Influence of age and sex on the longitudinal relaxation time, T1, of the lung in healthy never-smokers

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Running Title:
Lung T1 in healthy never-smokers

ABSTRACT

Purpose: As several studies have provided evidence that lung disease affects the T1 of the human lung, our purpose was to investigate the effect of age on the T1-relaxation time in the lungs of healthy never-smokers, including group difference between sexes.

Materials and methods: The Snapshot FLASH pulse sequence (inversion recovery with multiple gradient echo read-outs) was used to quantify lung T1 in 30 healthy never-smoking volunteers at 1.5 Tesla. Measurements were performed under breath hold of a tidal inspiration. Additionally, subjects underwent clinical MRI and pulmonary function tests. A linear regression model of T1 as a function of age and sex was tested.

Results: The slope of lung T1 at tidal end-inspiration as a function of age was statistically different between males and females ($p<0.001$). In a linear regression model of T1 as a function of age and sex, females have slope of -4.1 ms/year (95% CI=[-5.2,-3.0]) at $p < 0.001$, and males -0.064 ms/year (95% CI=[-1.2,1.1]) at $p=0.9$, with a whole model R^2 = 0.83.

Conclusion: The observed dependencies of lung T1 on age and sex are here attributed to a previously reported difference in blood T1 between sexes, and a previously reported decrease of pulmonary blood volume with increasing age. This may have implications for the interpretation of lung T1 measurements in both healthy individuals and patients.

INTRODUCTION

During recent years, the number of applications of magnetic resonance imaging (MRI) of the human lung has grown (1, 2). One MRI method which has gained interest for application in the lung is quantification of the water proton longitudinal relaxation time T1 (T1-mapping), and T1-mapping as the read-out for oxygen enhanced MRI, which has been shown to reflect the lung diffusing capacity(3, 4). T1-mapping and T1-mapping of oxygen enhancement in the lung have been applied to study healthy subjects (5–9), diffuse lung disease(10), cystic fibrosis(11, 12), asthma(13, 14), pulmonary hypertension(15), chronic lung allograft dysfunction(16) and in particular chronic obstructive pulmonary disease (COPD) (17–20). Considering that COPD alone is the third largest cause of death globally, surpassed only by cardio- and cerebrovascular disease (21), the interest in this non-invasive imaging technique is easy to appreciate. However, even though lung T1 has already been used as a read-out in clinical studies of for example COPD (20), no detailed data have been presented for a well-defined healthy cohort.

The ageing lung tissue of a healthy individual is characterized by increased compliance (less elastic recoil) – presumably due to changes in concentration or orientation of elastic fibers (22). A similar change in lung tissue compliance occurs in pulmonary emphysema which has already been shown to yield decreased lung T1(10). Several studies of COPD(17, 19, 20) have compared patients and control groups, where severe disease is often associated with a high age. If T1 is correlated to age, this will introduce a statistical bias, possibly obscuring important findings. Thus, there are several reasons to study a healthy cohort, evenly distributed between sexes and different age groups. The purpose of this study is to provide baseline lung T1 values of healthy never-smokers, male and female, between 20 and 70 years of age, and specifically investigate the effect of age on the native T1-relaxation time in the lung, including group differences between sexes, using a previously established method for T1-measurement.

MATERIALS AND METHODS

With approval from the Regional Ethical Review Board, 31 never-smoking healthy subjects (16 male, 15 female) between 20-70 years were recruited to perform a clinical lung MRI examination, whole lung T1 measurement and pulmonary function tests (PFT). Never-smokers were defined according to three criteria: a) never smoked daily for more than a month, b) smoking occasionally less than once a month, and c) reporting 0 pack-years of lifetime tobacco use. Subjects were provided with written instructions, signed informed consent, reported lifetime tobacco use, pulmonary health status and filled out a MRI safety sheet prior to the visit. The PFT was either made immediately after the MRI examination, or the next day. All MRI measurements were made on a 1.5 Tesla Siemens Magnetom AvantoFit (SIEMENS Healthcare, Erlangen, Germany), with an 18 channel body coil and a 32 channel spine matrix.

Clinical Images

In order to enable a radiological evaluation a clinical protocol preceded the T1 measurement. The clinical protocol included two full coverage coronal HASTE (Half-Fourier Acquisition Single shot Turbo Spin Echo) acquisitions during end-inspiration breath hold and during free breathing respectively, as well as an axial VIBE acquisition during end-inspiration breath hold. The images were examined by clinical radiologists with 10 (S.D.) and 5 (D.S.) years of experience.

T1-Measurements

Following the clinical MRI, a number of coronal T1-maps covering the entire lung volume were collected during tidal end-inspiration breath hold, resulting in 8 – 12 slices. All T1 measurements were made with the Snapshot FLASH pulse sequence(8) which is based on the Look and Locker sequence (23, 24). Following spin inversion, several gradient echo images are collected during magnetization recovery. Assuming sufficient time is left between measurements and the inversion pulse is homogeneous, the initial magnetization, steady-state magnetization and signal dynamics can be used to calculate the unbiased T1. Imaging parameters were as follow: matrix 128 x 64 zero filled to 256 x 256; 450 mm square field of view; 1.5 cm slice thickness; TE = 0.67 ms; TR = 3.0 ms; and flip angle = 7°. A non-selective hyperbolic secant pulse was used for spin inversion; and Hanning-windowed sinc pulses with 1.6 sidelobes were used for read-out. One T1 measurement is made in each slice; 16 gradient echo images with different inversion times are used in the calculation of the T1 map; and the total measurement time is 3.0 seconds per slice. Each T1 measurement was preceded by oral instructions for breath hold after a tidal inspiration and subjects were given at least 10 seconds of free breathing in between measurements, which is also assumed to be enough to restore magnetization equilibrium for T1 < 2000 ms.

Pulmonary Function Tests

Pulmonary function tests were performed at the clinical physiology department, using a Carefusion Masterscreen PFT and Body/Diffusion and the SentrySuite version 2.7 or higher (Carefusion Corporation, San Diego, CA). The parameters recorded in the PFT protocol were: height, weight, forced expiratory volume in 1 second (FEV_1), functional residual capacity (FRC), residual volume (RV), total lung capacity (TLC), vital capacity (VC), diffusion capacity of the lung for carbon monoxide ($D_{L,CO}$); and $D_{L,CO}$ adjusted for alveolar volume (k_{CO}).

Data Processing

Data processing was made in MatLab R2014b (MathWorks, Natick, MA). The lungs were segmented in 3D using a region growing algorithm ("regionGrowing", Daniel Keller, 2011) or, in case the first algorithm fails, a slice by slice k-means clustering algorithm with five clusters. Either segmentation method was applied on a 3D image stack, containing the last magnitude image from the series of collected gradient echoes, followed by manual removal of major vessels. Segmentations were considered successful when each slice clearly included the signal magnitude gradient representing the border of the lung. To calculate a single T1-value of the entire lung volume, a Gaussian curve was fitted to the histogram of all voxel values in the lung segmentation(7, 10). However, to speed up the calculation and identify the largest peak, the whole histogram was thresholded at the mean count of all bins, resulting in a very clearly defined peak as presented in
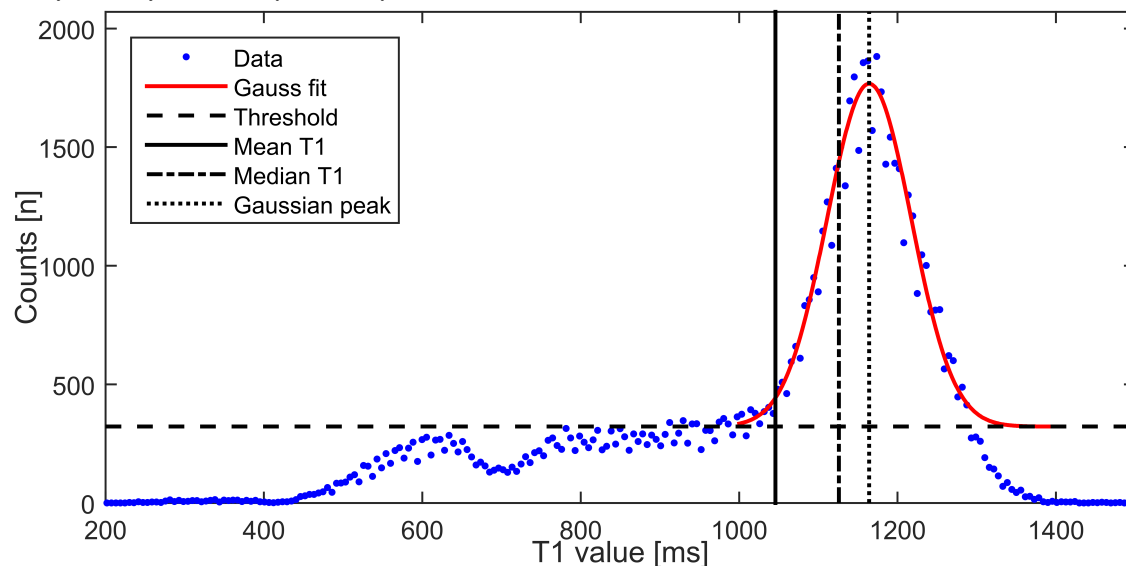

Figure 1. In the data processing, the standard deviation (SD) of the fitted Gaussian curve is used as a measure of intra-subject T1 variability.

Statistical Analysis

Statistical analysis was also performed in MatLab. For the included subjects with complete PFT (n=24), linear models on the form T1(x) = T1(0) + C*x as a function of age, sex, height, weight and PFT parameters were tested for the whole group, and separately for each sex. Analysis of variance (ANOVA) on the fit parameters were

performed to test which slopes, C, were significantly different between the sexes. The subsequent analysis was data driven and all linear models of pairs of variables with interaction terms, $T1(x*y) = T1(0) + C_x*x + C_y*y + C_{x,y}*x*y$, were tested. A selection of statistically significant models (p <0.01 due to multiple testing) were included in the results based on a post hoc threshold on the adjusted R^2 –value. Additionally, the Pearson correlation coefficients between all pairs of independent variables were calculated. Six subjects could, for logistical reasons, not perform PFT and could thus not be included in the aforementioned analysis. They are however included in the final models T1(age), as well as T1(age*sex), with a total of 30 included subjects. To highlight the detected group difference, a two sample Student's T-test was performed, comparing the lung T1 of young women (age<40) to all other subjects.

RESULTS

All subjects were able to complete the MRI examinations and T1-maps of good visual quality were produced, representative T1 maps in a central slice of four subjects are provided in
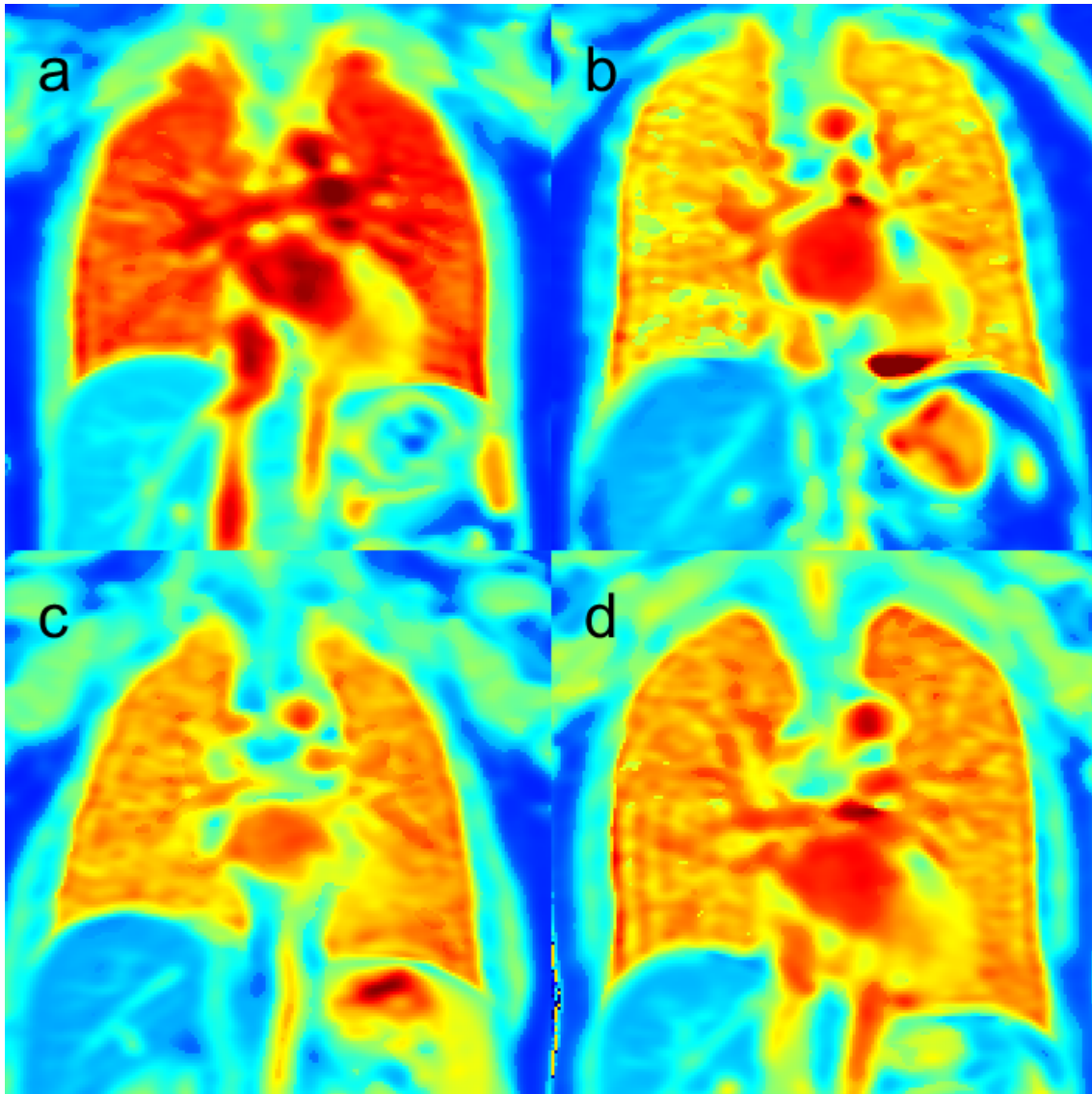
Figure 2. One subject was excluded from the entire study due to suspected pathological findings in the clinical images, and six subjects were not able to perform the PFT due to logistical reasons. Thus, 30 subjects are included in the final result and 24 subjects are included in the PFT analysis.

Segmentations were successfully performed on the 3D datasets from all subjects. The T1 histograms of all subjects were similar in shape and consist of a Gaussian shaped

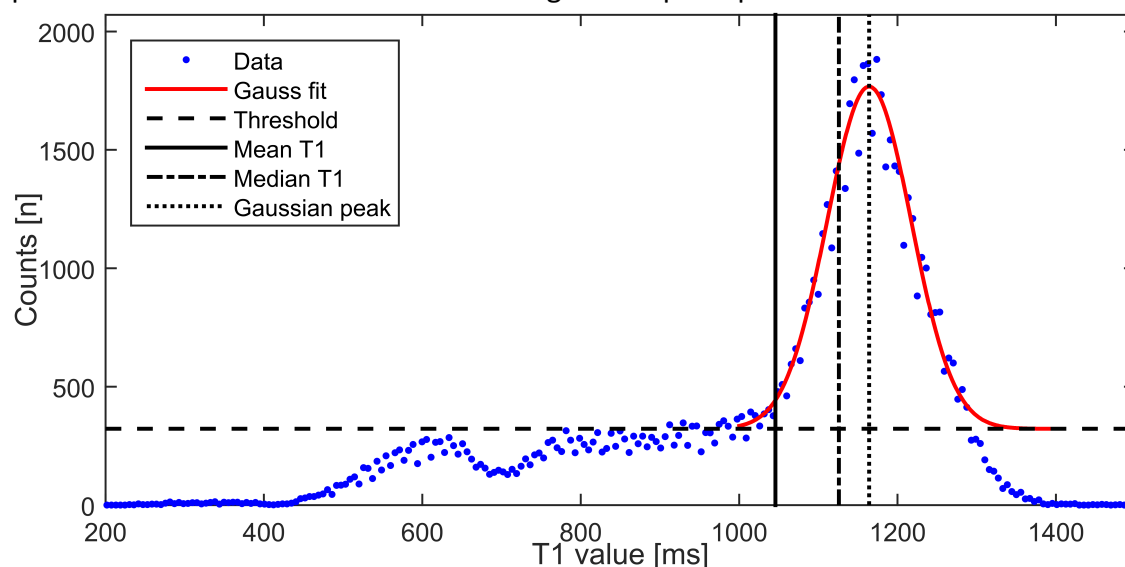peak with a tail on the low T1-side – a single example is provided in

Figure 1 which was representative of the group. The SD of the Gaussian curve (regarded as intra subject measurement uncertainty) was not significantly correlated (p >0.1) with any of the interesting variables sex, age or lung T1, which is a formal prerequisite for linear regression analysis. The width (SD) of the Gaussian T1-peak after thresholding was 49 +/- 8 ms (mean and SD) for the entire group (n=30).

Physiological data for the group with complete PFT (n=24) are presented in Table 1. Linear regressions of T1(x) as a function of all single parameters are provided in Table 2, for both sexes and for the whole group. Among the regressions for a single parameter, T1(RV) and T1(TLC) give the best fits for the whole group with R^2 ≥0.5 and slopes of -106 ms/l (95%CI [-146, -66]) and -37.7 ms/l (95% CI[-54,-21]) respectively. Only T1(RV) and T1(age) yield statistically different slopes between sexes (p<0.01), the other models did not (p>0.05).

Pearson correlation coefficients between independent variables are presented in Table 3. As expected, subject length is associated with all lung volumes. RV is highly correlated, r=0.70, with age, weight is moderately correlated with age, r=0.33. Both FEV_1 and k_{CO} are negatively correlated with age, with r=-0.37 and -0.47 respectively. The linear regression RV(age) yields a slope of 0.0226 liters/year (95% CI [0.01,0.03]) at p<0.001 for n=24.

Regression models of T1(x*y) was tested for all combinations of age, sex, height, weight, FEV_1, TLC, VC, FRC, RV, D_{LCO} and k_{CO}. Testing this many variables with interactions – most of them also correlated – yield several high (>0.5) R^2- values. However, the only models fulfilling the criteria of adj. R^2 >0.7 and p<0.01 were T1(age*sex), T1(RV*sex), T1(age*TLC) and T1(age*VC), which indicate that they describe the variation of lung T1 in the studied sample to a high degree. The model T1(age*sex) was robust to the addition of any PFT variables, or length or weight, as a confounder, in the sense that the adjusted 95 % confidence intervals for the male and

female slopes never overlapped, and that the slope estimates changed less than +/- 1 ms/y.

The linear regression model T1(age), also including the subjects without PFT data was statistically significant at p<0.01 and exhibited a negative slope of -2.3 ms/year (95% CI [-3.9,-0.7]) with R^2 = 0.2 (n=30). This means that there is a statistically significant negative relation, but the variable "age" alone is only accounting for 20% of the variability in the data.
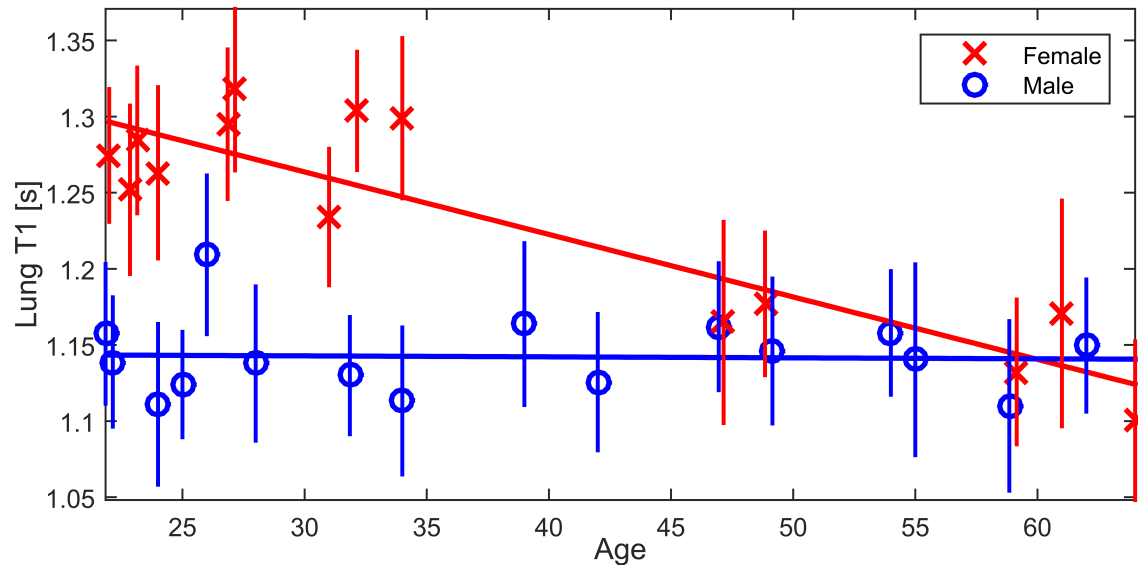
The linear model T1(age*sex) is presented in

Figure 3 with parameter estimates in Table 4. Whole model is significant at p<0.001 (n=30) with adjusted r^2 = 0.81 and r^2 = 0.83 which indicates that it accounts for most of the variation in the data. The slope of lung T1 at tidal end-inspiration as a function of age for women was found to be -4.1 ms/year (95% CI=[-5.2,-3.0]) at p < 0.001, and -0.064 ms/year (95% CI=[-1.2,1.1]) at p=0.9 for men, which indicates there was no correlation between age and inspired lung T1 for men. The slope of T1 as a function of age was different for men and women at p<0.001.

A Student's T-test revealed a significant difference of 136 ms (95% CI[114,159]) in lung T1 (p<0.001) between women of age<40, compared to all other subjects, according to

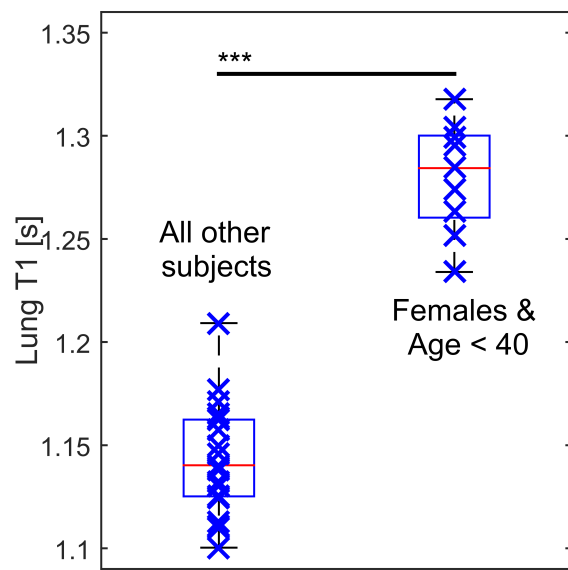Figure 4.

DISCUSSION

In this paper we present evidence that lung T1 measured during tidal end-inspiration is negatively correlated with age for women, but not for men, in a healthy non-smoking population. Overall, sex and age combined make the best fit to the T1 data from the studied group, followed by models including TLC, VC and RV. However, as confirmed by the present dataset, both TLC and VC are very dependent on sex, and RV is highly dependent on age, which means that all models T1(TLC*age), T1(VC*age), T1(sex*age) and T1(sex*RV) contain essentially the same information as T1(age*sex). The most informative two-parameter interaction model to describe lung T1 in healthy never-smokers is concluded to include only age and sex, which also has the highest correlation value of the tested models in this study.

The average T1 at end tidal inspiration in this study was 1185 ms (16 male, 14 females, mean age 38, TE=0.67 ms) which is well in line with the mean lung T1 reported in earlier publications by Stadler: 1199 ms (8 male, 2 female, mean age 30, end-inspiration, TE=1.4 ms) (7); Alamidi: 1053 ms (8 male, 4 female, mean age 63, TE=3 ms, free breathing, spin echo)(20) and Renne: 1250 ms (7 male, 5 female, mean age 28, TE 0.8, end-inspiration)(25).

To explain the present findings one must consider the intrinsic T1 of human blood, as well as the blood content of the lung. Triphan et al. has shown that the measured T1 for lung at conventional echo times are largely determined by blood T1(26).It is thus reasonable to assume that blood T1 is a major determinant of lung T1 at the TE of the present study (0.67 ms). A recent cardiac MRI-study by Piechnik et al. (2014) reported female left ventricular blood T1 on average 86 ms higher than in males, as well as a female specific decrease in blood T1 of 2 ms/year, after adjusting for hematocrit(27). It was also shown that male blood T1 increases from 30 years of age up to 65 (27); this can be explained by the main determinant of blood T1 - hematocrit (28), which is likely to decrease with falling testosterone levels (29, 30). Our own data can be used to confirm the generalizability of Piechnik's work – a circular region of interest was drawn in the left ventricle of the heart in the collected T1 maps, and the average T1 is presented as a function of age and sex in
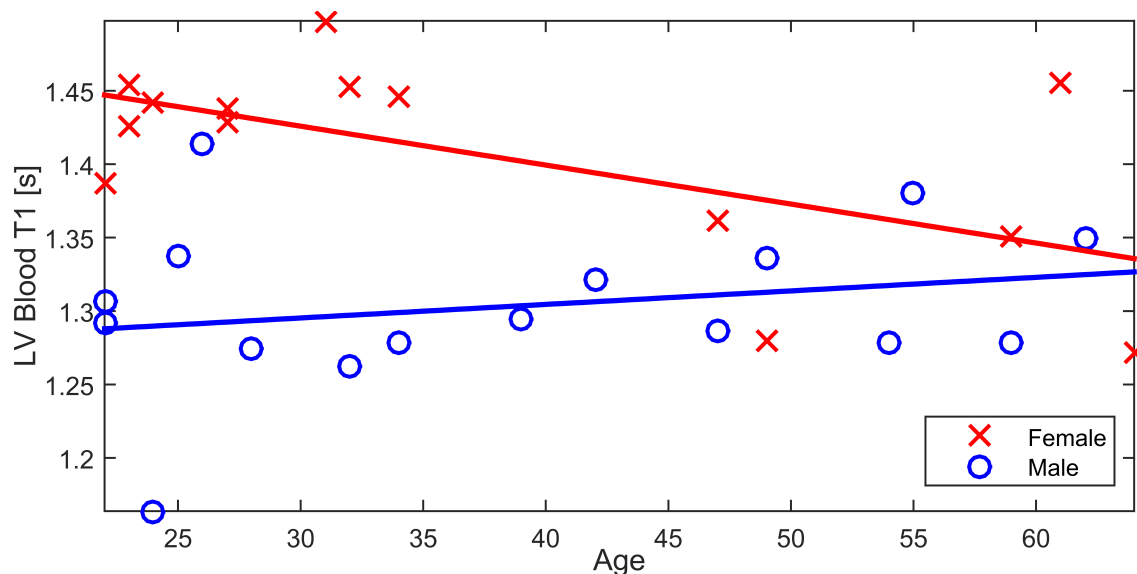
Figure 5. The slopes are -2.6 ms/year for females, and + 1 ms/year for males, indicating that the findings of Piechnik et al. also apply to the present study sample. The implication of this is that almost 50% of the age dependency in female lung T1 (-4.3 ms/year, present study) can potentially be attributed to blood T1 (-2.0 ms/year, Piechnik et al.).

However, one would expect to find an increase in lung T1 with age in males based purely on blood T1, therefore the pulmonary blood volume must also be considered. It has been shown that the inspiratory state influences lung T1, with the end-inspiration T1 on average being 134 ms lower than at end-expiration(7). It is also known that the volume adjusted pulmonary blood fraction is lower at inspiration(31) and that the lung parenchyma intrinsic T1 is lower than blood(26). The difference in lung T1 at inspiration and expiration may thus be attributed to differences in pulmonary blood volume. Moreover, there is also substantial evidence that pulmonary blood volume decreases with age(32), which, in light of the previously mentioned findings, would explain the observed decrease in lung T1 with age in our study. The underlying explanation for the age dependency of pulmonary blood volume may in turn be attributed to the age dependent loss of elastic recoil in the lung; leading to closure of small airways at larger lung volumes and a corresponding increase in RV with age. Elderly subjects will thus have tidal breathing in a more inspired state(22). In the current sample (n=24), RV increased with 0.02 liters/year, which is very similar to previously reported values (0.022 l/y for males, 0.016 l/y for females (33)). This would theoretically yield lower pulmonary blood fraction and a lower T1 in elderly subjects, as consistent with our findings.

Thus, combining the effects of blood T1 and pulmonary blood volume we can explain both the large negative slope of lung T1 observed in women, and the non-dependency between lung T1 and age observed in men; this assuming that the effects of blood T1 and decreasing blood volume are of approximately the same magnitude, but working in opposing directions in males.

A dependence of lung T1 on pulmonary blood volume would have consequences for the interpretation of measurements in patients, as pulmonary blood volume shows regional abnormalities in patients with interstitial lung disease (34, 35), acute and chronic pulmonary oedema (36, 37) and emphysema (32).

This work also highlights the importance of making age and sex balanced subject cohorts in studies in which T1 is a read-out and not generalize results to a population based on a limited group; such as young males. Researchers utilizing T1-measurements and OE-MRI should take into account the sex difference in baseline lung T1 in the inspired state, and may benefit from measuring changes in R1 instead of T1, since ΔR1 is not dependent on baseline T1.

A limitation of the present work is that measurements were performed on inspiratory breath holds only – a retrospectively gated, free breathing protocol, or separate measurements at inspiration and expiration would have provided more in depth data on the studied relationships. Moreover, the sample size of the present study was chosen from a power calculation based on an expected T1-age dependency for both sexes. A larger subject cohort would allow further multiparametric analysis on the data. To use the Gaussian curve's standard deviation as a measure of the intra-subject T1 variability is based on the expected heterogeneous distribution of T1 values in the supine position(38). The study was designed to test the T1(age) relationship, and a sex dependency was quickly established. The statistical methods are not used to choose a preferred model but only analyze the preferred model T1(age*sex), so no multiple comparisons correction has been made on the presented p-values. Adjusting for several variables were done to elucidate potential confounders, but the main result (i.e. the statistical significance of the sex dependency on the T1(age) relationship) , and the enfolding discussion, was not affected by the inclusion of adjusting variables. If more subject data can be pooled – for example in a multi-center study – a more advanced, multiparametric model can be applied to the data, analyzing T1 of different respiratory states as well as taking into account several PFT parameters, where RV may be an important confounder.

In conclusion, we have provided evidence that baseline lung T1 is dependent on age in women, but not in men. These findings are likely a combined effect of blood T1 and pulmonary blood volume, which are both decreasing with age in women, creating a concurrent effect on T1. For men, the effects of increasing blood T1 and decreasing pulmonary blood volume may cancel each other, to yield an apparent non dependency between age and T1. Additional studies are needed to further describe in detail the underlying determinants of lung T1 for men and women – lung T1 may be a complex function of blood T1 and blood volume as described here, or in addition be a function of the elastic recoil of the parenchyma. The present work underlines that much is still to be learned about the MR signal generation in the human lung, and that interpretation of lung T1 measurements should include a discussion about pulmonary blood volume.

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Table 1: Mean values and standard deviations of registered parameters for males and females with complete pulmonary function test (n=24).

	Male	SD	Female	SD
Number	13		11	
Age [y]	42.0	14	41.0	16
Height [cm]	182	6.0	166**	4.9
Weight [kg]	79.6	11	67.5**	9.0
TLC [l]	7.70	0.68	5.70**	0.66
RV [l]	2.12	0.36	1.68*	0.47
FRC [l]	3.98	0.57	3.01**	0.49
VC [l]	5.60	0.54	3.99**	0.42
FEV_1 [l]	4.18	0.60	3.19**	0.52
$D_{L,CO}$ (a)	11.2	1.6	7.75**	1.13
k_{CO} (b)	1.56	0.24	1.49	0.20
T1 [s]	1.14	0.03	1.22**	0.07

Student's t-test was used to test parameter difference between sexes:
* for p<0.05, ** for p<0.01.
a. [mmol/min/kPa]
b. [mmol/min/kPa/l])

Table 2: Linear regressions of T1(x) = T1(0) + C*x for n=24 with pulmonary function tests.

Regression Slopes:	Female C [ms/x]	95% CI	R^2	Male C [ms/x]	95% CI	R^2	All C [ms/x]	95% CI
Age [y]	-4.22	[-5.7 -2.7]	0.79***	-0.283	[-1.6 1.0]	0.00	-2.43	[-4.1 -0.7]
Height [cm]	-0.721	[-12 11]	0.00	-1.22	[-4.1 1.7]	0.00	-3.63	[-6.2 -1.1]
Weight [kg]	-4.43	[-9.5 0.7]	0.22	-0.990	[-2.4 0.5]	0.09	-3.25	[-5.2 -1.3]
TLC [l]	-53.7	[-125 19]	0.15	-25.8	[-47 -5]	0.35*	-37.7	[-54 -21]
RV [l]	-131	[-192 -70]	0.70**	-21.0	[-70 29]	0.00	-106	[-146 -66]
FRC [l]	-47.7	[-154 59]	0.003	-20.6	[-50 9]	0.10	-53.1	[-85 -21]
VC [l]	30.7	[-97 158]	0.00	-25.2	[-55 4]	0.17	-36.3	[-62 -11]
FEV_1 [l]	77.0	[-12 165]	0.22	7.94	[-22 38]	0.00	-15.7	[-53 22]
$D_{L,CO}$ a.	17.6	[-29 64]	0.00	-2.26	[-14 9]	0.00	-12.2	[-24 -0.6]
k_{CO} b.	204	[-24 432]	0.24	21.5	[-54 97]	0.00	57.1	[-71 185]

Model slopes and 95% CI are presented, as well as R^2 marked with whole model significance level: *p<0.05, **p<0.01, ***p<0.001. (Units: a. [mmol/min/kPa], b. [mmol/min/kPa/l])

Table 3: Pearson's Correlation coefficient for pairs of independent variables

	Age	Length	Weight	TLC	RV	FRC	VC	FEV$_1$	D$_{L.CO}$
Length	0.08								
Weight	0.33	**0.68**							
TLC	0.18	**0.90**	**0.63**						
RV	**0.70**	**0.52**	**0.50**	**0.71**					
FRC	0.19	**0.72**	0.32	**0.88**	**0.74**				
VC	-0.12	**0.90**	**0.55**	**0.94**	0.45	**0.78**			
FEV$_1$	-0.37	**0.71**	0.31	**0.68**	0.12	**0.56**	**0.84**		
D$_{L.CO}$	-0.18	**0.74**	0.45	**0.76**	0.32	**0.63**	**0.82**	0.59	
k$_{CO}$	-0.47	0.01	0.00	-0.08	-0.36	-0.17	0.06	0.01	**0.56**

Coefficients > 0.5 are bold.

Table 4: Estimates of final model T1(age*sex) for n=30, with 95% confidence intervals.

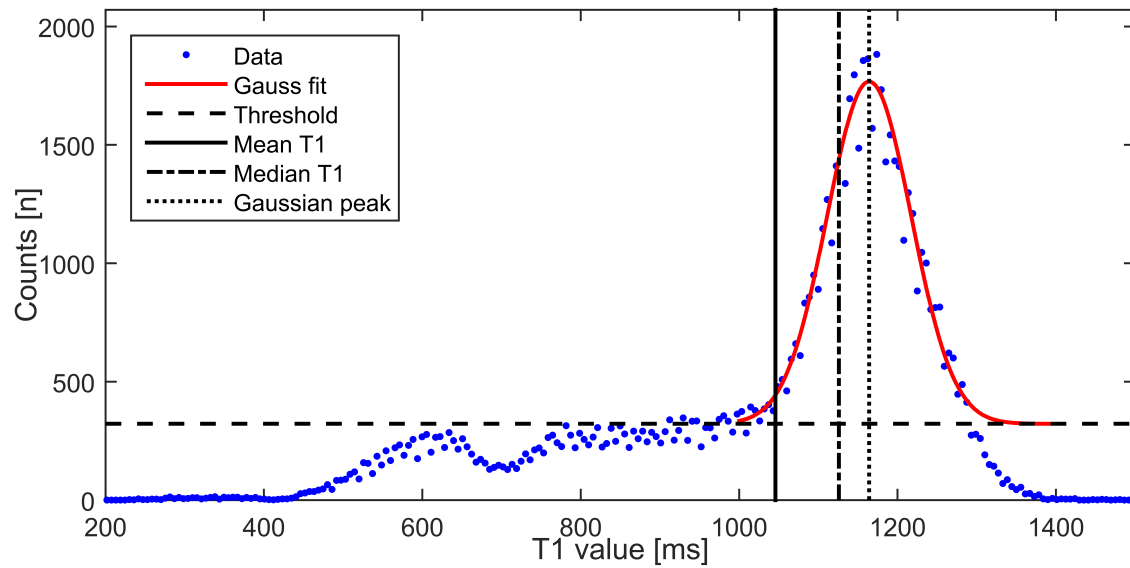|	Estimate	95% CI	p
Male			
T1(0) [ms]	1145	[1098 1190]	<1e-9
Slope [ms/y]	-0.064	[-1.2 1.1]	0.91
Female			
T1(0) [ms]	1387	[1342 1430]	<1e-9
Slope [ms/y]	-4.10	[-5.2 -3.0]	<1e-7

Figure 1: Histogram of T1-values from whole lung segmentation from a male subject, representative of the whole subject group. The peak of a Gaussian fit, after subtracting a threshold-value from the whole histogram, is defined as the T1 of the subject.
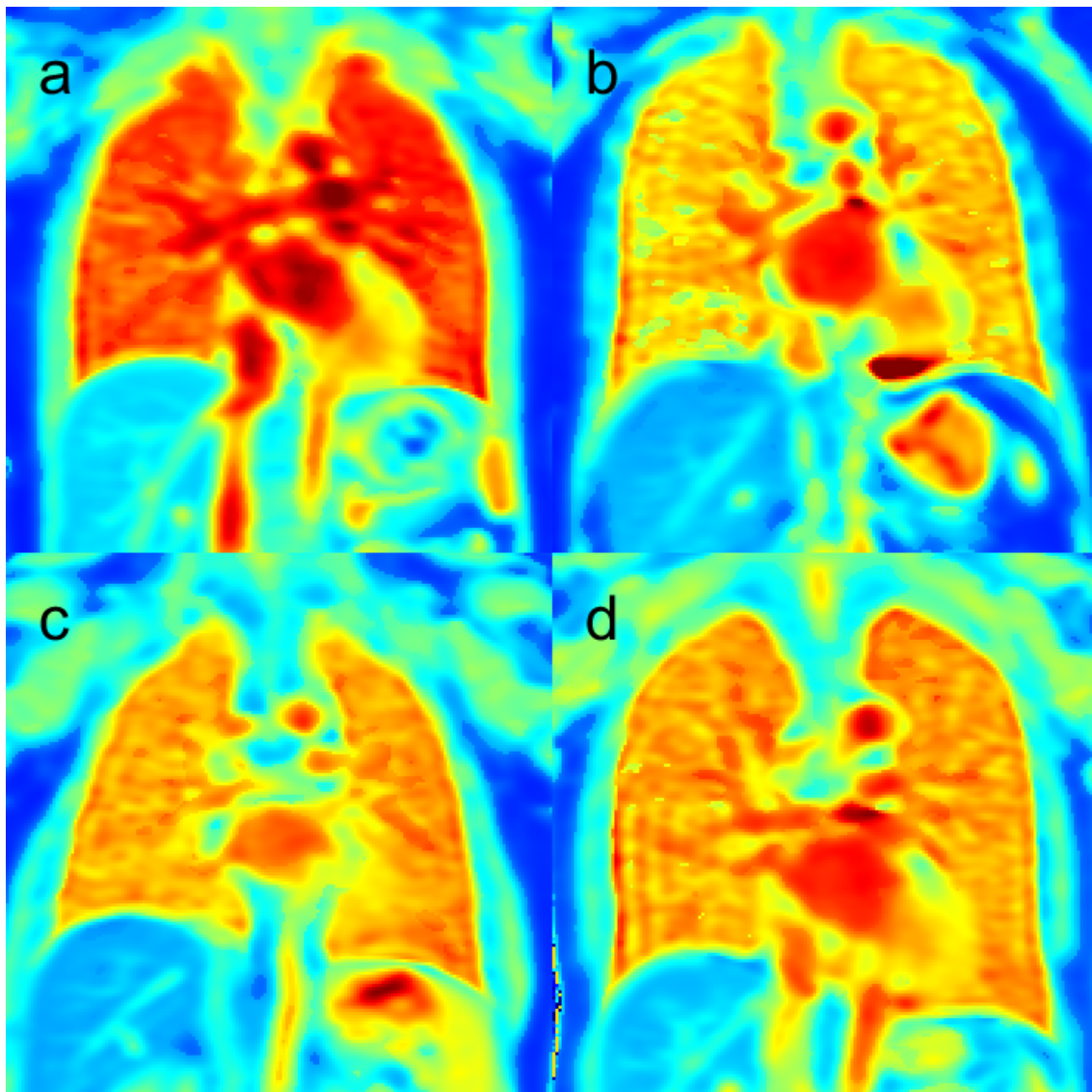
Figure 2: Representative T1 maps for (a) female age < 30, (b) female age >50, (c) male age<30, (d) male age>50. The contrast is adjusted for T1 = [0, 1600] ms.
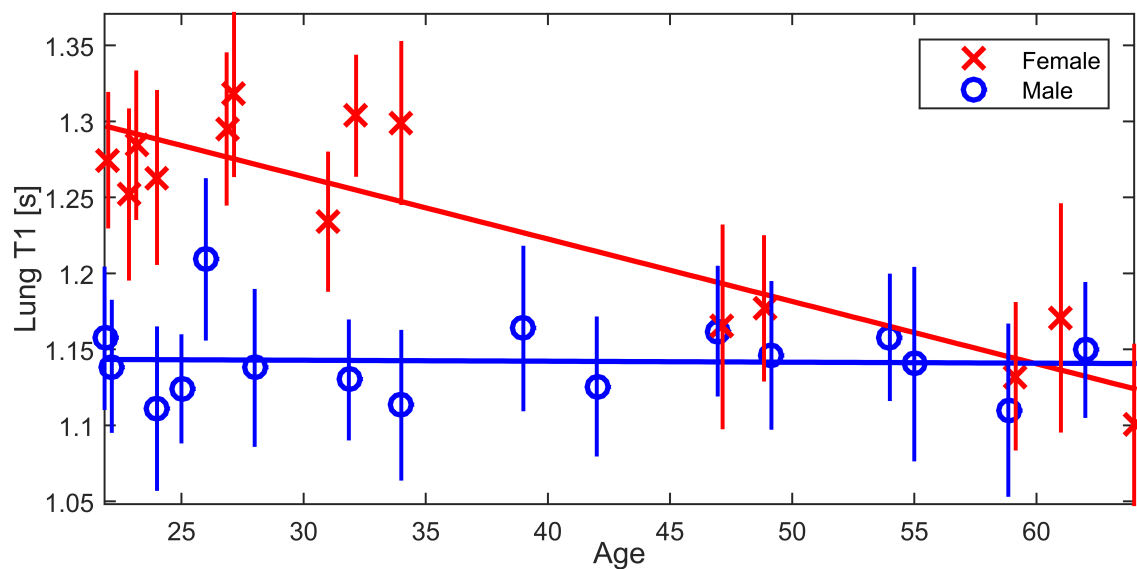
Figure 3: Plot of the final model of T1(age,sex) with separate regression lines for males and females, including the standard deviation of the Gaussian peak in the T1-histogram as error bars, for n=30. (Lateral offset of data points is only for visualization)
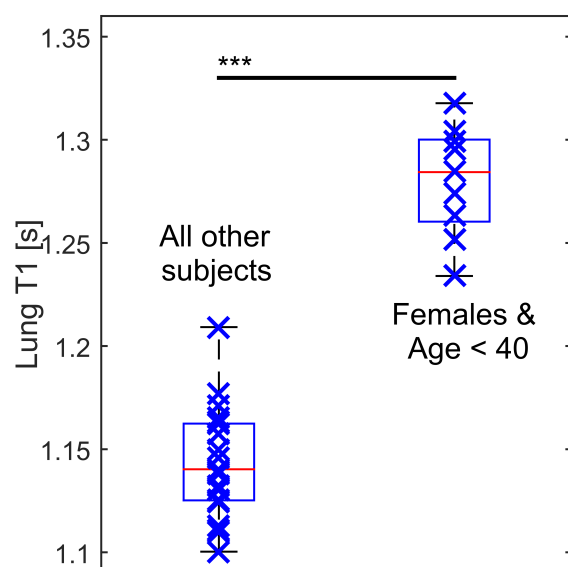

Figure 4: Student's t-test on lung T1 of women of age<40 (n=9), compared to the rest of the subjects (n=21), reveals a difference in mean lung T1 of 136 ms (95% CI[114,159]) at p<0.001.
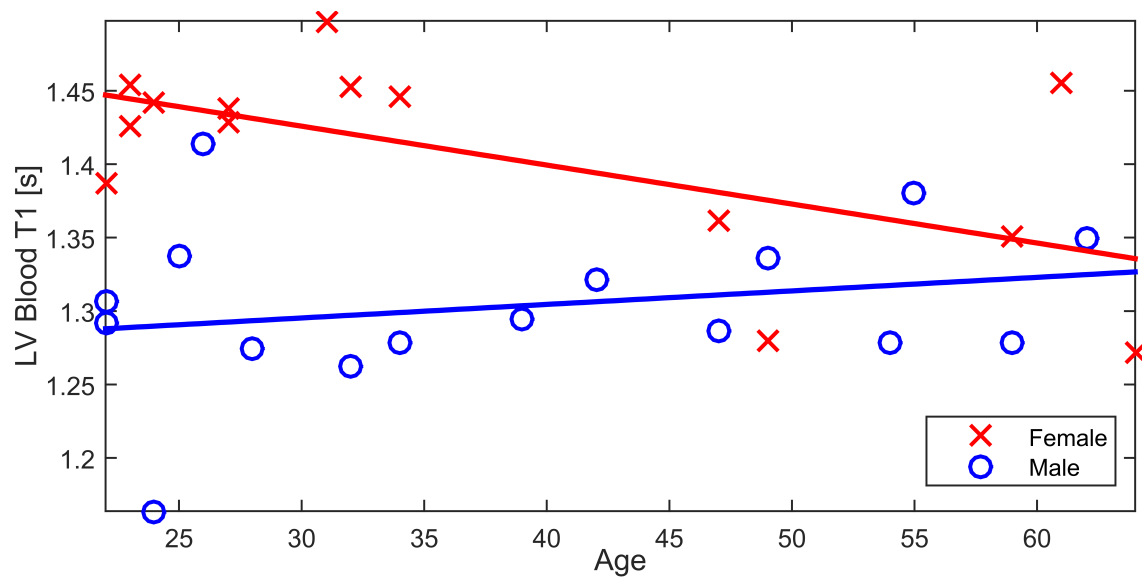
Figure 5: Mean T1 in circle drawn in the left ventricle of the heart as a function of age and sex. Although data is noisy (no cardiac triggering is used) male and female blood T1 exhibit different slopes as a function of age (p=0.035).