AR have a greater susceptibility for lung fluid accumulation relative to subjects homozygous for Gly at this position.

**Key Words: Genetics,Pulmonary Edema, Lung Water**

**Introduction**

There is a constant fluid flux in the lungs between the pulmonary capillaries, interstitial space, pulmonary lymphatics, and the alveoli with an estimated 3.8ml s/kg of total lung water in the normal human lung(49) . Although there appears to be a constant exchange of small amounts of fluid across the alveoli, the alveolar airspace must remain relatively free of fluid in order for adequate gas exchange to occur(18). There are multiple clinical and environmental conditions that may cause alterations in normal lung fluid balance. These include conditions such as congestive heart failure (CHF), where there is evidence that a low-level sub-clinical pulmonary edema may exist, even in stable, well managed patients(1, 17) Pulmonary  edema may also occur at high altitude where the onset of edema often becomes clinically significant after approximately 24 hours of exposure(14, 39).  With time, conditions such as CHF and exposure to altitude are contributory to changes in lung fluid balance; however, not all patients with CHF nor all sojourners to altitude develop pulmonary edema. This heterogeneity may be related to variability in genes involved in lung fluid regulation.

Fluid flux between the pulmonary capillaries, interstitial space, and lymphatics follow the forces described by Starling(46) .  If the hydrostatic pressure of the pulmonary capillaries is greater than the hydrostatic pressure of the interstitium, there will be an increased fluid transfer into the interstitial space. Lymph flow acts to remove fluid from the interstitial space which reduces the interstitial hydrostatic pressure and, therefore, plays an important role in preventing excessive alveolar fluid accumulation(22).  Fluid in the alveoli can increase if interstitial fluid accumulation

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becomes greater than interstitial removal. In addition to these factors regulating lung water, epithelial sodium channels (ENaC) on the apical portion of type II alveolar cells serve to actively clear fluid from the alveoli(13, 27, 32).  Patients with pseudohypoaldosteronism, a loss of function mutation of ENaCs, have been shown to have greater levels of lung water when compared to normal subjects, highlighting the importance of these channels in lung fluid clearance even in individuals without CHF and at sea-level(18).

The $\beta_2$ARs are found throughout the lung, in the airways, the pulmonary vasculature, and thepulmonary lymphatics  and have been shown to play a key role in lung fluid balance(28).  Stimulation of the $\beta_2$AR causes an increase in 3', 5' adenosine monophosphate (cAMP) and protein kinase A (PKA) which lead to an increase in the number of ENaCs on the apical portion of type-II alveolar cells, and the probability of an open ENaC(50).  It is also likely that $\beta_2$AR stimulation results in an increase in $Na^+K^+$ATPase activity on the basolateral portion of type-II alveolar cells through an increase in PKA(2, 27)  In addition, $\beta_2$AR stimulation leads to smooth muscle relaxation of the pulmonary lymphatics, which also play a key role in lung fluid balance, particularly in preventing excessive interstitial fluid accumulation. Therefore, the stimulation of the $\beta_2$ARs can prevent excessive accumulation of fluid in the lungs through several key pathways: (1) relaxation of the pulmonary lymphatics, reducing the hydrostatic pressure of the interstitium, (2) increasing the number and activity of the ENaCs on the apical portion of alveolar cells, and (3) enhancing theactivity  of $Na^+K^+$ ATPase on basolateral portion of these same cells. Highlighting the importance of these mechanisms, *in-vivo* work has demonstrated that over-

expression of the $\beta_2$AR induces fluid clearance from the in mice (7, 9). In addition, beta-agonists (including circulating catecholamines) increase lung fluid clearance in animals and reduce clinical symptoms of high altitude pulmonary edema (HAPE) in humans(20, 36).

The gene encoding the $\beta_2$AR has been sequenced and common polymorphisms have been described, including a glycine (Gly) substitution for arginine (Arg) at amino acid 16. Although th e most functional variant is an isoleucine substitution for threonine at amino acid 164, this occurs in only a small percentage of the population(23). Several previous studies have examined variation at amino acid 16 because of the frequency and functional importance of this polymorphism. Subjects homozygous for arginine of the $\beta_2$AR at amino acid 16 (Arg16) have been shown to have reduced receptor function when compared to subjects homozygous for Gly at this amino acid (Gly16), even in the presence of basal levels of circulating catecholamines, suggesting that differences in receptor density, rather than differences in desensitization may be driving this effect(4, 5, 8, 12, 40, 43, 45). It is likely, therefore, that genetic variation of this receptor at amino acid 16 might influence lung fluid clearance, particularly under conditions that cause elevations in catecholamines.

In the present study, we sought to determine if the Arg16Gly polymorphism of the $\beta_2$AR differentially influenced lung fluid balance in response to a rapid infusion of isotonic saline. Rapid intravenous saline infusion has previously been shown to reduce exercise capacity,challenge the ability of the lungs to regulate fluid , and result in airflow obstruction and increased airway responsiveness(19, 29, 31). We

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hypothesized that subjects homozygous for Arg at amino acid 16 would have greater

lung fluid accumulation when compared to subjects homozygous for Gly at this amino

acid.

**Methods**

*Protocol*

 The protocol was reviewed and approved by the Mayo Clinic Institutional

Review Board, all participants provided written informed consent prior to study, and

all aspects of the study were performed according to the declaration of Helsinki.

Twenty-nine non-smoking healthy subjects agreed to participate in the study and had

no exclusion criteria (cardiopulmonary abnormalities, pregnancy, inability to

exercise). Before performing the main protocol, subjects underwent screening tests

which included an incremental cycle ergometry test to exhaustion (to rule out

cardiovascular abnormalities), a pulmonary function test to rule out pulmonary

disease, a complete blood count (CBC, to rule out anemia) and, in women, a

pregnancy test.

 In addition to the screening visit, the study involved one separate visit to our

laboratory for a rapid infusion of isotonic saline.

*Saline Infusion*

 A rapid intravenous saline infusion was used to challenge the ability of the

lungs to handle fluid(19). The subjects were required to report to our laboratory in a

fasting state and all infusions were performed between 0700 and 0900. An 18-guage

venous catheter was inserted into a prominent antecubital vein for the infusion and

for blood sampling. Baseline measures of pulmonary capillary blood volume, Vc,

alveolar-capillary conductance, $D_M$, and CT-based lung density and tissue volume were obtained prior to the infusion. Saline was then infused intravenously (30 ml's/kg over ~15 minutes) while the subject was on the CT bed to optimize timing for post-saline CT measures. During the saline infusion oxygen saturation ($SaO_2$), blood pressure, heart rate (HR), and physical symptoms were continuously monitored and recorded every two minutes. Catecholamines (adrenaline and noradrenaline) were sampled pre and immediately post-saline from the venous catheter. Following the infusion, repeat assessment of Vc, $D_M$, lung density, and tissue volume were performed.

**Data Collection**

Beta-2 AR genotyping was PCR based according to methods of Bray(3). Buffy coat, obtained from whole blood collected on EDTA, was extracted using the Gentra Pure gene® DNA Isolation kit. Following extraction, DNA was treated with a Proteinase K solution in preparation for PCR. The PCR reaction was conducted according to standard methods, using the following primer sequences (for Arg16Gly): (forward) 5'-AGC CAG TGC GCT TAC CTG CCA GAC-3' (at -32) and (reverse) 3'-C<u>A</u> T<u>G</u>G GTA CGC GGC CTG GTG CTG CAG TGC –5', resulting in a PCR product 107 base pairs in length. The Arg16 subjects are represented by a single 107 bp band and the Gly16 subjects by 82 and 25bp bands. For Gln27Glu: (forward) 5'- CCC AGC CAG TGC GCT TAC CT-3' and (reverse) 3'- CCG TCT GCA GAC GCT CGA AC –5', resulting in PCR products 178 and 56 base pairs in length

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Catecholamines. Venous catecholamines (Adrenaline and noradrenaline) were assessed in the Mayo Clinic GCRC immunochemical core laboratory according to the methods of Sealey(38).

Measures of Pulmonary Capillary Blood Volume and Alveolar-Capillary Conductance:

Measurement of Vc and $D_M$ has previously required the use of at least two, but preferably three oxygen tensions. Recently Tamhane et al. have found that measuring the disappearance of nitric oxide (NO) in concert with carbon monoxide (CO) provides an accurate assessment of Vc and $D_M$ using just one oxygen tension(47). Triplicate maneuvers of the diffusing capacity of the lungs for carbon monoxide (DLCO) and nitric oxide (DLNO) were performed pre-saline and post-saline.

Diffusing capacities of the lungs for carbon monoxide and nitric oxide were assessed using the rebreathing technique with gases sampled on a mass spectrometer (Perkin-Elmer, 1100) and NO analyzer (Sievers Instruments, Boulder, CO) using custom analysis software. A 5-liter rebreathe bag was filled with 0.3% carbon monoxide ($C^{18}O$), 40 PPM NO (diluted immediately before the test in the bag from an 800 PPM gas mixture), and $O_2$. $C^{18}O$ was used instead of the more common $C^{16}O$ as the test gas because the mass spectrometer cannot distinguish $C^{16}O$ from $N_2$ (because of similar molecular weights). The volume of gas used to fill the rebreathe bag was determined by the tidal volume of the subject. Consistent bag volumes were assured using a timed switching circuit which, given a consistent flow rate from the tank, resulted in the desired volume(16). The switching circuit and tank were checked prior to each test for accurate volumes. At the end of a normal

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expiration (end-expiratory lung volume, EELV) the subjects were switched into the rebreathe bag and instructed to nearly empty the bag with each breath for 10 consecutive breaths.  The respiratory rate during the rebreathe maneuver was controlled at 32 breaths/minute with a metronome. Following each diffusing capacity maneuver, the rebreathe bag was emptied with a suction device and refilled immediately prior to the next maneuver.  For our lab, the coefficients of variation are 4.1% for the DLCO measure , 8.3% for the DLNO measures , 7.2% for $D_M$, and 6.4% for Vc.

<u>Measures of CT-based Lung Tissue Volume and Density:</u> All CT scans were performed on the same scanner (GE LiteSpeed spiral CT scanner, GE Healthcare). Initial slices were obtained for all scans which were 2.5mm thick with a 1.2mm overlap and then reconstructed to 1.25mm with a 0.6mm overlap. Prior to the baseline scan a scout scan was performed to determine the location and size of the lungs.  Marks were placed on the skin of the subject to indicate the anatomical location of the start of the scan and the table height was recorded to insure consistency with post-scans. The number of slices of the baseline scans was recorded in order to have consistent scans throughout the study.

*Normalization of Lung Volumes During Scanning*

Subjects were asked to breathe on a mouthpiece connected to a pneumotachometer which was integrated with a portable computer with custom analysis software in order to provide accurate lung volumes during the scans.  During the scans, the investigators observed the breathing pattern of each subject with the

pneumotachometer and instructed the subjects to the desired lung volume for the

breath-hold for imaging.  The system was calibrated using a 3-liter syringe prior to

each CT visit.  For the baseline scans, subjects were asked to hold their breath at

two lung volumes, total lung capacity (TLC) and EELV.  The post-saline scans were

performed at end-inspiratory lung volume (EILV).  To obtain consistent lung density

data while controlling for lung volume, the image data obtained post saline was

compared to values obtained by interpolating between the images obtained at TLC

and EELV during baseline scanning. This scanning strategy was performed to insure

accurate comparisons, because it is impossible to exactly match baseline and post

rapid saline infusion lung volumes, while minimizing the number of scans and amount

of radiation exposure to the subjects.

*Lung Density and Tissue Volume Analysis Using CT*

The CT images were submitted for analysis using custom image analysis

software (Pulmonary Analysis Software Suite (PASS), Physiological Imaging

Laboratory, Univ of Iowa, Iowa City IA). Using all of the slices from a subject's scan,

the software first segmented the images to separate lung tissue from surrounding

structures and the mediastinum as described previously(42). In each picture element,

lung density was assumed to be a linear combination of air (Hounsfield units, HU= -

1000) and lung tissue, which has density of water (HU=0). Thus, HU of -600 and -400

would represent 40% and 60% tissue, respectively. A histogram analysis of picture

elements within the lung tissue was performed to obtained mean lung density in HU

and tissue volume by summation among all elements in the lung fields. Lung tissue

volume and lung density data obtained from baseline images were plotted against lung volume to be used for linear interpolation to obtain data against which other images were compared.

*Estimation of Lung Water*

Tissue volume as assessed by CT includes lung tissue, blood, and water. Saline infusion likely causes changes in lung blood volume and water volume, but will minimally affect the volume of lung tissue. By combining our measures of tissue volume and Vc obtained from the lung diffusing capacity tests, we were able to estimate changes in lung water from baseline to post-saline using the following equation:

**Lung Water = (Pst TV-VcPst)-(IP TV-VcIP)**

Where Pst TV is the given tissue volume from the CT scan post-intervention (in mls), IP TV is the interpolated tissue volume at the post-intervention lung volume (in mls) from baseline CT scans, and Vc is the pulmonary capillary blood volume in mls at each time point, Pst is post intervention, IP is baseline.

*Data Analysis*

All statistical comparisons were made using the SPSS statistical software package (Chicago, IL). Group demographics were compared using an independent samples t-test with an alpha level of 0.05 to determine significance. The changes in the physiological parameters, pulmonary function, catecholamines, DLCO, $D_M$, Vc, tissue volume and lung water in response to saline infusion were compared using a repeated measures ANOVA with an alpha level of 0.05 to determine significance. The differences in the physiological parameters, pulmonary function, catecholamines,

DLCO, $D_M$, Vc, tissue volume and lung water  between the genotype groups at baseline and post-saline were compared using an independent samples t-test with an alpha level of 0.05 to determine significance. A Levene's test was performed before each comparison to determine equality of variance. Values are mean±SD.

**Results**

*Subject Characteristics*

Twenty-nine subjects (Arg16 n=14, 4 females; Gly16 n=15, 3 females) who had no exclusion criteria (cardiopulmonary abnormalities, inability to exercise, use of cardiovascular or pulmonary medications) participated in the study.  There were no differences between genotype groups in age, weight, body mass index (BMI), or peak oxygen uptake (peak $VO_2$) (age=30±7 vs. 30±8 years, weight=76±15 vs. 82±11kg, BMI=25±4 vs. 25±4kg/m$^2$, $VO_2$ peak= 37±7 vs. 40±7ml/kg/min (103±15 vs. 105±11 % predicted), for Arg16 vs. Gly16), but the Gly16 group was taller than the Arg16 group (174±7 vs. 181±8cm for Arg16 and Gly16, respectively).

*Saline Infusion*

On average, 2210 mls of salinewas  infused in the Arg16 group while 2380 mls was infused in the Gly16 group (over 17 minutes for both groups). The most common symptom reported during the saline infusion was a chest tightness that averaged 1.7 on a scale of 0 to 4. Saline infusion caused a similar increase in HR in both groups (Arg16= 25±7, Gly16=18±5 % change from baseline, p>0.05), but there were no changes in $SaO_2$.  There were increases from baseline in adrenaline and noradrenaline, with no differences between genotype groups (adrenaline=283±117% vs. 252±118%; noradrenaline=129±137% vs. 99±123%, for the Arg16 and Gly16

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groups, respectively)(figure 1). The Arg16 group had greater decreases in $D_M$

compared to the Gly16 group (figure 2). Alveolar-capillary conductance corrected for

Vc fell more in the Arg16 group compared to the Gly16 group (figure 3). This

condition also resulted in increases in lung tissue volume, density, and estimates of

lung water in both groups, with the greatest increase in lung water in the Arg16 group

(lung water=90±66mls vs. 48±144mls, $p < 0.05$)(table 2).

**Discussion**

This study suggests that subjects homozygous for the Arg allele at amino acid

16 of the $\beta_2AR$ have an increase in lung fluid accumulation relative to subjects

homozygous for Gly at this position during an intervention that has previously been

shown to challenge the ability of the lungs toregulate  fluid.  This is evident since the

Arg16 subjects had lower conductance of the alveolar-capillary membrane, and

higher lung tissue volume and estimates of lung water following saline infusion

relative to the Gly16 subjects.

*Lung Fluid Balance*

While it is established that certain clinical and environmental conditions can

cause alterations in lung fluid balance and lead to alveolar fluid accumulation, less is

clear about the amount of alveolar fluid in the healthy normal lung(1, 14, 17, 39)  .

Evidence of lung fluid flux into the alveoli even in healthy subjects has been

demonstrated by Karem et al. who reported that subjects with

pseudohypoaldosteronism (a loss of function mutation of the ENaCs) have more lung

fluid than those without this mutation(18).  Because the ENaCs influence lung fluid

clearance from the airspace, this study suggests that fluid must transfer into the

airspace in humans even with normal pulmonary vascular resistance and pulmonary capillary integrity. The ENaCs have been localized to type-II alveolar cells and play a key role in alveolar fluid clearance allowing for $Na^+$ and water transfer from the alveolar space into the cell and are primarily regulated by the $\beta_2$ARs(13, 32).

*$\beta_2$ARs and Lung Fluid clearance*

Several studies have shown the importance of the $\beta_2$ARs in lung fluid regulation(7, 11, 26, 30).  Immediately following birth, stimulation of the $\beta_2$ARs clear amniotic fluid from the lungs, stimulated by circulating epinephrine(10).  Additionally, the $\beta_2$ARs clear fluid from the lung following acute lung injury and sepsis, and have been shown to reduce the clinical and radiographic signs of high-altitude pulmonary edema in adults(11, 36, 37, 48).  Animal models have suggested that stimulation of the $\beta_2$ARs in the lung stimulates lung fluid clearance even in healthy animals(7, 34) The $\beta_2$ARs have been localized to lymphatic smooth muscle tissue and stimulation of these receptors on the pulmonary lymphatics leads to smooth muscle relaxation, dilation of the lymphatic vessels, and movement of water away from lungs(15, 24, 51).  It is possible, therefore, that the observed differences between the genotype groups in lung fluid accumulation in the present study were a result of dilation of the pulmonary lymphatics and a concurrent increase in lung lymph flow, reducing the hydrostatic pressure of the interstitium.

*Limitations*

A potential limitation to studying alterations in lung fluid balance in humans is the difficulty assessing small changes in lung water.  We have used state of the art methods combining CT imaging at controlled lung volumes (anatomical assessment)

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and measures of gas transfer (DLCO and DLNO, physiological assessment) to estimate changes in lung water in healthy humans.  Both of these independent measures demonstrated consistent findings; that rapid saline infusion resulted in lung fluid accumulation characterized by an increase in lung density and a widening of the alveolar-capillary membrane, even when corrected for changes in pulmonary capillary blood volume.  A limitation of CT scanning is the inability to separate blood from tissue and water (because of similar Hounsfield units). Therefore, in the present study, we calculated the estimated changes in lung water by combining tissue volume from CT scanning with Vc measured from the DLCO/DLNO technique. Because Vc only measures the amount of blood that is in contact with the alveoli, it is possible that this is not a true representation of the amount of blood in the entire lung. We did simultaneously measure cardiac output (reported in (45)) and found that the Gly16 subjects had a higher cardiac output both before and following the saline infusion. Because essentially all of the blood from the heart goes to the lungs, and because we found a lower tissue volume and lung density in the Gly16 group, it is possible that we underestimated the difference in lung water between the genotype groups.

In the present study, we sought to determine differences in lung fluid balance based on genetic variation of the $\beta_2$AR at amino acid 16. Although the interest in the phenotypic effects of single-nucleotide polymorphisms is considered by some to have less of an impact than haplotype analysis, genetic variation of the $\beta_2$AR at amino acid 16 appears to have powerful physiologic and clinical implications (8, 12, 21, 40, 41, 43-45).  Another polymorphism of this protein that is frequently studied and

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suggested to have an effect on receptor function is amino acid 27. There is significant

linkage disequilibrium between amino acids 16 and 27, therefore, when the two

polymorphisms are combined they important predictors of the variation along the rest

of the $\beta_2$AR gene(6). Based on this, we provide data on several of our indices of lung

water according to variation at amino acids 16 and 27 (table 1). Although not

powered for statistical analysis of amino acid 27 , there appears to be a primary effect

at amino acid 16, with a secondary effect at position 27. Although it will be important

in future studies to consider variation at multiple sites with larger groups, smaller

mechanistic studies are essential to understanding mechanisms behind larger,

population based, less mechanistic studies(21).

It is not possible from the current study design to determine the contribution of

lung fluid accumulation versus active fluid clearance to the observed genotype-

related differences in lung water. To determine if there are differences between the

genotype groups in active fluid clearance, a minimum of two separate time points

measuring differences in lung water would have been necessary. Previous work has

demonstrated that basal alveolar fluid clearance is around 8-12% over 4 hours in

humans and increases to 14-28% in the presence of terbutaline or catecholamines

(33, 35). In the present study, the infusion time averaged 17 minutes, the final CT

scan was taken at minute 21 and the DLCO/DLNO measures were taken at minute

30 (given the transit time to the physiological core laboratory). In addition, there was

a significant elevation in venous epinephrine which likely accelerated alveolar fluid

clearance (25). We feel, therefore, that the genotype-related differences in lung water

are most likely explained primarily by $\beta_2$-mediated changes in the lymph flow from the lung and active fluid clearance from the alveolar airspace.

**Implications and Conclusions**

Lung fluid regulation is challenged in a number of clinical and environmental conditions. Patients with heart failure and individuals who travel to high-altitude may be particularly susceptible to pulmonary edema; however, not all patients with heart failure nor all sojourners to high-altitude develop pulmonary edema despite similar clinical or environmental conditions.  This would suggest that genetic variation in the regulatory proteins important in lung fluid balance may influence the susceptibility to pulmonary edema. Although the pulmonary edema that occurs in heart failure and with exposure to high altitude develops over  a longer time period than that which was used in the present study, it is likely that similar mechanisms involved in regulating lung fluid are challenged in these conditions. The results of the present study suggest that subjects homozygous for Arg at amino acid 16 of the $\beta_2AR$ demonstrate greater lung fluid accumulation compared to subjects homozygous for Gly following rapid fluid loading.  Future, larger studies are needed in patients with heart failure or in sojourners to high altitude where prolonged elevations in catecholamines may accentuate the differences between genotype groups due to a proposed enhanced susceptibility to receptor desensitization in the Arg16 subjects.

## Acknowledgments

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Table 1. Changes in Lung Diffusing Capacity, Density and Tissue Volume with Rapid Saline Infusion According to Genetic Variation at Amino Acids 16 and 27.

| | Pre Saline | | | | Post Saline | | | |
|---|---|---|---|---|---|---|---|---|
| | Arg16 | Gly16 | | | Arg16 | Gly16 | | |
| | Gln27Gln | Gln27Gln | Gln27Glu | Glu27Glu | Gln27Gln | Gln27Gln | Gln27Glu | Glu27Glu |
| **n** | 14 | 5 | 5 | 5 | 14 | 5 | 5 | 5 |
| **DLCO (ml/min/mmHg)** | 23±3 | 29±4 | 28±2 | 32±7 | 22±7 | 27±2 | 28±3 | 34±10 |
| **DM (ml/min/mmHg)** | 29±6 | 34±6 | 42±4 | 37±3 | 24±6 | 33±8 | 41±7 | 37±14 |
| **DM/Vc** | 0.45±0.24 | 0.44±0.30 | 0.77±0.40 | 0.40±0.23 | 0.28±0.17 | 0.42±0.44 | 0.74±0.37 | 0.33±0.25 |
| **Lung Density (HU)** | -796±25 | -798±4 | -814±32 | -784±24 | -738±24 | -749±12 | -763±25 | -748±19 |
| **Lung Tissue Volume (mls)** | 772±130 | 950±47 | 854±150 | 1041±180 | 873±133 | 1007±51 | 902±176 | 1070±158 |

Values are mean±SD. DLCO= Diffusing Capacity of the Lungs for Carbon Monoxide, $D_M$= Alveolar-Capillary Conductance, DM/Vc= Alveolar Capillary Conductance Corrected for Pulmonary Capillary Blood Volume, HU= Hounesfield Units.

**Table 2. Changes in Lung Tissue Volume (from Computed Tomography) and Estimated Lung Water Following Saline Infusion According to Genotype.**

|                                 | Arg16 | Gly16 |
|---------------------------------|-------|-------|
| Lung Tissue Volume (%change)    | 13±3  | 3±3*  |
| Estimated Lung Water (%change)  | 15±3  | 4±4*  |

Values are mean±SEM. *p<0.05 Arg16 vs. Gly16.

**Figure Legend**

**Figure 1.** Changes in catecholamines following saline infusion according to genotype. The top graph represents adrenaline while the bottom graph represents noradrenaline. The grey bars (▫) represent the Arg16 group, while the black bars (■) represent the Gly16 group. $^\dagger$p<0.05 compared to baseline.

**Figure 2.** Change in DLCO and components following saline infusion according to genotype. The grey bars (▫) represent the Arg16 group, while the black bars (■) represent the Gly16 group. *p<0.05 Arg16 vs. Gly16

**Figure 3.** Change in alveolar-capillary conductance corrected for pulmonary capillary blood volume in response to saline infusion. The dashed line with open diamonds represents the Arg16 group, while solid line with filled squares represents the Gly16 group. *p<0.05 Arg16 vs. Gly16.

Figure 1. Changes in Catecholamines Following Saline Infusion According to Genotype.
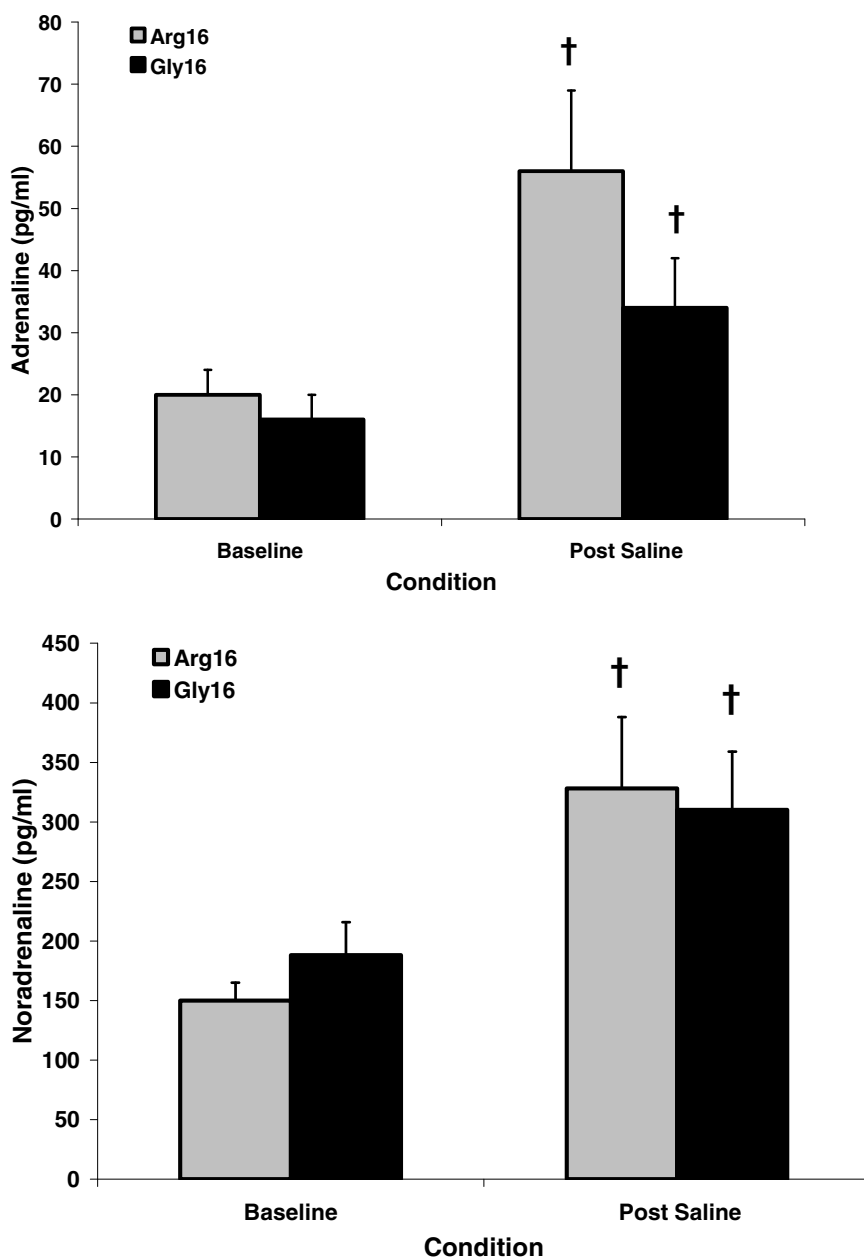


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Figure 2. Change in DLCO and Components following Saline Infusion According to Genotype.
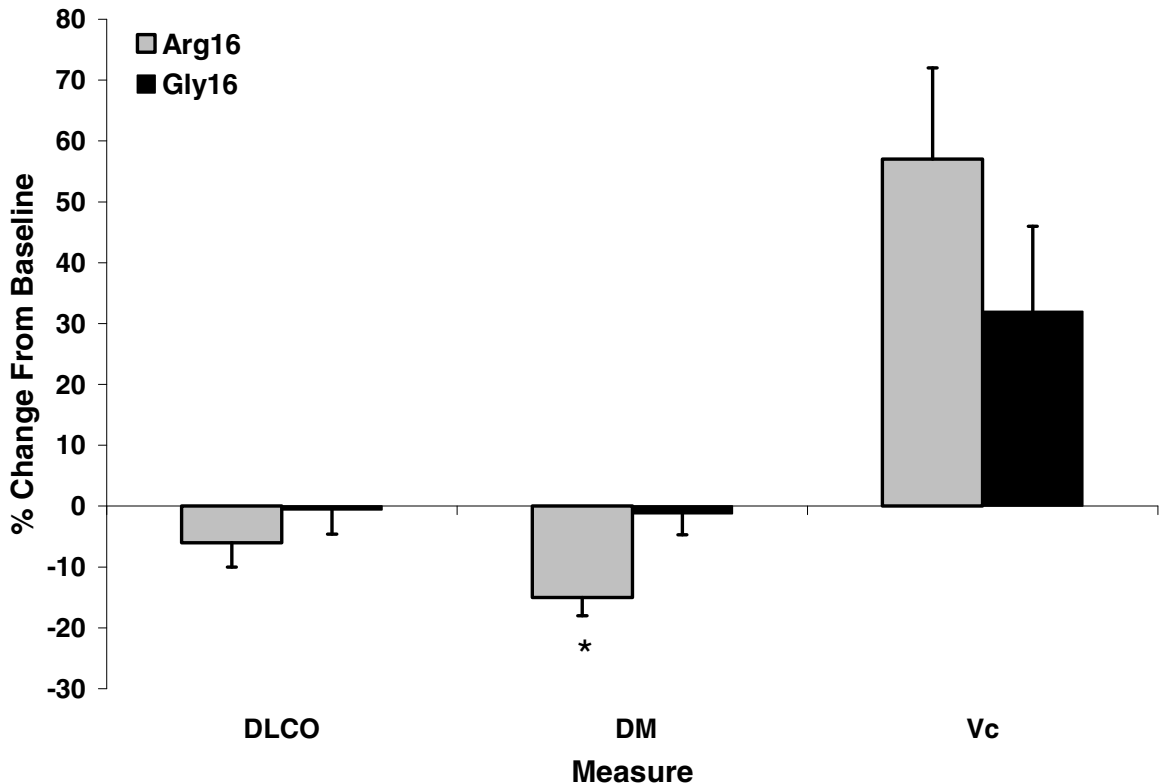
Figure 3. Change in Alveolar-Capillary Conductance Corrected for Pulmonary Capillary Blood Volume in Response to Saline Infusion.