CHALMERS
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Thoracic and Total Body Bioimpedance Measurements

Individual variability, Repeatability and Reproducibility

Master's thesis in Biomedical Engineering

SAMIRA GHOMIAN

Department of Biomedical Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2015

Master’s Thesis SSYX04/2015

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Supervisor: Rubén Buendia, Chalmers University of Technology
Examiner: Bengt-Arne Sjöqvist, Department of Signals and Systems

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Department of Signals and Systems
Division of Biomedical Engineering
Chalmers University of Technology
SE-412 96 Gothenburg
Telephone +46 31 772 1000

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This thesis is dedicated to my lovely daughter Sahel

who brighten my everyday.

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SAMIRA GHOMIAN
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Chalmers University of Technology

Abstract

Electrical bioimpedance (EBI) is defined as the opposition of biological tissue to the flow of electric current. Furthermore EBI is a potential diagnostic and monitoring tool for hidden traumatic injury. Finally, bioimpedance technology is ideal for the pre-hospital setting because it is portable, quick, non-invasive and cost effective.
The most common injury as consequence of a road crash is chest blunt trauma and it is often hidden. Thus there is a need of tools for non-invasive detection and monitoring of thoracic injury. Whether abnormal values of thoracic EBI would be found in thoracic trauma patients, measuring EBI in the ambulance would potentially uncover hidden trauma.
In this thesis thoracic EBI in healthy subjects was characterized in order to determine the normal values of thoracic EBI in healthy individuals. Because the biggest challenge in order to discriminate between healthy people and thoracic trauma patients may lay in the inter-individual variability, the ratio between right and left sides of the thorax was also characterized. Nevertheless, different spectra of the bioimpedance values of different configurations of the body, such as total body, thoracic transversal, right side of thorax, left side of thorax and the ratio between the right side and the left side of the thorax, have been characterized in 16 healthy individuals at 50 kHz single frequency. Each single frequency measurement contain three features of the Bioimpedance measurement, resistance, reactance and phase angle.
As expected, the parameter exhibiting less variance was the phase angle ratio between both sides of the thorax which value is 1.03±0.1 degrees. Therefore, ratio of thoracic impedance might be a promising parameter for hidden thoracic injury detection and monitoring.
Moreover, repeatability and reproducibility as dependency of the results on the operators was determined using paired Student T-tests and Bland-Altman plots. Thoracic as well as total body EBI proved being highly repeatable and reproducible. Finally measurements observed in the morning, and in the evening were compared performing one-sample Student T-test and resulted in a non-statistically significant difference.

Keywords: Electrical bioimpedance, Thoracic trauma diagnostics, prehospital care.

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Samira Ghomian

List of Abbreviations

CI	Confidence Interval
EBI	Electrical BioImpedance
EBIS	Electrical BioImpedance Spectroscopy
ECF	ExtraCellular Fluid
ICF	IntraCellular Fluid
TB	Total Body
TBF	Total Body Fluid
TH	Thoracic Transversal
SF-EBI	Single Frequency- Electrical Bioimpedance
R	Resistance
RL	Right side of thorax (Right Lung)
RL:LL	Ratio between both Sides of thorax (Ratio between both sides of Lung)
X	Reactance
LL	Left side of thorax (Left Lung)

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1. Theoretical Frame

1.1. Electrical properties of biological tissue

Biological tissue primarily consists of Extracellular fluid (ECF) and cells. The cells contain organelles, intracellular fluid (ICF) and cell membrane. The ECF is composed of the medium surrounding the cells and the extracellular space (1).

As both ICF and ECF contain ions, they are considered as electrolytes. These ions can move freely and are capable of transporting electrical charge on both sides of the cell membrane. It enables the cumulating of charges, which cause different dielectric properties of the cell (2) And (3).

The dielectric properties of tissues are characterized by, not only the dielectric behavior of the cell membrane and the surrounding ionic fluids, but also the polar molecules, proteins, and macromolecules in the intracellular and extracellular area, which are too large to move in the presence of an electrical field. Nevertheless, these pieces can spin and position the dipole along the gradient of the electrical field (4).

An electrical field perform a movement of charges when applied to a biomaterial. This displacement is not instantaneous, and various charged molecules or proteins take different times. Polarization is maximized when the measuring frequency is enough low and all charges have sufficient time to change position. The polarization and permittivity come down with increasing frequency. This dependency of time may be characterized by introducing the concept of relaxation. Relaxation time is relevant to the polarization mechanism. Electronic polarization is the most rapid mechanism, with relaxation in the higher MHz and GHz regions as it is shown in Figure 1 (4) And (5).

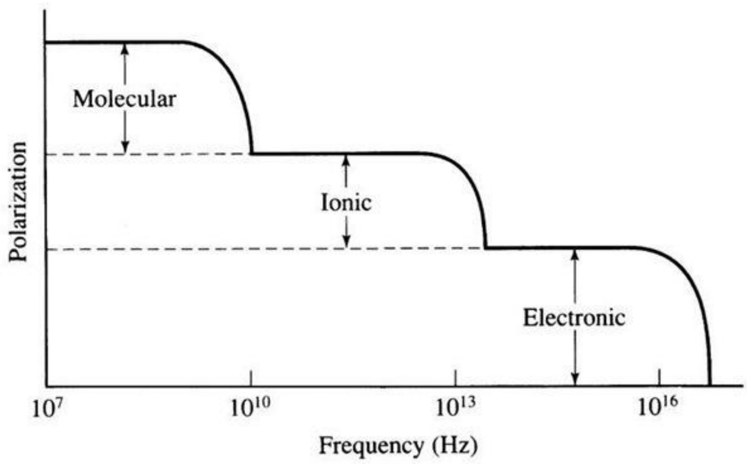


***Figure 1*:** Polarization mechanism in different frequency regions (5)

Dispersion is the frequency dependence of the permittivity according to the laws of relaxation; and permittivity is the corresponding concept of relaxation as a function of frequency. Tissues have finite ionic conductivities proportional and symmetric with the nature and extent of their ionic content and ionic mobility. Figure 2 shows permittivity and conductivity as a frequency function for muscle tissue and its distribution windows. Table I illustrates different distributions (3).

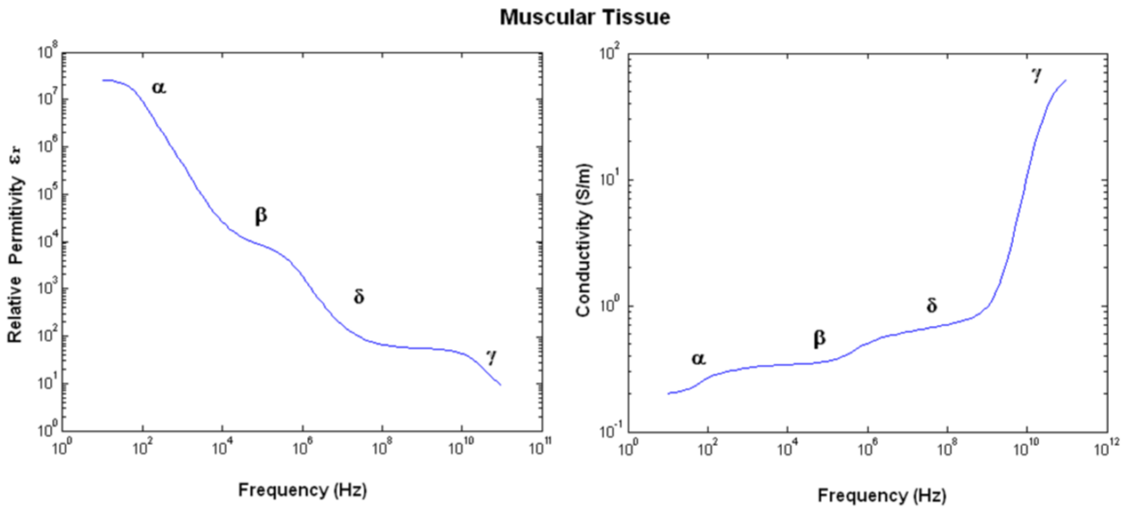


Figure 2: Permittivity and conductivity as a function of frequency (3)

Table I: Frequency ranges of the different dispersions (3)

Dispersion	**Frequency Ranges**
alfa	mHzs - kHzs
Beta	0.001 - 100 MHz
delta	100 MHz - 5GHz
gama	0.1 - 100 GHz

1.2. Electrical Bioimpedance

Electrical bioimpedance (EBI) is defined as the opposition of biological tissue to the flow of electric current. Since a large portion of the body is containing water and ions, generally the current is conducted through the body. The body can be divided in non-conducting components which correspond to the bones, components with low conductivity as fat mass, and conductive components as muscles, blood, organs and all components containing water and ions (3), (6) And (7).

Generally bioimpedance is measured by injecting a low level of electrical current through the tissue in order to excite passive tissue and sensing the voltage generated by the opposition of the tissue to the current. In tetra polar measurements the stimuli in the form of current is injected through a pair of electrodes that are attached on the tissue, and the response in the form of voltage is be sensed using another pair of electrodes, (3), (6) And (7).

Cell membranes behave as capacitors. At high frequencies the current passes through these capacitors and the result is dependent on the tissue and the liquids both inside and outside the cells. Otherwise at low frequencies the membranes impede the current flow and the result is dependent only on liquids outside the cell. Therefore, bioimpedance increase with decreasing frequency as they are inversely proportional to the reference current, which is kept constant, and directly proportional to the electrical field (3) And (8).

1.3. EBI measurements

Considering each section of a body like a cylinder is an ideal model of a tissue, the impedance Z is obtain by Equation 1.

Equation 1: $$Z = \rho L / A$$

Figure 3 shows a tissue example with electrodes around that, the outer ones are for injecting the current and the inner electrodes are to subsequently pick up the voltage (3) And (9).

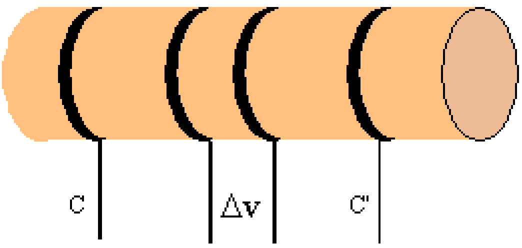


***Figure 3*:** A tissue example with electrodes around (8)

In the formula above, ρ is the resistivity of the tissue, L is the distance between the voltage sensing electrodes, and A is the cross-sectional area. Generally two methods are being used in EBI measurements, Electrical bioimpedance spectroscopy (EBIS) and single frequency electrical bioimpedance (SF-EBI) (3).

EBIS consider frequency spectra with multiple frequency measurements and SF-EBI is just one frequency. This frequency is often 50 kHz (3).

1.4. Four electrode measurements

There are different configurations using 2, 3 and 4 electrodes to perform an EBI measurement, in this experiment the 4 electrode configuration shown in Figure 4 was examined.

According to the Figure 4, an amperometric approach is made to apply the current with two electrodes and the voltage drop is sensing performing the two other electrodes. Note that applying a voltage and thus sensing the electrical current can be approached as the voltametric approach (3) And (10).

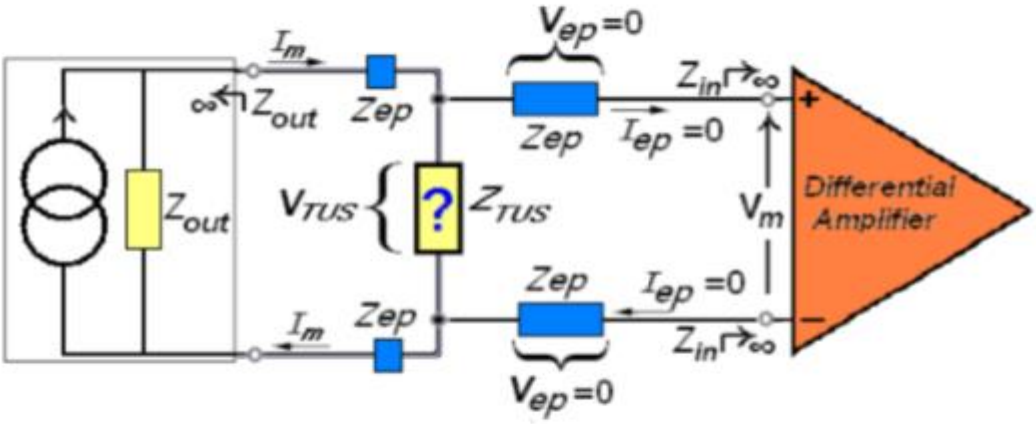


***Figure 4* :** 4 electrode configuration diagram (3)

According to the measurement model pictured in Figure 4, since electrode polarization impedance (Z_{ep}) is small enough compare to the input impedance of the circuit, the effect of electrodes on the measurement will be eliminated. Also, since input impedance (Z_{in}) is large enough then electrode polarization current (I_{ep}) is negligible and consequently electrode polarization voltage (V_{ep}) is considered null. Thus measured impedance (Z_{mean}) = tissue under study impedance (Z_{TUS}). Following equations 2 – 5 are shown for a comprehensive understanding (3) And (10).

Equation 2: $$Zm = \frac{Vm}{Im}$$

Equation 3: $$Vep = IepZep$$

Equation 4: $$Vm = Vep + Vtus + Vep = Vtus + 2\,Vep$$

Equation 5: $$Zm = Ztus$$

1.5. SF-EBI for tissue assessment

The different spectra of the Bioimpedance are Resistance (R) that is directly related to tissue resistivity, Reactance (X) which indicate cell membrane density and function and Phase Angle (PA) indicating cell membrane vitality. All 3 spectra are related existing a high correlation between X and PA. They can be observed at Figure 5, (11).

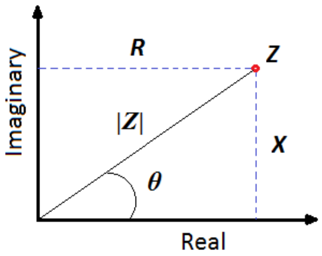


Figure 5: Complex SF-EBI measurement

Healthy membranes cause a phase difference in the transit of voltage and current; accordingly the bigger the PA, the better condition the cell membranes and decreases in PA return damaged membrane (11). Therefore PA decreases with inflammation, tissue damage and cell loss. This way, conventional whole-body Bioimpedance measurements at 50 kHz using hand-to-foot electrode placements manifested that phase angle is a great predictor of morbidity and mortality in many types of chronic disease as well as an independent predictor of inflammation (12).

Reactance (X) provides similar information as PA. It also decreases with tissue damage and cell loss. However it is more related to cell membrane density than to cell membrane vitality. Both X and PA would decrease with tissue damage and thus with chest trauma. However PA is expected to be a more sensitive indicator (12).

Finally it was concluded that "*Regional Bio-impedance has a promising predicting and monitoring value in diseases affecting skeletal muscle*". Besides, measurements in younger and professional FC Barcelona players reveal that injury to muscle causes represented decreases in *R*, X and PA and that these variations are related to the severity of the injury, (11), (13), (14) And (15).

1.6. Accuracy and precision of the measurements

Measurements are subjected to various sources of errors for several reasons, which cause the measured value to differ from the true value. In fact, every time a measurement is repeated with an instrument, some different results are obtained. Basically, the error can be divided in systematic and random error (16), (17) And (18).

Random errors are statistical swings in the measured data set usually caused by operator's inability to measure certainly in the same way and keep the conditions identical in order to get the exactly equivalent result. Otherwise systematic errors, are caused often due to a flaw of the device, which remains throughout the whole experiment or from mistakes the operator makes during the entire experiment, (16), (17) And (18).

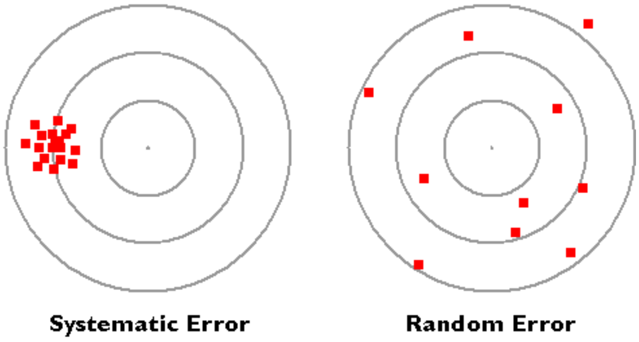


Figure 6: Geometric definition of random error and systematic error (18)

As it is illustrated in Figure 6, Systematic errors are almost constant in size and/or direction while random errors differ both in magnitude and direction.

Precision is related to random errors, and accuracy is related to the systematic error. The accuracy of a measurement is the degree of closeness of measurements to the true value, and the precision indicate reliability (11), (19) And (20). This is visualized in Figure 7.

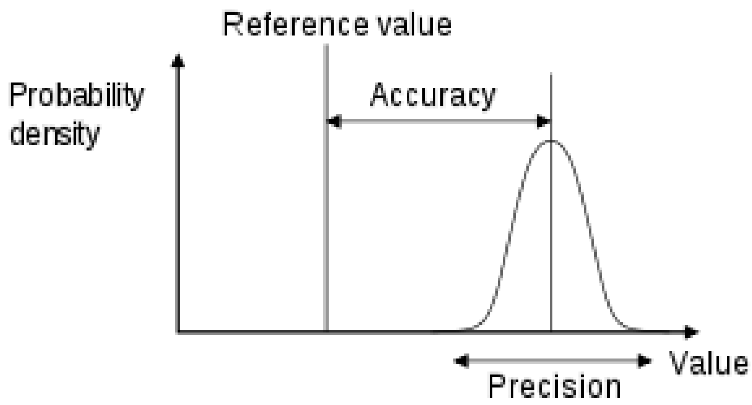


Figure 7: Visual explanation between accuracy and precision (21)

1.7 Repeatability and Reproducibility

Repeatability of measurements is the variation in repeated measurements made on the same item under identical conditions. This means that measurements are taken by the single instrument or method, the same observer or rater, in a short period of time. It follows that variability in measurements made on a same subject in a repeatability study can be ascribed only to errors due to the measurement process itself (22) And (23).

Reproducibility refers to the variation in measurements under changing conditions. The changing conditions may be due to different measurement methods or instruments being employed, and different independent observers or raters. Reproducibility can be defined as the variance in the mean of measurements obtained in the mentioned conditions, (22) And (24), see Figure 8.

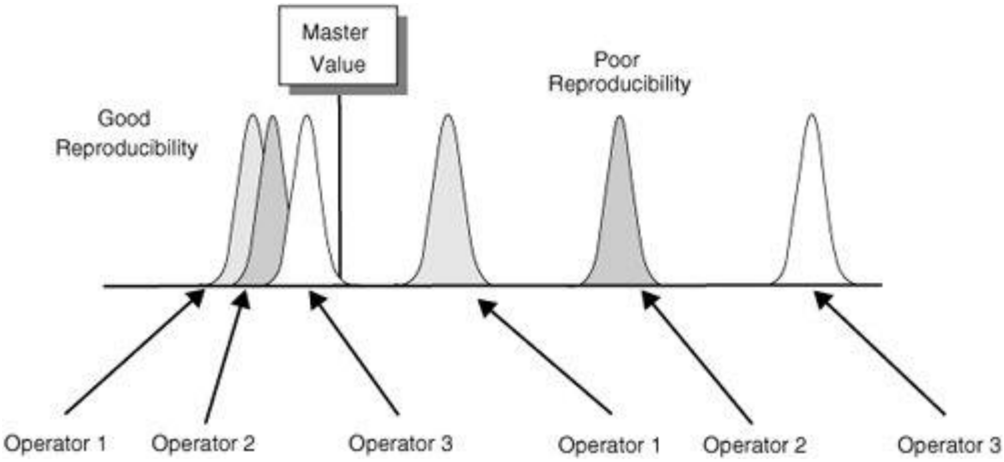


Figure 8**:** Geometric definition of reproducibility (25)

1.7. Statistical methods

1.7.1 Student T-Test

The statistical hypothesis test used in current experiment is the student T-Test both paired and unpaired. Student T-Test is generally used to compare the mean of two groups in order to find out if there is a significant difference between them; in other words, if two data sets might belong to the same population or not (26).

The unpaired *t*-test is used when we want to know if our sample comes from a particular population but we do not have full population information available. A paired T-Test is used to compare two population means where you have two samples in which observations in one sample can be paired with observations in the other sample (27) And (28).

The formula for t-test is given below:

Equation 6: $$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Where:

$\overline{X}_1$= Mean of first group of values
$\overline{X}_2$= Mean of second group of values
S_1 = Standard deviation of first group
S_2 = Standard deviation of second group
n_1 = Total number of values in first group
n_2 = Total number of values in second group.

The standard deviation formula is:

Equation 7: $$S = \sqrt{\sum \frac{(X - \overline{X})^2}{n-1}}$$

Where:

X = Values given

$\overline{X}$=Mean

n = Total number of values (29)

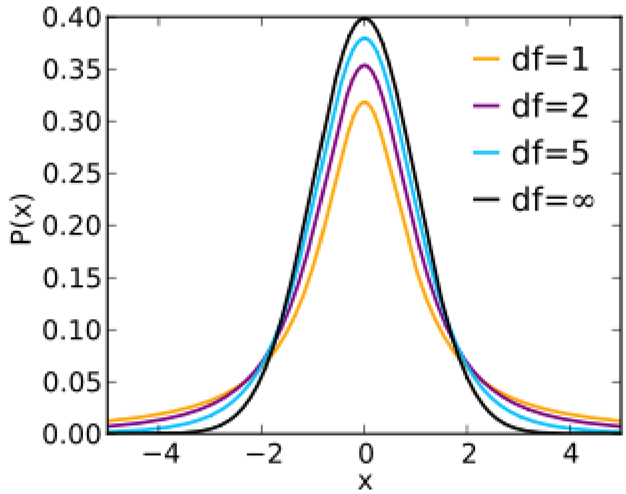


Figure 9**:** T- distribution curve, which is a function of the degrees of freedom (30).

Figure 9 shows an example of t- distribution curve, which is a function of the degree *of* freedom. *Df* can be described as the number of values that are free to vary. These values are the independent part of data is involved in calculation. It identifies the accuracy of the sample population, such as the larger df, larger the possibility of the whole population to be sampled accurately. The formula for degrees of freedom is given below (29) And (30).

$$df = n_1 + n_2 - 2$$

1.7.2. Bland-Altman

Bland–Altman plot or Difference plot is a method of data plotting used in assessing the agreement between two measurements techniques or instruments, for an example see Figure 10 (31). The horizontal axis of the plot represents the average of each pair of measured data and the vertical axis the difference between them. By other words, each pair on the plot is represented as A = ((x_1+x_2)/2, (x_1-x_2)).

The Bland–Altman method visualizes the average between two measurements methods (bias) and 95% limits of agreement (CI= 1.96 std). Such as the 95% limits include 95% of differences between the two measurement methods. (31) And (32).

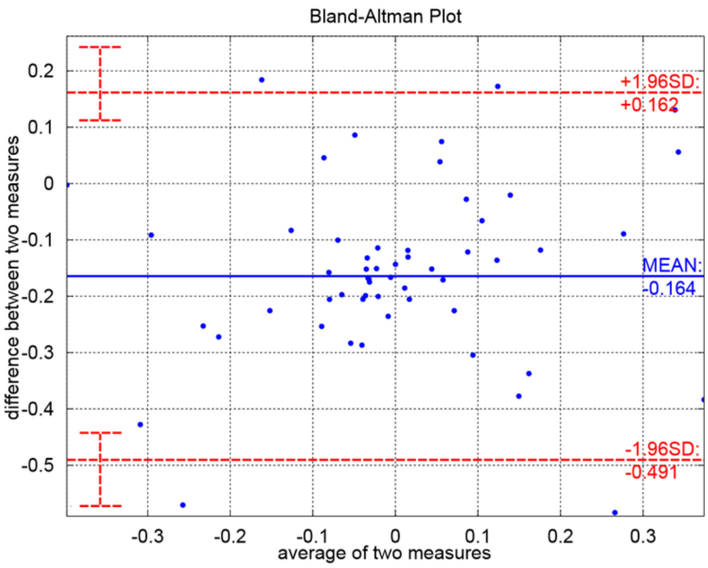


Figure 10**:** Bland altman plot

According to 95% limits of agreements, around 95% of the time the difference between 2 measured data, lies whithin the CI lines.Generally in a bland altman plot, the size of bias and the width of the limits of agreement (CI) are remarkable in order to assess accuracy and precision. Such as a high bias means a low accuracy. The variance is a measure of the precision as a high variance means low precision (31) And (32).

2. Introduction

Electrical Bioimpedance (EBI) is the opposition of biological tissue to the flow of electric current. Therefore it is a direct measurement of the electrical properties of biological tissues. Furthermore, it is a function of the applied electrical frequency and the resistive and capacitive properties of tissue components. This technology offers a gentle, noninvasive, cost effective, and efficient alternative method for the estimation of body composition. Body composition includes concepts like fluids volume, body cell mass or fat mass. Since it is quick, portable and easy to use, the technology has a great potential in diagnosis for pre-hospital monitoring and ambulance applications.

In this way, electrical bioimpedance (EBI) technology is a potential diagnostic and monitoring tool for traumatic injury. In addition to traditionally monitored EBI parameters, like basal impedance and impedance variations, the EBI Phase Angle (PA) measurement offer a novel opportunity to non-invasively detect hidden trauma (11) and (12). Moreover low PA has been demonstrated to be a good independent predictor of inflammation (12). The reason of this prediction power is that healthy membranes cause a certain phase change between the measured voltage and the injected current -the greater the PA the healthier the cell membranes and decreased PA reflects an impaired membrane function.

Apart from PA other spectra of the impedance are Resistance (R), that is directly proportional to tissue resistivity and inversely related to extra cellular fluid, and Reactance (X) that indicates cell membrane density and function. Measuring Bioimpedance in a pre-hospital setting like in an ambulance or a crashed vehicle with special attention to PA could uncover injuries when they do not present external symptoms of severe trauma.

The detection power of Bioimpedance for traumatic injury may be highly hindered by inter-individual variability. This way thoracic impedance values difference between individuals may be larger than differences within the same person before and after suffering thoracic trauma. In order to overcome this problem the ratio between right and left side of the thorax can be considered because the inter-individual variability of this ratio should be much smaller.

The goal of this thesis is to characterize thoracic measurements in a healthy population. This characterization includes the evaluation of repeatability and reproducibility of the measurements. The ratio between the bioimpedance values of the right and left side of the thorax in healthy individuals as well as Phase Angle (PA) receives more attention.

This study was conducted on 16 healthy people 9 female 7 and male aged 26-74. The Bioimpedance values of different configurations of their body (total body, thoracic transversal, right lung and left lung) was measured at single frequency, 50 kHz by two independent operators. The observed values of each individual is recorded and based on them the bioimpedance values will be characterized. In addition repeatability and reproducibility of bioimpedance measurements in mentioned configurations will be determined and discussed according to assessment of dependency of the results to the operator who performed the test. Apart of that, influence of time the experiment performed, 5 in the morning 11 in the evening, will be evaluated.

3. Materials and Method

3.1. Subjects Characteristics

Only healthy subjects were included in the study. Their characteristics are presented in the following table.

Table II: Subjects characteristic

	All (16 people)	Males (7 men)	Females (11 women)
	mean ± std	mean ± std	mean ± std
Age	33,5 ± 12,6	32,7 ± 8,2	34,0 ± 15,4
Weight (kg)	70,8 ± 15,4	87,1 ± 17,1	62,7 ± 4,6
Height (cm)	171,1 ± 7,3	176,3 ± 5,7	167,6 ± 6,2

3.2. EBI Device & Electrodes

There are a variety of devices utilizing bioimpedance measurements in the medical devices market. They inject an extremely small electrical current (200 – 800 µA) in the patient body and subsequently record the voltage drop produced by this current and the opposition of the body to it. Simultaneously the phase shift between alternating current and voltage is measured, indicating the cell mass capacitive component (33).

The device used was the *Arkern BIA 101 Anniversary*. EBI of different body segmentations at single frequency (50 kHz) was measured. The result of measuring is visualized in the display in the form of Resistance (R) in Ohms, Reactance (X) in Ohms and Phase angle (PA) in degrees. The device was calibrated prior to each set of measurements using the supplied test circuit. It simulates the electrical properties of the body and assures the accuracy of the device, cables and connections within the tolerance range. Fnally, BIA electrodes supplied by *Akern* were used.

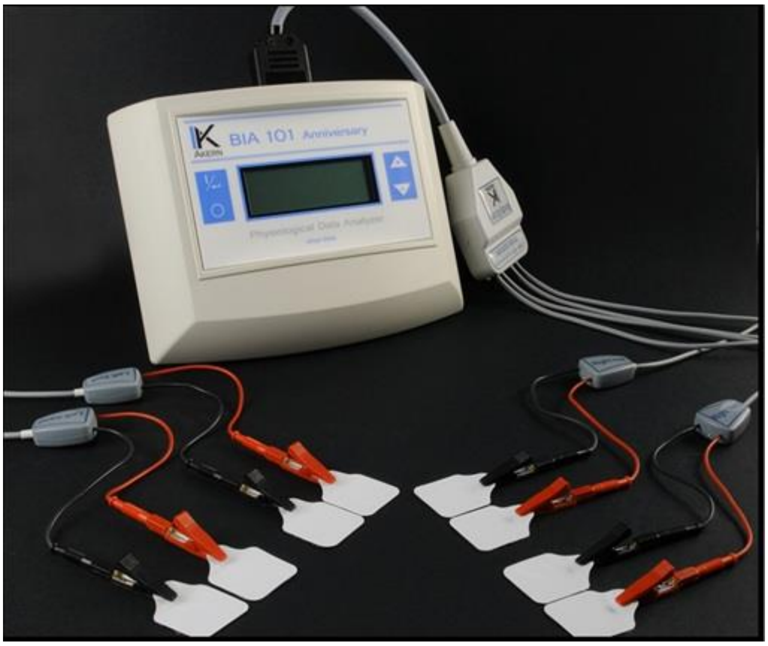


***Figure 11*:** BIA 101 anniversary including cables, connections and electrodes

***Figure 12*:** BIA electrodes (33)

3.3. Measurements Set-Up

Tetra polar measurements were performed on the subjects using 4 different configurations by two different operators:

1. Total body, Right Side Wrist to Ankle
2. Thoracic Transversal
3. Longitudinal left lung
4. Longitudinal right lung

Operators measured the subjects independently twice each time and record the results. The measurement processes were done in different places, Borås university, Chalmers university , MedTech West co. at Sahlgrenska hospital and SAFER co. Similar conditions were kept during the experiments.

Placing electrodes on the subject's body and device calibration were performed at the beginning of the measurement session. Thereafter both operators independently connected the device to relevant electrodes for each segmentation and recorded the results. When bioimpedance value of all segmentations were measured and before repeating it again, the subject was asked to walk a bit or stand up for 5 minutes (relaxing time) in order to avoid the gravity effect on body fluids and try to keep the condition and situation similar over the whole experiment time. After that the other operator starts to measure independently and repeated the same procedure. The whole process for each individual took about 45 minutes; including the four electrodes configurations, recordings and relaxing times. The subjects were asked to breathe normally during the recording time and remove any metallic objects. The measurement set up includes sixteen electrodes on the subject's body. The subject was asked to lie in supine position. Figure 14 illustrates the electrode placement.

3.3.1. Total body

Measurement of total body bioimpedance is the most commonly used method for estimating whole body composition and in our case, it was such a reference to compare and evaluate with other body segments bioimpedance measurements.

According to the Figure 14 yellow electrodes were attached in order to measure total body bioimpedance value. The outer electrodes applies small electrical current and the inner ones measures the voltage drop. The placement and the distance between electrodes is recommended by the device supplier and we followed that as well.

The current electrode on the hand was being placed under the first joint of the middle finger and the other one on the foot was being placed on the base of the second toe. The voltage electrode on the hand was being attached under an imaginary line cutting the ulnar head (bone of little finger side of wrist) and the other one on foot was being attached under an imaginary line cutting the medial malleus (bone on big toe side of ankle).

Total body RS-WA measurements are traditionally on the right side and the distance between each pair of electrodes was keep around 5cm as recommended.

3.3.2. Thoracic transversal

In this segment both lung and cardiac region bioimpedance has been measured and the electrode locations are on the anterior upper thorax as it is shown in the Figure 14 by blue electrodes. The upper ones are voltage electrodes and current electrodes are the lower ones. In order to have an observed pattern for all our subjects we defined the placement of these electrodes according to imaginary line passes Xiphoid extension (lower end of sternum). The electrodes were placed on the upper and lower of the lateral sides of imaginary line and the distance between each pair was considered about 2 cm.

3.3.3. Left side of the thorax

Cardiac, left lung and left thorax bioimpedance is being measured in this segment. In order to cover the whole mentioned region one pair of electrodes were located under the middle of Clavicle bone and the other pair was placed just above the imaginary line passes Navel on the lateral back side. The distance between electrodes are about 2 cm, the outer electrodes are current electrodes and inner ones are voltage electrodes. This placement are shown in the Figure 14 by red electrodes.

3.3.4. Right lung or right side of the thorax

The electrodes were allocated similar to the left lung measurement but on the opposite side of the body as it shows in the Figure 14 by black electrodes.

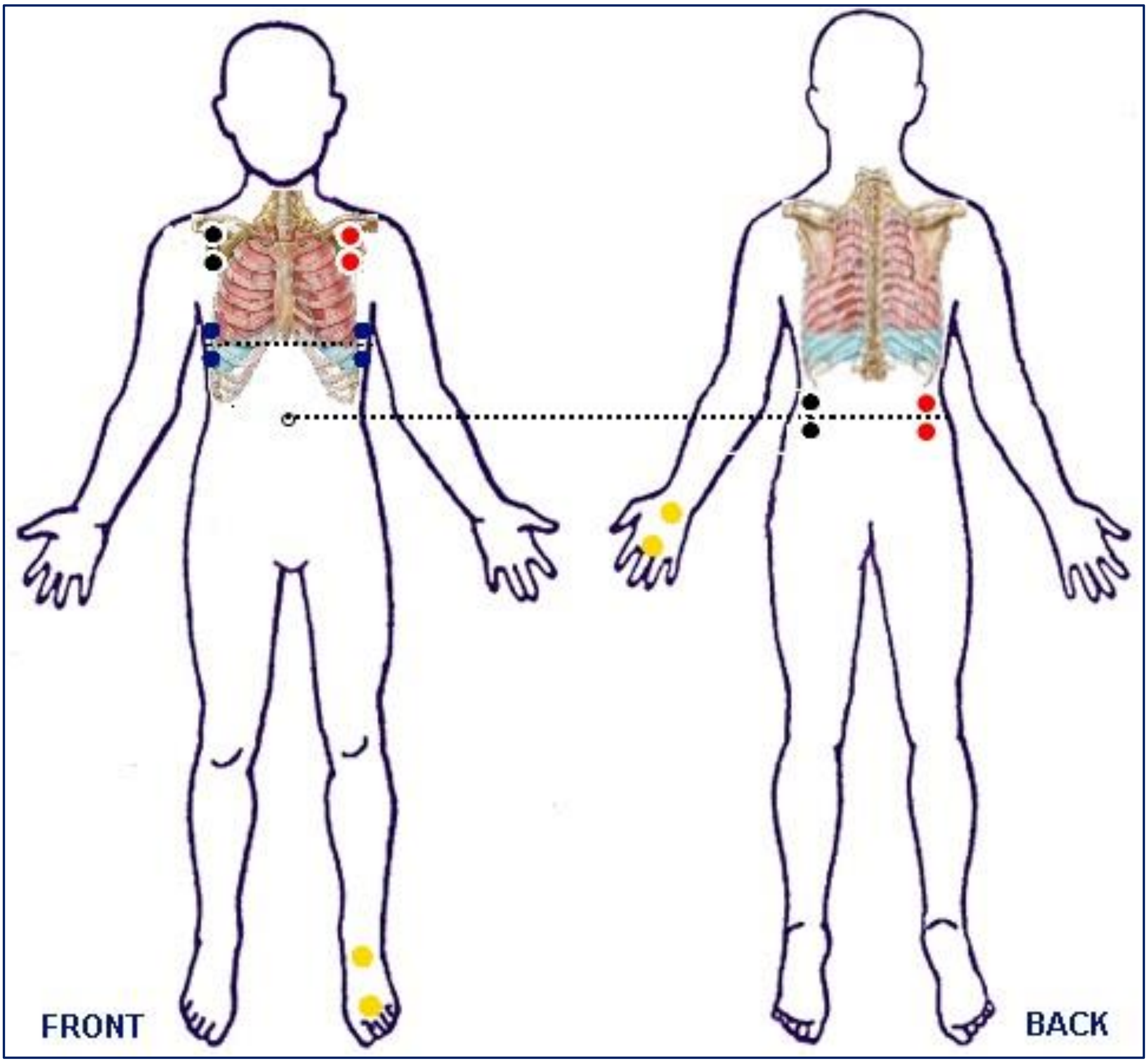


Figure 13**:** Electrodes placements

Since the measurement is not sensitive to small changes in electrode locations (34), (35) And (36). The locations can be slightly modified. Table III gives a short explanation of the Figure 14.

Table III: Explanation of Figure 14

Measurement	Color of electrodes	Distance between
Total body	Yellow	5 cm
Thoracic transversal	Blue	2 cm
Left side of thorax	Red	2 cm
Right side of thorax	Black	2 cm

In this document the different configurations were abbreviated as follow:
TB: Total body
TH: Thoracic transversal
RL: Right side of thorax
LL: Left side of thorax
RL:LL: Ratio between both sides of thorax

3.4. Statistical Methods

The Bland-Altman plot was used to compare two measurement techniques. In this graphical fashion the differences between the two methods are plotted against the averages of them. In this graph three horizontal lines are being drawn as well; Bias line which shows the mean of differences and the two others are the limits of agreement which are defined as the bias plus and minus 1.96 times the standard deviation of the differences (31) And (37). The p-value according to the student T-Test was also used.

4. Results

The first statistical result is the mean and standard deviation (std) of our observations in order to characterize the range for each feature of each bioimpedance measurement configuration. Second result regards to repeatability, which the repeatability of results repeated by a single operator in a short period of time, has been determined. The third result is about reproducibility between operators. Two different operators measured under identical conditions. Finally bioimpedance measured values have been divided into two different categories based on the time experiment was done, morning or afternoon, and their differences subjected to the T-test.

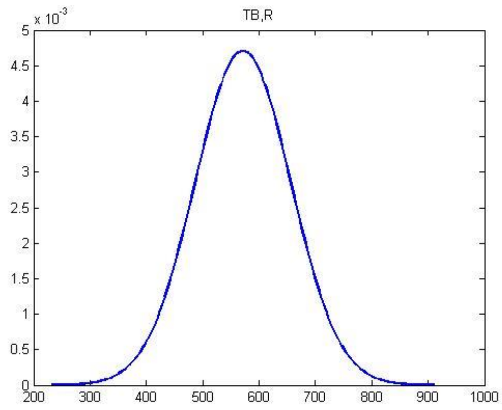
4.1. Assessment of data distribution

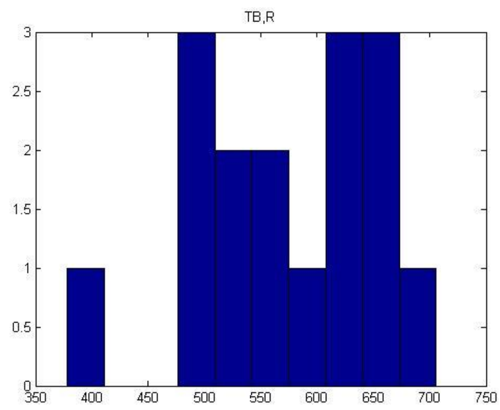
At the beginning, the mean and std for each body segment, regardless of the operators, have been calculated in order to characterize the range as well as figuring out how much the values we got are spread around the mean; table IV shows the results. Since the Gaussian curve and histogram plot illustrate normal distribution and data distribution, Figure 15 containing the Gaussian curves and histogram graphs to display the spread and the closeness of the measurements.

Note that in a histogram plot, the length of bars indicates the frequent of a data. Also the width of Gaussian curve indicates the closeness of the data under study.

Table IV: Data distribution

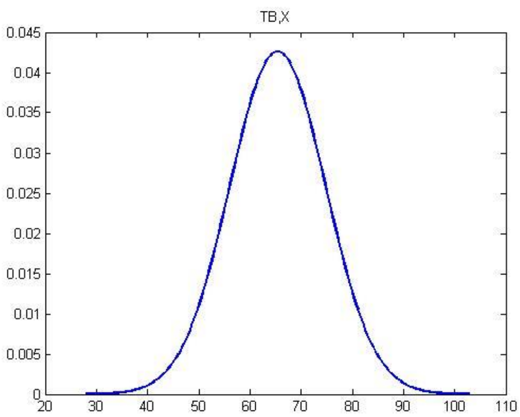
		mean	*± std*	*Percent std from the mean*
Total body	*R*	571,752	± 83,448	14,6%
	X	65,567	± 9,222	14,1%
	PA	6,581	± 0,774	11,8%
Thoracic transversal	*R*	41,366	± 9,443	22,8%
	X	8,494	± 2,084	24,5%
	PA	11,956	± 3,048	25,5%
Right side of thorax	*R*	55,671	± 14,829	26,6%
	X	8,657	± 2,407	27,8%
	PA	9,292	± 2,914	31,4%
Left side of thorax	*R*	54,622	± 12,73	23,3%
	X	8,629	± 1,428	16,5%
	PA	9,43	± 2,631	27,9%
Ratio between Right side and left side of thorax	*R*	0,994	± 0,108	10,9%
	X	1,026	± 0,142	13,8%
	PA	1,032	± 0,105	10,2%

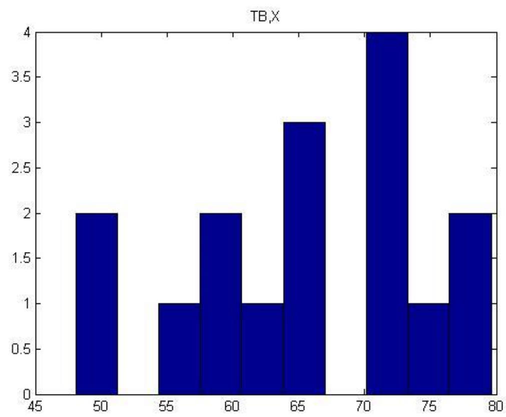




a) *Normal distribution for the value of R in all total body measurements*

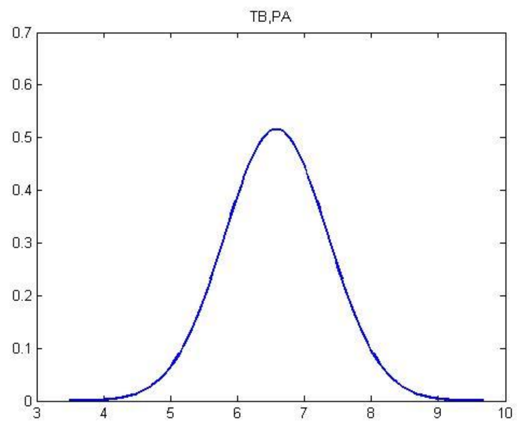
b) *Data distribution for the value of R in all total body measurements*

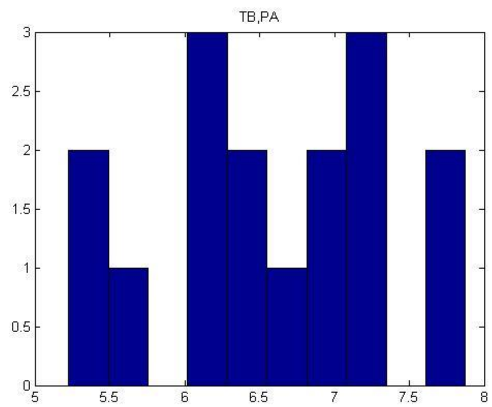




c) *Normal distribution for the value of X in all total body measurements*

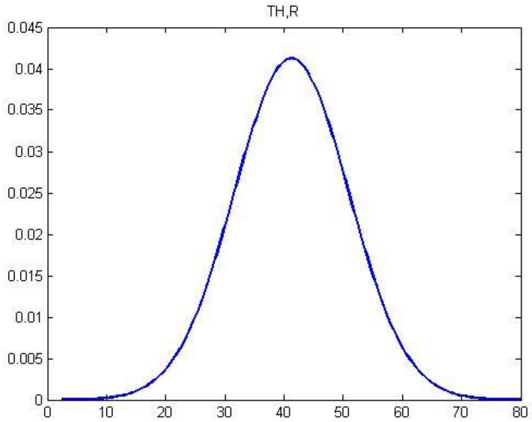
d) *Data distribution for the value of X in all total body measurements*

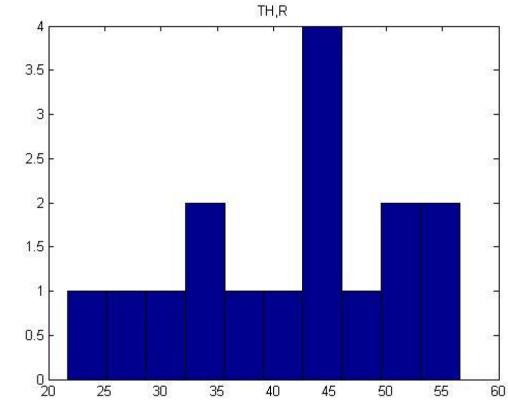




e) *Normal distribution for the value of PA in all total body*

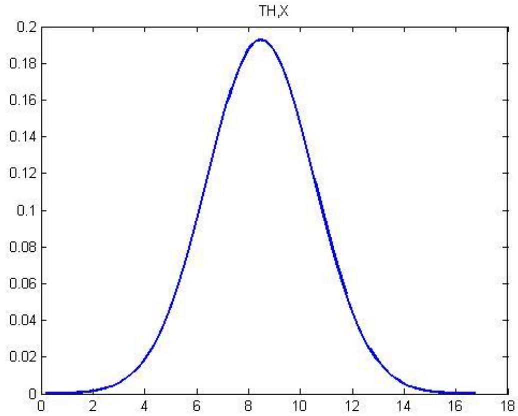
f) *Data distribution for the value of PA in all total body measurements*

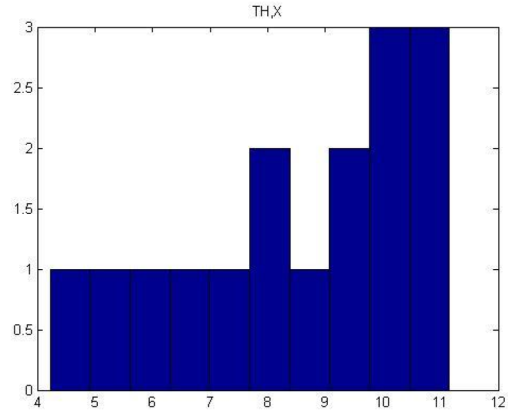




g) Normal distribution for the value of R in all thoracic transversal measurements

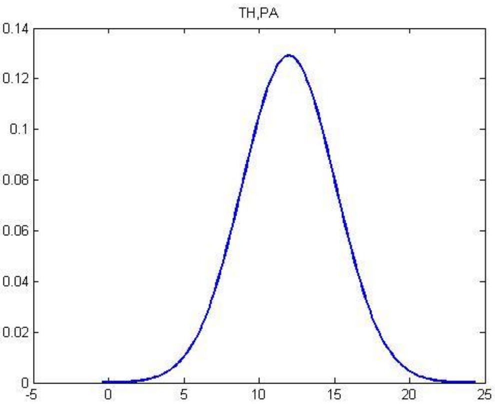
h) Data distribution for the value of R in all thoracic transversal measurements

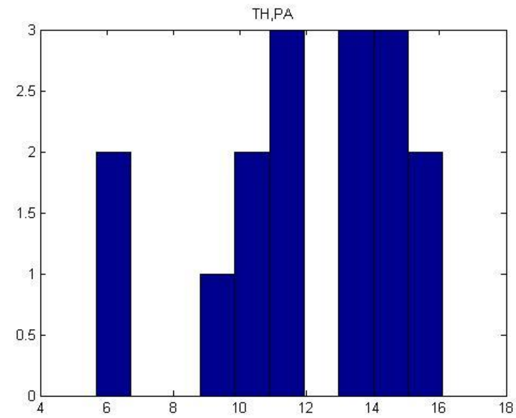




i) Normal distribution for the value of X in all thoracic transversal measurements

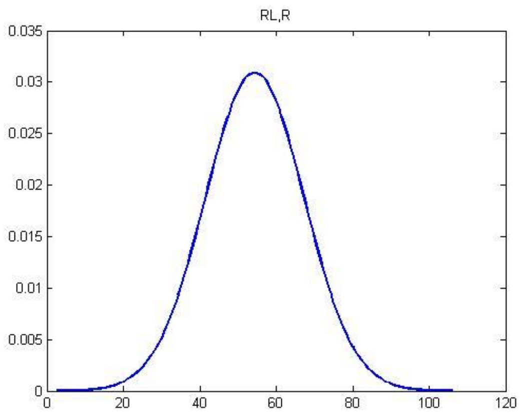
j) Data distribution for the value of X in all thoracic transversal measurements



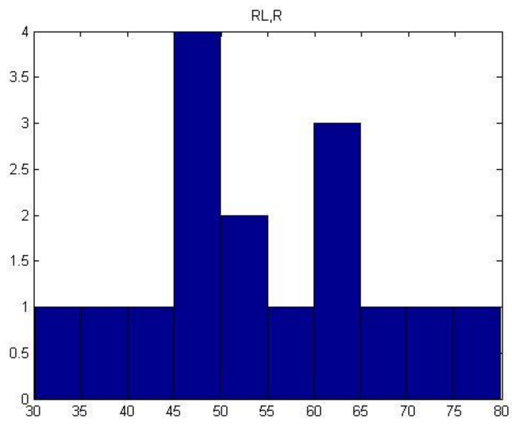


k) Normal distribution for the value of PA in all thoracic transversal measurements

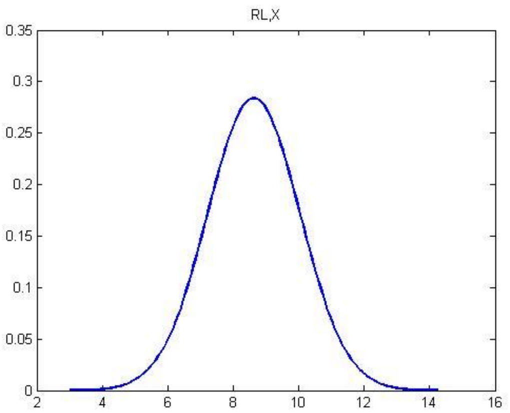
l) Data distribution for the value of PA in all thoracic transversal measurements



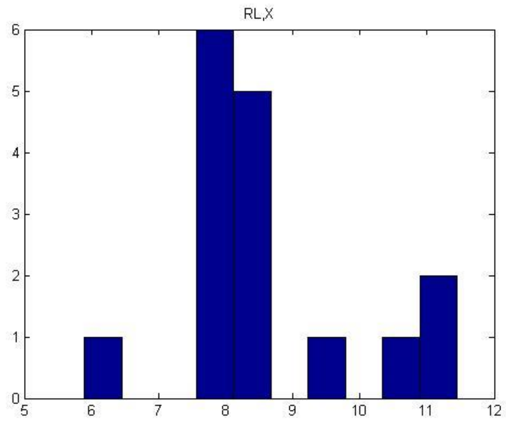
m) Normal distribution for the value of R in all the right side of thorax measurements



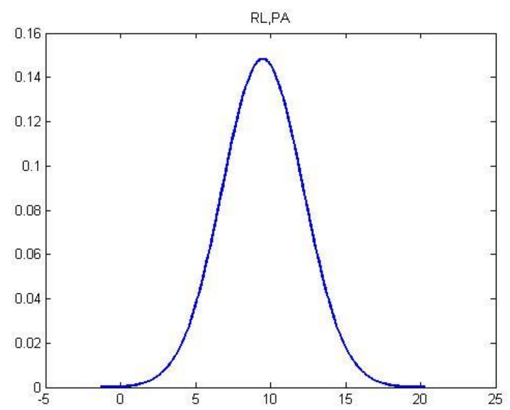
n) Data distribution for the value of R in all the right side of thorax measurements



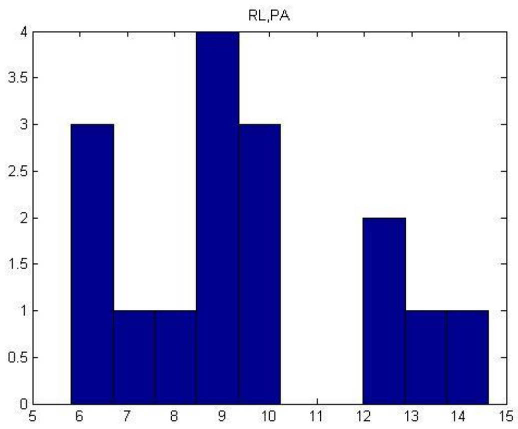
o) Normal distribution for the value of X in all the right side of thorax measurements



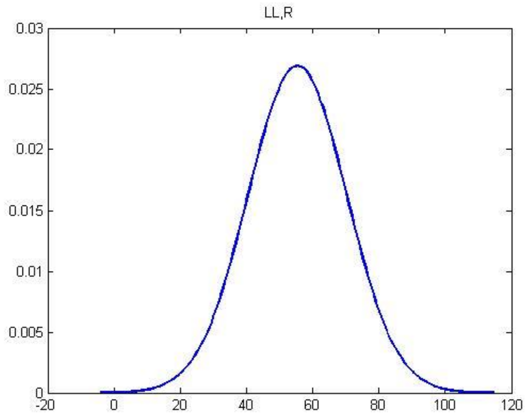
p) Data distribution for the value of X in all the right side of thorax measurements



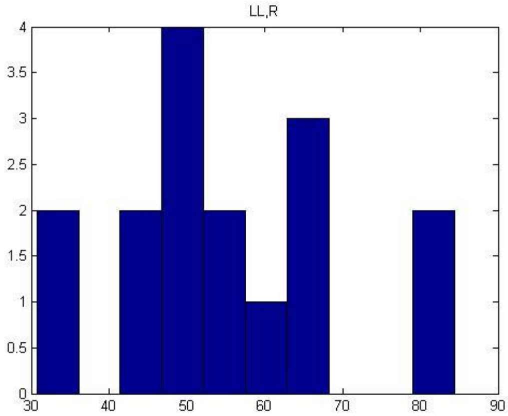
q) Normal distribution for the value of PA in all the right side of thorax measurements



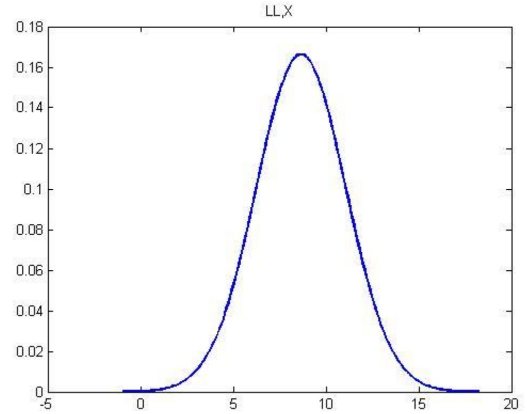
r) Data distribution for the value of PA in all the right side of thorax measurements



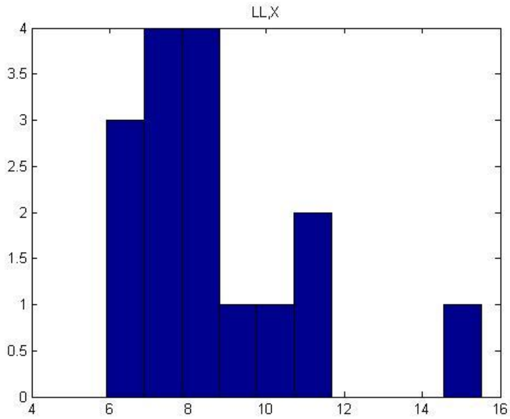
s) Normal distribution for the value of R in all the left side of thorax measurements



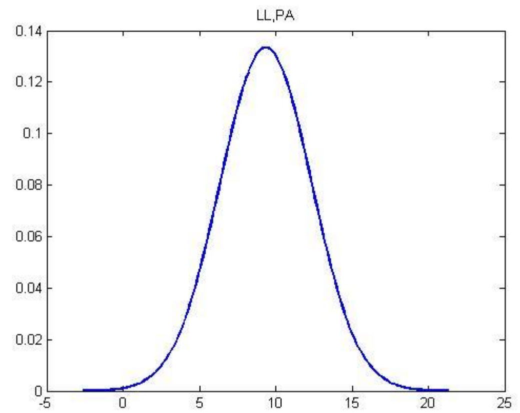
t) Data distribution for the value of R in all the left side of thorax measurements



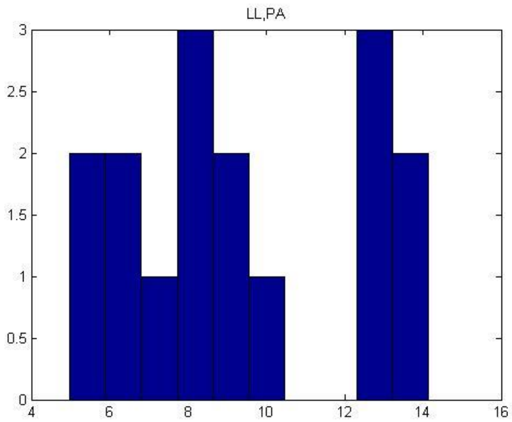
u) Normal distribution for the value of X in all the left side of thorax measurements



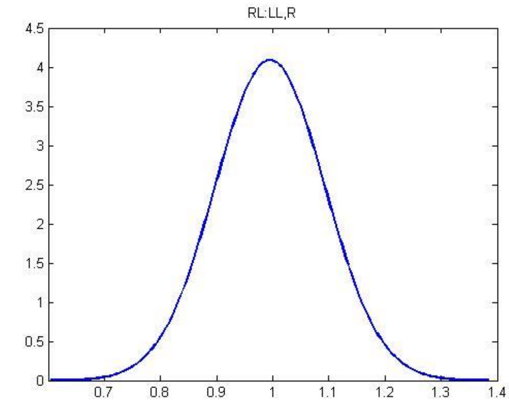
v) Data distribution for the value of X in all the left side of thorax measurements



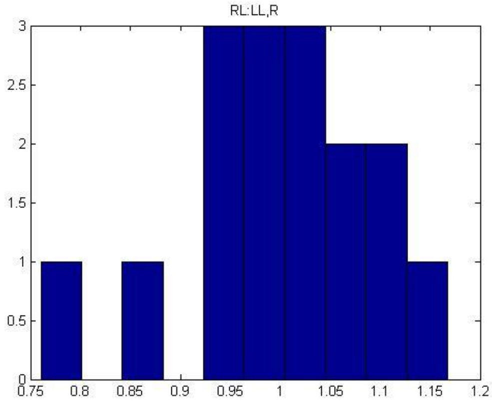
w) Normal distribution for the value of PA in all the left side of thorax



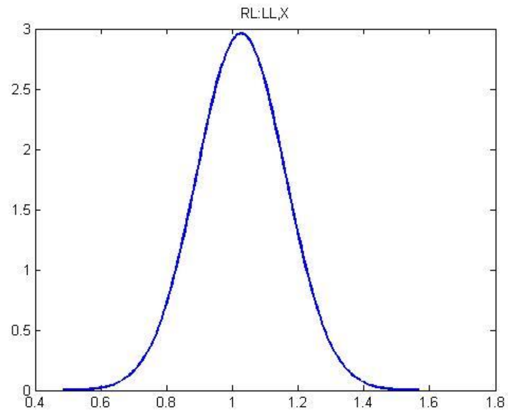
x) Data distribution for the value of PA in all the left side of thorax measurements



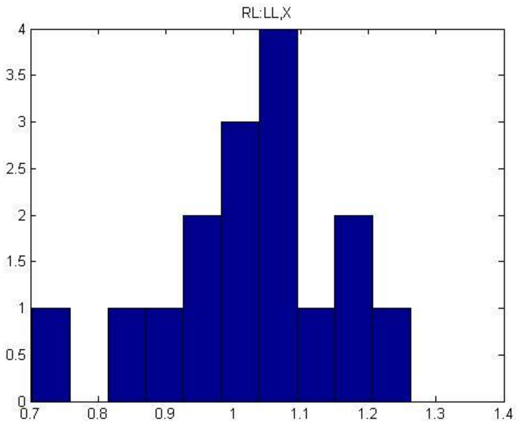
y) Normal distribution for the value of R in all the ratio between right side and left side of thorax measurements



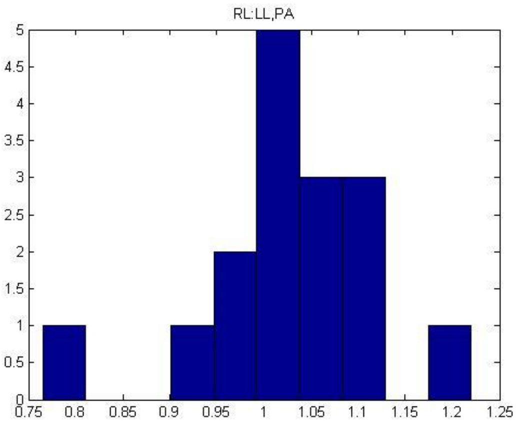
z) Data distribution for the value of R in all the ratio between right side and left side of thorax measurements



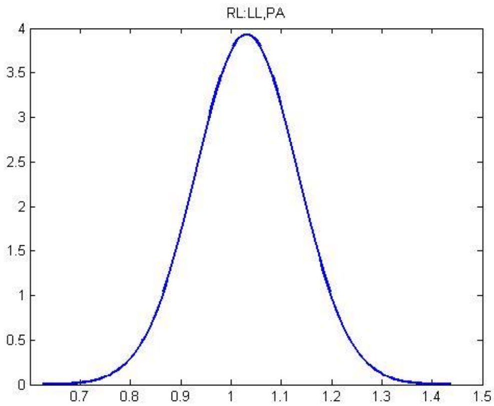
aa) Normal distribution for the value of X in all the ratio between right side and left side of thorax measurements



bb) Data distribution for the value of X in all the ratio between right side and left side of thorax measurements



cc) Normal distribution for the value of PA in all the ratio between right side and left side of thorax measurements

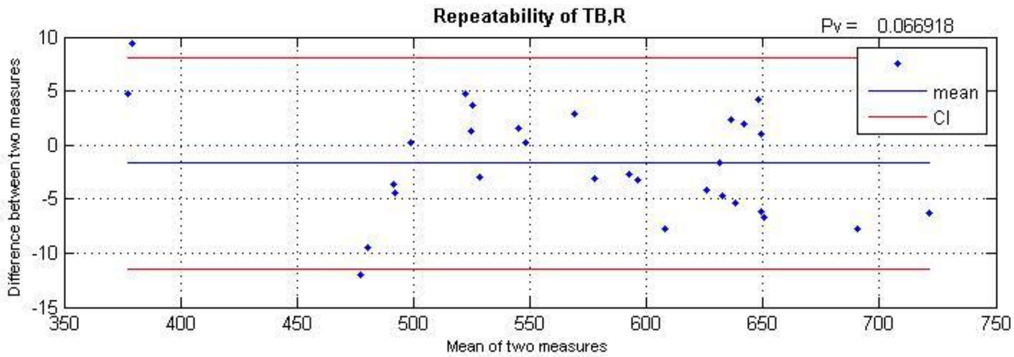


dd) Data distribution for the value of PA in all the ratio between right side and left side of thorax measurements

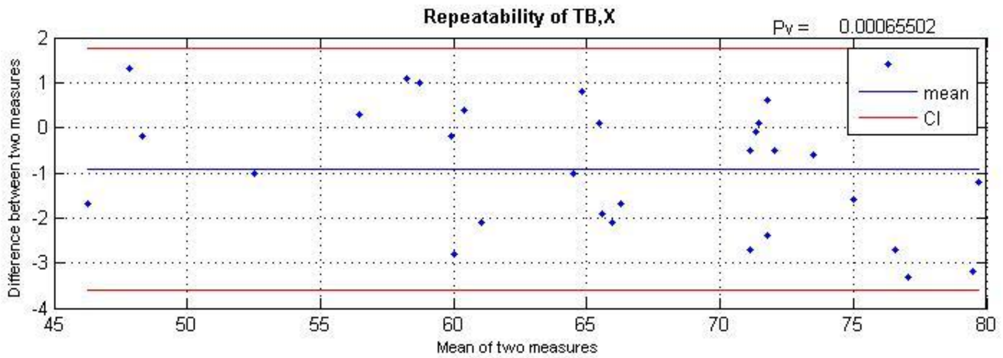
Figure 14: Plots a-dd, normal distribution and data distribution of every spectra in each body segment

4.2. Assessment of repeatability

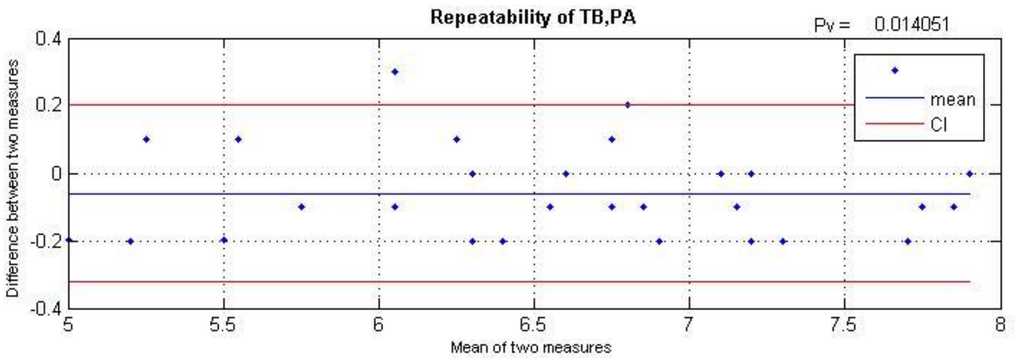
Here the repeatability of measured values by a single operator has been studied, and the results are illustrated in the bland altman graphs in continue, Figure 15. The bias and CI have been shown in each plot, and p-value is written additionally as well in order to identify the significance of the values.



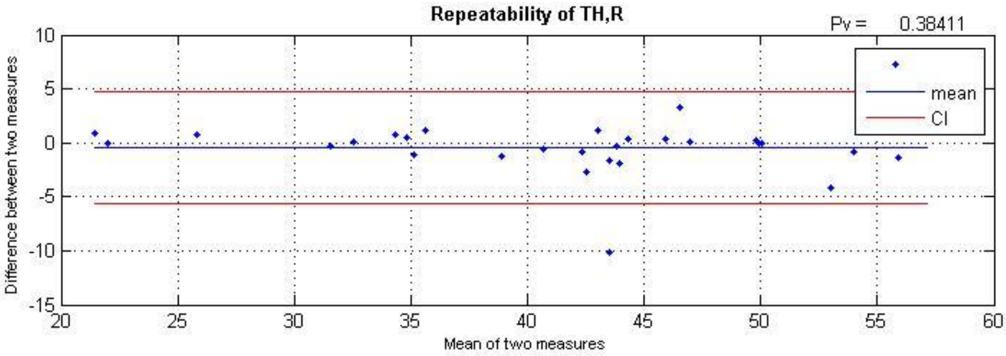
a) Repeatability of measured value R in total body measurements



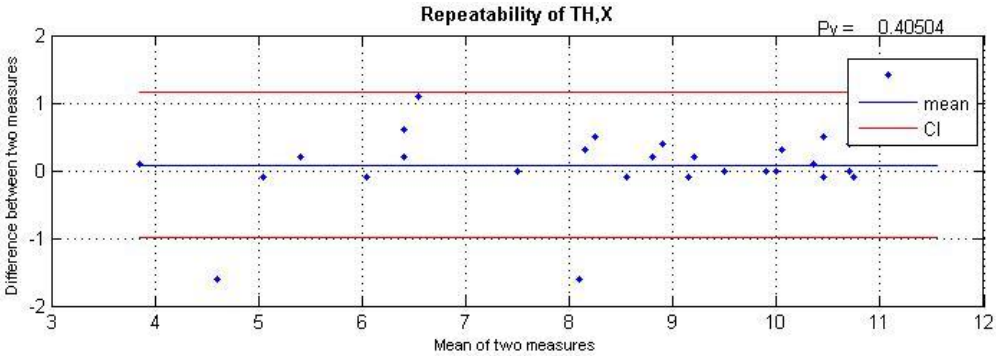
b) Repeatability of measured value X in total body measurements



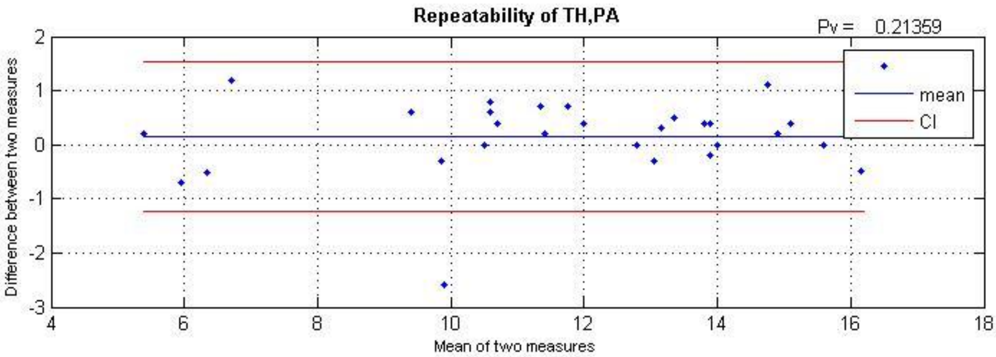
c) Repeatability of measured value PA in total body measurements



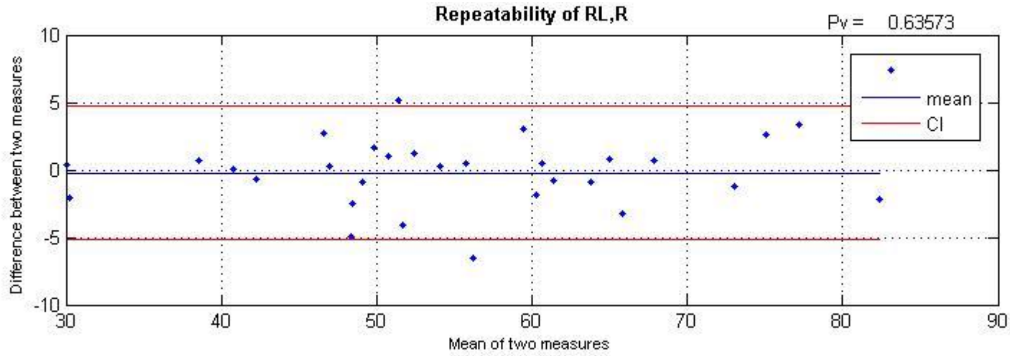
d) Repeatability of measured value R in thoracic transversal measurements



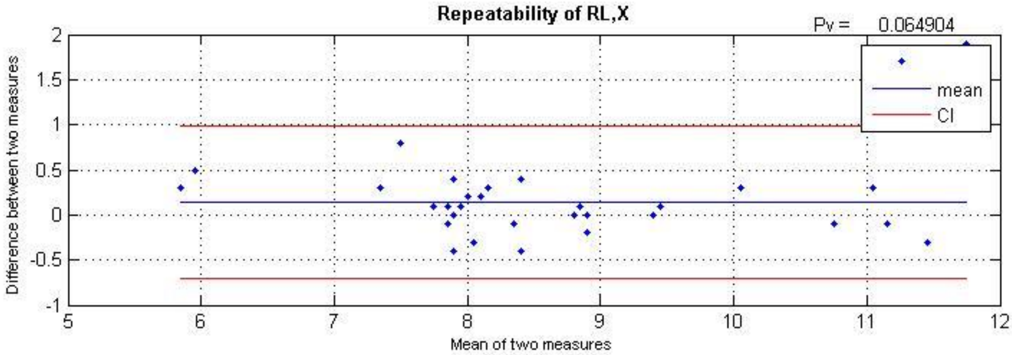
e) Repeatability of measured value X in thoracic transversal measurements



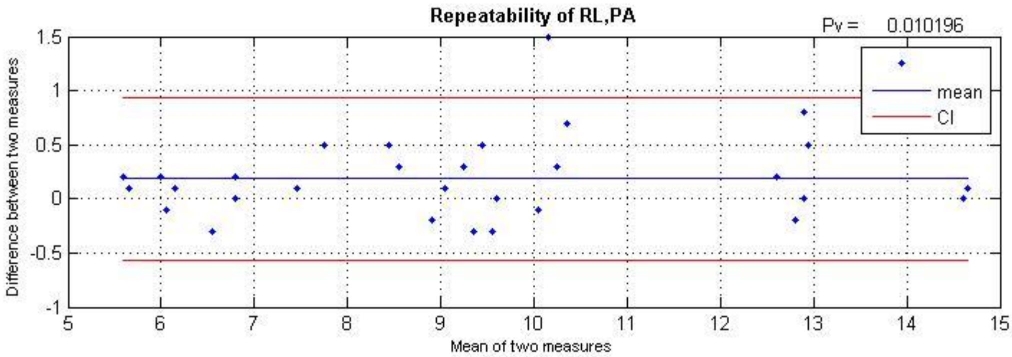
f) Repeatability of measured value PA in thoracic transversal measurements



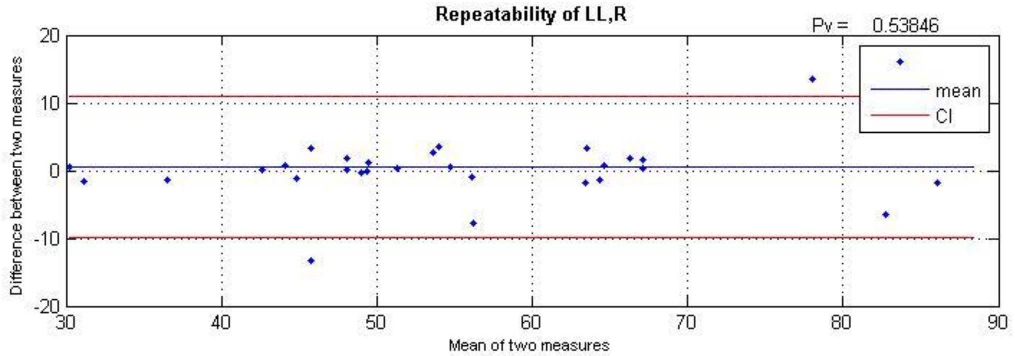
g) Repeatability of measured value R in Right side of thorax measurements



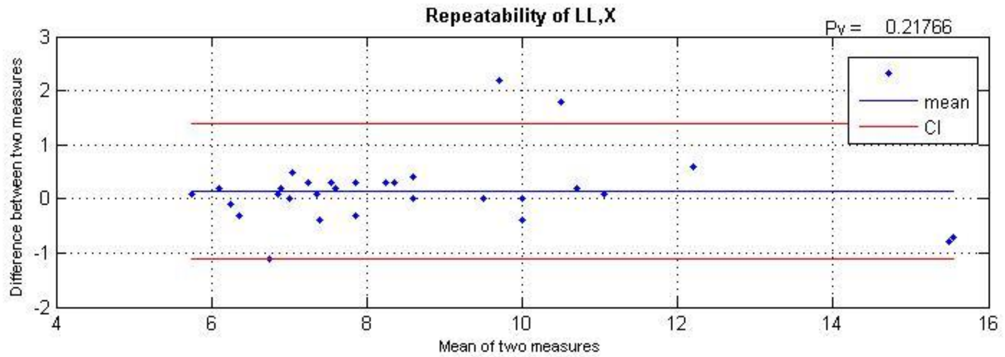
h) Repeatability of measured value X in Right side of thorax measurements



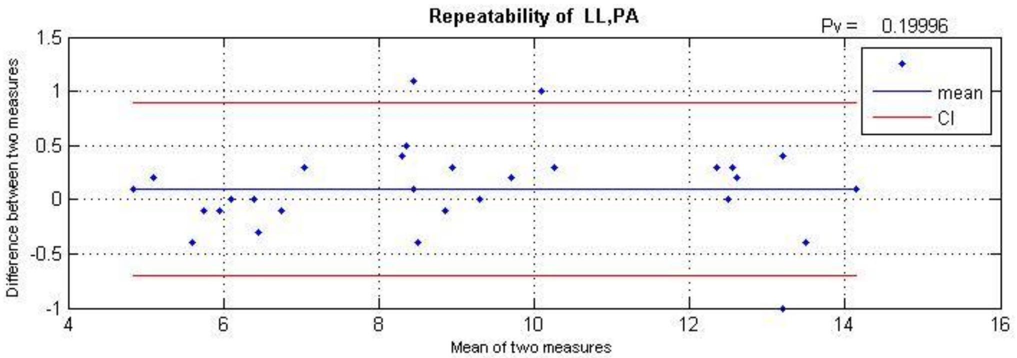
i) Repeatability of measured value PA in Right side of thorax measurements



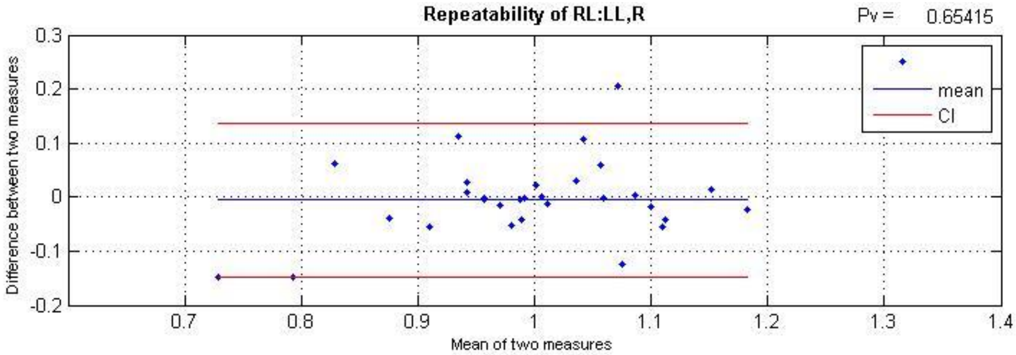
j) Repeatability of measured value R in Left side of thorax measurements



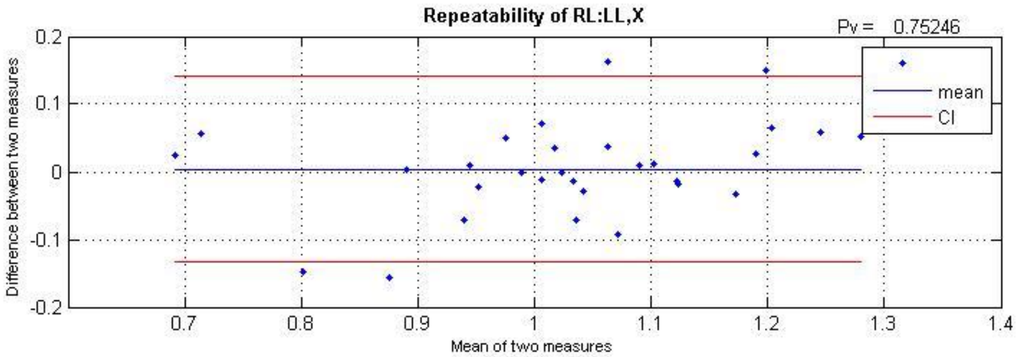
k) Repeatability of measured value X in Left side of thorax measurements



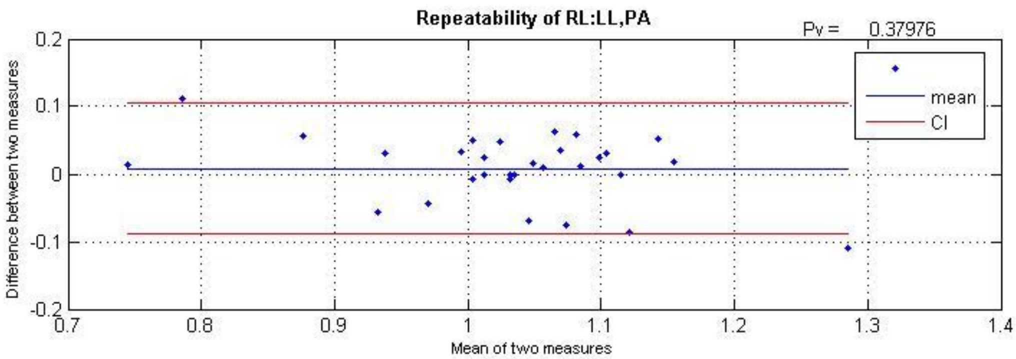
l) Repeatability of measured value PA in Left side of thorax measurements



m) Repeatability of measured value R in ratio between right side and left side of thorax measurements



n) Repeatability of measured value X in ratio between right side and left side of thorax measurements



o) Repeatability of measured value PA in ratio between right side and left side of thorax measurements

Figure 15**:** Plots a-o, Bland altman plots to assess repeatability of every measured value in each body segments

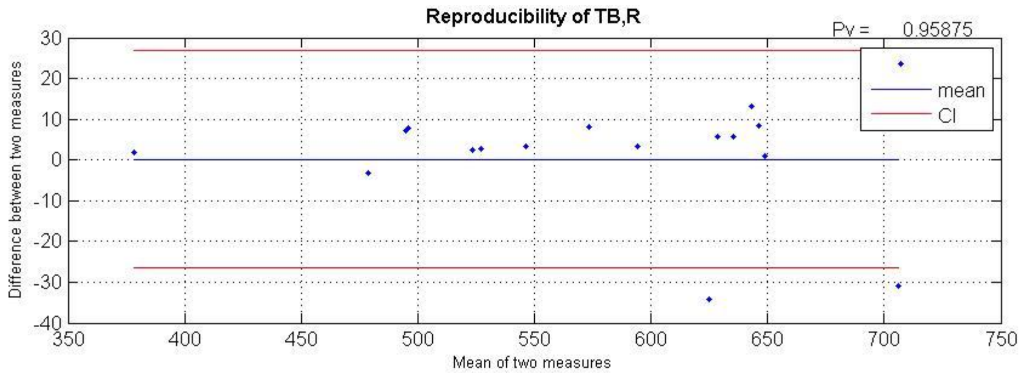
In order to have a summarized result from the studied repeatability of observed values and the Bland-Altman graphs, the table below, Table V, has prepared containing the value of Bias ± CI and p-value for every parameter in each body segment and as well as the ratio between LL and RL measured values.

Table V: Bias ± CI, p-value and mean values according to repeatability assessment

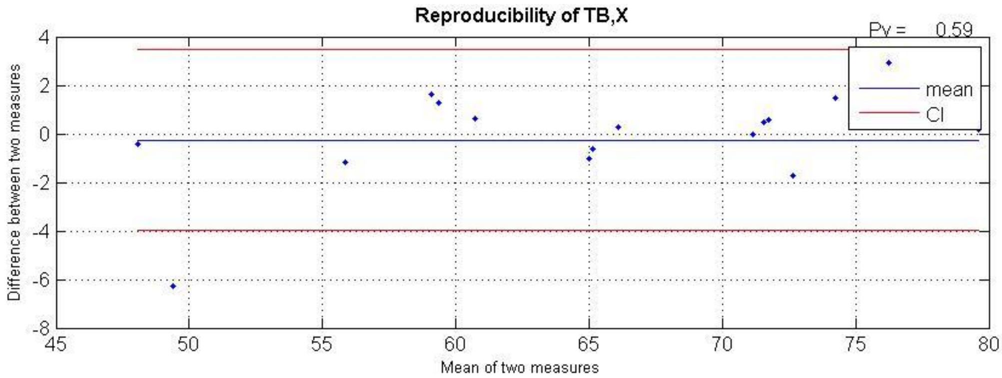
		p- value	*Bias*	*±CI*	*Mean Values*
Total Body	*R*	0,0669	-1,671	± 9,7870	571,752
	X	0,0006	-0,9161	± 2,6828	65,567
	PA	0,0141	-0,0613	± 0,2617	6,581
Thoracic transversal	*R*	0,3841	-0,4167	± 5,1649	41,366
	X	0,405	0,0833	± 1,0803	8,494
	PA	0,2136	0,16	± 1,3783	11,956
Right side of thorax	*R*	0,6357	-0,2129	± 4,9541	55,671
	X	0,0649	0,1452	± 0,8435	8,657
	PA	0,0102	0,1839	± 0,7467	9,292
Left side of thorax	*R*	0,5385	0,5806	± 10,3904	54,622
	X	0,2177	0,1419	± 1,2551	8,629
	PA	0,2	0,0935	± 0,7949	9,43
Ratio between Right side and Left side of thorax	*R*	0,6541	-0,0058	± 0,1419	0,994
	X	0,7525	0,0039	± 0,2758	1,026
	PA	0,3798	0,0078	± 0,0970	1,032

4.3. Assessment of reproducibility

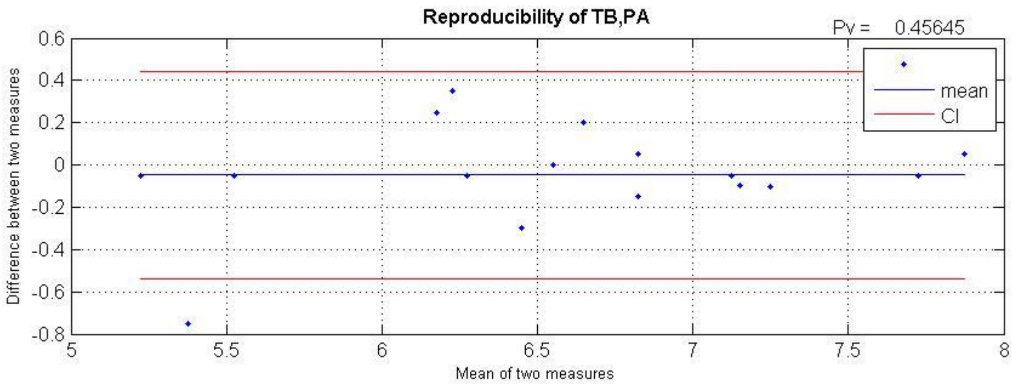
Here the reproducibility of our discovered values by two different operators has been studied, as it has been explained before, it includes assessing the agreement between observed values by two independent operators. The result of the study has been shown in the form of bland altman plots containing the values of Bias, CI and p-value, Figure 16.



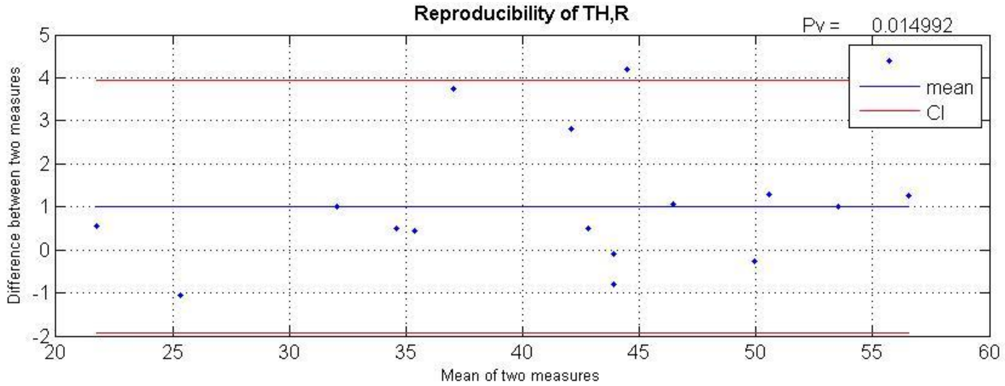
a) Reproducibility of measured value R in total body measurements



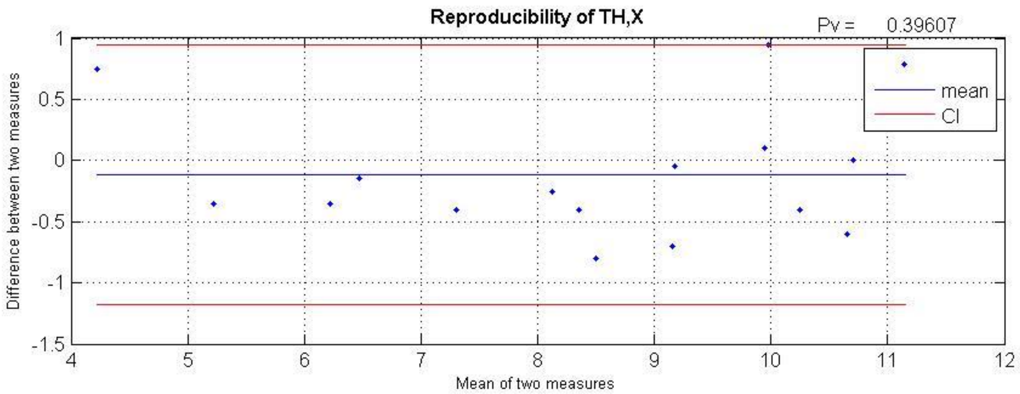
b) Reproducibility of measured value X in total body measurements



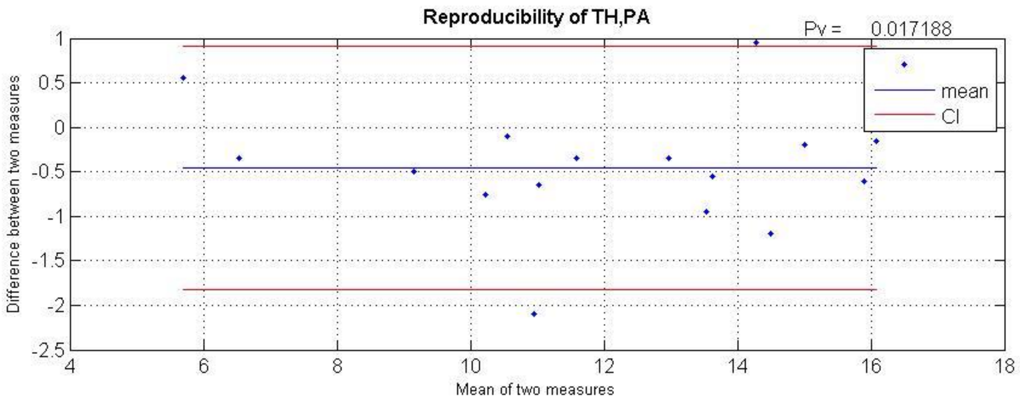
c) Reproducibility of measured value PA in total body measurements



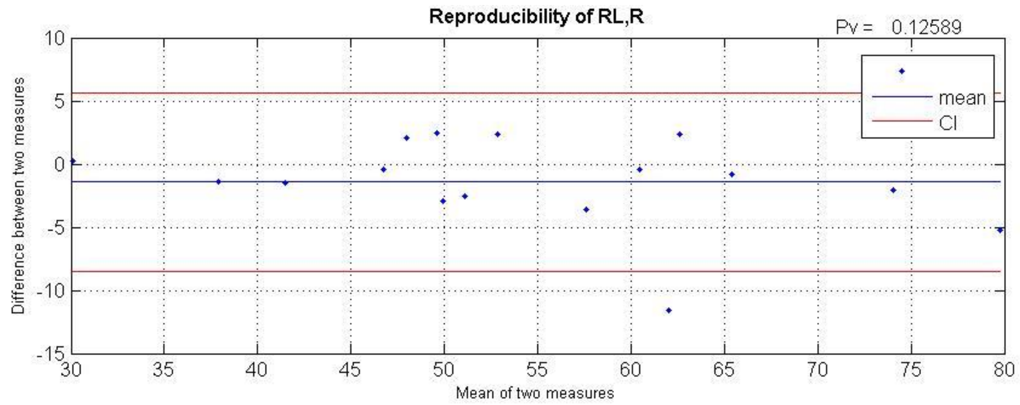
d) Reproducibility of measured value R in thoracic transversal measurements



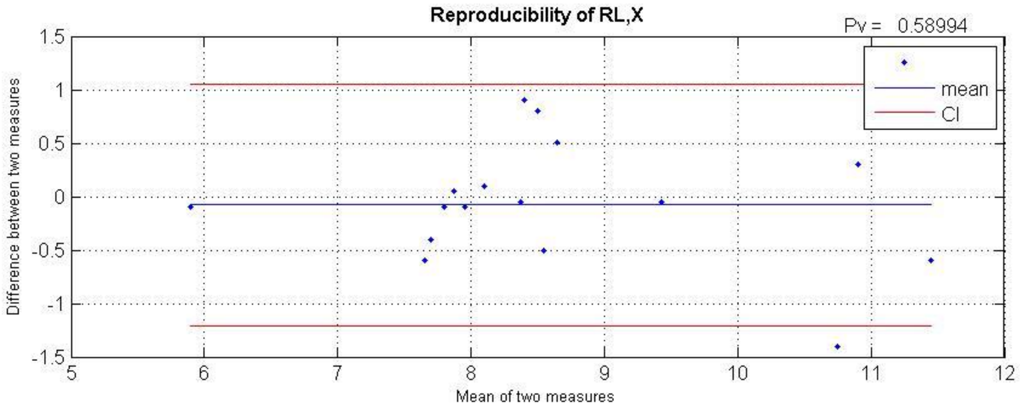
e) Reproducibility of measured value X in thoracic transversal measurements



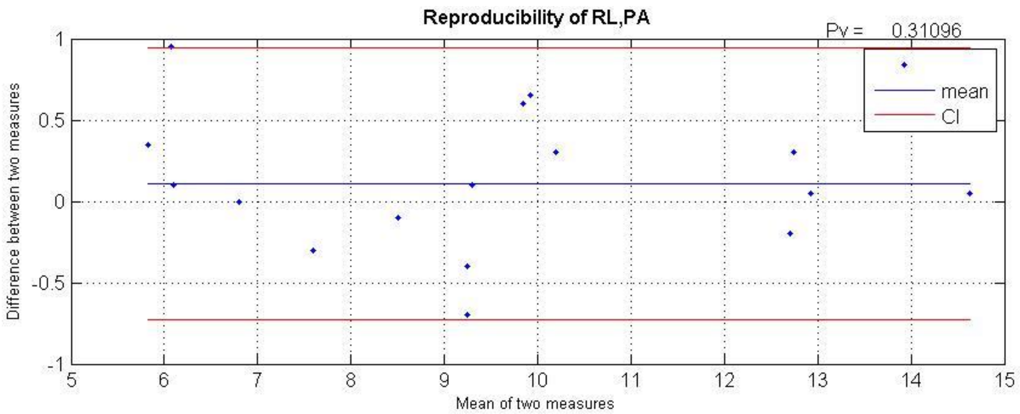
f) Reproducibility of measured value PA in thoracic transversal measurements



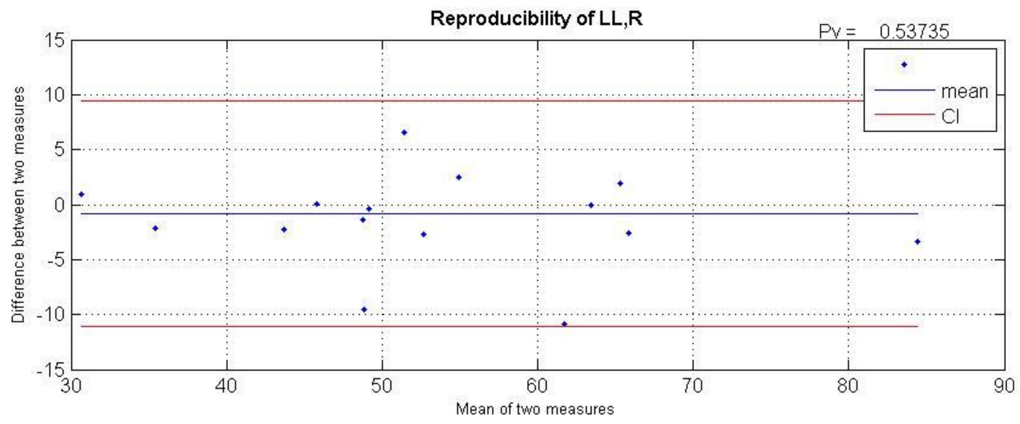
g) Reproducibility of measured value R in right side of thorax measurements



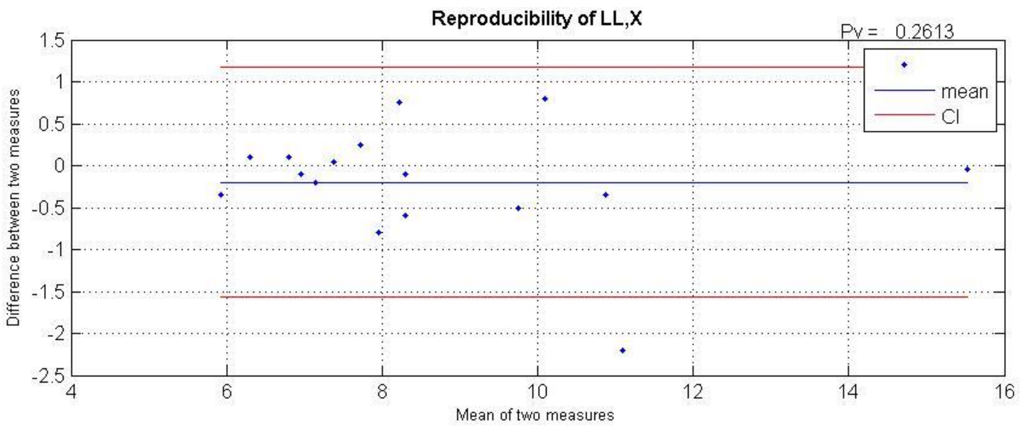
h) Reproducibility of measured value X in right side of thorax measurements



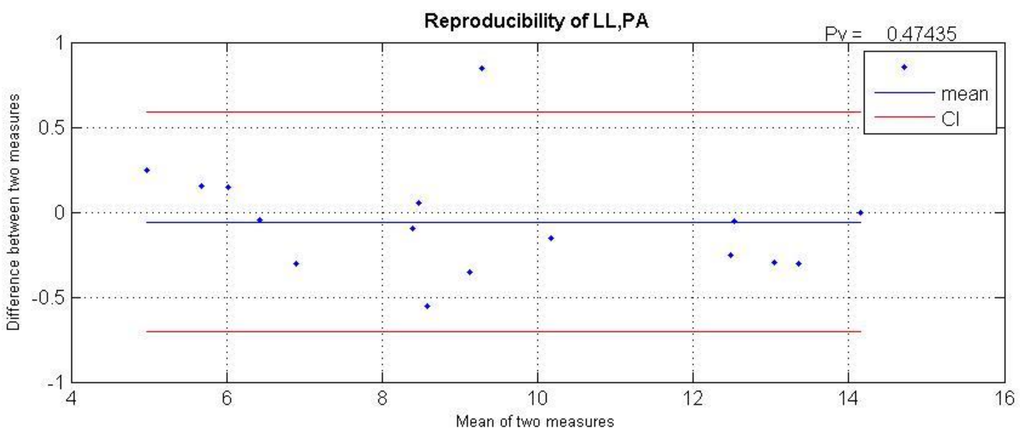
i) Reproducibility of measured value PA in right side of thorax measurements



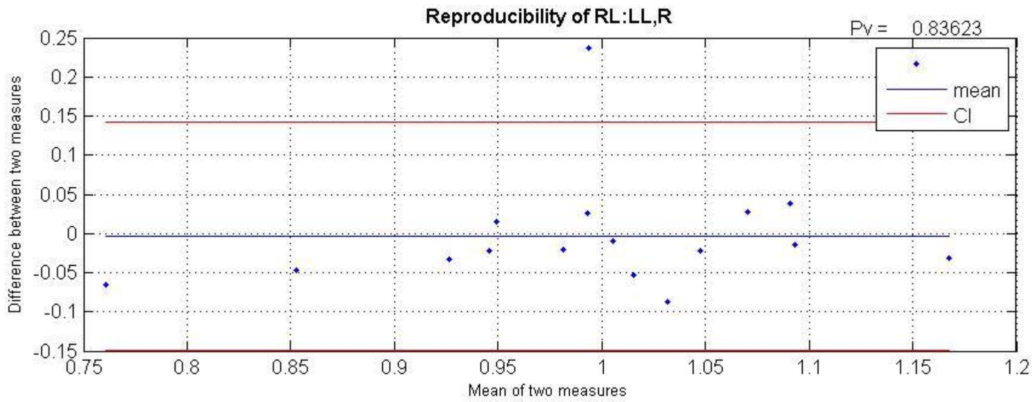
j) Reproducibility of measured value R in left side of thorax measurements



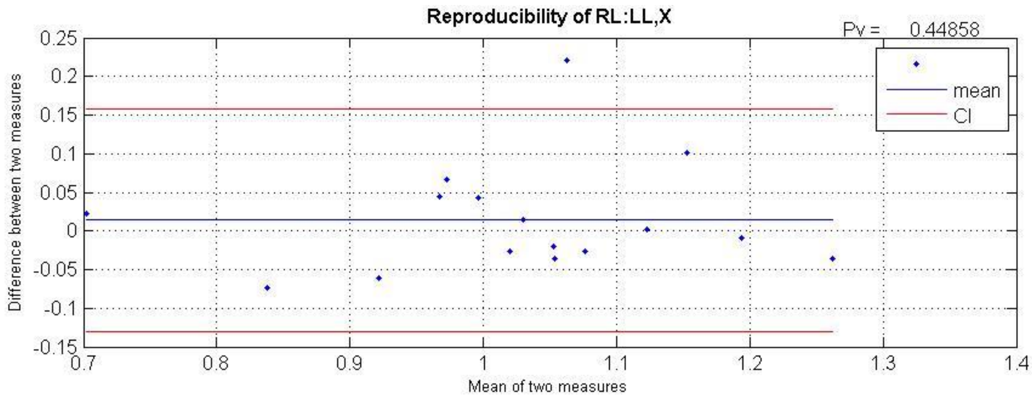
k) Reproducibility of measured value X in left side of thorax measurements



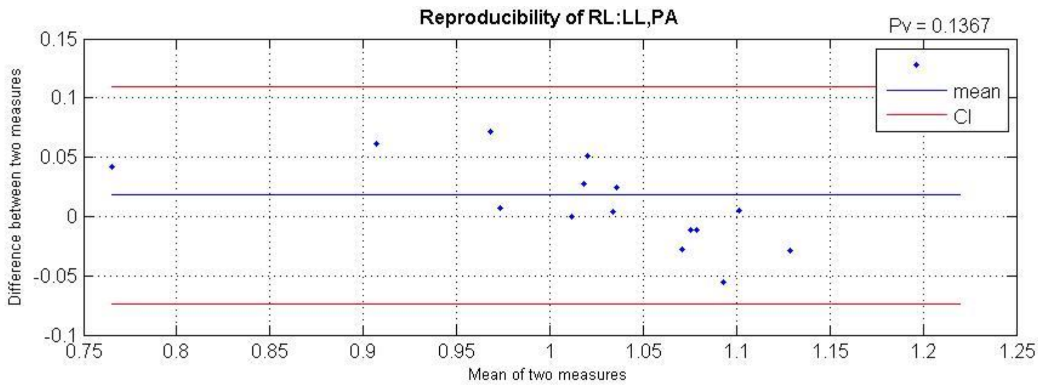
l) Reproducibility of measured value PA in left side of thorax measurements



m) Reproducibility of measured value R in ratio between right side and left side of thorax measurements



n) Reproducibility of measured value X in ratio between right side and left side of thorax measurements



o) Reproducibility of measured value PA in ratio between right side and left side of thorax measurements

Figure 16: Plots a-o, Bland altman plots to assess reproducibility of every measured value in each body segments

Table VI has been added below containing the information of Bias, CI and p-value related to each body segment's parameter.

Table VI: Bias ± CI, p-value and mean values according to reproducibility assessment

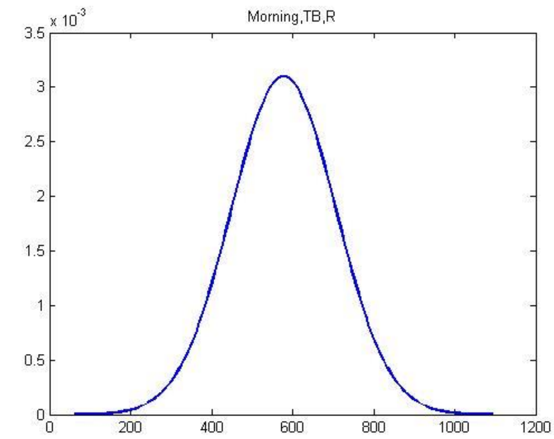
		p- value	*Bias*	*±CI*	*Mean Values*
Total Body	*R*	0,9587	0,175	± 26,6172	571,752
	X	0,59	-0,2563	± 3,7231	65,567
	PA	0,4564	-0,0469	± 0,4905	6,581
Thoracic transversal	*R*	0,015	1,0094	± 2,9401	41,366
	X	0,3961	-0,1156	± 1,0588	8,494
	PA	0,0172	-0,4563	± 1,3628	11,956
Right side of thorax	*R*	0,1259	-1,4375	± 7,0953	55,671
	X	0,5899	-0,781	± 1,1349	8,657
	PA	0,311	0,1094	± 0,8344	9,292
Left side of thorax	*R*	0,5373	-0,8125	± 10,2962	54,622
	X	0,2613	-0,2	± 1,3706	8,629
	PA	0,4744	-0,0594	± 0,6473	9,43
Ratio between Right side and left side of thorax	*R*	0,8362	-0,0038	± 0,1456	0,994
	X	0,4486	0,014	± 0,1443	1,026
	PA	0,1367	0,018	± 0,0917	1,032

4.4. Morning vs afternoon student T-Test assessment

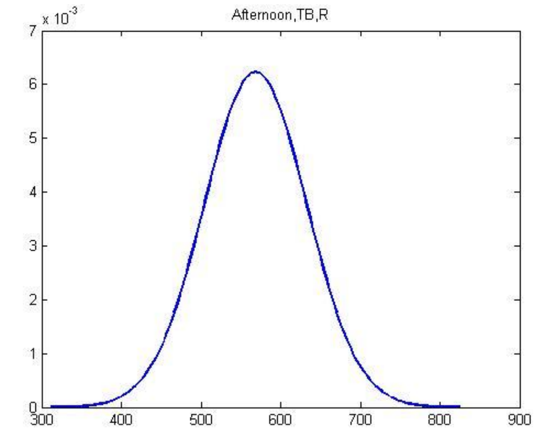
P-value returned by student t-test has been presented in the table VII, in order to show the correlation between the data observed in the morning and the one recorded in the evening. Moreover Gaussian curves were provided to display normal distribution of the measurements in the morning and in the evening separately.

Table VII: Student T-test results

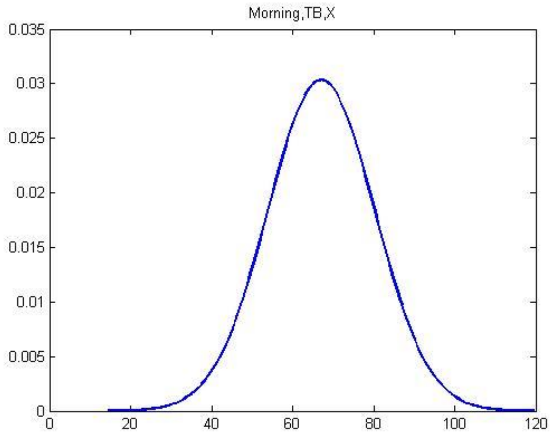
	TB			*TH*			*RL*			*LL*			*RL:LL*		
	R	*X*	*PA*	*R*	*X*	*PA*	*R*	*X*	*PA*	*R*	*X*	*PA*	*R*	*X*	*PA*
p- value	0,69	0,51	0,5	0,31	0,08	0,38	0,86	0,46	0,81	0,49	0,27	0,81	0,03	0,17	0,98
Mean morning	577,87	67,08	6,72	37,03	6,94	10,82	54,68	8,12	9,13	50,86	7,57	8,98	1,07	1,09	1,01
Mean Afternoon	568,48	64,66	6,52	43,18	9,17	12,51	54,22	8,86	9,64	57,41	9,15	9,52	0,96	0,99	1,04



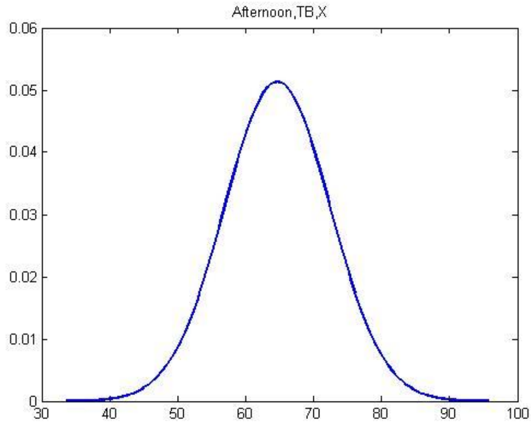
a) Normal distribution for the value of R in total body measurements performed in the morning.



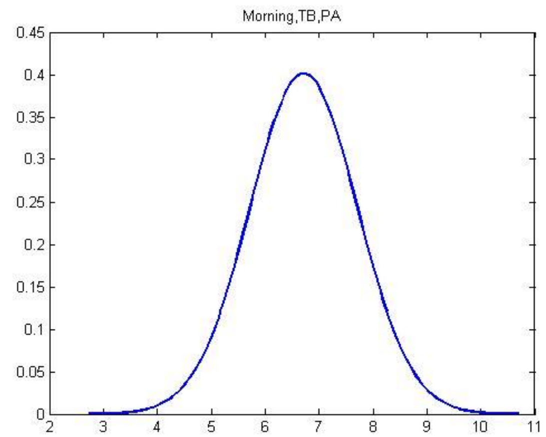
b) Normal distribution for the value of R in total body measurements performed in the afternoon.



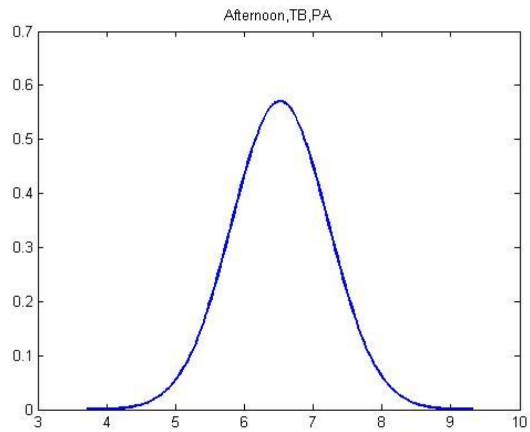
c) Normal distribution for the value of X in total body measurements performed in the morning.



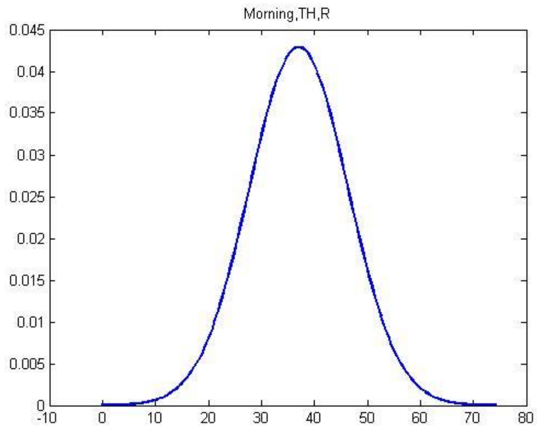
d) Normal distribution for the value of X in total body measurements performed in the afternoon.



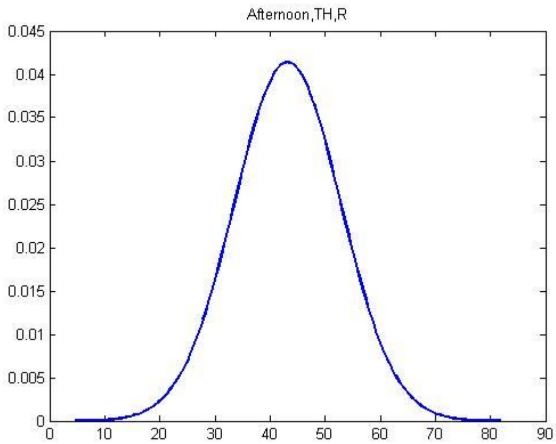
e) Normal distribution for the value of PA in total body measurements performed in the morning.



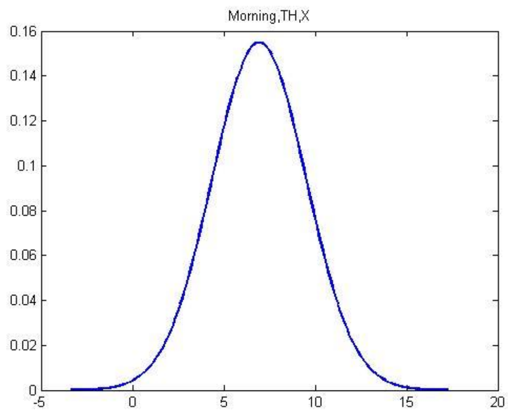
f) Normal distribution for the value of PA in total body measurements performed in the afternoon.



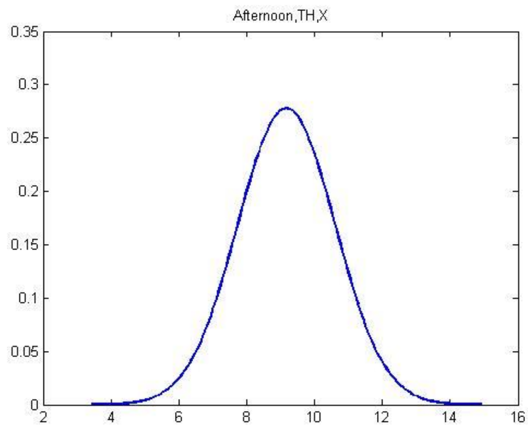
g) Normal distribution for the value of R in Thoracic transversal measurements performed in the morning.



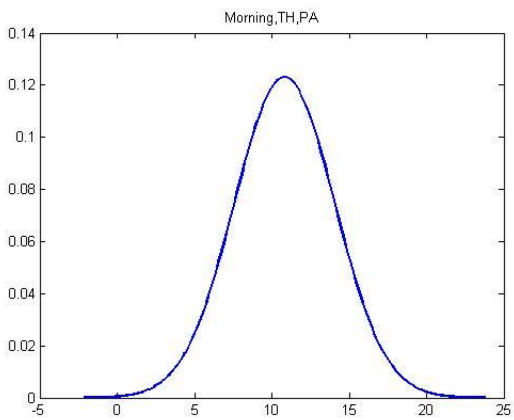
h) Normal distribution for the value of R in Thoracic transversal measurements performed in the afternoon.



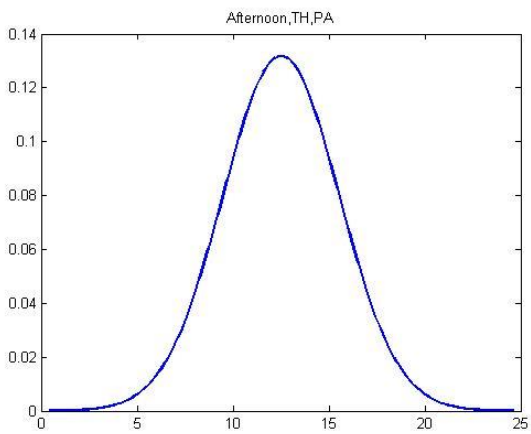
i) Normal distribution for the value of X in Thoracic transversal measurements performed in the morning.



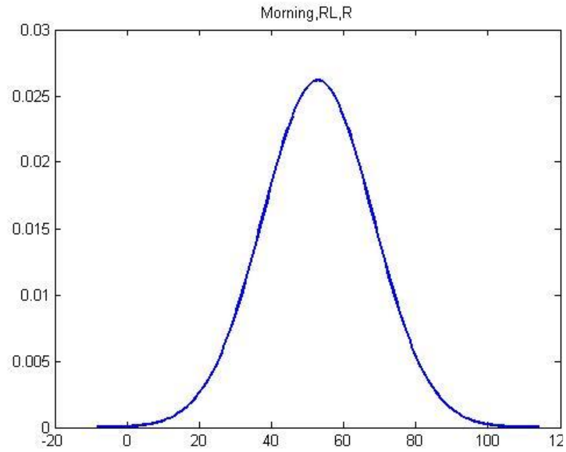
j) Normal distribution for tshe value of X in Thoracic transversal measurements performed in the afternoon.



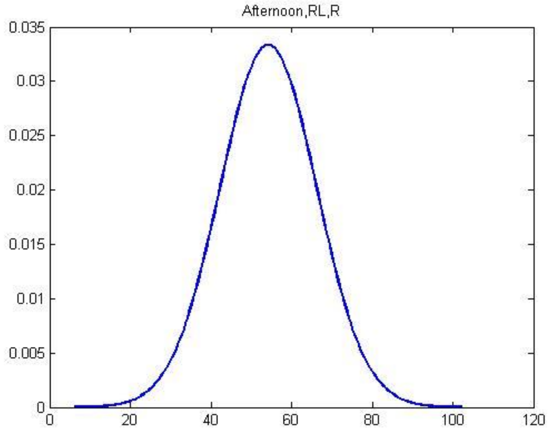
k) Normal distribution for the value of PA in Thoracic transversal measurements performed in the morning.



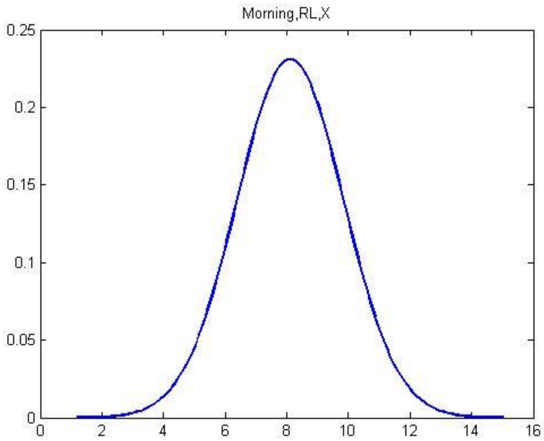
l) Normal distribution for the value of PA in Thoracic transversal measurements performed in the afternoon.



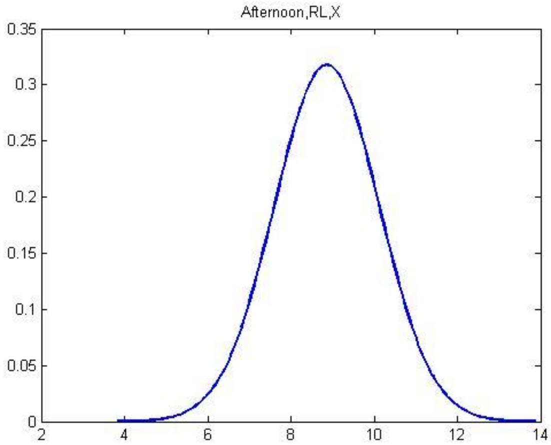
m) Normal distribution for the value of R in Right side of thorax measurements performed in the morning.



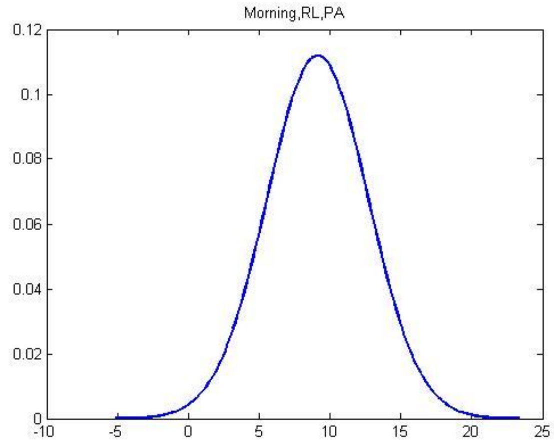
n) Normal distribution for the value of R in Right side of thorax measurements performed in the afternoon



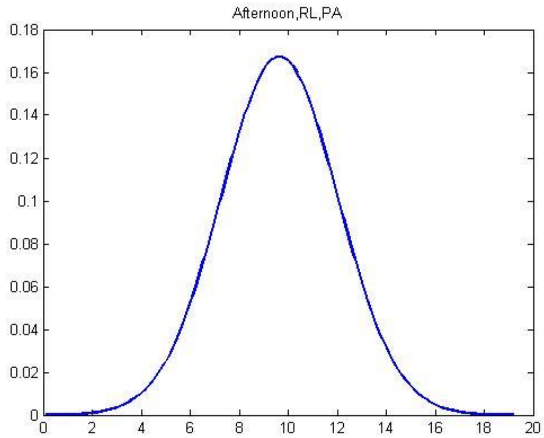
o) Normal distribution for the value of X in Right side of thorax measurements performed in the morning.



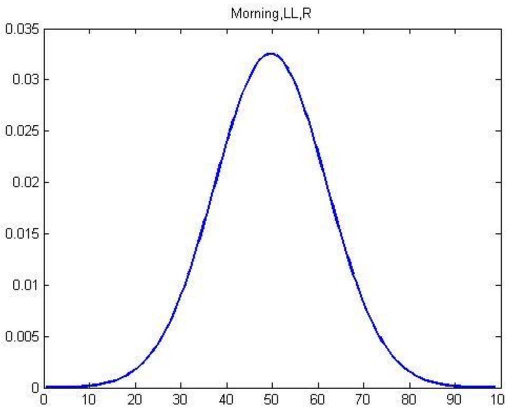
p) Normal distribution for the value of X in Right side of thorax measurements performed in the afternoon



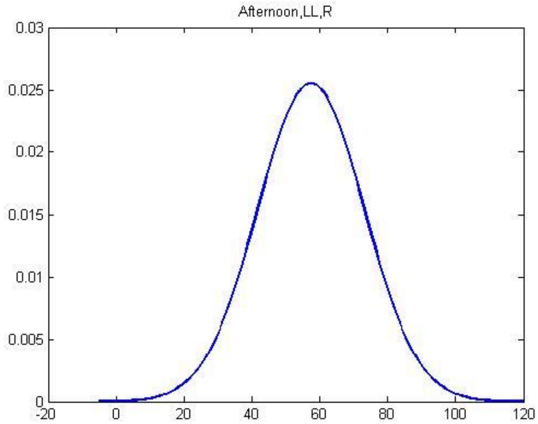
q) Normal distribution for the value of PA in Right side of thorax measurements performed in the morning.



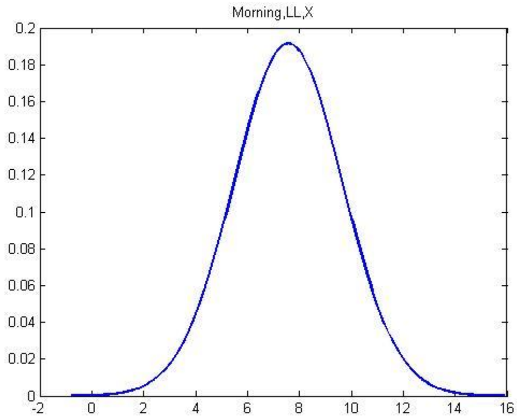
r) Normal distribution for the value of PA in Right side of thorax measurements performed in the afternoon.



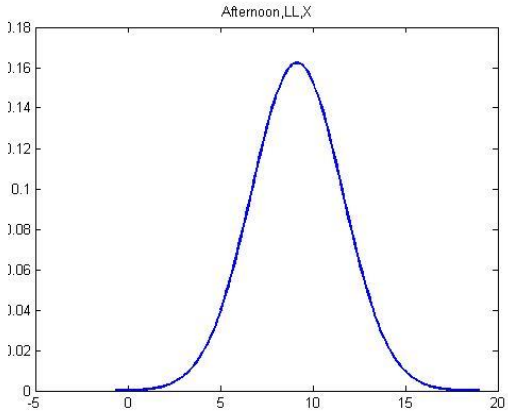
s) *Normal distribution for the value of R in left side of thorax measurements performed in the morning.*



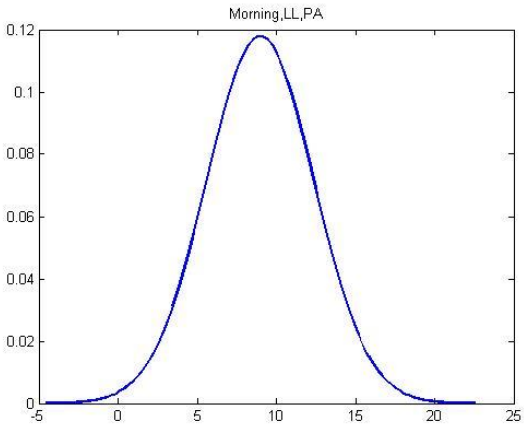
t) *Normal distribution for the value of R in left side of thorax measurements performed in the afternoon*



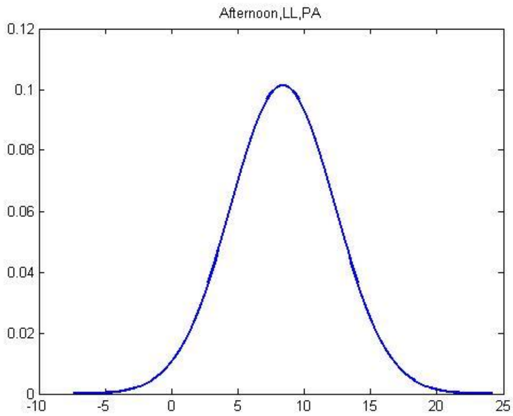
u) *Normal distribution for the value of X in left side of thorax measurements performed in the morning.*



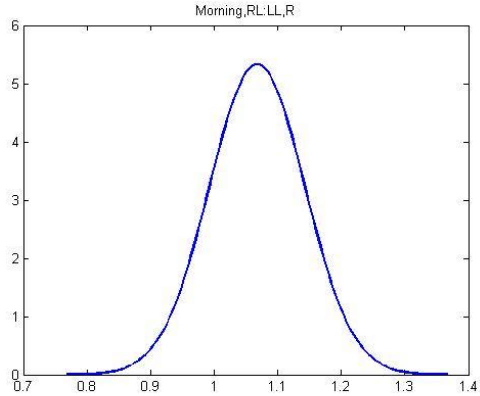
v) *Normal distribution for the value of X in left side of thorax measurements performed in the afternoon..*



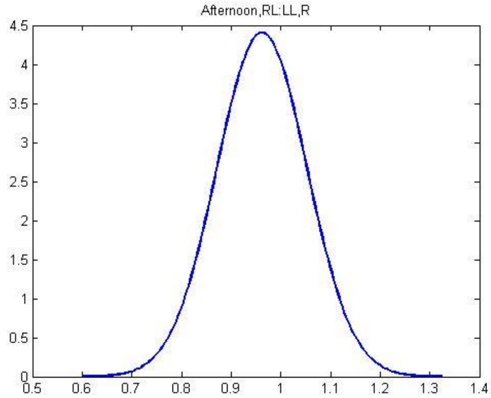
w) *Normal distribution for the value of PA in left side of thorax measurements performed in the morning.*



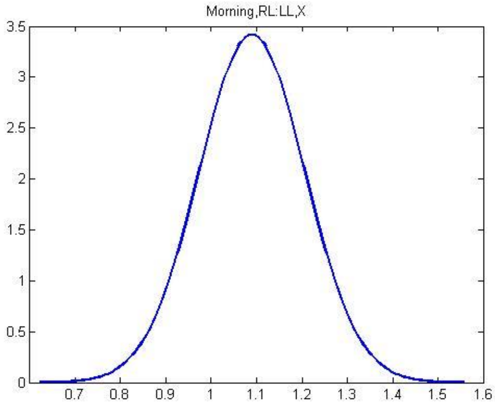
x) *Normal distribution for the value of PA in left side of thorax measurements performed in the afternoon..*



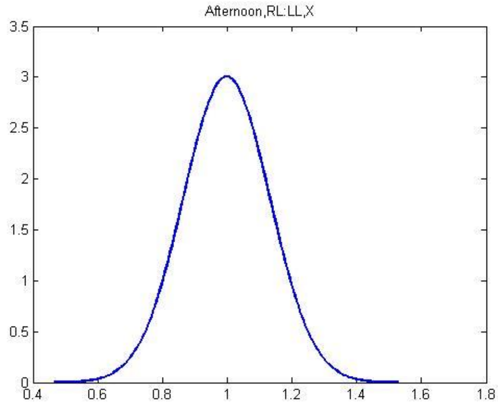
y) Normal distribution for the value of R in Ratio between left side and right side of thorax measurements performed in the morning.



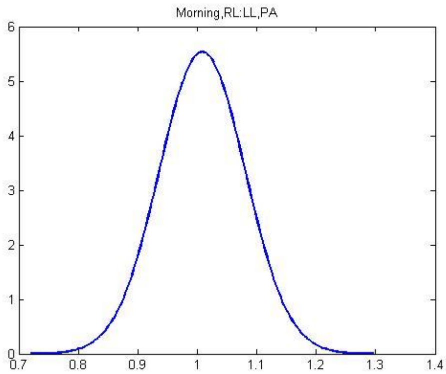
z) Normal distribution for the value of R in Ratio between left side and right side of thorax measurements performed in the afternoon.



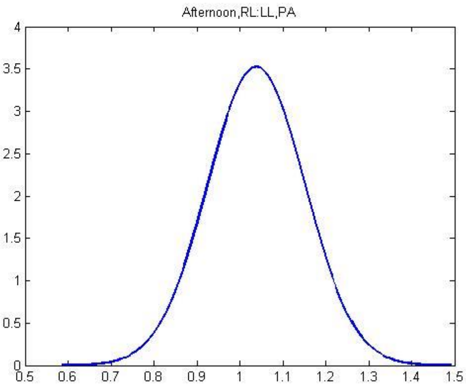
aa) Normal distribution for the value of X in Ratio between left side and right side of thorax measurements performed in the morning.



bb) Normal distribution for the value of X in Ratio between left side and right side of thorax measurements performed in the afternoon.



cc) Normal distribution for the value of PA in Ratio between left side and right side of thorax measurements performed in the morning.



dd) Normal distribution for the value of PA in Ratio between left side and right side of thorax measurements performed in the afternoon.

Figure 17**:** Plots a-dd, normal distribution of measured value in the morning vs in the afternoon

5. Discussions

5.1. Data distribution

The typical values for the different configurations based on our population were characterized. According to table IV a standard deviation from the mean of around 13% of the mean value can be observed for every spectra in total body measurements. For thoracic transversal measurements, the deviation is around 25%. That value is repeated in longitudinal thoracic measurements for both sides. Nevertheless, the ratio between right and left side reduce this variability to 11% what was the purpose of calculating the ratio. Finally, the histograms mark that normal distributions can be assumed in every spectra.

5.2.Repeatability and reproducibility

In this section, results shown in chapter 4.2, 4.3 are interpreted. Bland-Altman plots were used to test variability in measurements taken in the same conditions with 1 and 2 different observers. First, differences between the first and second measurements taken by an observer were compared. Afterwards, the averages of both measurements taken on a subject at a certain configuration by an observer are compared to the analogous average of both measurements taken by another observer.

The size of bias, pointing to the average of differences between two measurements and thus the systematical difference, was studied. Referring to both, table V and table VI, Bias values are mostly close to zero. The bias calculated in the table in comparison with the range of the data measured shown in table IV can be considered low.

P-value shows the statistical significance of the bias. That means how likely is that the bias occurred by chance and thus there is not systematical difference. It's a number between 0 and 1. By tradition if this value is less than 0.05 then the bias is considered real.

Referring to the tables V-VII all p-values we got are almost bigger than 0.05. It indicates that the biases found were not significant enough, and the small sample size in this experiment can be considered as one of the reasons for that.

The scatters around bias was also reviewed. This is the random difference expressed in terms of limits of agreement (CI). Some information about a range of possible values is given referring to table V and VI; which 95% of the time a measured value is lying in that range.

5.3.Differences between morning and afternoon

Finally the measurements observed in the morning, and in the evening were compared in order to study how time of the day affected the results. This difference was not found statistically significant.

6. Conclusion

For the conducted project, the bioimpedance value of several parts of the body, such as total body, thoracic transversal, right side of thorax, left side of thorax and the ratio between both sides of thorax, were characterized on 16 healthy individuals.

In addition, repeatability and reproducibility in terms of dependency of bioimpedance measurements in mentioned configurations have been determined. According to the results high repeatability and high reproducibility were concluded.

7. Future work

It’s recommended to increase the sample size much more than 16 people in order to get more information and finding more significance difference between the observations and on the other words making the results more statically significant.

Furthermore, assessing repeatability and reproducibility test based on two distinct devices in different times would be informative.

It would also be interesting to assess the effect of temperature, age, gender, weight, height on the measured bioimpedance values (38) And (39).

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