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Bioimpedance measurement of specific body resistance

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Dissertation submitted to the Faculty of Electrical Engineering of
Czech Technical University in Prague
in partial fulfillment of the requirements
for the degree of
Doctor
in the branch of study
Artificial Intelligence and Biocybernetics
of study program Electrical Engineering and Informatics
2015

I would like to dedicate this thesis to my loving beautiful wife, my parents,
my sister and her partner for their support during my whole academic career.

Acknowledgements

I would like to thank to my supervisor Lenka Lhotska for her help with thesis and all problems I was ever solving. I would like to thank my colleagues and Department of Cybernetics for stimulating workspace and atmosphere.

Abstract

My work aims at bioimpedance and body composition parameters evaluation. Bioimpedance and BIA (bioimpedance analysis) serve in many biomedical applications as a technique for estimation of body composition parameters such as hydration, percentage of fat mass, fat free mass, etc. The applications of bioimpedance are listed in state-of-the art chapter, which describes bioimpedance, body composition parameters and body composition evaluation techniques in general and also presents basic applications in evaluation of body composition by various methods. In my work I used bioimpedance and BIA as a tool to for solving problems that are connected with this method and as a method for tracking of changes in body composition parameters. The measurements I have done are proving that bioimpedance and especially evaluation of electrical properties of a tissue are good estimators of followed processes. Whole work is describing bioimpedance measurements from starting conditions to comparison with DXA method and proposing new equations for calculation of body fat % in Czech population finishing with data collection in specific field and proposing of new pilot model. The results show that the bioimpedance is a very good method for following of electrical parameters that are directly connected with body composition values. This work adds new results in a field of measurement correctness, propose a new equation for calculation of body fat that were compared with known equations and is presenting entirely new approach in monitoring of gestation process. Obtained results were tested and compared with other state of the art methods and approaches.

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List of abbreviations

BMI … body mass index

BIA … bioimpedance analysis

TBW … total body water

ICW … intracellular water

ECW … extracellular water

FFM … fat free mass

DXA … dual-energy X-ray absorptiometry

LBM … lean body mass

%BF … body fat percentage

FM … fat mass

MM … muscle mass

TBF … total body fat

WHR … waist hip ratio

WHO … world health organization

3-C model … three compartment model

2-C model … two compartment model

BIS … bioimpedance spectroscopy

CPE … constant phase element

1. *Introduction*

In my work I have focused on bioimpedance of human body, body composition evaluation and issues that are connected with bioimpedance. This approach to body composition (bioimpedance and bioimpedance analysis) is one of the present frequently used methods. The bioimpedance analysis (BIA) and bioimpedance spectroscopy (BIS) methods are capable to provide quantitative information about crucial body composition elements such as total body water (TBW), fat mass (FM), fat free mass (FFM), muscle mass (MM), reactance, resistance, etc.

Bioimpedance represents a very important concept in body composition evaluation. This method is very engaging. BIA body composition compartments could be estimated without an invasive approach and the method is fast and relatively cheap. These facts support bioimpedance application in cases where other methods are not suitable or can be harmful to patients.

Currently many methods of bioimpedance, BIA and BIS (bioimpedance spectroscopy) are used (single/multi frequency, dual/multi electrode, different placements of electrodes) in medicine and body composition evaluation. Some approaches provide good measuring results, however, other measuring methods of bioimpedance are only informative and should be treated this way. Lack of standardization, imprecise information about patients and misuse of measuring machines can distort outcome values of these methods. Sometimes values can be poorly interpreted which can also lead to bad classification of patient's body composition.

Another current problem is a lot of equations that are used for calculation of body composition compartments. This can lead to faults in values that are calculated by these equations. However, lots of research about equations was done in the past and we can state that present equations for calculation of body composition have good results with minor differences between each other.

The issues connected with the measurement and evaluations of body composition are the main objective I focus on.

First objective of the PhD thesis is to find out whether different medium in between electrodes and contact body part influences the measurement and to create a standardized protocol of conditions for BIA assessment that will be able to include all possible effects. This will lead to minimizing errors and take into account different conditions and other effects.

Second objective is to perform experiments with the aim to compare two devices that are high tech equipment in bioimpedance composition assessment. This will help to find out whether these devices are interchangeable and results from various devices and laboratories can be compared.

Third objective of my dissertation is the comparison of InBody machine with DXA method. DXA method is stated to be a gold standard in body composition evaluation. It will show how precise and trustworthy the values obtained by devices in laboratory are.

Fourth objective of my PhD thesis is design and realization of the bioimpedance measurement in pregnant women with the aim to discover possible use of bioimpedance as a method for pregnancy progress visualization and control.

Final objective is design of a new, more precise model of body composition based on the bioimpedance measurement and its justification for measured data. Methodology of the measurement and evaluation procedure is proposed as well.

1.1. Aims of the thesis

The main aim of the thesis is to design and experimentally verify a methodology of bioimpedance measurement and evaluation. This aim requires a detailed analysis of measurement conditions, comparison of several approaches to body composition analysis and evaluation of experimental measurements. This implicates more detailed goals that are defined bellow. The whole PhD thesis is structured to map possible problems that can occur during measurements continuing with data evaluation and unique experiment. The goals of the PhD thesis were proposed as:

1) Analysis of the problem of standard measurement conditions
    a) The goal is to prove that external conditions (creams and moistures) do not represent key issues in measuring of bioimpedance
2) Comparison of two approaches - bioimpedance and DXA and creation of the more precise equation for body fat % calculation fitting better to the Czech population
    a) The goal is to find, discuss and verify differences and similarities of body composition measurements by bioimpedance and DXA approaches
    b) The second goal is to create a new more precise equation for calculation of body fat % in Czech population
3) Experimental measurements as a necessary basis for a new model representing resistance changes during pregnancy
    a) The goal is to describe BIA electrical and body composition parameters during the whole gestation period in the Czech population that was not discussed and done before
    b) The second goal is to observe changes of body composition and bioelectrical parameters during pregnancy
4) The final goal is to create a model that will interpret changes of resistance during gestation process to substantially change the current diagnostic methodology.

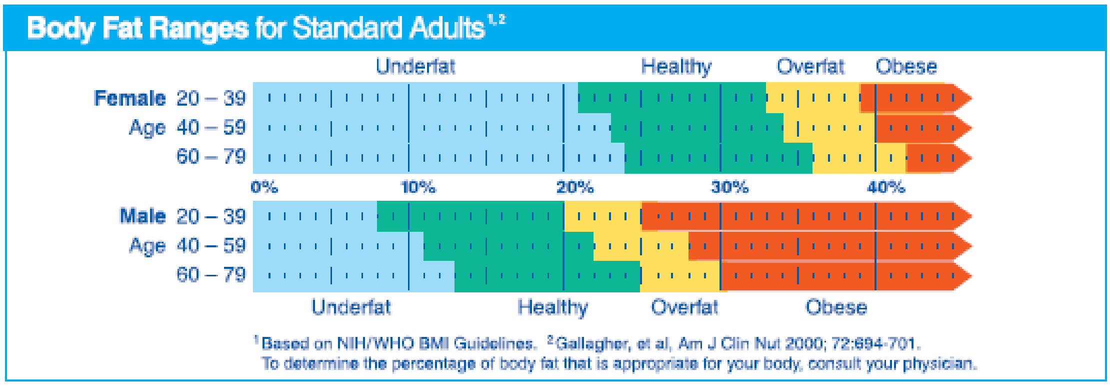
2. *Principles of bioimpedance and body composition analysis*

These days one of the greatest medical issues around the globe is obesity. Much co-morbidity is connected with this ´disease´, e.g. high blood pressure, diabetes, renal problems, and psychological problems (feeling of inferiority because of fatness). These co-morbidities play a huge part in medical care today (cardiovascular operations, insulin treatment, joints problems, etc.). It can be said that these diseases are more dangerous for a patient than obesity itself. In addition to health problems, these diseases represent enormous economic problem.

In some cases it is difficult to say whether a patient is obese or, to be more precise, whether a patient has more FM (fat mass) than is the healthy state consensus. When obesity problem is not obvious (patients do not have visually increased body partitions where fat stores), the patient can have fat stored in his/her abdomen, legs or arms. This case means that fat surrounds vital organs such as heart, liver, kidney, etc. and this is much worse scenario than visible body fat.

Therefore BIA is a good technique for body fat estimation because it discovers not only the visible fat but also the hidden fat. Direct impedance of patient could be measured and then value of body fat can be obtained. Thus examiner has quick information about patient’s health state in dependence on specific body composition parameters.

One of the very important body composition parameters is body fat percentage. This parameter is estimating patient`s amount of fat that should be reaching specified levels. Obtained value is then compared with standard ranges marked in special tables provided by WHO (world health organization). Thanks to this table of ranges it can be decided about the patient health state and treatment can start if needed. Body fat ranges are shown in Figure 1.



¹Based on NIH/WHO BMI Guidelines. ²Gallagher, et al, Am J Clin Nut 2000; 72:694-701.
To determine the percentage of body fat that is appropriate for your body, consult your physician.

Figure 1: Ranges of fat mass (FM) from the WHO guidelines

2.1. *Methods for body composition estimation*

There are lots of methods for body composition estimation these days. From basic evaluation methods which are using only basic anthropometric measurements such as BMI, hydrostatic weighing, continuing with more sophisticated methods such as: anthropometric measurements and skinfold thickness, to the most technically and difficult: bioimpedance, DXA (DXA - Dual-energy X-ray Absorptiometry) and Air Displacement Plethysmography Technology (ADP). All these methods are used for body composition estimation with different level of precision. Next paragraphs will explain and describe possible methods of body composition, their advantages and disadvantages and fields of use.

2.1.1. Body mass index – BMI parameter

A very popular and publicly well-known parameter for body composition evaluation is the Body Mass Index (BMI). As a single parameter indicating the degree of obesity (or slimness) of a patient is this parameter widely used. Advantage of BMI is simplicity of this parameter. The examiner needs only patient`s basic anthropometric values and can get results describing patient`s obesity status.

This parameter was invented by a Belgian polymath Adolphe Quetelet in 1840s. BMI is used as a standard for recording obesity statistics and as a descriptor of body composition and patient`s health state by WHO and other parties [1]. However, this parameter cannot tell anything about the body composition and fat distribution of an examined person. Some studies find that BMI is a non-specific indicator of body composition in healthy adults [3, 4, 5].

BMI is a calculated parameter that uses patient’s height and weight and is calculated as follows:

$$BMI = \frac{weight\ (kg)}{height^2(m)} \tag{1}$$

The obtained value is compared with the International Classification of adult underweight, overweight and obesity provided by WHO and according to this table the patient is classified into one of health status categories (Figure 2). A problem can occur in different populations such as Western Pacific Region (Figure 3). To overcome this problem other classification table than WHO`s International standards was provided by the Japanese Society for the Study of Obesity and WPRO (2000) (The Steering Committee of the Regional Office for Western Pacific Region of WHO. In this standard BMI ranges are different from international WHO classification to be more suitable for Western Pacific Region population.

Classification	**BMI(kg/m^2)**	
	Principal cut-off points	**Additional cut-off points**
Underweight	<18.50	<18.50
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	18.50 - 24.99	18.50 - 22.99 23.00 - 24.99
Overweight	≥25.00	≥25.00
Pre-obese	25.00 - 29.99	25.00 - 27.49 27.50 - 29.99
Obese	≥30.00	≥30.00
Obese class I	30.00 - 34.99	30.00 - 32.49 32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49 37.50 - 39.99
Obese class III	≥40.00	≥40.00

Figure 2: Classification of body composition in dependence on BMI value (Adapted from WHO 1995, WHO 2000 and WHO 2004).

NHLBI (1998)			**WPRO (2000)**	
WHO(2000) Classification	**Terminology**	**BMI (kg/m^2)**	**Classification**	**BMI (kg/m^2)**
Underweight	**Underweight**	< 18.5	**Underweight**	< 18.5
Normal range	**Normal**	18.5 – 24.9	**Normal range**	18.5 – 22.9
Preobese	**Overweight**	25 – 29.9	**Overweight at risk**	23 – 24.9
Obese I	**Obese I**	30 – 34.9	**Obese I**	25 – 29.9
Obese II	**Obese II**	35 – 39.9	**Obese II**	≥ 30
Obese III	**Obese III**	≥ 40		

Figure 3: NHLBI (1998): The Obesity Task Force of the National Heart, Lung and Blood Institute, USA

BMI is a good statistical tool for evaluation of huge number of observed patients. This method allows examiner to use means of correlation between groups that are related by general mass and estimating adiposity [85]. One of the problems with BMI is accuracy of obtained data and pertinent misclassification in specific class.

A problem can occur for example in athletes or muscular body types because muscle mass contributes to BMI more than fat. This can result in the case when two patients have the same BMI but entirely different body composition (muscular athlete and obese patient - weight and height are the same, this means the same BMI). This can lead to misclassification of patient to overweight or obese level. Difference in body composition can cause biases also in classification of children and elderly. BMI can underestimate adiposity in these cases with less lean body mass [86].

In general, BMI overestimates adipozity in those with more lean body mass (e.g. athletes) and underestimates excess adiposity in those with less lean body mass. And therefore BMI values can be insufficient for body composition evaluation. One successful attempt was to include BMI in the prediction models [40] to account for differences in body shape.

Even if BMI represents an important epidemiological tool, as evidenced by the study by Ancel Keys [8], when considering a single individual, it cannot be considered a reliable diagnostic tool to define the degree of obesity that is necessary to define the intensity of the clinical interventions (nutritional, psychological, rehabilitation, surgical, and pharmacological interventions) that can be applied to the overweight or obese patient. But further medical examination is needed to estimate the degree of obesity and health risk.

Generally, the body mass index is suitable for recognizing trends within sedentary or overweight individuals because there is a smaller margin for errors. Good results are also obtained when BMI is used in large sets of patient`s data correlating with other variables. BMI should be considered a complementary value in body composition evaluation. To use only BMI can cause misinterpretation of the patient`s health state.

2.1.2. Anthropometry and body composition

Anthropometry is a basic method for estimation of patient`s body parameters. This method is one of the oldest methods used in evaluation of human body parameters. It covers a wide variety of measurements such as: body size, structure, and composition. It is very important to be aware of these parameters and to be able to measure them during time differences of patient`s treatment or life (depending on actual observations).

Values of weight, height, skinfold thickness measurements and waist-hip measurements are the most important values for body composition and evaluation of health state of a patient. Examiner is able to determine patient's fat mass, muscle mass, body type, etc. from these values.

The most basic measurements in anthropometry are weight and height. These parameters are easily obtained by usage of fundamental equipment (scale and meter). From these obtained values BMI can be calculated and according to this the first basic health state parameters of the body can be estimated. Weight and height are used in all following methods for body composition evaluation, which is why these basic parameters are one of the most crucial parameters describing human body essence. Another important parameter in anthropometrical body parameters evaluation is waist to hip ratio, (WHR), which is the ratio of the circumference of the waist to that of the hips.

WHR has been used as an indicator of the health status of a person and as an indicator of the risk of developing serious health conditions (body composition values referring to obesity). It is not a direct measurement of body composition but it is one of the indicators that alert to patient`s changes in body composition and obesity problems. According to WHO procedure, WHR should be measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch-resistant tape that provides a constant 100 g tension. Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor [95]. WHR is then calculated as:

$$WHR = \frac{waist}{hip} \tag{2}$$

The obtained value is then compared with standards displayed in table (Figure 4) and the patient`s health status is determined.

	acceptable		**unacceptable**		
	excellent	**good**	**average**	**high**	**extreme**
male	< 0.85	0.85 - 0.90	0.90 - 0.95	0.95 - 1.00	> 1.00
female	< 0.75	0.75 - 0.80	0.80 - 0.85	0.85 - 0.90	> 0.90

Figure 4: Table of WHR from WHO standards

This simple measurement method has good results for estimating of patient’s health status. WHO uses this method as a predictor of obesity, which, in turn, is a possible indicator of other more serious health conditions.

As a third anthropometrical measurement can be considered skinfold thickness measurement. These measurements have been commonly used for body fat estimation. The development of skinfold measurement is a result of investigations for easier and less expensive method for body composition estimation. Skinfold thickness and body circumference is then used for prediction of body composition in special equations [96, 97]. These equations are the most accurate for people with average values of body fat. Otherwise, they tend to be less accurate in athletes, obese, young and old people.

For skinfold thickness measurement method a special tool, called a caliper, is needed. It is used for measuring thickness of subcutaneous fat. The examiner grasps the skin between the thumb and forefinger about 1cm from the measurement site following the natural fold of the skin. He lifts the skin up from the muscle in exact places (e.g.: triceps skinfold, abdominal skinfold, suprailiac skinfold, thigh skinfold), uses the caliper and waits for 4 seconds before reading the caliper.

Four seconds delay is due to fat compressibility. This procedure is repeated ten times (Pařízkova) or four times (Durnin, Wormesle) depending on the approach. Possible measurement sites of body are displayed in Figure 6. According to obtained values body density can be calculated as follows Figure 5:

Age (r)	**Sex**	**Equation**
9 – 12	M	D = 1,18 - 0,069 . log x
9 – 12	F	D = 1,16 - 0,061 . log x
13 – 16	M	D = 1,205 - 0,78 . log x
13 – 16	F	D = 1,205 - 0,78 . log x
17 – 45	M	fat% = 28,96 . log x - 41,27
17 – 45	F	fat% = 35,572 . log x - 61,25

Figure 5: Equations for body density by Pařízkova, D is body density and x is sum of ten skinfolds [Riegerová a kol., 2006]

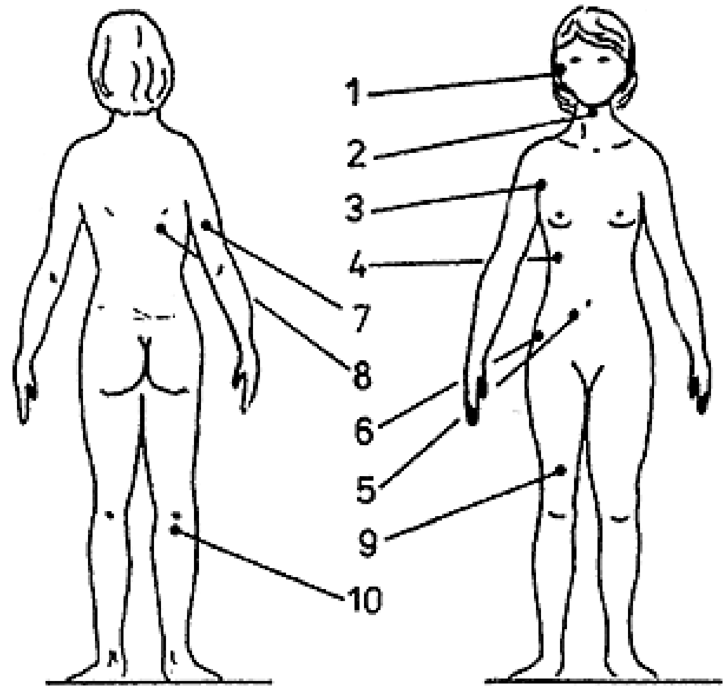


Figure 6: Places of skinfold measurement

[Riegerová J a kol. Aplikace fyzické antropologie v tělesné výchově a sportu 2006]

From the values of body density body fat % can be calculated by using Siri equation [91]. This method is a bit obsolete for body composition evaluation but still used. Major disadvantages of this method are: need for a precise and regular calibrated caliper, discomfort for a patient, need for precise measuring places (a wrong caliper placement can bias the whole procedure), and a good cooperation patient – examiner. This method is also very time-consuming and sometimes used equations can be inaccurate. Despite these disadvantages and discomfort of a patient, this method is used for body composition evaluation. Some doctors and body evaluation specialists are even using this method despite having a new and more precise equipment in their laboratories.

2.1.3. Hydrodensitometry - Hydrostatic Weighing

Hydrodensitometry or underwater weighing is one of the classic measurements of body composition. This method is based on Archimedes' Principle and uses basic physiological properties of tissues (body fat is less dense than water - density of fat = 0.9007 g/cm^3, it increases patient`s buoyancy while the fat-free mass - density of fat-free = 1.100 g/cm^3, which has a density greater than water, makes patient to sink).

It usually involves the use of a specially constructed tank in which the subject is seated on a suspended chair or frame. After the patient`s immersion, sensitive and continuous measurement of underwater mass is applied. Total expiration of the patient is necessary and remaining residual volume, water temperature, and estimation of gut volume is taken into account. All measurements and residual lung volume correction (decreased by underwater weight) are done and body density is obtained as follows:

$$Body\ Density = \frac{dry\ weight}{\left[\left(\frac{(dry\ weight - wet\ weight)}{water\ density}\right) - RV - 0.1\right]} \tag{3}$$

weight (kg), residual volume - RV (l), 0.1 represents an estimated volume (l) of gas in the human gastrointestinal tract [92].

However, body density is not a preferred value for nutritionists and other health professionals working with body composition [93]. More appropriate value for them is body fat % value. Calculation of body fat % from body density measurements is based on the assumption that the body is composed of two homogenous components: fat and fat-free tissue each having consistent densities.

This assumption allows calculating body fat % from obtained body density. To calculate body fat % two equations can be used [91, 92].

Siri equation (general population):

$$fat\% = \left(\left(\frac{4{,}57}{Body\ Density}\right) - 4{,}142\right) * 100 \tag{4}$$

Brozek equation (lean and obese individuals):

$$fat\% = \left(\left(\frac{4{,}95}{Body\ Density}\right) - 4{,}50\right) * 100 \tag{5}$$

After obtaining body fat %, we can assume better understanding of patient`s body composition.

The main limitation of this two-component model (fat and fat free mass) is possibility to estimate only two body parameters. In reality the body is composed of more components than just of fat and fat free mass. This leads to a finding that total body mass and circumference measurements have shown closer correlations with skinfold measurement than with percentage of body fat estimated by the hydrostatic technique [94].

The equipment required to carry out underwater weighing is expensive. The tanks are mostly located at research institutions, and usually there is not an easy access for the general population.

This method may underestimate body fat percentage of athletes as they tend to have denser bones and muscles than non-athletes, and may overestimate body fat percentage of elderly patients suffering from osteoporosis.

It was also shown that in extreme obesity cases this method is not appropriate. Therefore, this method is mostly appropriate for population that does not have extreme values of body fat. The other disadvantages are as follows: the subject must cooperate, exhale completely, and the tank must be filled with water. It follows that this method is not suitable for obese, pregnant, elderly, or disabled subjects.

Nevertheless, underwater weighing has been widely used to test the body density and in the past it was a criterion measurement for other indirect measurements.

2.1.4. Dual-energy X-ray absorptiometry - DXA

Dual X-ray absorptiometry (DXA or previously DEXA) method was originally mentioned as a tool for measuring bone mineral density. Recently, DXA is often used as one of the golden standard for body composition methods.

DXA scans are used primarily to evaluate bone mineral density (osteoporosis). Another possible use of this method is to measure total body composition and fat content with a high degree of accuracy [87].

DXA represents a 3-C model to estimate body composition values. DXA technique divides the body into 3 compartments: fat, bone mineral, and all fat-free mass that does not include bones. Hence DXA is not subject to errors caused by variations in bone density among different ethnicities as 2-C models.

DXA method uses absorption of two X-ray beams with different energy levels by tissue (fat, bone mineral, and fat-free soft tissue have different absorption properties). This absorption is then used for calculation of specific body values and bone density, so estimates of body composition can be obtained by scanning the entire body.

The whole measurement process took about 20-25 minutes in the past, now scans can take about 5-10 minutes (in cases of smaller regions of interest). The decrease of time needed for examination decreased errors caused by movement of the measured patient and makes everything more convenient.

Several different types of DXA systems are available, but they all operate on similar principles. A radiation source is aimed at a radiation detector that is placed directly opposite to the site to be measured. The patient is placed on a table in the path of the radiation beam and measurement is started. The source/detector assembly is then scanned across the measurement region. The attenuation of the X-rays is measured with highly sensitive detectors positioned at a fixed distance from the scan bed and above the anterior surface of the body [53] Figure 7. After measurements are done, the attenuation of the radiation beam is determined and is related to the BMD (bone mineral density) [88, 89].

Figure 7: DXA – patient`s examination

The greatest disadvantage of this method is exposure to ionizing radiation (<5μSv) [54]. Though the amount of radiation for a scan of recent DXA devices is only a fraction of that received from a standard chest X-ray. It is comparable to the amount received on a transcontinental airline flight. Hence, this DXA method produces less radiation than other methods.

Other disadvantages of this method are the time and software. With a change of software the outcomes can differ (software upgrades can change the algorithms that the device uses to calculate body composition.). It takes about 20 minutes to obtain whole body DXA scan the procedure (depending on the equipment used and the parts of the body being examined) and the patient has to stay in the supine position without any major movement.

That is why this is not appropriate technique for small children (because of movement and X-ray exposure). This means that the examiner must pay attention to detail in positioning. When DXA studies are performed incorrectly, it can lead to major mistakes in diagnosis and therapy [90].

Another source of error is the same error that manifests in all 2-compartment models: the hydration of fat-free mass. A 5% variation in fat-free mass hydration can change DXA-determined body fat percentage by nearly 3% [134].

Some studies have compared DXA to 4-C models. When considering group averages, DXA have results with errors of 1-2%. However, like with all other body fat testing techniques, individual error rates can be higher. The error rates vary between studies and machines used. The error can be in range from 4% in one study [135] up to 8-10% in another study [136]. Also sex, size, fatness, and disease state of subjects can influence accuracy of DXA [129].

DXA represents a 3-C model, with its error rates comparable to hydrostatic weighing. Like other techniques, DXA performs well when looking at group averages, but errors can occur when looking at individual measurements. Individual error rates tend to be around 5%, although some studies have shown error rates higher. When considering change over time in individuals, error rates have been reported around 5%.

In general, if all standard measurement conditions are applied and positioning of the patient is correct, this method is considered to be very precise in body composition evaluation. However, due to X-ray this method represents certain non-negligible problems for some specific patients such as pregnant women or children.

2.1.5. *Air Displacement Plethysmography*

Whole-Body Air Displacement Plethysmography (ADP) is a recognized and scientifically validated densitometry method to measure human body composition. This is an alternative approach that uses pressure-volume relationships for body composition estimation.

The basics of this method are similar to hydrostatic weighing, but this densitometry technique uses air displacement rather than water immersion. This technique had its limitations in technical difficulties in adjusting deviations in humidity and temperature for air near body and hair. These problems were partly overcome by a new BOD POD system (Life Measurements Instruments, Inc., Concord, CA) which increased precision and accuracy.

Air-displacement plethysmography offers several advantages over other mentioned body composition methods. It is quick, comfortable, automated, noninvasive, and safe measurement process, that is able to measure e.g., children, obese, elderly, and disabled persons.

This method uses indirect measurement of patient`s volume by measuring the volume of air he/she displaces inside an enclosed chamber (plethysmograph). This means that body volume is measured when a patient sits inside the chamber.

Body volume is calculated indirectly by subtracting the volume of air remaining inside the chamber when the subject is inside from the volume of air in the chamber when it is empty. The BOD POD determines body volume by measuring changes in pressure within closed a device. The air inside the chamber is measured by applying relevant physical gas laws. The used Boyle’s Law states that at a constant temperature, volume (V) and pressure (P) are inversely related:

$$\frac{P_1}{P_2} = \frac{V_2}{V_1} \tag{6}$$

where P_1 and V_1 stands for one conditions of volume and pressure and P_2 and V_2 for other.

The first condition presents an empty BOD POD chamber and P_2 and V_2 presents condition when the patient is sitting inside the measuring chamber. The Bod Pod is functionally divided into two chambers (schema of a bod pod is described in Figure 8): a test chamber (for the subject) and a reference chamber. The internal volumes of these chambers are ~450 and 300 L, respectively.

When the volume is increased in one of the chambers, it is decreased by the same amount in the other chamber, and vice versa. The pressure in each of the two chambers responds immediately to this volume change, and the magnitude of the pressure changes indicates the relative size of each of the chambers. The pressure change is roughly ±0.5 cm H_20, and is rarely noticeable to the subject (comparable to the change in pressure while moving from the first floor to the second floor in an elevator).

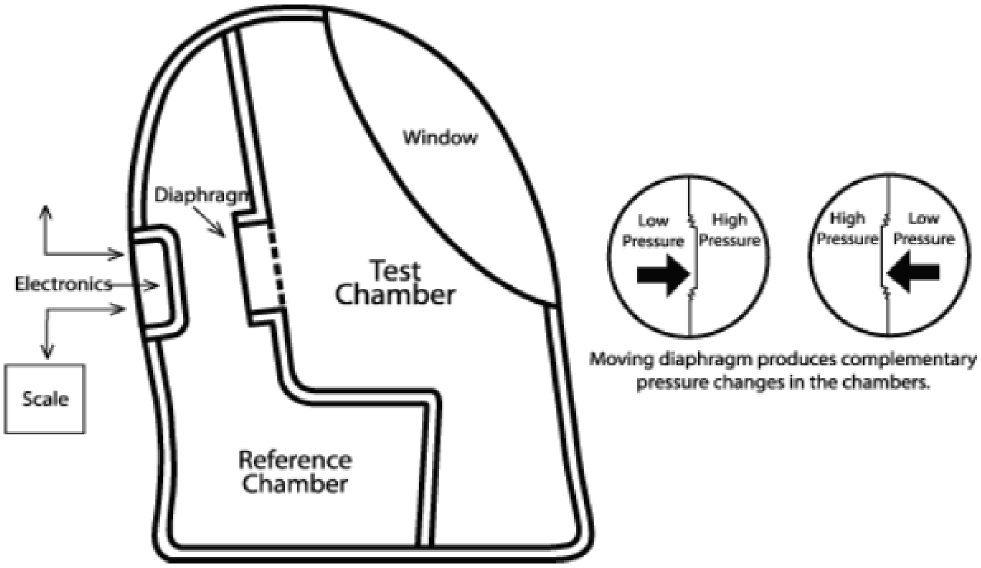


Figure 8: Schema of Bod Pod functionality
[Altered from http://mobilebodyanalysis.com]

Nowadays BOD POD uses adiabatic properties of air in chambers (air in the chambers is allowed to compress and expand). This means that there is not any strict requirement of temperature and humidity levels. Boyle’s Law is then replaced by Poisson’s Law, which describes the pressure-volume relation under adiabatic conditions as follows:

$$\frac{P_1}{P_2} = \left(\frac{V_2}{V_1}\right)^{\gamma} \tag{7}$$

where γ is the ratio of the specific heat of the gas at constant pressure to that of constant volume and is equal to 1.4 for air [115].

One of the problems is that some volume of air is maintained in isothermal conditions (near skin or hair, and in clothing). This phenomenon can be partially removed if the subject wears a tight-fitting swimsuit and swim cap.

In the next procedure step the effect of isothermal air near the skin’s surface is estimated by calculating a surface area artifact SAA (L). For the final body volume calculation V_{TG} (thoracic gas volume) must be estimated to overcome possible inaccuracy. After obtaining measured body volume $V_{m,}$ correction for SAA and V_{TG} is done. Final body volume (all values are in liters) is calculated as follows:

$$V = V_m - SAA + 40\%V_{TG} \tag{8}$$

Body density for further evaluation is calculated with the use of body volume and body mass (measured during the procedure) as follows:

$$\text{Density} = \frac{Body\ mass}{Body\ volume} \tag{9}$$

After density of the body is determined, the relative proportions of body fat and lean body mass are calculated (2-C model). With all these known results the whole body composition can be calculated by use of 2-C models by Brozek or Siri [91, 92]. Alternatively, body density can be used in 4-C models of body composition. The final results of Air Displacement Plethysmography done in BOD POD normally consist of fat %, fat free mass and additional values required for calculation of body composition (Figure 9).

Body composition results

% FAT	15.2	%
% FAT FREE MASS	84.8	%
FAT MASS	34.846	lb
FAT-FREE MASS	194.483	lb
BODY MASS	229.329	lb
BODY VOLUME	90.000	L
BODY DENSITY	1.111	Kg/L
THORAIC GAS VOLUME	4.500	L

Figure 9: Body composition results obtained by BOD POD

[Altered from www.cosmed.com]

The BOD POD has a high level of accuracy (the manufacturers indicate that the general error range of the BOD POD is 1-2% -the same as hydrostatic weighing), ease-of-use, and fast test time makes this approach relatively favored measuring option. One of the biggest advantages of the BOD POD compared to underwater weighing is that the Bod Pod does not require getting wet, and it is well-suited for special populations such as children, obese, elderly, and disabled persons.

Air Displacement Plethysmography technique has some problematic aspects such as calibration (must be done before every measurement), technical complexity and patient`s cooperation. Nevertheless, Air Displacement Plethysmography and especially the BOD POD is a reliable and valid technique that can quickly and safely evaluate the whole body composition (the most often body fat and fat free mass) in a wide range of subject types, including those who are often difficult to measure, such as the elderly, children, and obese individuals.

2.1.6. Bioimpedance vector analysis

Bioimpedance vector analysis (BIVA) method has a great potential in the future. This method uses only impedance vectors and does not take into account models and equations that are used for body composition evaluation. The BIVA was developed by Piccoli et al. [55, 56, 57] and its advantage is that BIVA includes only error of measurement and variability of subjects. Vectors of reactance and admittance are plotted in R-Xc plane Figure 10. Individual vectors are compared with reference 50%, 75%, and 95% tolerance ellipses calculated in the healthy population of the same gender and race [58]. Ellipse can vary with age and body size [59].

Major axes interpret hydration status and minor axes stand for tissue mass. A unique application of BIVA is the discrimination of fat and fluid changes in obesity. In comparison with healthy adults with normal BMI, obese individuals have shorter vectors and similar phase angles.

Obese adults with edema have significantly shorter vector length than the normal weight and otherwise healthy obese adults, and significantly lower phase angle [2, 60]. This method can be very interesting in the future but nowadays further evaluation and better understanding is needed.

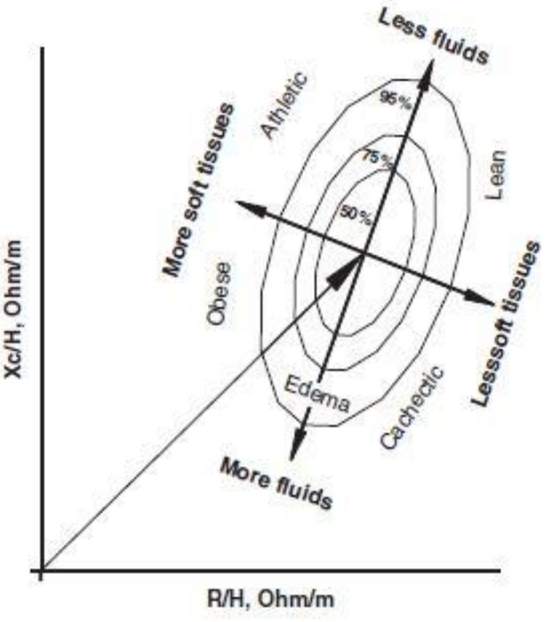


Figure 10: BIVA vector plot

[S. Grimnes and Ø. G. Martinsen, Bioimpedance and Bioelectricity Basics]

2.1.7. *Body Impedance Analysis (BIA)*

The Body Impedance Analysis (BIA) represents one of the methods for classification of body composition and classification of body tissue values. BIA method uses small current (μA) injected into the patient`s body. According to this, voltage is measured and electrical properties of body are used for body composition evaluation. This method is very popular because it is noninvasive, fast, portable and inexpensive. These parameters give a good background for this method to be widely used. This method will be described in detail in Chapter 2.3.

2.2. *Human body composition parameters*

From the point of electrical view, the living organism consists of intra and extracellular fluids that behave as electrical conductors, and cell membranes that act as electrical condensers and are regarded as imperfect reactive elements. Because of different tissue parameters: permittivity and conductivity different body tissues have different electrical values. Basic electrical tissue characteristics were described by Gabriel et al. [108] and chosen tissues are listed in Figure 11. Hence this impedance of specific tissue differs a lot. This phenomenon can be used to estimate bioimpedance and further in BIA method.

	σ [S/m]	**ε_r**
Skin	0.065836	15357
Bone	0.020791	227.64
Fat	0.024414	92.885
Muscle	0.36185	8089.2

Figure 11: Permittivity εr and conductivity σ values at 100 kHz from

[Lisa Rothlingshofer Monitoring Change of Body Fluid during Physical Exercise using Bioimpedance Spectroscopy and Finite Element Simulations]

For example fat tissue has very high resistance (smaller conductivity) because it consists mainly of lipids and almost no water. Muscle mass or fat free mass (excluding bone mass) on the other hand is composed mainly of water and therefore this has small resistance (bigger conductivity). These major differences (muscle mass have conductivity approximately ten-times higher than fat mass) in impedance are used for estimation of body composition values (total body water - TBW, extracellular water - ECW, intracellular water - ICW, etc.). Mainly four or five values are used to estimate body composition, such as TBW (composed of

ICW and ECW), fat mass, fat free mass and muscle mass sometime bone mass (minerals) is incorporated in estimations. Sometimes the fifth/sixth parameter (not obtained from BIA) is used: resistance index H^2/Z (H stands for height and Z is impedance) which is connected with TBW [18]. Other values obtained with BIA method can be body cell mass (BCM), Adipose Tissue Mass (ATM), Fat Tissue Index (FTI - defined as the quotient of ATM/Height^2 [kg/m^2]), proteins, etc. These values (excluding bone mass) are not so often used and precision can be biased.

In body composition evaluation there are many models of tissue description used. Beginning with a simple diagram of body composition model that divides body tissue into just two parts 2-C (two compartment model) model and continuing with more sophisticated and better descriptive models 4-C (four compartment model) and 5-C (five compartment model). Models are shown in Figures 12 [7] and 13. With these models the whole body tissue can be divided into several parts. According to these dimensions of these parts nutrition health status can be described. These parameters describe body property and are much more precise than BMI.

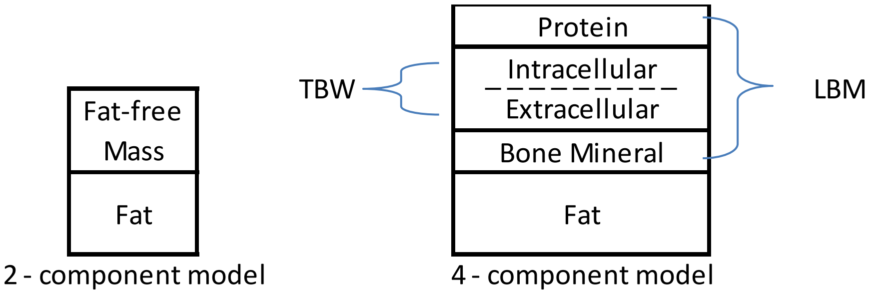


Figure 12: Body composition models 2-C and 4-C
[Modified from Chanchairujira T, Mehta RL. “Bioimpedance and Its Application.”]

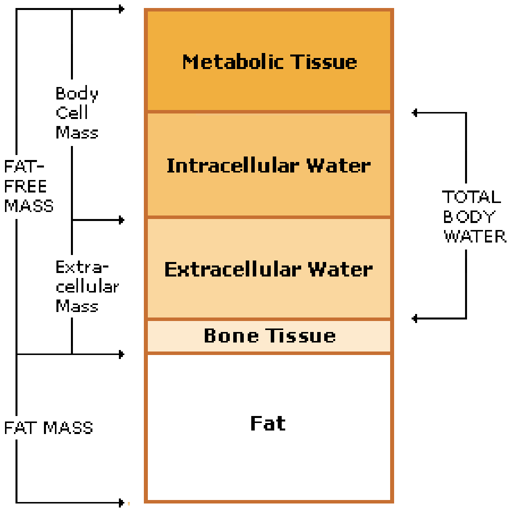


Figure 13: Body composition models 4-C another possibility

Basic two-component model of human body composition takes into account only fat mass and fat free mass. This 2-C model is widely used in body composition techniques: hydrodensitometry, anthropometrical measurements, and basic evaluation). However, this model has its limitation.

The first limitation of this model is that it takes into consideration of only two body composition parameters. This can lead to inaccurate evaluation of the body composition. Hence, division of body tissue into only two tissues does not describe the real body composition. Because there are no incorporated values such as bone mineral, muscle mass etc., which means for example if only fat free mass will be considered there is no description of muscle mass. This is a problem because the assumption that the fat free mass has constant composition can resolve not to incorporate it into muscle mass, which means that we can obtain a bit of fat mass. This can result in misclassification by this model.

Another limitation of the 2-component model other than division of body composition into two values is limitation in discriminating differences in body composition when factors such as physical activity, illness, and aging affect a person. Nevertheless, the 2-C model is very suitable for young adult white males [38].

All the problems encountered by using the 2-C model can be solved by using more complex models that take into consideration a wider range of body composition parameters.

These multi compartment models (3-C, 4-C, 5-C) of body composition consider also properties of fat free mass and other values respectively. This leads to separation of all fat free mass parameters into categories of: TBW (consist of ICW and ECW), muscle mass (stands for protein), bone mass and mineral mass. By using these multi compartment models better understanding of human body can be obtained and better outcomes in further treatment can be concluded. In case of using estimation only for fat free mass it can contain ascites, which can result in misclassification in some hydration problems.

If the body is divided into more composition parameters it is easy to discover which parameter is out of optimal range and a precise treatment can be started. Multi compartments models have an advantage in exact description of a specific tissue and after estimation of specific body composition values an individual treatment can be prescribed. Nevertheless, multi compartment approach has also disadvantages which are mainly: need of calculation for lots of characteristics and possible danger in biases.

2.2.1. *Total body water*

Total body water is the whole body water content that is considered to be 72.3% of the whole body fat free mass volume [19, 20, 21]. This status does however change during the life. For example a healthy infant`s TBW is 80–83% of the FFM, this percentage then decreases rapidly over the next 3–5 years until the hydration fraction reaches that observed for adults [101]. Also time period of a day and other factors such as temperature and humidity can have influence on actual TBW.

To accurately predict the volume of total body water, the mixture effects need to be considered because the relationship between R and body water volume is nonlinear [23, 24]. The value of TBW consists of two parts: ICW (intracellular water) and ECW (extracellular water). From these values a simple equation can be stated to calculate TBW as follows:

$$TBW = ECW + ICW \tag{10}$$

If the examiner wants to obtain the total body water, high frequency current should be used (high frequency current penetrates cells and TBW is measured. More is explained in Chapter of ECW). At high frequencies ECW and ICW contribute to the conductive medium and all other items in the body form restrictive material [25]. This was previously supported by Patel et. al. [26] and Van Loan et al. [27]. Some of the total body water equations were discussed in [10]. De Lorenzo et al. [25] postulated equation of total body water volume as follows:

$$V_{tnm} = \left(\frac{\rho_\infty K_b H^2 W^{\frac{1}{2}}}{R_\infty D_b^{\frac{1}{2}}} \right)^{\frac{2}{3}} \tag{11}$$

where ρ_∞ is a mean TBW resistivity and was declared to be 104.3 ± 7.9 Ωm for men and 100.5 ± 7.8 Ωm for women, ρ_{50} was found to be 128.44 Ωm for men and 123.01 for women [21], H is a height in meters, K_b=4.3 is shape factor, D_b= 1.05 kg/l is body density which can differ between individuals but generally is in range from 1-1.07 kg/l [29].

For estimating TBW at a specific frequency of 50 kHz (the most common for single frequency measurements) and use of directly measured resistance another equation was formed. Krusher et al. [30] in his work used to calculate the volume of TBW equation as follows:

$$V = 0{,}5561 \frac{H^2}{R_{50}} + 0{,}955W + 1{,}726 \tag{12}$$

the same equation for men and women, where H [cm] presents height and W [kg] presents weight, R stands for resistance at 50 kHz.

By measuring another group of people new terms of equation were derived by Hannan at al. [31]. By using these new parameters equation was defined as follows:

$$V = 0{,}446\frac{H^2}{R_{50}} + 0{,}126W + 5{,}82 \tag{13}$$

H, R and W have the same meaning as in (11) and (12).

Both these previous equations do not consider difference between men and women. Hence equation by Lukaski and Bolonchuk [32] is mentioned to describe gender differences that can conclude to differences in obtained outcome. By using sex differentiation in their equation Lukaski and Bolonchuk postulated their equation as follows:

$$V = 0{,}372\frac{H}{R} + 3{,}05sex + 0{,}142W - 0{,}069age + 4{,}98 \tag{14}$$

Deurenberg et al. [82] have proposed a similar equation using sex as one of the parameters as follows:

$$V = 6{,}53 + 0{,}3674\frac{H^2}{Z_{50}} + 0{,}1753W - 0{,}11A + 2{,}83sex \tag{15}$$

where A is the age in years, sex is 0 for men and 1 for women, H is height in cm and W is weight in kg.

Other equations for BIA can be found in various works publicized during a long time period [10, 33, 34, 35, 36, 37, 38, 39].

2.2.1.1. *Extracellular water*

Extracellular water is one component of TBW and usually indicates all body fluid outside of cells. This fluid is composed of blood plasma, interstitial fluid, and transcellular fluid and it has high amount of Na^- and low in K^+ ions. The resistivity of extracellular water is close to that of saline and the accrual resistivity is about 40 Ωcm. The amount of extracellular water is normally ~ 60% of TBW. This body fluid also helps to control the movement of water and electrolytes throughout the body.

There is a strong correlation in ECW and TBW so the ECW/TBW ratio is tightly regulated.

To obtain precise ECW parameter bioimpedance measurements should be performed at low frequencies <1 kHz. This is because of cell membrane which can be replaced (in the circuit theory) to a capacitor. This leads to phenomena that capacity of cell membrane does not allow current to penetrate into cells and current does not penetrates into cells. Thus, only ECW is measured. Vice versa if a high frequency current is used, then the current penetrates cell membranes and total body water is measured. A detailed description is shown in Figure 14.

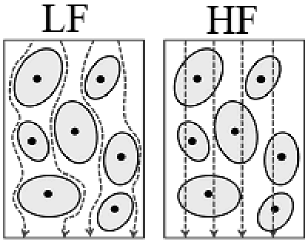


Figure 14: Frequency of used current and cell membranes penetration

Because of technical reasons, impedance meters using surface electrodes are normally limited to a frequency range of 5–1000 kHz, and ECW resistance (Re) and the TBW one (R∞) must be calculated by extrapolation to zero and infinite frequencies, respectively [106]. After this assumption ECW is calculated with the use of impedance at zero frequency and approximation of the human body as a sum of 5 cylinders (limbs and trunk). The final equation was described in Jaffrin et al. as follows [19]:

$$V_e = k_e \left(\frac{H^2 + W^{1/2}}{R_e} \right)^{2/3} \tag{16}$$

Van Loan et al. [18] suggested to take $k_e = 0.306$ for men and 0.316 for women, when V_e is in liter, H in cm, weight in kg and R_e in Ω, Nevertheless, this equation does not incorporate the whole body impedance, hence this whole body impedance equation was incorporated in work of other authors. For example Hannan`s et al. [31] used in his equation single frequency impedance parameters to obtain the ECW parameter. He discussed possibilities to use both bioimpedance complex parameters reactance and resistance at frequency of 50 kHz.

With this approach the volume of ECW can be calculated as follows:

$$Ve = 0.0119\frac{H^2}{X_{50}} + 0{,}123\frac{H^2}{R_{50}} + 6.15 \tag{17}$$

where X_{50} is reactance at 50 kHz and R_{50} is resistance at same frequency and H is a height.

Sergi`s et al. [81] added to his equation difference in sex parameter. He presented his equation as follows:

$$Ve = -5{,}22 + 0{,}2\frac{H^2}{R_{50}} + \frac{0{,}005}{X_{50}} + 0{,}08W + 1{,}9 + 1{,}86sex \tag{18}$$

where sex 0 was for men and 1 for women, H is height , R - impedance and W - weight have the, X_{50} stands for reactance at 50 kHz.

All these equations are possible to use for calculation of ECW volume. Examiner has to decide which will be better for his specific needs and obtained data.

2.2.1.1. *Intracellular water*

Intracellular water usually indicates all body fluid inside of cells. The ionic composition of ICW depends on cells in which ICW is considered. Thus, ionic characteristics of ICW are not known and mean resistivity cannot be determined as for ECW. Nevertheless, it can be said that ICW mainly contains K^+ ions with concentration ~160 mequiv./l.

To determine ICW volume equation of TBW calculation or impedance parameters can be used. For estimation of ICW volume by impedance values intracellular resistance R_i must be calculated. This approach assumes that R_e and R_i are in parallel Figure 15. After this assumption R_i can be calculated by using total resistance R_{Tot} and extracellular resistance R_e as follows:

$$R_i = \frac{R_e R_{Tot}}{R_e - R_{Tot}} \tag{19}$$

where total resistance is R_{Tot} and extracellular resistance R_e.

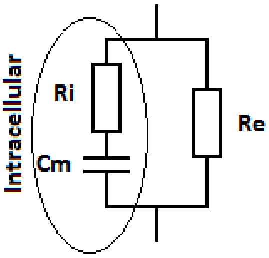


Figure 15: Model for ICW calculation assuming Re and Ri are in parallel

Because the ICW compartment is represented by R_i and a capacitance of membrane C_m R_i cannot be used as the ICW resistance unlike R_e for ECW. Thus ICW volume (V_i) is calculated a bit differently. One of ICW equations was presented by De Lorenzo et al. [25] where volume of ICW was calculated by the use of resistances and volume of extracellular fluid a follows:

$$\left(1 + \frac{V_i}{V_e}\right)^{5/2} = \frac{R_e + R_i}{R_i}\left(1 + K_p \times \frac{V_i}{V_e}\right) \tag{20}$$

Van Loan et al. [27] have suggested values of K_p of 3.82 for men and 3.40 for women.

De Lorenzo et al. used in his research a measurement criterion and derived equation as follows [113]:

$$V_i = 12{,}2 + 0{,}37065 \times \frac{H_t}{R_{ICW}} - 0{,}132 \times age + 0{,}105 weight \tag{21}$$

R_{ICW} - intracellular resistance, 1 for men and 0 for women.

2.2.2. Fat free mass

Fat free mass also called Lean Body Mass (LBM), is the total amount of nonfat (lean) parts of the body. It consists of approximately 73% water, 20% protein, 6% mineral, and 1% ash. This tissue consists of all body composition parts that do not include fat (see Figure 12, 13) and mainly stands for muscles, proteins, tendons and all tissues in internal organs.

Changes of FFM are associated with an increased risk of some chronic diseases and it also changes during the life. The FFM peaks during 35- to 44-year old men and in 45- to 54-year old women. After this period the amount of FFM is declining.

The difference between the middle aged population and older population can be critical. It was reported that mean FFM was 8.9 kg or 14.8% lower in men older than 85 year than in men 35 to 44 year old and 6.2 kg or 14.3% lower in women older than 85 year than in women 45 to 54 year old [106].

The amount of FFM is considered to be directly correlated with health and longevity [41] and it is an important predictor of survival in some critical illnesses and malignancies [42]. One on the basic FFM equations is based on anthropometric values of body. According to this, fat free mass equation can be derived by using TBW. Thus, FFM can be calculated as an easy coefficient of TBW:

$$TBW = 0.732FFM \qquad (22)$$

This calculation can be used because the hydration coefficient of human body is proposed to be 0.732 [83, 10].

Nevertheless, this basic equation can be easily overcome by bioelectrical impedance analysis (BIA). Bioimpedance has shown to be more accurate for determining leanness or fatness in humans [105]. Equation that was used bioimpedance was proposed by Lukaski et al. [43] and the fat free mass was calculated as follows:

$$FFM = 0{,}827\frac{H^2}{R} + 5{,}21 \qquad (23)$$

where H is height and R is resistance.

This equation was later extended by Van Loan et al. [44]. In his work he used sex of a patient as an important parameter to obtain the final FFM. His equation was postulated as follows:

$$FFM = 0{,}51\frac{H^2}{R} + 0{,}33W + 1{,}69sex + 3{,}66 \qquad (24)$$

where H is height and R is resistance, W is a weight, sex is 1 if men and 0 in women

Recently a new equation for FFM was used by G. Kyle at al. [106] for fat free mass in their study on a big set of patients of ages 15 through 95. This equation also incorporates both imaginary parts of impedance. FFM is calculated as follows:

$$FFM = -4.104 + (0.518 * height^2 resistance) + (0.231 * weight) + (0.130 * reactance) + (4.229 * sex) \tag{25}$$

where sex = 1 if men and 0 if women, used frequency 50 kHz,

Shumei S et al. [38] incorporated in his equation a 4-C body composition model with known constant densities for each component. In his work he proposed an equation that is bioimpedance dependent on a single frequency (50 kHz). His final prediction of race-combined equation was sex specific divided into two separated equations for female population and male population. The equation was defined as follows:

Male

$$FFM = -10,68 + 0,65 \frac{height^2}{resistance} + 0,26\, weight + 0,02\, resistance \tag{26}$$

Female

$$FFM = -9,53 + 0,69 \frac{height^2}{resistance} + 0,17\, weight + 0,02\, resistance \tag{27}$$

where FFM is in kg, height^2/resistance is in cm^2/Ω, and resistance is in **Ω**.

Although these final BIA race-combined prediction equations did not perform as well in the blacks as in the whites, it is still a very good equation for FFM estimation. Another advantage of this equation is incorporation of the resistance coefficient that takes into account resistance of the patient (non-calculated value). This equation was used for a successful evaluation of a big cohort of patient with satisfactory outcomes.

All previously mentioned equations have incorporated directly the measured resistance, which positively increases the outcome value of FFM.

Overall FFM is one of the very important parameters of body composition evaluation. According to its value, the examiner can conclude if the patient is progressing in his desired treatment. Sometimes there can be problems with substitution of FFM for body muscle mass which can lead to misclassification of the patient`s treatment, especially during the weight reduction treatment.

2.2.3. *Muscle mass*

Muscle mass or sometimes skeletal muscle mass (SMM) is a part of a fat free mass that stands for muscles and is regarded as a significant indicator of overall physical strength. For an average adult male is this value up of 42% of body mass and for an average adult female is this value up to 36% of body mass [102]. The density of mammalian skeletal muscle tissue is ~1.06 kg/liter. It is composed primarily of water amount of 72~73% muscle mass and protein (actin, myosin), which constitutes most of the non-aqueous portion of muscle. Muscle mass has been an important focus of the nutritionists. Human body has three types of muscle tissues.

Skeletal muscle or "voluntary muscle" is anchored by tendons to bone and is used to effect skeletal movement such as locomotion and in maintaining posture. This type of muscle mass is possible to be controlled by the patient`s mind and is the most represented from all three muscle types.

Smooth muscle or "involuntary muscle" is found within the walls of organs and structures such as the esophagus, stomach, intestines, etc. Unlike skeletal muscle, smooth muscle is not under conscious control.

Cardiac muscle is also an "involuntary muscle" but is more akin in structure to skeletal muscle, and is found only in the heart.

There are variations of equations for calculating of muscle mass [104], it mainly depends on values that were measured. For certain anthropometric values and bioimpedance by Lee et. al. [103] anthropometry based predictive equation can be used:

$$\begin{aligned} SMM\,(kg) = {} & Ht_m(0{,}244 \times BM) + (7{,}8 \times Ht) \\ & + (6{,}6 \times gender) - (0{,}098 \times age) + (ethnicity - 3{,}3) \end{aligned} \tag{28}$$

Ht_m, height (m), Ht height (cm), BM body mass (kg), Gender: male=1, female=0; ethnicity: Asian=1,4, African-American=1.2, White=0, R^2=0.86, SEE=2.6

This equation was reported to have the best results from other anthropometry- based equations [104].

Muscles contain a big amount of ECW in which Na^+ and Cl^- ions are dissociated. This is an optimal medium for current conduction. Hence muscle mass has smaller impedance than fat mass which is very important in bioimpedance body composition analysis. Due to these electrical properties of muscle mass, bioimpedance can be easily used to obtain to impedance of a body and additional anthropometric body measurements. Jansen et. al. [105] derived a prediction equation with use of single frequency resistance as follows:

$$SMM\ (kg) = \left[\left(\frac{H_t}{R} \times 0{,}401\right) + (gender \times 3{,}825) + \left(age \times (-0{,}071)\right)\right] + 5{,}102 \tag{29}$$

where Ht is height (cm); R is resistance in ohms 50 kHz; Gender men = 1 women = 0, age is in years. The R^2 and SE of estimate of the regression equation were 0.86 and 2.7 kg (9%), respectively.

The advantage of equation that uses bioimpedance is fewer variables (smaller possibility of measurement error). It is sufficient to know only height from anthropometric measurements, gender and age to obtain the same value of muscle mass as would be obtained by non bioimpedance equation. Nevertheless, bioimpedance equation does not incorporate ethnicity, this can play an important part in muscle mass estimation.

Muscle mass is one of the important markers that are followed in obesity treatment. Because the goal of body weight loss procedure is mainly to decrease the body fat of a patient which basically means the decrease of overall weight. Nevertheless, in this procedure patients are told not just to decrease their daily consumption of calories but also to increase their movement activity. Hence, this increase of muscle mass should be reported, and because muscle mass is denser than fat mass, a slight increase in overall body weight can be reported although decrease in body fat is noticeable.

Thus, changes in body muscle mass must be reported to conclude if there is not any error in treatment and if there is increase in body muscle mass. Because in some cases there cannot be just decrease in body fat but also in muscle mass, and it can be very problematic.

Hence, this muscle mass is a very important marker of a successful treatment and should be closely observed.

2.2.4. Body fat

Body fat mass is an adipose tissue of a body. This tissue plays a big role in the human body. It can be divided into two parts: essential body fat and storage body fat. Body fat serves as an energy storage of the organism, is maintains life, reproductive functions of an organism, it also serves as a thermal insulation of organs, and myelin tissue that covers nerves is body fat as well. It is one of the most important markers of the patient`s health status. According to the value of body fat patient is classified to a specific group Figure 16.

Body fat differs in dependence on sex, age and race of a patient (table of fat % in dependence on sex and age Figure 17). The age difference is very important because body fat % changes during the life of a person. Also difference in women`s and men`s percentage of body fat is significant. Hence, it is necessary to know exactly patient´s gender and age to determine the correct range of optimal body fat %.

Changes during the life are from 10%-15% of body fat for infants at birth followed by an increase up to 30% during the middle decade of life and then followed by a decline in elderly patients. Maximum percentage of body fat is then between the ages of 50 - 60 [114]. Progress of body fat in kg during the life across races is described in Figure 16.

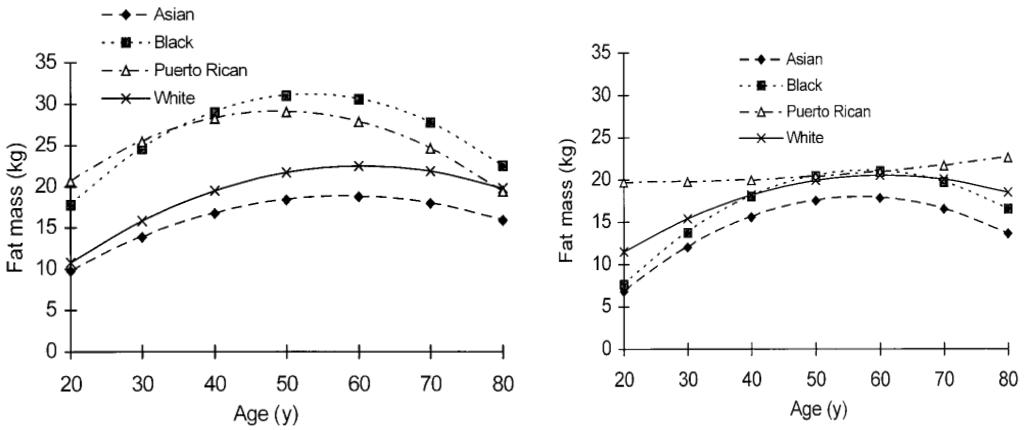


Figure 16: Changes of fat mass in dependence on age in women and men

[Mott et al. Relation between body fat and age in 4 ethnic groups. Am J Clin Nutr]

The percentage of essential body fat for women is in the range of 10–13% of body weight and is greater than that for men whose ranges are lower 2–5%. This is because of the demands of childbearing and other hormonal functions that are different in both genders. The essential fat is the level below which physical and physiological health would be negatively affected. The second fat tissue - the storage fat consists of fat accumulation in adipose tissue and partly of fat that protects internal organs. This tissue also stores an amount of energy for human organism. The final body fat percentage is a sum of both these fat tissues. In Figure 17 there are shown percentages of body fat for specific type of athletes in comparison with average men and women.

Classification	**Women (fat %)**	**Men (fat %)**
Essential fat	10-13%	2-5%
Athletes	14-20%	6-13%
Fitness	21-24%	14-17%
Average	25-31%	18-24%
Obese	32% and higher	25 and higher

Figure 17: Ranges of body fat including essential body fat for specific groups of sport active men and women with comparison of average population

From the above mentioned it clearly shows that the value of body fat is a very important parameter across all groups of population. Hence, according to the percentage of body fat obesity and patient`s health status is relatively easily determined. Patient`s body fat percentage should exceed essential body fat but on the other hand this parameter should be within healthy ranges.

Increase in body fat % is the first and important indicator of obesity and risks such as arteriosclerosis and other severe diseases connected with obesity (Figure 18). Because of this fact obesity is described as an increase of accumulated adipose tissue. With increase of body fat it is likely for the patient to suffer from various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis [111].

		Women		
Age	Under fat	Healthy	Overweight	Obese
20 – 40 years	Under 21%	21 – 33%	33 – 39%	Over 39%
41 – 60 years	Under 23%	23 – 35%	35 – 40%	Over 40%
61 – 79 years	Under 24%	24 – 36%	36 – 42%	Over 42%

		Men		
Age	Under fat	Healthy	Overweight	Obese
20 – 40 years	Under 8%	8 – 19%	19 – 25%	Over 25%
41 – 60 years	Under 11%	11 – 22%	22 – 27%	Over 27%
61 – 79 years	Under 13%	13 – 25%	25 – 30%	Over 30%

Figure 18: Body fat ranges for men a women basic distribution to groups according to age and body fat percentage [110]

It is crucial to know the percentage of body fat to decide about the health state. There are lots of possible ways to determine body fat, starting with basic anthropometric measurements continuing skinfold measurements and hydrodensitometry to obtain body density and then use Siri or Brozek equation for body fat estimation. Another usable equation for total body fat was proposed by Shumei S et al. [38] where parameters obtained by DXA method are used:

$$TBF = 2{,}513BV - 0{,}739TBW + 0{,}947\frac{BMC}{1000} - 1{,}79(weight) \tag{30}$$

where BV is body volume (L) calculated as a body weight/body density, BMC is total body mineral content [g] from DXA.

Heitmann [112]: incorporated in his equation bioimpedance parameters and sex parameter as follows:

$$BF = 14{,}94 - 0{,}079\,\frac{{H_t}^2}{R_{50}} + 0{,}818 \times weight - 0{,}231 \times H_t - 0{,}064 \times sex \times weight - 0{,}077 \times age \tag{31}$$

where sex 1 for men, 0 for women.

There are more equations for body fat estimation [10] that were derived by various authors. Hence, body fat is one of the most important parameters for body composition estimation.

2.3. History of bioimpedance

Electrical properties of tissues were described firstly by Hermann et al. in 1870s.
In 1928 Cole proposed an expression for the impedance at DC and infinite frequency. He also proposed CPE (constant phase element) that is used in his electrical model. Cole was the first who plotted a complex imminence (term that stands for impedance and admittance) in the Wessel diagram, sometimes called the Cole – plot. He was one of the first who presented the three-component model (two resistors and one capacitor) in two different configurations.

In coming years, Thommasset [6] performed the first studies that used electrical impedance measurements as an index of total body water (TBW). He used two subcutaneously inserted needles. After Thommasset Hoffer et al. and Nyboer [8, 9] introduced in their studies the four-surface electrode BIA technique.

In 1950 Schwan revealed frequency dependence of muscle tissue capacitance and interpreted a relaxation phenomenon. He also introduced low frequency precise measurements and four-electrode technique.

In 1970s BIA method was established. The relationships between the impedance and the body water content (BWC) of the body have been made. Afterwards a lot of single frequency BIA analyzers were commercially available. And in the 1990s several multi-frequency analyzers were introduced.

More recently segmental BIA machines have been used because this approach can overcome the problem of trunk values. This area has a small contribution to the total body resistance (R), but a big contribution to the total body mass. A segmental approach is useful tool for body composition assessment considering also these body disproportions.

Recently a wide variation of device can be found in a field of bioimpedance (bipedal, bimanual, tetra polar) which are capable of various measurements using a single or multi frequency approach. This leads to the fact that bioimpedance has an important role in various fields of medicine, body composition evaluating, pregnancy observation, biomaterials, bioimpedance spectroscopy, bioimpedance tomography, etc.

2.4. *Basic principles of bioelectrical impedance*

Impedance (Ω) is a ratio between a current and a voltage that applies in both alternating current (AC) and direct current (DC). From this statement bioimpedance can be described as a passive electrical property of biological materials to oppose electrical current. If we consider only basic setup this simple method needs only two or more electrodes (one CC – current carrying and PU – pick up) and an injection of current to work.

Basic concept of bioimpedance is impedance **Z** [ohm, Ω] which is as follows:

$$\mathbf{Z} = \mathrm{R}^2 + {X_e}^2 \tag{32}$$

or admittance

$$Y = \frac{1}{Z} \tag{33}$$

These values express relationship between an AC sinusoidal current and AC sinusoidal voltage (it is the complex ratio of the voltage to the current in an alternating current circuit). Impedance extends the concept of resistance to AC circuits, and possesses both magnitude and phase. In DC driven circuits resistance and impedance are the same. Hence, there is a lack of phase difference between the voltage and the current.

Bioimpedance is a complex value because a biomaterial not only opposes current flow but also adds phase-shift of the voltage with respect to current in time domain [10]. Impedance of body tissue consists of two values: resistance (R) and reactance X or Xc.

Resistance R is a real part of impedance, it describes tissue values of current opposition and does not change with frequency. Reactance X (presents induction of voltages in conductors self-induced by the magnetic fields of currents - inductance, and the electrostatic storage of charge induced by voltages between conductors - capacitance) is imaginary part of impedance and presents capacitive properties of body tissue (value influenced by cell membranes - capacitor) [10] and this value changes with frequency. These capacitive properties of body tissue are due to cells which have poorly conducting membranes. These two values are bounded together with the phase angle φ (see Figure 19) as follows:

$$\varphi = arctg\,\frac{X}{R} \tag{34}$$

Interpretation of this formula is that the phase angle can show a relative distribution of fluids. Its value can differ in theory from 0 (no cell membranes) to 90° (cell membranes only). In clinical use the phase angle can be very useful, because it should respond to ECW/ICW ratio that should be a sensitive measurement of malnutrition or illnesses [16]. Length of the vector of impedance **Z** can be considered as inversely related to fluid volume [2].

Bioimpedance is defined as the frequency domain ratio of the voltage to the current. The magnitude of bioimpedance values represents the ratio of the voltage amplitude to the current amplitude. Whereas the phase represents the phase shift by which the current lags the voltage. Using Ohm`s law impedance is expressed as follows:

$$V = IZ = I|Z|e^{jarg(Z)} \tag{35}$$

The magnitude of the impedance |Z| stands for resistance (the drop in voltage amplitude across an impedance) Z for a given current I. The phase factor shows that the current lags the voltage by a phase of θ= arg (Z) Figure 19.

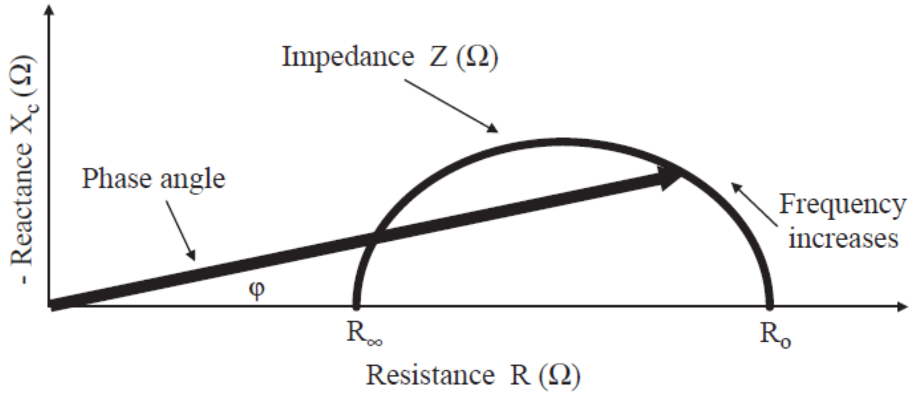


Figure 19: Geometric interpretation of impedance (Z), resistance (R), reactance (Xc) and phase angle φ, changes of Z depending on frequency

[Ursula G. Kulda, ect. , Bioelectrical impedance analysis part I: review of principles and methods]

In biological tissue there are lots of cells with membranes that have the effect of frequency dependence of bioimpedance. Hence, with a higher frequency impedance it is lower and vice versa (see Figure 20). This gives us also information about the tissue, membrane structures and intracellular and extracellular liquid distribution. The frequency also determines measurements, because with specific frequencies different tissue properties can be measured.

If we do not consider frequency and take into account only pure resistive values of tissue then only resistance is measured.

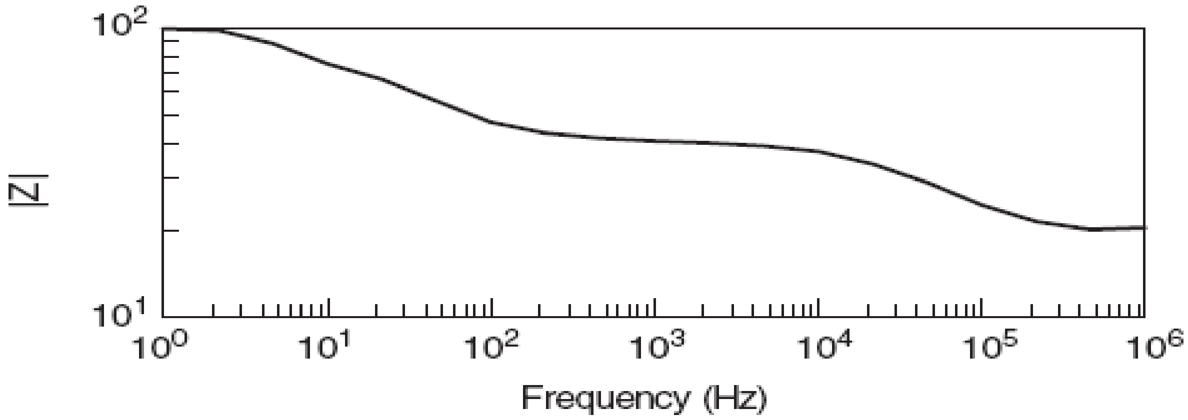


Figure 20: Changes of impedance in dependence on frequency
[S. Grimnes and Ø. G. Martinsen, Wiley Encyclopedia of Biomedical Engineering]

The resistance value is dependent on material's proportions and properties. If we consider basic ideal model of a homogeneous conductive material (see Figure 21) its resistance can be expressed as follows [11]:

$$R = \frac{\rho L}{A} = \frac{\rho L^2}{V} \tag{36}$$

where L is length of material, A is cross sectional area, ρ [Ω.m] is material resistivity and V is volume of the material.

This model can be used for a basic understanding of bioimpedance. It can be used as the first model of parts of human body that can be in the circuit theory replaced with five cylinders. The body should be divided into five parts (parts behave as if they are in series with each other, with shorter and thicker segments contributing less to the total R) that represent limbs and trunk. With each tissue having different resistivity figure 21 resistance can be calculated with the knowledge of proportions and resistivity. This will be a case when the human body is homogenous and tissue does not include cells that are surrounded by a cell membrane that is mainly composed of lipids.

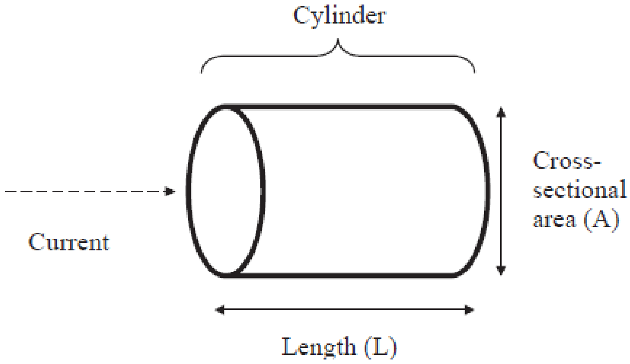


Figure 21: Basic model of cylindrically shaped tissue

[Ursula G. Kulda, ect. , Bioelectrical impedance analysis part I: review of principles and methods]

However, the reality is much more complicated. The body does not consist of homogenous material as mentioned above. Every tissue has its own specific resistivity (ρ) thus this R of a single segment can differ a lot. We must consider tissue to be homogenous at the macroscopic level. This approximation must be made, because tissue at its microscopic value is not homogenous. At the microscopic level there must be considered all tissue parts such as blood, lipids, plasma, etc. Single cells should also be incorporated into this consideration.

Because of all these facts, the whole impedance should be considered not only a real part, but also an imaginary part. Reactance is a part of bioimpedance that stands for capacitive properties of cell membranes. Cell membranes consist of a layer of nonconductive lipophilic material interposed between two layers of conductive molecules. They behave like tiny capacitors (storing energy). Reactance in the body reflects the strength of this capacitance. Since intact cellular membranes are contained primarily within body cell mass, the reactance of the body is proportional to the amount of body cell mass.

With consideration of all parameters of body tissue, better electrical models can be used and better description of the body can be done. By obtaining the basic electrical property of a body composition, values can be calculated using various equations.

3. Bioimpedance measurements and body composition models

This chapter of a PhD thesis describes theoretical background of electrical current in patient`s body, models used for calculation of body parameters and instrumentation used for measurements of body composition. Every section of this chapter is meant to increase theoretical background and awareness of possible approaches, instrumentation and other important facts for further assumptions.

The first section deals with basic principles of electrical current in human body to cover bioelectricity that is a basic of bioimpedance with specification of impedance of specific body regions. This section is followed by electrical analogies and models that are modeling tissue for calculating and estimation of bioimpedance (reactance and resistance).

The following sections describe several possible approaches how to measure bioimpedance by various devices and frequencies.

I discussed the possibility of bioimpedance usage in very special body status in women – pregnancy. In this part I formulate several important assumptions for the practical experiment.

At the end of this chapter I discuss electrical safety in bioimpedance measurements (it is very important to know the exact electrical boundaries that are safe to be used) and other possible applications of BIA in medicine.

3.1. Electrical current in tissue

An electric current is a flow of electric charge for which a medium is needed. This charge can flow in three types of environment: metal, electrolyte, plasma. Electrons in the metal medium involve no transport of metal ions and thus this transport of electrons is very fast and current flow is unlimited.

On the contrary electron flow in electrolyte (human body) requires the whole movement of ions. Hence the transport in electrolyte is slower than in metal. The transport of ions has also one disadvantage: ion generation in electrodes with inverse charge is connected with this phenomenon. This can lead to destruction of cells in body tissue. That is why AC is used for bioimpedance measurements, which ensures the change of polarity.

From the point of circuit theory, human body can be considered as a huge electrolyte. If a body tissue is simplified it is composed of major form of water and ions. Human body consists of a lot of ions, e.g. Na+, K+, Cl-, and Ca+. These ions are dissociated in water and therefore human body leads electrical current. Without this phenomenon no bioelectricity would exist and bioimpedance could be measured.

If bioimpedance is to be measured, electrical AC current is needed. Applying electrical current with a current carrying electrode voltage can be measured at signal pick up electrode and with use of the Ohm’s law impedance can be calculated as follows:

$$\mathbf{Z} = \frac{V}{I} \tag{37}$$

Alternating current (ac) should be used to prevent iontophoresis and to detect the phase shift between voltage and current [15]. With this phase shift, phase angle can be calculated as an important part of bioimpedance.

The frequency of applied electrical current is very important because impedance changes with the frequency and phase angle as well. The signal penetration through the body tissue (cell membranes) also depends on the frequency. It was mentioned that the body tissue composes of cells and each cell has a membrane. The membranes are mainly composed of lipids (lipid bilayers). These membranes have a high capacitance and low but complicated pattern of conductivity. Hence, they behave as a capacitor. Model of a cell with electrical analogies is shown in Figure 22.

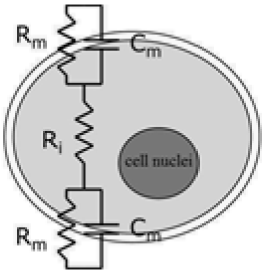


Figure 22: Electrical model of a cell R_m – resistance of membrane, C_m – capacity of membrane, R_i- resistance of intracellular water

This has the effect that at a low frequency an alternating current or direct current signal passes around the cell, and only a small part of the cell interior contributes to the current flow. In this case extra cellular volume or ECW is measured.

At high frequencies the cell membrane as a capacitor lets the current through (despairing of the cell membrane effect). The current flows directly through the tissue (cells) according to the local ionic conductivity. With the use of high frequency effect, the total volume can be measured. Different paths of the current in dependence on frequency are shown in Figure 23.

Usage of high frequencies can be very important because there are a lot of membranes in the body. Generally, it can be said that current flows through TBW and ECW in dependence on their conductivity and volumes [22].

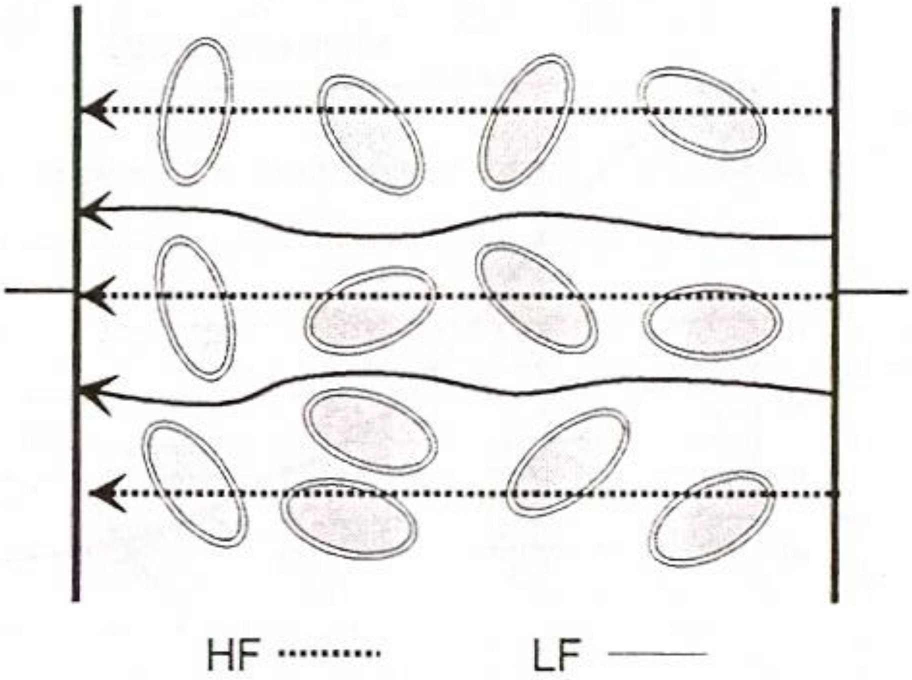


Figure 23: Flow of current through tissue dependence on frequency
[S. Grimnes and Ø. G. Martinsen, Bioimpedance and Bioelectricity Basics]

With the use of high frequencies the whole body content is measured to obtain all parts of the hydration status that consist of TBW, ICW and ECW. The frequency also determines the depth of current penetration. With the use of higher frequencies the measurements will reach deeper layers of a tissue [17]. The use of specific frequencies has an important role in determination of the body composition values. Some studies [26, 31, 10] discussed the use of various frequencies for special cases. With the use of various frequencies only some parts of body composition can be measured.

In general, human body tissue is a very anisometric medium because of the existence of cell orientation, macro membranes (peritoneum, abdominal membrane, pericard, etc. – that can have an influence on current flow) and organs. All these reasons can influence the current flow.

There are defined more than 30 types of tissues according to tissue electrical properties [108]. This means that the variations in heterogeneous tissues can cause interfaces, separating regions of different properties to trap or release electrical charge as stimulus potential is changed.

The time lag between the stimulus potential and the change in charge in these interfaces creates a frequency (f)-dependent Z (i.e., dispersion). The dispersion found in the low-frequency (LF) radio range (1 kHz to 100 MHz), which is of interest for predicting ECW and ICW volume, is known as Maxwell Wigner β-dispersion for the dielectric interfaces. This dispersion is caused by cell membrane capacitance (C_m) [28].

If we consider human body as a non-homogenous tissue it is clear that different parts of human body should have different properties [25]. This takes into account body resistivity (if the body is considered to be purely resistive) and body segment propositions. Because of this in different body parts current flow differs. In particular body segments resistance differs in dependence on tissue properties (resistivity cross section area and length). All these properties change resistance of specific segments. Using equation 36 resistances can be calculated. Some of the typical resistances of the body are shown in Figure 24.

These typical values depend mainly on proportions of the particular body part and occurrence of specific tissues. Also skin contribution and current constructional effects caused by small electrodes have to be considered, which is not in this Figure 24.

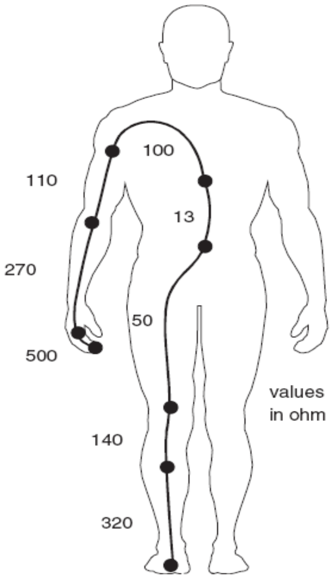


Figure 24: Typical values of resistance of human body compartments (the values represent resistance between the marked points)

[S. Grimnes and Ø. G. Martinsen, Wiley Encyclopedia of Biomedical Engineering]

Figure 24 also shows that various segments contribute differently to whole body resistance. Whereas segments such as trunk and chest have negligible influence to resistance (small values due to high amount of conductive matter – body fluid, point to small contribution to whole resistance), segments such as fingers, under arm and leg contribute to resistance a lot. Higher resistance may be also due to relatively small cross sectional areas and presence of a highly non-conductive tissue such as a bone, which dramatically increases resistance.

3.2. *Bioimpedance tissue models*

For better understanding and application of bioimpedance or admittance, body tissue must be described by pure electrical models. Every electrical property can be described by a basic electrical component: a resistor or a capacitor.

Later in Cole`s work, a constant capacitor was replaced by CPE (Constant Phase Element - not a physical device but a mathematical model) [116]. Because bioimpedance is a measurement of passive properties of a tissue resistive and capacitive properties of a tissue can be described by an admittance model that should be used if components (resistor and capacitor) are parallel. Admittance of this model is described as follows:

$$\mathbf{Y} = G + j\omega C \qquad \varphi = \arctan\left(\frac{\omega C_p}{G}\right) \qquad |\mathbf{Y}|^2 = G^2 + (\omega C)^2$$

where j is phase angle indicating to what extent the voltage is time-delayed, G is parallel conductance [S], and C_p parallel capacitance [F]. The term ωC_p is capacitive susceptance.

On the contrary, the impedance model should be used if components are physically in series (resistor and capacitor). Impedance of this model is inverse to the admittance equation $\mathbf{Z} = \mathbf{Y}^{-1}$ described as follows:

$$\mathbf{Z} = R - \frac{j}{\omega C} \qquad \varphi = arctan\left(\frac{-1}{\omega R_s C_s}\right) \qquad |\mathbf{Z}| = R^2 + \left(\frac{1}{\omega C}\right)^2$$

where $-1/\omega C_s$ is capacitive reactance, R is resistance and j is imaginary unit.

The values of the series (R_s, C_s) components are not equal to the parallel ($1/G$, C_p) values which results into impedance counted as followed:

$$\boldsymbol{Z} = R_s - \frac{j}{\omega C_s} = \frac{G}{|\boldsymbol{Y}^2|} - \frac{j\omega C_p}{|\boldsymbol{Y}^2|} \tag{38}$$

This shows the importance of a chosen model. The impedance model should be used in case when the components are physically in parallel: R_s and C_s are both frequency-dependent whereas G and C_p are not. Implicit in these equations is the notion that impedance is a series circuit of a resistor and a capacitor, and admittance is a parallel circuit of a resistor and a capacitor. Measurement results must be given according to one of these models [116]. The serious issue is which model should be used.

Some of the basic electrical models of biological tissue in vivo can be seen in Figure 25. The model a) (left side of Figure 25) takes into account behavior of biological tissue as a resistor and a capacitor in series connection. The second model in Figure 25 is Fricke's model that involves arranging R and capacitance in parallel connection (see Figure 25b) [10]. In this model resistance of intracellular water (ICW) and extracellular (ECW) are parallel and X_c presents a cell membrane that separates these two environments.

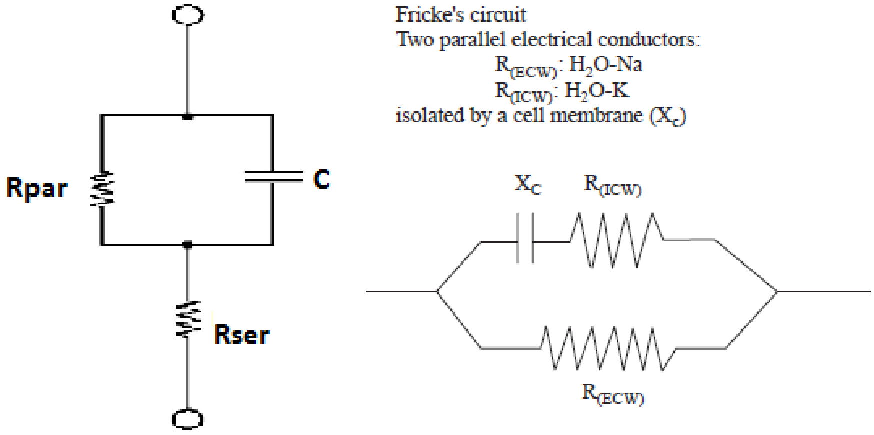


Figure 25: Basic electrical models of body tissue a) series and b) parallel connections

[Ursula G. Kulda, ect. , Bioelectrical impedance analysis part I: review of principles and methods]

However, the human tissue is much more complex. Therefore, better and more accurate models have been described, such as Debye circuit [12] and Cole-Cole circuit model [13] (see Figure 26). The impedance of Debye model is as follows:

$$Z = R_\infty + \frac{1}{G_{\mathrm{var}} + G_{\mathrm{var}} j\omega\tau_Z} \qquad \tau_Z = \frac{C}{G_{\mathrm{var}}} \tag{39}$$

where Z is complex impedance [Ω]; R_∞ is resistance [Ω] at infinity frequency, j is imaginary unit, ω is angular frequency [1/s], τ_Z is the characteristic relaxation time constant [s] of the circuit corresponding to a characteristic angular frequency $\omega_Z = 1/\tau_Z$, C is parallel capacitance [farad, F], and G_{var} is independent parameter of conductance [siemens, S].

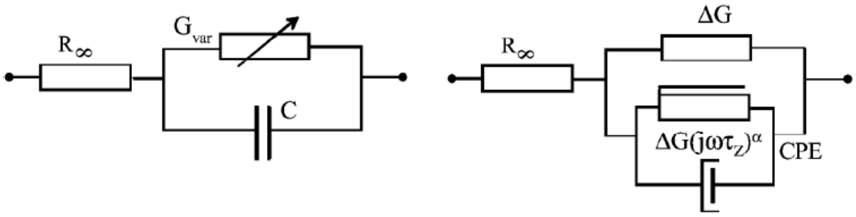


Figure 26: Bioimpedance models: left - Debye model with ideal components, right – Cole-Cole circuit model. More general model with the ideal capacitor is replaced by a constant phase element (CPE) with frequency dependent components

[S. Grimnes and Ø. G. Martinsen, Cole electrical impedance model a critique and an alternative]

The Cole frequency dependence equation for the tissue or cell suspension is [14] as follows:

$$\mathbf{Z} = R_\infty \frac{R_0 - R_\infty}{1 + (j\omega\tau_Z)^\alpha} \tag{40}$$

where R_0 is resistance at low frequencies, τ_z is characteristic time constant of the system corresponding to a characteristic angular frequency $\omega_Z = 1/\tau_z$, and α is exponent. The product $\omega\tau_z$, and $(j\omega\tau_Z)^\alpha$ represents a CPE along as α is constant because

$$j^\alpha = \cos(\frac{\alpha\pi}{2}) + j\sin(\frac{\alpha\pi}{2}) \qquad\qquad \alpha = \frac{\varphi_{CPE}}{90^\circ} \tag{41}$$

CPE can be altered with a resistor and a capacitor, both frequency dependent (no ideal) so that the phase φ_{CPE} becomes frequency independent. The capacitance of this element is then:

$$C_{CPE} = \frac{\Delta G}{\omega}(\omega\tau_Z)^\alpha \sin(\frac{\alpha\pi}{2}) \tag{42}$$

3.3. *Electrodes*

Electrodes play a very important role in bioimpedance measurements. It is a place where electronic conduction changes into ionic conduction. There are basically two types of electrodes: a current carrying electrode and a pick up electrode sometime the third measuring/current carrying electrode is considered that are used for measurements Figure 27.

Electrodes are in contact with the skin (in case of non-invasive approach), or can be inserted directly into the tissue (in case of invasive approach – not used for BIA method). It is crucial to use the right sets of electrodes to avoid the influence of electrode polarization impedance. In case that only dry samples are measured, 2-electrode system can be easily used. However, wet, ionic samples are prone to errors and special precautions must be taken.

Nevertheless, in bioimpedance theory, the materials are considered to be wet, with double layer. This means that polarization effects will occur on the metal surfaces. This phenomenon introduces errors into the measurements. However, these errors can be reduced by introducing 3- or 4-electrode systems Figure 27.

Three most common electrode systems are: bipolar lead (electrodes are equal which means that every electrode is measuring and current carrying), tetra polar (with three electrodes, the first one is current carrying, the second one is a signal pick up electrode and the third one is a measuring and current carrying electrode) and quadrupolar (with four electrodes two are current carrying and two are signal picking) [11]. These systems are shown in Figure 27. The four electrode system is usually stated not to be prone to errors from electrode polarization impedance [61, 62, 63].

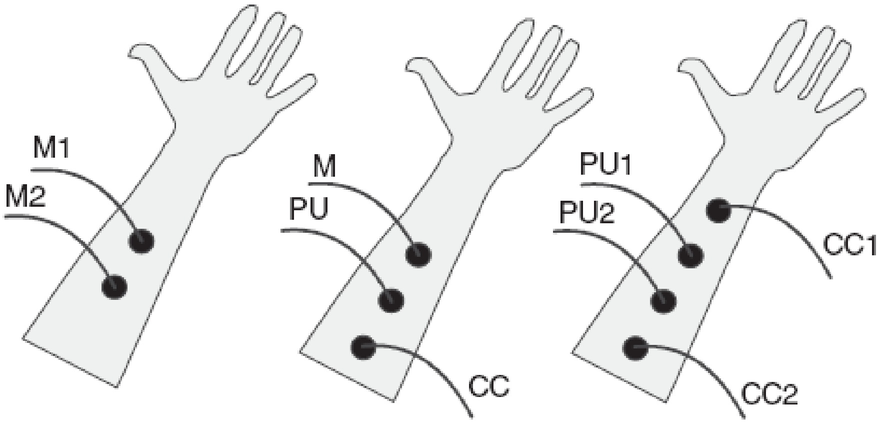


Figure 27: Electrode systems: bipolar, tetrapolar and quadrupolar
[S. Grimnes and Ø. G. Martinsen, Wiley Encyclopedia of Biomedical Engineering]

There are lots of electrode designs, but in bioimpedance and BIA the most common electrodes are: skin surface electrodes or metal electrodes. Design of skin surface electrodes is well described e.g. in [64]. Also a material of an electrode is important in electrode design. There are various metals, polymers and carbon that can be used for electrode production. Every material is more suitable for specific measurements for example Tanita MC 180 MA device uses for feet: stainless steel and for handgrips plated electrodes (mechanically strong, noncorrosive, highly DC polarizable and noisy, very alloy dependent) [64]. In special cases invasive electrodes can be used as well but it is not very common to use them because then BIA loses its advantage of noninvasiveness.

Concerning electrodes, it is very important to know that the sensitivity field of an electrode system can vary. If we use four electrode systems for a direct current resistance measurement the sensitivity will be [61]:

$$S = \frac{\mathbf{J}_1 \cdot \mathbf{J}_2}{I^2} \tag{43}$$

where $\mathbf{J_2}$ is current density between two current carrying electrodes and $\mathbf{J_2}$ is current density between signal pick up electrodes, I is current.

A positive value for sensitivity means that if resistivity of this volume element increases, a higher total resistance will be measured. Another result of a higher value for sensitivity is a greater the influence on the measured resistance.

On the other hand, a negative value for sensitivity means that increased resistivity in that volume gives a lower total measured resistance [61]. For example in a four-electrode system there are typically small volumes with negative sensitivity between the CC and voltage PU electrodes.

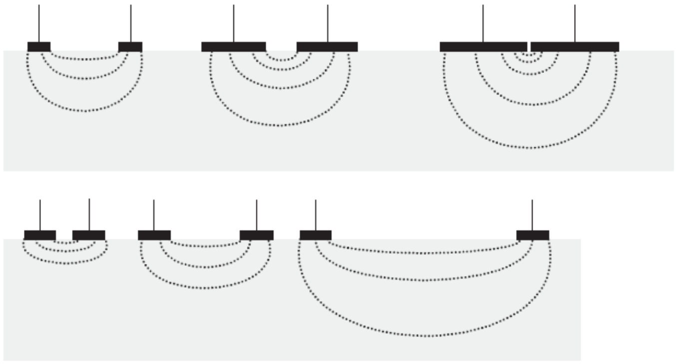


Figure 28: Depth of measurements in dependence on electrode dimensions and spacing [S. Grimnes and Ø. G. Martinsen, Bioimpedance and Bioelectricity Basics]

The analysis of the sensitivity field of an electrode system is of vital importance for the interpretation of measured immittance because sensitivity is also influenced by dimensions of applied electrodes Figure 28.

From equation (18) the resistance in a small volume can be calculated using tissue resistivity ρ [61]:

$$R = \int_V \frac{\rho \mathbf{J}_1 \cdot \mathbf{J}_2}{I^2} dv \tag{44}$$

with the use of reciprocity theorem (change of PU and CC electrodes does not change R - electrodes and voltage PU electrodes can be interchanged without any change in measured values) taken into account then resistance can be calculated as follows:

$$R = \int_V \frac{\rho \mathbf{J}^2}{I^2} dv \tag{45}$$

Sensitivity calculations can be utilized equally well for two-, three- or four-electrode systems. In each case you must identify the two electrodes used for driving an electrical current through the material and the two electrodes used for measuring the potential drop in the material [61].

Design of an electrode can differ a lot according to all properties of tissue and possible materials. One of the designs that are have been recently used is a skin surface electrode (SSE). This electrode is usually made with a certain distance between the metal part and the skin where the area of contact between the metal and the electrolyte determines the polarization impedance.

Other electrodes can be as clicks that are used for clipping onto the patent’s limbs. Recently, the most used electrodes for BIA devices are metal electrodes attached to plastic grips or metal electrodes fitted in a plastic pack on which the patient stands. Electrodes such as SSE require an experienced examiner to be placed in correct places.

3.4. *Devices for body composition measurements*

There is recently a huge variance of devices for body composition evaluation using BIA method. The two basic criteria for dividing the devices into groups are: first one takes into account number of electrodes that are used for measurements and the second one takes into account number of used frequencies.

If we consider first criterion (number of used electrodes) we can divide devices into groups of two, four and eight electrode systems.

Two electrode devices are those that use hands - hand to hand (patient holds electrodes in his/her hands) or feet - feet to feet [45, 46, 47] (patient stands on electrodes). Another device from this group is Bodystat device that is using four electrode systems with placement of electrodes on hand and leg (electrodes are located one on arm and another on leg).

First two systems (hand to hand and feet to feet) have just limited predictive ability because with hand to hand system only upper part of a body is measured and this approach does not take into account legs and abdomen. The same problem is with feet to feet measurement where only lower part of a body is measured. This has problematic aspect that parts of a body that have bigger percentage of TBW contribute less to measurements. Hence leg to arm localization (Bodystat) is better, because whole body impedance can be measured. This is one advantage of hand – feet electrode system shown in Figure 29.

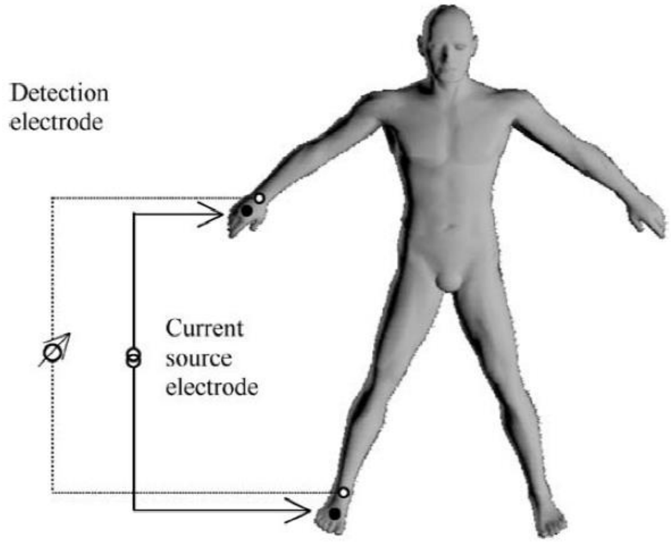


Figure 29: Hand to leg electrode system

[Ursula G. Kulda, ect. , Bioelectrical impedance analysis part I: review of principles and methods]

Four and eight electrode devices [48] are much better for obtaining body composition values. With this option it is possible to measure not only the whole body impedance but also a segmental impedance and composition of every part of human body (typically – hands, feet and torso). Hence much more accurate reading can be obtained. Segmental BIA is performed by attaching additional electrodes to wrist/hands and feet on both sides of a body [49]. Other placements of electrodes have been described in various publications [50, 51, 52].

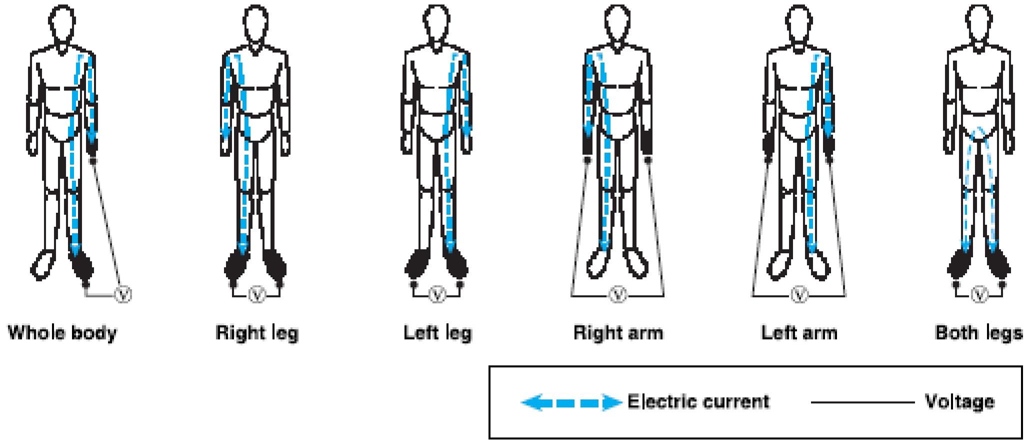


Figure 30: Measuring specific segments of body
[Tanita manual]

Measurements of specific segments of a body are than done using specified electrodes as a current carrying and voltage measuring. For example an electric current is supplied from electrodes at the ends of the fingers on both hands and the toes on both feet, and the voltage is measured at the ball of the thumbs on both hands and at the heels of both feet [137]. Principle of different electrodes usage (8-electrode method) is shown in Figure 30.

If we consider second criterion devices can be divided into groups according to used frequencies for measurements: single frequency device, multifrequency devices.

Single frequency device uses generally frequency of 50 kHz with an electrode localization that can differ. With these devices we are strictly speaking about measuring not TBW but weighted sum of extracellular water (ECW) and intracellular water (ICW) resistivity (~25%) [10]. This method allows estimation of FFM and TBW but it is impossible to measure ICW and ECW separately. It is a good method for estimation of absolute FFM and TBW in normally hydrated patients [50]. It was also discovered that single frequency approach was more accurate and less biased for TBW in critically ill subjects [117]. Nevertheless, single frequency bioimpedance measurements are not valid under conditions of significantly altered hydration [10].

Multi frequency devices are using different frequencies mainly 5, 50, 250, 500 and 1000 kHz. Lower and higher frequencies do not make sense because it has been reported that use of lower frequencies than 5 kHz and higher than 1 MHz means bad reproducibility especially of reactance [31]. Advantage of this method is improvement of estimation of fat free mass and body fat. This method is capable of direct determination of ICW and ECW.

Most common manufacturers of BIA devices are Tanita, Biospace (InBody device), Omron (majority of their devices are only capable to measure basic values) and Bodystat. These manufacturers offer wide variety of devices with different measurement possibilities from basic devices to complex and high-ends. Every device and its accessories should be chosen according to experiment design and expected outcomes.

3.5. Body composition and bioimpedance in pregnancy

Pregnancy is a time of development of one or more offspring. This state is divided into three trimesters. Whole pregnancy normally takes 40 weeks after start of the last normal menstrual period. During this period woman`s body composition changes dramatically because of development of embryo and fetus in her womb. Precise description of pregnancy is described elsewhere. The major visual change of woman`s body is an increase of her weight. This is not only due to body fat deposition that occurs physiologically but also by increase of TBW and other body composition parts [68, 138]. The weight gain can differ in dependence on woman and pregnancy stage and is only partly related to the weight of the baby. The Institute of Medicine recommends an overall pregnancy weight gain for normal weight (BMI 18.5–24.9), of 11.3–15.9 kg having a singleton pregnancy. Women who are underweight (BMI of less than 18.5), should gain between 12.7–18 kg. Women who are overweight (BMI of 25–29.9) are advised to gain between 6.8–11.3 kg. Obese women (BMI>30) should gain between 5–9 kg [118]

The major contributors to the weight gain are products of maternity (fetus, placenta, and amniotic fluid), uterine and breast tissue, body water (intracellular and extracellular water), and maternal fat [67]. Although, the fetus, placenta and amniotic fluid represent the most important contributors, the plasma volume, which reaches the highest values in the second trimester of pregnancy, participates to the total body water increase during pregnancy as well [68, 138]. This can have influence on TBW and by tracking TBW progress pregnancy can be observed.

Bioimpedance with its good results of body composition estimation can play important role in pregnancy observation. Hence this method is very easy to use and can cause no harm to a pregnant woman whereas other methods such as CT, X-ray, DXA or ADP are not usable for body composition. Also some electric properties of a pregnant woman can be very crucial for birth of an infant.

Valensice et al. discussed in their paper that an evaluation of variations in TBW in each of the 3 trimesters of pregnancy can provide important data about the maternal physiologic adaptation to pregnancy [66].

Ghezzi et al. found that the reduction of the body resistance in the second trimester of pregnancy is independently associated with the birth weight. He also discussed that both the TBW and ECW are predictors of the birth weight only in the second trimester of pregnancy and not later in gestation. Hence this can mean that the second trimester of pregnancy influences the fetal and neonatal well-being [68].

Nonetheless, according to all these publications there should be done more research regarding woman`s pregnancy and body composition estimation through BIA method.

3.6. Electrical safety in bioimpedance measurements

The safety of a patient is a very important parameter in all kinds of measurements. Hence in bioimpedance is this problem much more important. With use of electrical current as a major measuring element patient`s overall and electrical safety must be granted. Basic national and international standards (IEC, UL, VDE, MDD (the European Medical Device Directive)) must be followed to reduce the risk of hazardous currents flowing through the patient under normal conditions. Even if there is a small possibility of a fault, condition of patient´s safety shall be secured.

If 2- and 3-electrode systems are used, it is usually possible to operate with current levels in the microampere range (μA). These levels of current correspond with applied voltages around 10 mV rms. These levels may be safe for most applications and direct cardiac applications [64].

With use of 4-electrode system, the measured voltage for a given current is smaller, and for a given signal-to-noise ratio, the current must be higher, often in the lower mA range. For measuring frequencies below 10 kHz, this is unacceptable for direct cardiac applications. Low frequency (LF) mA currents may also result in current perception by neuromuscular excitation in the skin or deeper tissue. Dependent on the current path, these LF current levels are not necessarily dangerous, but are unacceptable for routine applications all the same [64].

The perception of a current through human skin is dependent on frequency, current density, effective electrode area (EEA), skin site/condition and current duration is also a factor. Thus small currents and frequencies in kHz ranges are used for bioimpedance measurements.

The maximum sensitivity of our nervous system with the use of a sinusoidal wave is in the range 10–1000 Hz. At a lower frequency (< 10 Hz) there is a risk of non-reversible electrolytic effects. At frequency range from 1 kHz the sensitivity is strongly reduced and with continuous frequency increase – frequencies from 100 kHz no perception remains. Thus the frequencies levels are so high that electric stimulation is overcome by the heat effect of the current.

This means that devices used for bioimpedance measurements must have all certificates. In general, all bioimpedance devices should use current in μA and frequencies should not decrease less than 1 kHz.

3.7. Other possible applications of BIA in medicine

BIA has various possibilities of applications in biomedicine and medicine. Electric current that is used in BIA (maximally 800µA) is noninvasive and safe. There are many devices on the market today that use bioimpedance mainly for body composition. However, other applications of bioimpedance and BIA can be found:

- Measurements of TBW in woman during pregnancy [65, 66, 67]
- Observation of pregnancy and estimation of birth weight [68]
- Prediction of health status of the patients [69, 70, 71]
- Bioimpedance spectroscopy BIS [72, 73]
- Bioelectrical impedance vector analysis [74, 75, 76, 77]

It has been proved that bioimpedance is a very useful technique for estimation of body composition and patient health status. Also in case of obesity this approach can be very handy because it is able to discover precise body composition without use of invasive or ionization methods.

4. Design and realization of experiments

This chapter describes design and successive realization of experiments, in which theoretical assumptions were tested. Experimental part of dissertation work is divided into four parts. The first part is focused on changes of body composition parameters in dependence on external conditions, to discover whether various conditions have influence on measurement results.

The second part is dealing with the issue whether BIA method has comparable outcomes with golden standard DXA method, to discover whether the results from both methods are comparable. So the BIA method can be used when DXA is contraindicated.

In the third part there is evaluated problem of different comparable devices used for measurements of bioimpedance values. To specify whether different measuring devices can have the same results from measured parameters and if these devices are interchangeable.

Last part is focused on clinical application of BIA. BIA method was used in group of pregnant women for measuring and monitoring changes of body composition during gestation process.

Whole experimental part of dissertation work is divided into several sections that are following: influence of various conditions on measurements results, compare DXA to bioimpedance and evaluate results obtained from different devices and focus on application of BIA in clinical research.

The performed experiments have served for practical verification of proposed methodology described in chapter 5.

4.1. Changes of body composition parameters in dependence on external conditions

Based on theoretical information that are mentioned and described in previous chapters I concluded how the bioimpedance data should be analyzed and if external conditions such as temperature, humidity, hydration, etc. can have an influence on obtained data.

There exist wide varieties of external conditions that can affect obtained values of BIA method. Some of these conditions are discussed and standardized in NIHT protocol. However, it was never described in literature if currently easily accessible and often used moistures and lotions in European Union and Czech Republic (to cover conditions that can occur in various laboratories in Europe) for hand and feet treatment do have influence on measured body composition values obtained by bioimpedance method. All the specific creams and moistures were chosen after a discussion with doctors and other specialists regarding their experience. Selection was than increased by Indulona cream that is one of the most used creams in the Czech Republic and by an Avon cream from Poland.

In the first part of the thesis, I observed different external conditions (skin-to-electrode impedance) to discover if application of a specific moisture or cream will have an effect of changing bioimpedance and body composition values. The standard protocol by NIHT [127] mentions cleaning hands and feet with alcohol before bioimpedance measurements. But the question is if this procedure is really necessary and if the alcohol (that dries out the skin and changes normal conditions) cannot be excluded from the preparation procedure.

The manual of Tanita device does not include information that the patient’s hands and feet should be treated with special care before measurements. The only information in the manual is that the electrodes should be cleaned. Because of this fact the influence of different media between electrodes and hands should be discussed. These moistures with specific composition can increase or decrease the conductivity, sweat channel throughput and different values can be measured. Hence, the exact influence of specific environments should be described, and possible influences should be discussed.

For this first task I used a tetrapolar bioimpedance device Tanita MC 180 MA (Tanita Co., Ltd, Japan) that was stationed in the Gerstner Laboratory the Czech Technical Universityin Prague Karlovo namesti. Tanita is four an electrode multi-frequency device that allows to measure at four frequencies: 5 kHz, 50 kHz, 250 kHz and 500 kHz. The four electrodes approach can be used for measuring separated segments of the human body and in my case to avoid electrode polarization error [61]. Measurements were taken in standing position, each measurement was repeated twice and averaged for possible measurement error overcome.

In initial setup subjects' food intake was taken into account because in Deuberger et al. food influence was discussed [79]. Also temperature was considered because in some studies influence of temperature was discussed although Garby et al. [80] showed that a change of temperature from 24 to 35°C does not affect impedance values. Measurements were taken in standard condition with no pressure on electrodes to prevent a change in outcome values [78].

Twelve subjects in the age group of 25 to 62 years were included in the monitoring. The height was measured by stadiometer and weight was measured by build in scale in Tanita device. BMI was calculated and mean average value 22 ± 4.44 kg/m2was obtained. All individuals were people having average (no obesity or extreme slimness) values of biological parameters.

The patient was asked to step barefoot on a scale (feet electrodes) and hold hand electrodes. Hands were in vertical position along the body with no contact to body during measurement. Every measurement was performed twice and final value was averaged. Before every change of conditions scale was cleaned with alcohol towel and then dried with paper towel. Patient`s hands and feet were washed and dried with paper towel. After this cleaning process new conditions were applied.

For verification of proposed goals I designed a methodology of standard measurement conditions. The following experiment was a practical evaluation of this designed methodology.

All body composition parameters were extracted directly from the Tanita device by USB in RAW txt format without changes.

All individuals were measured in the following conditions: 1. normal conditions (application of no environmental change, patient state in time of arrival), 2. application of water (normal water used from laboratory water supply), 3. solution saline (Baxter Czech, NaCl 0,9%, pH=5,5), 4. EEG gel (Eci electro-gel, Electro-Cap International, Inc., USA), 5.hand cream with oil (Indulona Universal, Zentiva, k.s., Hlohovec, Slovenská republika) hand application, 6. oil free hand cream (Nivea Creme, Biersdorf, Germany) hand application, 7. hand cream with oil hand and foot application, 8. oil free hand cream hand and foot application, 9. oil free hand cream and water based foot cream (Foot works active cooling gel, Avon Co. Poland). To overcome measurements errors each measurement was doubled and average values were used.

For better description all conditions were put into table number 1.

Measurement number	Condition
1	normal conditions
2	application of water
3	solution saline
4	EEG gel
5	hand cream with oil hand application
6	oil free hand cream hand application
7	hand cream with oil hand and foot application
8	oil free hand cream hand and foot application
9	oil free hand cream and water based foot cream

Table 1: Specification of conditions

I was observing majority of values that are measured and obtained by Tanita device. These were TBW %, body fat %, Fat free mass, muscle mass, fat mas (kg) and impedance (real part - resistance) exact description of all followed parameters in in next chapter.

4.1.1. Result and discussion of external conditions changes

All measurements were compared with first experimental setup (hand and feet were cleaned and dried with paper towel).

Obtained basic parameters for specific body composition are displayed in table 2:

Parameter	Average	St. dv.
Age	30.48	10.11
Height	176.10	6.72
Weight	71.07	15.16
BMI	22.00	4.44
TBW%	58.58	7.76
Body fat %	18.56	9.96
Muscle mass	51.86	14.42
Fat free mass	56.98	11.27
Impedance 5 kHz	711.59	126.96
Impedance 50 kHz	629.06	126.03
Impedance 250 kHz	564.50	117.35
Impedance 500 kHz	540.85	106.05

Table 2: Basic parameters of observed group

Changes of important body composition parameters: TBW, body fat %, muscle mass, fat free mass are shown on figures 31, 32, 33 and 34. All the data were displayed in graphs to show trend of changes.

Statistical analysis that was performed across whole experimental setup for important obesity marker body fat % and important hydration marker TBW % showed that there was statistically (P=0.05) significant change only for measurement number nine (body fat % and TBW % change) and for measurement number three (only TBW % change). These setups correspond to conditions of oil free hand cream and water based foot cream for measurement - number nine and solution saline for measurements condition number three. Only these two setups had statistically significant difference from standard setup. Other measurement setups had no statistically significant (P=0.05) change from starting conditions. This was rather unexpected result. Hence statistically significant change was expected in highly conductive EEG gel. However this expectation was not proved.

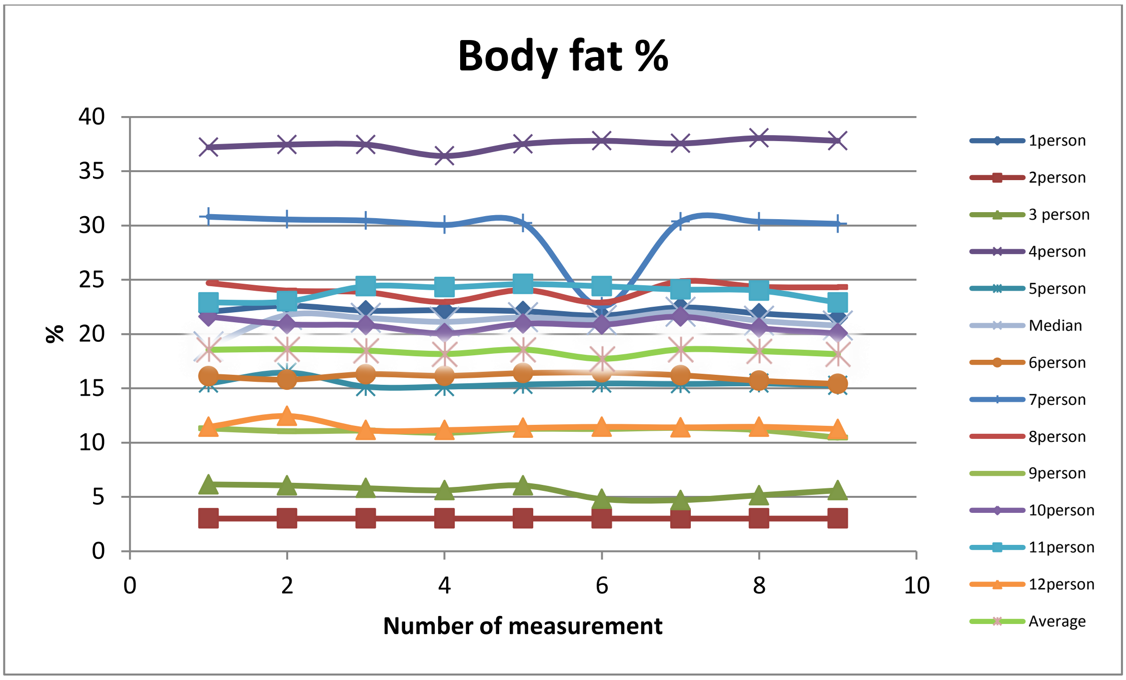


Figure 31: Changes of body fat in all twelve subjects in dependence on conditions

Figure 31 illustrates changes of body fat % in dependence on conditions. Measurements of fat% can be divided into three groups according to percentage (fat under 15% fat % from 15% to 25% and fat over 25%). Change was recorded only in condition number nine. However, in results of person number seven was reported ~9% decrease in body fat % in condition number six (oil free hand cream hand application). This significant decrease is pointing out that the oil free cream hand application can in some patients result in increase of conductivity. This can lead to biased results in body fat % that stands for non-conductive tissue in body.

Nevertheless, this change was reported only in one case. Hence overall results were not influenced by this case and no statistically significant change was reported for measurement number six.

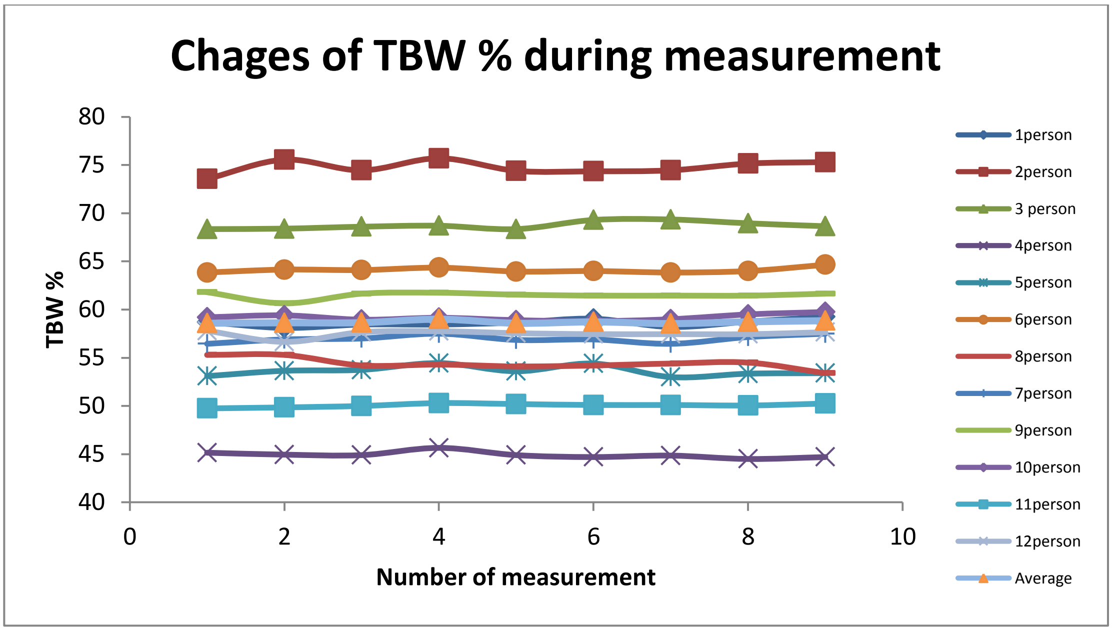


Figure 32: Changes of total body fat in all twelve subjects in dependence on conditions

Figure 32 illustrates changes of TBW % in dependence on conditions of measurement. Measurements showed normal distribution of TBW % in my tested sample (normal values Female: 45–60%, Male: 50–65% based on Tanita internal Research). Only in one case TBW % value was higher than optimal range. In this case average TBW % of a subject was 74.76 % this can be due to person`s low percentage of fat and high percentage of lean mass that is mainly composed from water and protein. Significant changes were proved in change of condition number nine and three.

Change of conditions in measurement number nine was also statistically significant for ICW and fat mass kg value.

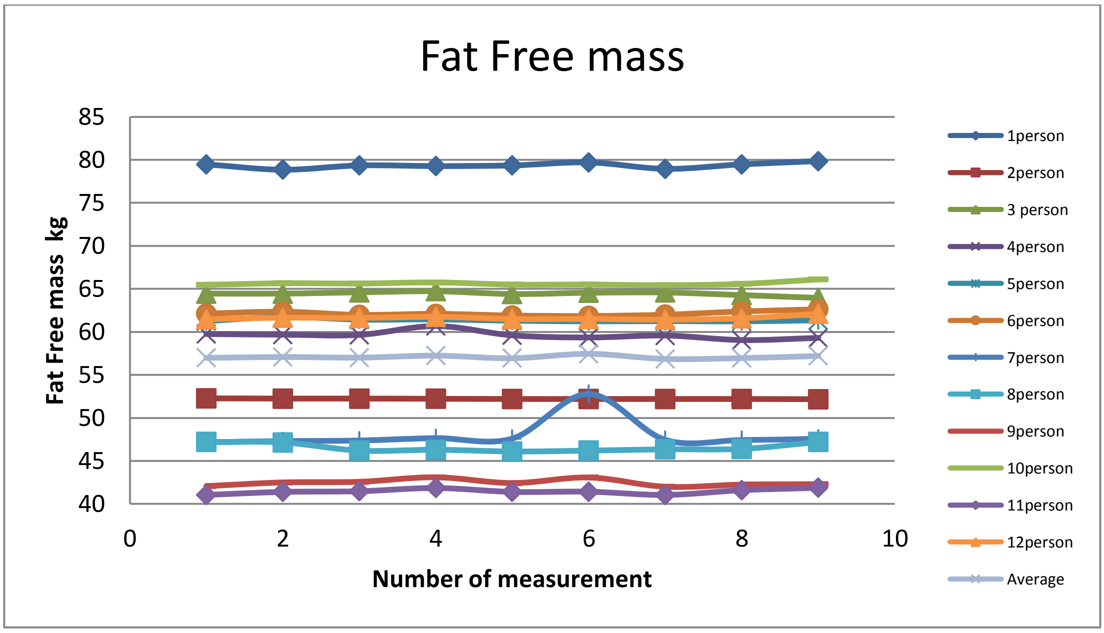


Figure 33: Changes of fat free mass in all twelve subjects in dependence on conditions

Figure 33 shows differences of fat free mass kg that were obtained during changes of conditions. I discovered that application of moistures and creams resulted in no statistical significant change (P=0.05) for fat free mass kg parameter. Overall changes of fat free mass kg were so small that they did not exceeded one kg.

However, in case of patient number seven and condition number six there was reported increase in fat free mass ~10kg.

This change is directly connected with previously mentioned decrease in fat %. Because with decrease in fat there should be increase in fat free mass. The same change was also reported in muscle mass kg shown in Figure 34.

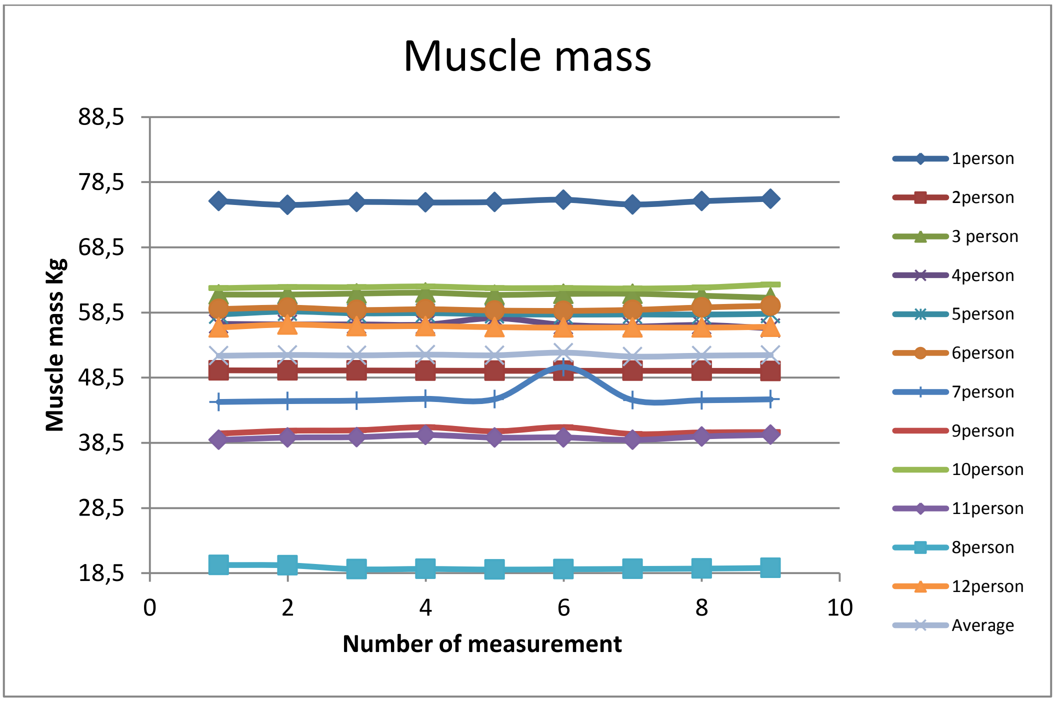


Figure 34: Changes of muscle mass in all twelve subjects in dependence on conditions

Figure 34 shows changes of fat free mass during measurement environment changes. For muscle mass body composition value no statistical significant change was discovered. Same results were observed for ECW body composition value.

In global, statistically significant changes (P=0.05) for body composition data that are not directly measured were observed maximally in two conditions (TBW %, body fat %, ICW and fat mass). These conditions were number four and nine, although more statistically significant changes were expected across condition changes.

In second part of this measurement direct measured values were considered. This value is a representation of pure resistance of body composition part. Real part of impedance – resistance was observed on all four frequencies.

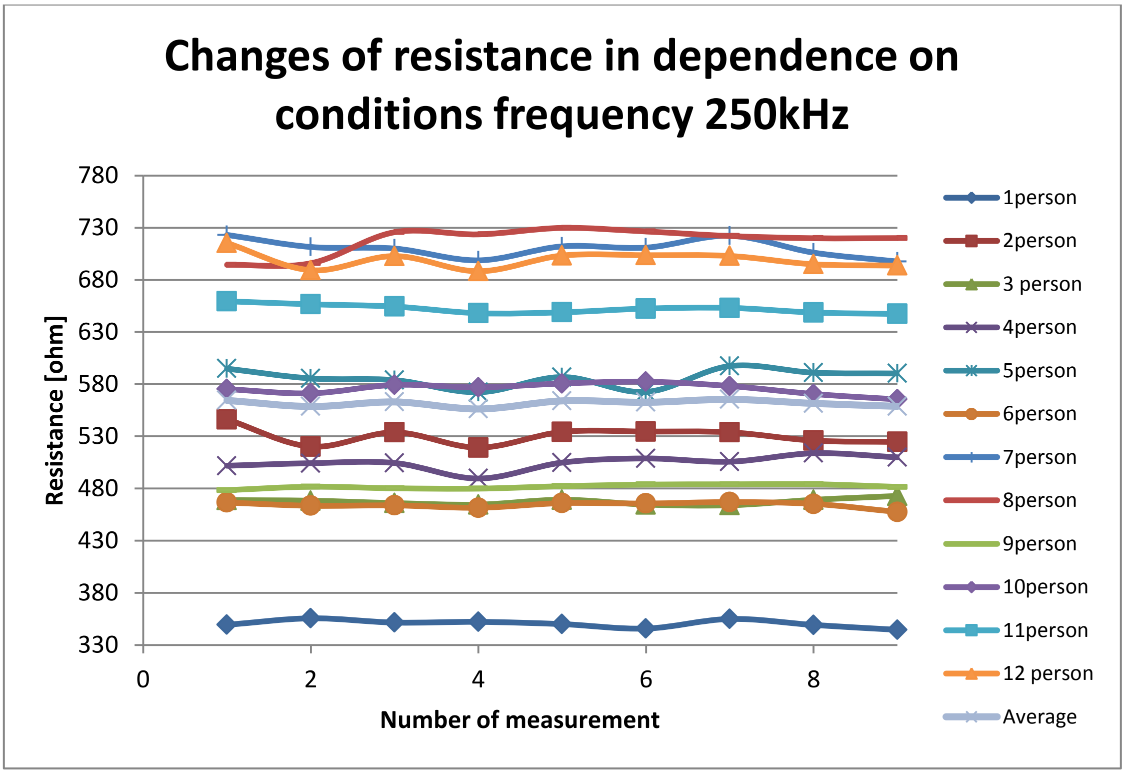


Figure 35: Changes of resistance (250 kHz) in all twelve subjects in dependence on conditions during pregnancy

Figure 35 shows changes of resistance measured on 250 kHz for all twelve subjects (trends are represented for better description). The frequency of 250 kHz was chosen to be displayed because it had best correlation with calculated/obtained body composition parameters. Results from other frequencies are described in text with no graph.

Frequency 5 kHz and 50 kHz showed statistical significant changes only in measurement number four (EEG gel) which differed from indirect body composition values (TBW %, ICW, body fat %). Whereas in frequency 250 kHz changes showed in measurement number two which was observed for no other reactance or indirect body composition value. For frequency 500 kHz were discovered no statistically significant changes at all.

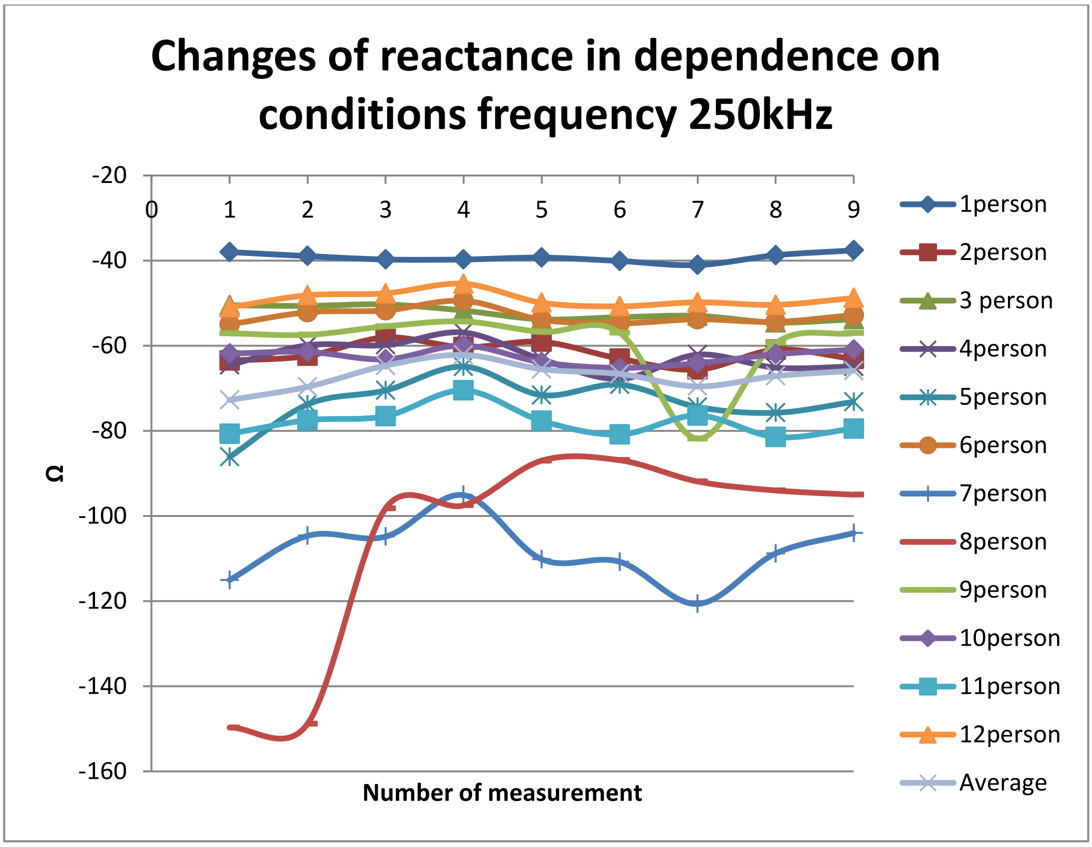


Figure 36: Changes of reactance (250 kHz) in all twelve subjects in dependence on conditions during pregnancy

Figure 36 shows changes of imaginary part of an impedance – reactance. Although changes in some subjects were notable (changes are well seen on a figure 36) - subject seven (~13% biggest difference in measurement number four) and subject number eight (~37.5% biggest difference in measurement number six) for frequencies 5 kHz and 50 kHz no statistical significant (P=0.05) changes were proved.

Frequencies 250 kHz and 500 kHz showed statistically significant (P=0.05) changes for condition number two (application of water) and condition number four (EEG gel). Other conditions had no statistically significant change.

The first part of section 4.1 shows that there were significant changes only for few condition changes. Most changes were observed for condition number two and four for directly measured values and number nine for obtained/calculated values. Which were water – condition two, EEG gel - condition four and application of oil free hand cream and water based foot cream – condition nine. All these three conditions were characterized by increase of conductance.

Nevertheless, it is interesting and not expected that condition number two – water had statistically significant change and condition number three – solution saline didn`t. This could be because with application of water that contains ions are dissociated ions that were on palms and conductivity rises. Thus interface palm – electrode changes and outcome results differs. However, it is unexpected that with applications of solution saline (0.90% w/v of NaCl, about 300 mOsm/L or 9.0 g per liter) that should have more Na and Cl ions than water showed no statistically significant change. This can be due to composition of water that contains more dissociated ions (Ca^{2+}, Mg^{2+}, Fe^{3+}, NH^{4+}, NO_{2-} a NO_{3-}) than solution saline.

Increase of values for EEG gel application was expected and was proved for directly measured values. However, 500 kHz resistance, 5 kHz and 50 kHz reactance showed no change. This is an unexpected outcome. Hence all frequencies should register this increase of conductivity and changes should be significant.

Moreover, changes of conditions that were significant for directly measured values were not statistically significant for calculated/obtained values. Although it was expected that the conditions with statistical significance would be in all cases the same.

4.1.2. Conclusion - influence of different conditions on measured values

The experiment showed that direct and calculated body composition parameters obtained from bioimpedance (BIA) can be biased by application of various moistures and lotions. This fact occurred only in case of application of highly conductive gels and moistures. Oily creams and moistures did not statistically changed outcome values for direct and calculated/obtained values.

The standard conditions that are considered for patient status (hydration, temperature or feasting) [119,120,121] should be followed. No statistically significant changes were observed in range of small frequencies (5 – 50 kHz). This concludes that no special treatment of contact areas should be needed. In a case that the creams or lotions were not applied exactly before measurement and were not highly conductive this conclusion can be done for the whole used frequency range. Although at NIHT 1996 [127] was recommended to prepare places of electrodes application with alcohol. Nevertheless, my experiment concluded in this study didn`t approved that places that are in contact with electrodes should be treated with alcohol otherwise every measurement would be biased. This leads to conclusion that cleaning with an alcohol may be not necessary in a case that the patient has not visually wet or sweaty hands (presence of EEG gel on subject`s palms is highly improbable).

However, to obtain always the same objective results of body composition values it is recommended to comply standardized protocol of measurements. Because only by complying with standard measuring conditions there can be achieved the same results within various clinical centers.

4.2. Comparison of body composition evaluation approaches DXA and bioimpedance - BIA

In this part of the thesis I considered precision of BIA method to prove whether this method can be compared with golden standard in body composition evaluation which is DXA. In this approach was considered only body composition values that are calculated/obtained from BIA method. Hence resistance and reactance are not comparable with DXA because there is no such outcome.

All the bioimpedance body composition values were measured and obtained using a four-electrode device InBody 720 (Biospace Co., Ltd., Korea). With measuring range of frequencies 1 kHz, 5 kHz, 50 kHz, 500 kHz and 1 MHz. BIA measurement was performed under standard conditions – in the morning on an empty stomach after defecation and urination. The temperature in the room where the measurement was performed was 22°C and relative air humidity was constant.

The outcome of measurement was a protocol containing the amount of intracellular water – ICW in liters, extracellular water – ECW in liters, total body water - TBW in liters, lean body mass - LBM in kilograms, the amount of body fat (BF in kg and %), BMI value (kg/m2), the amount of visceral fat (VF in cm^2) and bio-impedance values at frequencies of 1, 5, 50, 250, 500 kHz and 1 MHz for the right and left arm, for the right and left leg and for the trunk.

The second used device for measuring the body composition in my comparative study was DXA 7682 device. This device uses the principle of dual-energy X-ray absorptiometry. The measurements were performed at the clinic of the University Hospital in Hradec Králové at the "Osteocentrum" Department. The measurement was performed under standard conditions. The result of the measurement was a protocol containing the values for total body fat in %, the total amount of body fat in kilograms, the total amount of lean body mass in kilograms. A group of 75 persons with the average age of 35.3 ± 11.1 and with the average BMI of 25.5 ± 3.1 kg/m^2 participated in my experiment. The above mentioned group involved clinically healthy persons without any chronic disease, or acute clinical disease.

For further statistical analysis the basic group was divided into a group of women, which included 50 females with the average age of 39.4 ± 10.8, average BMI of 27.8 ± 3.6 kg/m^2, and a group of men, which included 25 males with the average age of 40.5 ± 12.4, average BMI of 26.6 ± 1.4 kg/m^2.

For next statistical assessment the basic group was then divided according to the age categories into as follows:

a) group below the age of 30 (21 persons)

b) group at the age of 30-40 (36 persons)

c) group over the age of 40 (18 persons)

The aim of the above mentioned division was to monitor changes in body composition depending on the age of observed subjects.

The bio-impedance measurement was performed in the morning on an empty stomach after urination. Before the actual bioimpedance measurement the body weight in kilograms and body height in meters was determined by anthropometric procedure. The value of BMI was automatically calculated and recorded by the device from the measured values.

Each bio-impedance measurement was performed twice; the average value was calculated and used for statistical assessment. The outcome of bio-impedance measurement was a protocol including basic characteristics of body composition and data extracted directly from the device via RS-232 port.

After BIA method measurement, the measurement of body composition was performed using DXA method within half an hour. The observed subjects were without any physical or psychic load during the period between the measurements. They were given no food or liquids, and they did not urinate during this period. The conditions in the rooms for measurements were equal.

4.2.1. Results and discussion of BIA and DXA comparison

Outcome values that were measured during the body composition assessment using DXA and BIA methods are shown in the following tables.

All monitored continuous quantities have approximately normal distribution, thus they are presented by the average and standard deviation. In pairs of monitored quantities the correlation coefficient r, its significance p and the coefficient of determination r^2 in % (it shows what percentage of variability in "dependent quantity" can be explained by the linearity of the relationship between "dependent" and "independent" quantity) were calculated using correlative analysis.

Tables 3-11 show outcomes of correlations which were established using DXA and BIA methods. Tables 3-5 show outcomes of correlations of body fat (adipose tissue) in %. Tables 6-8 shows outcomes of correlations of adipose tissue in kg and the Tables 9-11 shows the amount of fat free mass (lean body mass)in kg in the group as a whole and in the group of men and women.

Outcomes of correlations of body fat (adipose tissue) % - Tab. 3-5

Quantity	Average	Minimum	Maximum	SD
BF BIA (%)	35.7	18.2	48.6	7.6
BF DXA (%)	36.3	21.7	46.2	6.7
r	0.9452			
p	p<0.001			
r^2 (%)	89			

Table 3: Group as a whole, 75 persons – basic data

Quantity	Average	Minimum	Maximum	SD
BF BIA (%)	39.3	32.8	48.6	5.1
BF DXA (%)	39.7	33.3	46.2	4.2
r	0.9146			
p	p<0.001			
r^2 (%)	84			

Table 4: Group of women – basic data

Quantity	Average	Minimum	Maximum	SD
BF BIA (%)	26.7	18.2	31.7	5.1
BF DXA (%)	27.6	20.7	31.0	2.9
r	0.7272			
p	0.101			
r^2 (%)	53			

Table 5: Group of men – basic data

Outcomes of correlations of adipose tissue in kg – Tab. 6-8

Tab. 4 Group as a whole, 75 persons – basic data

Quantity	Average	Minimum	Maximum	SD
BF BIA (kg)	30.6	15.0	50.0	7.9
BF DXA (kg)	30.9	17.6	45.2	6.8
r	0.9588			
p	p<0.001			
r^2 (%)	92			

Table 6: Group as a whole, 75 persons – basic data

Quantity	Average	Minimum	Maximum	SD
BF BIA (kg)	32.1	23.7	48.0	7.8
BF DXA (kg)	32.3	23.9	45.2	6.8
r	0.9765			
p	p<0.001			
r^2 (%)	95			

Table 7: Group of women – basic data

Quantity	Average	Minimum	Maximum	SD
BF BIA (kg)	26.9	15.0	32.9	7.4
BF DXA (kg)	27.6	17.6	34.2	6.0
r	0.8820			
p	0.020			
r^2 (%)	78			

Table 8: Group of men – basic data

Outcomes of the amount of lean body mass – Tab. 9-11

Quantity	Average	Minimum	Maximum	SD
LBM BIA (kg)	55.6	40.4	84.1	12.4
LBM DXA (kg)	55.0	40.2	82.0	12.2
r	0.9820			
p	$p<0.001$			
r^2 (%)	96			

Table 9: Group as a whole, 75 persons – basic data

Quantity	Average	Minimum	Maximum	SD
LBM BIA (kg)	48.8	40.0	56.3	5.2
LBM DXA (kg)	48.4	40.2	58.7	5.8
r	0.9405			
p	$p<0.001$			
r^2 (%)	88			

Table 10: Group of women – basic data

Quantity	Average	Minimum	Maximum	SD
LBM BIA (kg)	72.4	63.2	87.0	8.1
LBM DXA (kg)	71.2	62.3	82.0	7.6
r	0.9164			
p	0.010			
r^2 (%)	84			

Table 11: Group of men – basic data

To determine the connection between the classification of obesity using BMI and the amount of body fat in % or kg and the fat free mass (lean body mass) in kg, the Phi coefficient was chosen and its significance was stated similarly as in the correlation coefficient.

A statistically significant dependence of BMI and body fat in % or kg was proved for both used methods – DXA and BIA (Tab. 12 and 13). Simultaneously, a statistically significant positive correlation between BMI and adipose tissue was proved using BIA method. Similar significant correlations were proved in the group of men and in the group of women.

No statistically significant dependence of BMI and the amount of body fat in % and kg was proved in both used methods in connection with the age in monitored persons.

BMI	BF (%)	BF (kg)	LBM
Phi	0.5223	0.8744	0.0942
P	0.015	$p<0.001$	0.685
r^2 (%)	27	76	1

Table 12: BMI correlations in DXA method – group as a whole, LBM – lean body mass

BMI	BF (%)	BF (kg)	LBM	VF (cm^2)
Phi	0.6111	0.8760	-0.0009	0.7302
P	0.003	$p<0.001$	0.997	0.001
r^2 (%)	37	77	0	53

Table 13: BMI correlations in BIA method – group as a whole, LBM – lean body mass, VF – visceral fat

The next part of results was focused on solving the relationship between different measurement methods. Based on experimentally obtained results assumptions and equations representing the relationship between DXA and BIA method were proposed.

Linear regression was used for calculation of s

This concludes that results of body composition measurement obtained by DXA and BIA are identical no matter which method I use (DXA or BIA).

Based on the performed analysis I propose the equations presented in Table 14. These equations of relationship between the outcomes of BF in %, BF in kg and lean body mass in kg determined by using DXA and BIA methods were calculated and tested to obtain desired relations. The equations are based on application of linear regression.

% BF INB = -3.09102 + 1.06955 * % BF DXA
kg BF INB = -3.69403 + 1.10873 * kg BF DXA
kg LBM INB = 0.593886 + 1.000284 * kg LBM DXA

Table 14: The equations of linear regression

The linear regression was also used to determine the dependence of BMI on the percentage of body fat, on the amount of body fat in kg and lean body mass in kg measured by using DXA and BIA methods**.** The proposed outcome equations are shown in Tables 15 and 16.

BMI = 20.57855 + 0.24620 * % BF DXA
BMI = 16.86447 + 0.40869 * kg BF DXA
BMI = 28.15766 + 0.02468 * kg LBM DXA

Table 15: Dependence of BMI on the percentage of body fat, on the amount of body fat in kg and lean body mass in kg measured by using DXA

BMI = 20.41847 + 0.25458 * % BF BIA
BMI = 18.67096 + 0.35408 * kg BF BIA
BMI = 29.52784 - 0.00024 * kg LBM BIA

Table 16: Dependence of BMI on the percentage of body fat, on the amount of body fat in kg and lean body mass in kg measured by using BIA

After discussion with obesity specialists a %BF model was considered. This new approach included a variable to reflect the difference in male and female body constitution. Because of this gender composition specification was added into the %BF model. By adding the gender variable an increase in accuracy of the obtained model was observed as well. The new and more complicated model included gender of a patient, weight and height of a patient. Final representation of this model is proposed as

$$\boxed{\%\mathrm{BF} = -21{,}91 + \left(1{,}569 * \frac{weight}{height^2}\right) + (10{,}62 * \mathrm{gender})}$$

where weight is in kg, height is in meters, gender is 0 for male, 1 for female

The new model has SEE of 3.6 %BF and $R^2 = 0.7688$. This model was evaluated in a group of 39 persons with various body composition types and values. Validation of this model showed a very good estimation for body fat in males and females at all ages.

It was also observed that even persons with low body fat percentage (from 18%) were evaluated correctly. However, in athletic subjects the prediction formulas overestimated results of %BF. Nevertheless, it was only in subjects that had body fat under 16.5 % which is athletic or fitness range in male population and have to be processed with caution.

A comparison with the equation [142] was done. Results obtained from my new equation had better estimation of %BF in 76.9 % of results than values obtained by Deurenberger [142].

A statistical assessment and a correlation analysis between individual values of body impedance have been performed, which were measured at the frequencies of 1, 5, 50, 250, 500 kHz and 1 MHz in individual body parts – the right arm, the left arm, the right leg, the left leg, trunk), and individual items of body composition – body fat in kg and %, lean body mass in kg, the amount of visceral fat in cm^2, TBW, ICW and ECW in liters. No statistical significance was proved in any item on any used frequencies and in any measured body parts.

A high statistical significance was proved in the percentage of body fat –$p<0.01$ at the frequencies of 5 and 250 kHz, and in fat mass (adipose tissue) kg at the frequency of 5 kHz. A statistically significant negative correlation was proved in the values of TBW, ICW and ECW at the frequencies of 1, 5, 50, 250 and 500 kHz.

It can be concluded from my data evaluation the best frequency for measuring body fat % or kg is frequency of 5 kHz. The best frequency for measuring TBW, ICW and ECW is also 5 kHz. However, to obtain TBW and ECW separately, we have to use two frequencies: low frequency of 5 kHz for ECW and high frequency of 500 kHz for TBW.

Considering the discrepancy of measured outcomes which followed from the correlation analysis, it is impossible to recommend a single frequency of measurement that is suitable for measuring the body impedance.

A similar conclusion can be also estimated for measuring in individual body parts. The outcomes of performed extensive statistical analysis lead to a conclusion – it is necessary to use a multi-frequency measurement with multi-electrode array to measure the body impedance and to assess subsequently the body composition, which means the proportion of individual body tissues (BF, lean body mass, TBW, ECW, and ICW). The use of multi-frequency and multi-electrode device provides a precondition for decreasing the measurement error.

A statistically significant correlation in BF in kg and in % was proved in the body composition measurement in the group as a whole and in the group of women. The same situation was observed in both the groups in assessment of LBM in kg.

No correlation in BF in % and in kg was registered between the outcomes determined by using DXA and BIA methods in body composition in men. A significant correlation concerning the significance level of 0.01 % was registered in LBM in all the groups.

Established differences in statistical significance between men and women can be probably caused by sex differences. An important role also plays a lower number of monitored men. Lots of authors point out two possible impacts of higher physical load in men on promoting the blood circulation in muscle mass as a possible cause influencing the body composition measurement by using the method of body impedance measurement [144].

The current body weight significantly influences the health state of a particular person. To determine the total risk of health impairment it is necessary to perform not only the assessment of current body weight, but also the assessment of body composition aimed at individual body tissues.

Lots of methods are used to assess the body composition. In the comparative study the outcomes of body composition measurements determined by using BIA method were compared with the outcomes of body composition measurements determined by using DXA method.

A statistically significant positive correlation was proved at the significance level of $p<0.01$. The correlation coefficient reached the following values: in body fat in % - 0.9452, in adipose tissue kg - 0.9588 and in lean body mass the value of 0.9820. In assessing the amount of adipose tissue by using BIA method a lower amount of adipose tissue was proved in comparison with DXA method – 35.7 % versus 35.3 %, 30.6 kg versus 30.9 kg. Other authors also show analogous outcomes in the literature. [130, 131]. Lots of authors show an opposite trend in persons with obesity – BMI >30 kg/m^2.

They establish a higher amount of body fat using BIA method in comparison with the amount of body fat determined using DXA method. The above mentioned situation is justified by possible technical difficulties concerning DXA method (if the body volume is higher – BMI over 30 kg/ m^2 , the body of the examined person can be out of the scanned field, simultaneously it is necessary to realize that it comes to increasing of photon absorption with increasing of fat layer thickness) [145,146]. The value of average BMI in monitored persons was lower than 30 kg/ m^2 in the assessed comparative study. The above mentioned situation was not observed.

4.2.2. Conclusions of BIA and DXA comparison and estimation of new BF % equation

The relationship between DXA and BIA approaches calculated by using the linear regression proved in the study that measuring the amount of BF in % and in kg and the amount of lean body mass in kg is the same in both of the measurement approaches.

Proving the relevance of outcomes leads to conclusion that it is possible to use the approach of body impedance measurement in practice on a larger scale, and that both approaches can be interchanged. The advantage of using the bioimpedance (BIA) approach is a significantly smaller load in the monitored person, from the mobility of examination even outside the clinical workplace which is specially adapted and at least but not last from a significantly lower purchase cost of the equipment for measuring the bio-impedance in comparison with the equipment for measuring the DXA.

A lower price for one examination of a particular person also plays its role. Some authors proved not only the relationship between BIA and DXA methods, but they also take into account the possibility of measuring the adipose tissue using calliperation. [147, 148].

The measurement of body impedance enables to calculate not only the amount of body fat in % and kg, the amount of lean body mass, but also the amount of TBW**,** ICW and ECW**.**

A new linear model for estimation of body fat % from measurable anthropometric values was proposed. This model takes into consideration easily accessible values and gives specialists another tool for estimation of subject`s body composition. This helps the doctors directly compare body fat percentage with the guidelines and obtain more detailed information on health status.

The new approach was able to provide valid estimation for %BF of 17% and higher. For lower %BF ranges (athletic and fitness) overestimated results were reported. However, these ranges require special models that will take into consideration the special composition of these subjects. This will be a point of interest in continuation of my studies.

Results from the new model were compared with results obtained by Deurenberg. It was reported that results obtained by the new model were more accurate in 76.9 % than results obtained by Deurenberg. This leads to the conclusion that the new model has better results in the Czech population than Deurenberg and should be used for direct estimation of %BF from measured anthropometric values.

4.3. Changes of body composition and bioimpedance during pregnancy

Pregnancy is one of the most specific time period in women`s life. During this period body composition of a woman should dramatically change and these changes of specific body composition can conclude specific results.

In the fourth part of the experimental section of the thesis changes of body composition values and bioimpedance values were observed in pregnant women during gestation. All the values were measured by Tanita MC 180 MA device that was stationed at the University Hospital in Brno at the Clinic of Obstetrics and Gynecology.

Every subject had been previously attending medical consultation at University Hospital in Brno. Before the beginning of measurements every woman was medically examined by an obstetrician and a specialist to be included into the study. An informed consent had to be also signed to participate in the study.

Initially 49 women were included in my study. Nevertheless, thirty women were excluded from final evaluation because of only one measurement during whole gestation. Other nine women were excluded because of no regular measurements.

From the final count seven women were measured in all three trimester and three were measured in the second and third trimester. The measured women were at the age of 33.6 ± 3.69 and had BMI of 28.71 ± 8.21 kg/m^2.

Data were recorded directly from the device by an enclosed program in RAW format. USB connector and standard USB 1,5m cable was used for data transfer. Electrodes were cleaned by alcohol towel after every measurement to overcome possibility of measurement error. Measurements were done at hospital`s office by educated nurse. Every woman read and agreed with informed consent. All the measurements were done at the time of subject`s regular medical examination during pregnancy.

The measurement was taken in standing position with hands near the body holding the electrodes and after every measurement the electrodes were cleaned and patient`s hands were cleaned by alcohol towel as recommended in NIHT guidelines [127]. Although previous measurements presented in this dissertation work didn`t prove overall bias of measured values. All the measurements were taken twice and for the final evaluation average values were used.

4.3.1. Results and discussion of body composition changes during pregnancy

Results are divided into two parts. In the first part there is described difference between the first and third trimester and in the second part there are described differences between second and third trimester.

From the calculated/obtained values were monitored: Weight (kg), Body fat %, Fat mass (kg), Fat free mass (kg), Muscle mass (kg), TBW (kg), TBW%, Intracellular water (kg), Extracellular water (kg), ECW % to get the whole description of body composition.

From directly measured (without calculation) values were monitored: Reactance and Resistance (5 kHz, 50 kHz, 250 kHz and 500 kHz). All the obtained values are considered for the whole body observation.

In the first set of Tables 17-20 there are described calculated/obtained values in the first and third trimester. All parameters are represented by average values with SD. Tables 17 and 18 describe differences between the first and third trimester. For better understanding in Table 19 differences of body composition in absolute values (increase of body weight, body fat and TBW) are included. Table 20 shows relative differences in percentage between the first and the third trimester.

First trimester	Average	SD
Weight kg	75.53	17.10
Body fat %	35.11	4.51
Body fat kg	26.80	11.32
TBW kg	33.76	8.39
TBW %	47.59	5.93

Table 17: The selected calculated/obtained values first trimester measurement

Third trimester	Average	SD
Weight kg	86.41	17.16
Body fat %	34.23	5.66
Body fat kg	30.14	9.95
TBW kg	40.39	5.69
TBW %	47.34	4.00

Table 18: The selected calculated/obtained values third trimester measurement

Difference Table	Difference
Weight kg	10.88
Body fat %	-0.89
Body fat kg	3.34
TBW kg	6.63
TBW %	-0.25

Table 19: The selected calculated/obtained differences between the first and third trimester

Difference Table	Percentage
Weight kg	14.41%
Body fat %	-2.52%
Body fat kg	12.46%
TBW kg	19.63%
TBW %	-0.53%

Table 20: The selected calculated/obtained differences between the first and third trimester in percentage

Values from the first and the third trimester from seven subjects that were measured were compared and a statistically significant (P= 0.05) change was found. The change was reported for all subjects between the first trimester and the third trimester. Changes were reported in these calculated/obtained values: Weight (kg), Fat mass (kg), Fat free mass (kg), Muscle mass (kg), TBW (kg), Intracellular water (kg), Extracellular water (kg). On the contrary no statistical significance was found for values that are recorded in percentage: Body fat %, TBW%, ECW %.

This can have very important consequences because the percentage of TBW and body fat are one of the most important parameters to determine the patient`s health state. Other result was that both the constituents of total body water (ECW and ICW) changed.

Another finding was that ICW kg average difference (2.98 kg) was higher than ECW (2.3 kg) which I would expect vice versa. Hence it would be expected a bigger increase in ECW. This could be because of amniotic fluid. The amount of this liquid changes during gestation and can reach up to 2000 ml [132]. This would point to an increase in ECW rather than to an increase in ICW.

There was reported statistically significant increase in body weight parameter. The average increase in weight was 10.88 kg which was for selected group of women with average BMI 28.71 ± 8.21 (overweight in terms of BMI evaluation) at the upper border of recommended weight gain. Recommended weight gain is for this group 7-11.5 kg [128] during pregnancy although in some studies was observed increase of 15 kg [125].

For the second set of observations in the first experimental setup directly measured values were monitored. Resistance values are shown in Table 21 and 22. The obtained resistance values are represented by average values and standard deviation. Differences of reactance between trimesters are then shown in Table 23.

In observed values there were monitored and discovered statistically significant changes. In all measured parameters (resistance and reactance at all frequencies) a statistically significant decrease (P=0.05) was reported, although only a decrease in resistance was expected. Hence, reactance stands for capacitive properties of a body (cell membranes) and due to an increase in ICW, an increase in reactance was expected as well.

First trimester	Average	SD.
Resistance 5 kHz	725.03	90.02
Resistance 50 kHz	645.42	80.65
Resistance 250 kHz	576.60	69.64
Resistance 500 kHz	560.47	66.37

Table 21: The selected directly measured values for the first trimester

Third trimester	Average	SD.
Resistance 5 kHz	597.93	70.85
Resistance 50 kHz	535.91	61.66
Resistance 250 kHz	481.69	53.95
Resistance 500 kHz	469.97	52.28

Table 22: The selected directly measured values for the third trimester

Difference table	Difference (Ω)
Resistance 5 kHz	127.1
Resistance 50 kHz	109.5
Resistance 250 kHz	94.91
Resistance 500 kHz	90.50

Table 23: Difference between the first and third trimester resistances

Decrease in both impedance constituents can indicate that the decrease of impedance in general is more dependent on both the values rather than only on resistance values. Hence, an expected decrease of resistance should manifest more. Overall average decrease in resistance values across all frequencies was 16.78 ± 0.06 % while reactance decrease was 26.96 ± 1.45 %. This indicates that there should be discrepancy in cell mass increase.

After comparison of the calculated/obtained body composition values with directly measured reactance and resistances different trends were found. Whereas the calculated/obtained body composition values from the first to third trimester (excluding values that are in percentage) increased, directly measured values decreased and the decrease was in general greater than in the calculated/obtained values.

In general, an increase in all body composition parameters during gestation indicates that gestation was physiological. Hence, during pregnancy body fluids and mother`s body constitution changes dramatically.

Increase in TBW kg was expected because of amniotic fluid. Increase in weight is mainly composed of fetus (3500 g human fetus contains about 2500 ml of water, 350 ml of which are in the vascular compartment, 1000 ml in the intracellular space, and the remainder in the extracellular space [133]) and body fat of a women. Hence, the woman`s body is preparing for breastfeeding of an infant. Overall increase in body fat kg of the observed group was 3.34 kg which corresponds with other studies [126].

In the second observations there were monitored changes between the second and the third trimester in the same calculated/obtained values as in the first part of the experimental setup. For this observation three more women were included, which meant 10 women all together. The observed values were the same as in the first measurement setup. As calculated/obtained values were observed: Weight (kg), Body fat %, Fat mass (kg), Fat free mass (kg), Muscle mass (kg), TBW (kg), TBW%, Intracellular water (kg), Extracellular water (kg), ECW %. Chosen parameters are shown in Tables 24 and 25.

Second trimester	Average	SD
Weight kg	73.87	15.99
Body fat %	32.26	8.10
Body fat kg	24.61	10.59
TBW kg	35.10	4.36
TBW %	49.08	6.42

Table 24: The selected calculated/obtained values first trimester

Third trimester	Average	SD
Weight kg	82.30	15.58
Body fat %	33.10	5.15
Body fat kg	27.77	9.03
TBW kg	39.23	4.99
TBW %	48.34	4.09

Table 25: The selected calculated/obtained values third trimester

After the final evaluation the differences nearly in all monitored parameters were found. The Second observation showed again differences between the second and third trimester. Significant differences (increase) were discovered in: Weight (kg), Fat mass (kg) Fat free mass (kg), Muscle mass (kg), TBW (kg), Intracellular water (kg), Extracellular water (kg). Once again there was no statistical significance in values recorded in percentage of body fat %, TBW%, and ECW %. Differences between trimesters are shown in Tables 26 and 27.

Difference Table	Difference
Weight kg	8.42
Body fat %	0.84
Body fat kg	3.16
TBW kg	4.13
TBW %	-0.74

Table 26: Table of differences between second and third trimester selected calculated/obtained values

Difference Table	Percentage
Weight kg	11.40%
Body fat %	2.60%
Body fat kg	12.85%
TBW kg	11.77%
TBW %	-1.51%

Table 27: Table of percentage differences between the second and third trimester selected calculated/obtained values

When results from the first and the second observation setup in selected calculated/obtained values were compared, it was discovered that body fat % changes were nearly similar. Although for the first observation the values it was for the whole pregnancy and in the second case the values were only a difference between the second and third trimester.

This describes and corresponds to the fact that the biggest body composition changes in pregnancy are mainly from the second trimester to the third trimester/before delivery, when the fetus grows the most rapidly and increases of amniotic fluid is the biggest.

In the second observation setup (second and third trimester) directly measured values were observed. Resistance values are shown in Tables 28 and 29. Absolute differences of average in directly measured values between the second and third trimester are shown in Table number 30.

Also in this setup for resistance and reactance values statistical significant (P=0.05) decrease on all used frequencies was discovered. Average decrease of resistance across all frequencies was 84.71 ± 8.88 Ω which presented 13.57 ± 0.09% decrease from second trimester to third trimester. Nevertheless, smaller decrease was expected due to the growth of fetus. That should represent new cell mass with specific electrical values.

Second Trimester	Average	SD
Resistance 5 kHz	714,87	91,05
Resistance 50 kHz	642,83	87,80
Resistance 250 kHz	577,98	83,47
Resistance 500 kHz	562,86	64,31

Table 28: The selected calculated/obtained values third trimester

Third Trimester	Average	SD
Resistance 5 kHz	618,55	79,34
Resistance 50 kHz	555,97	73,16
Resistance 250 kHz	499,08	66,33
Resistance 500 kHz	486,11	64,31

Table 29: The selected calculated/obtained values first trimester

Difference Table	Difference (Ω)
Resistance 5 kHz	96,33
Resistance 50 kHz	86,86
Resistance 250 kHz	78,91
Resistance 500 kHz	76,75

Table 30: Table of differences between the second and third trimester resistance values

For a better description and comparison between all three trimesters changes in resistance were observed between the first and second trimester. When comparison of frequency of 50 kHz between the first and second trimester was done there was discovered a statistically significant difference although the average change was only 3.26 ± 0.024%.

This is a huge difference in comparison with the change between the second and third trimester where the average change for the same group of women was 14.33 ± 0.085 %. According to this information the changes in first trimester are not so dramatic in a resistance values.

This can point to the conclusion that measurements done in the first trimester does not have enough evidence strength to prove some relevant results for following the gestation progress. Nevertheless, values obtained in the first trimester have an important role in observing of the gestation progress. This means that the measurements in the first trimester are very important for difference of monitoring.

For better description and visualization of the progress in body composition parameters during the whole gestation process, linear interpolation of basic calculated/obtained composition values for sixteen women (every woman had minimally three measurements) was calculated. These changes are displayed in Figure 37 and 38.

These graphs show a constant increase in all parameters recorded in kg (excluding patient No. 65 that had a specific course of pregnancy and at the end was excluded from further evaluation). In parameters recorded in % no increase is noticeable. This all corresponds to the finding that values reported in % did not have statistically significant changes in any of my measurements.

This was due to an overall increase in body weight of measured patients. Hence, values recorded in percentage could not be statistically changed. Percentage of the calculated/obtained values remains nearly the same during the whole gestation.

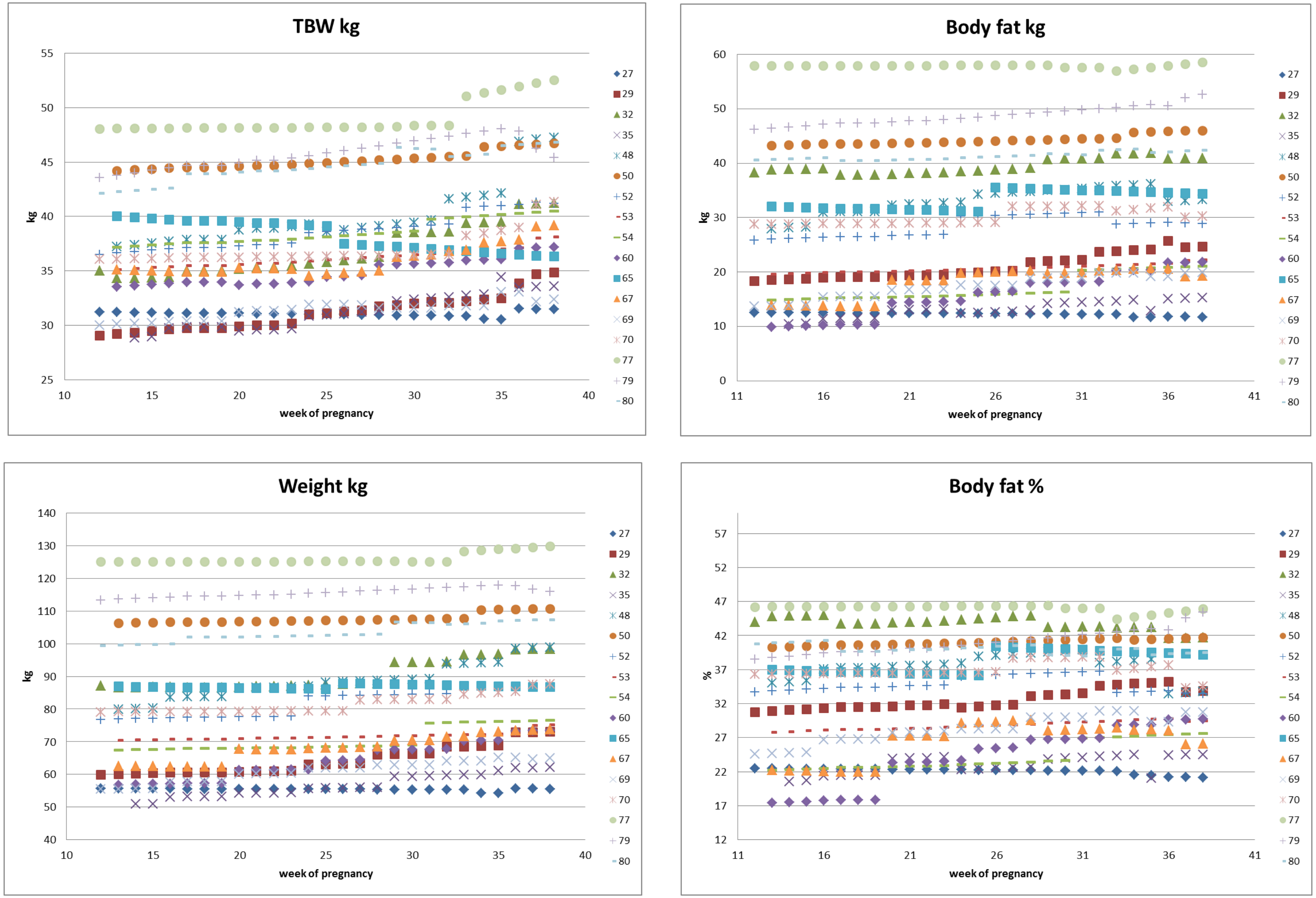


Figure 37: Changes of body composition parameters interpolation I

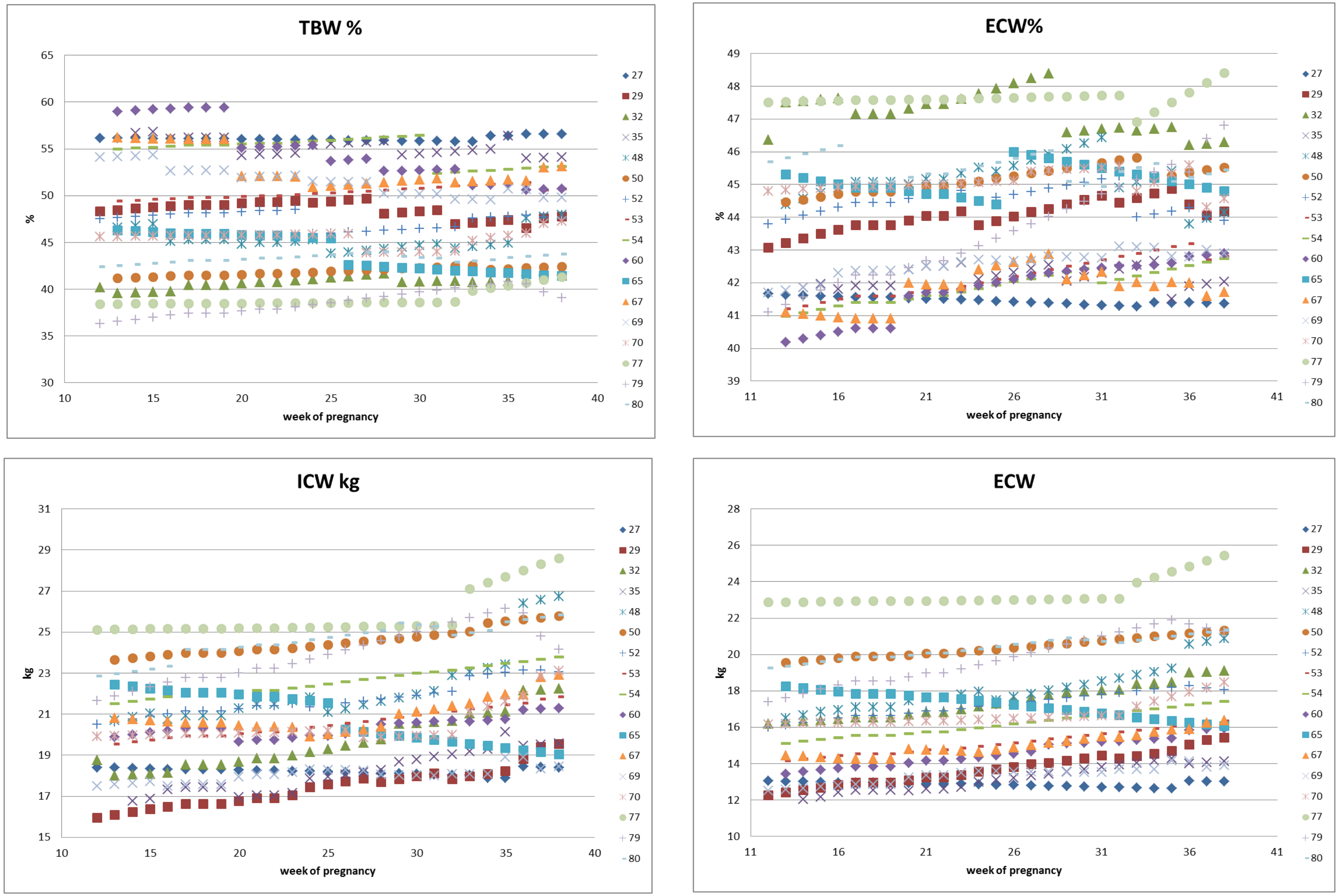


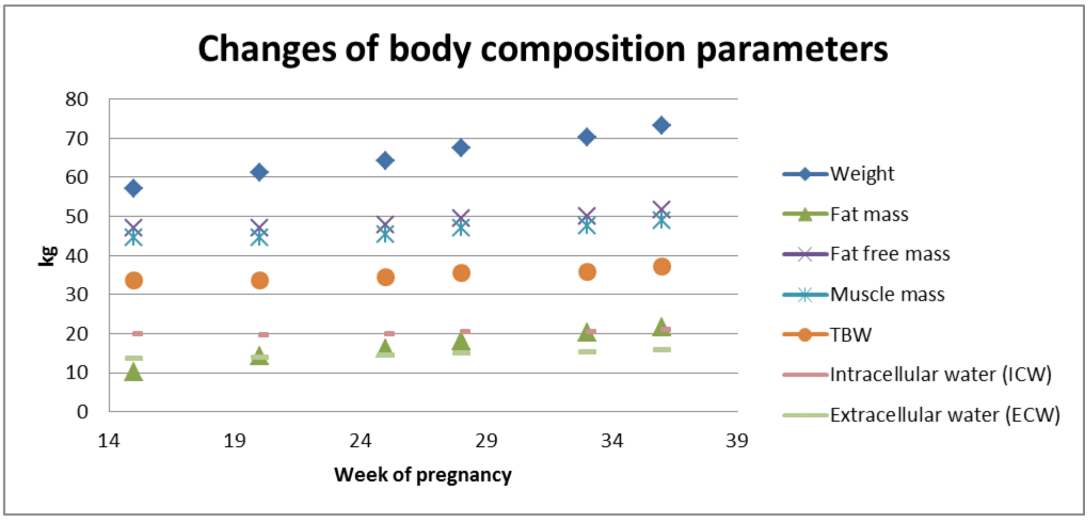
Figure 38: Changes of body composition parameters interpolation II

To point out individual gestation process a woman (id number 60) with significant changes in all body composition results was chosen. This woman was observed during the whole pregnancy with intervals of observations between three to five weeks. This observation frequency allowed tracking of changes for all calculated/obtained and directly measured changes.

All the changes of important parameters (weight, body fat, TBW, ICW, ECW, muscle mass and fat free mass) are displayed in Figure 39.

During pregnancy of this woman I could also observe a great increase in weight. In this case most of the weight gain was represented by body fat mass. The body fat increase was 11,65 kg (increase of 115,92%) which resolved in overall increase of body fat 68,18% from the first measurement

This can cause that the change of percentage of body fat would be probably statistically significant in a group of women with the same body fat increase.



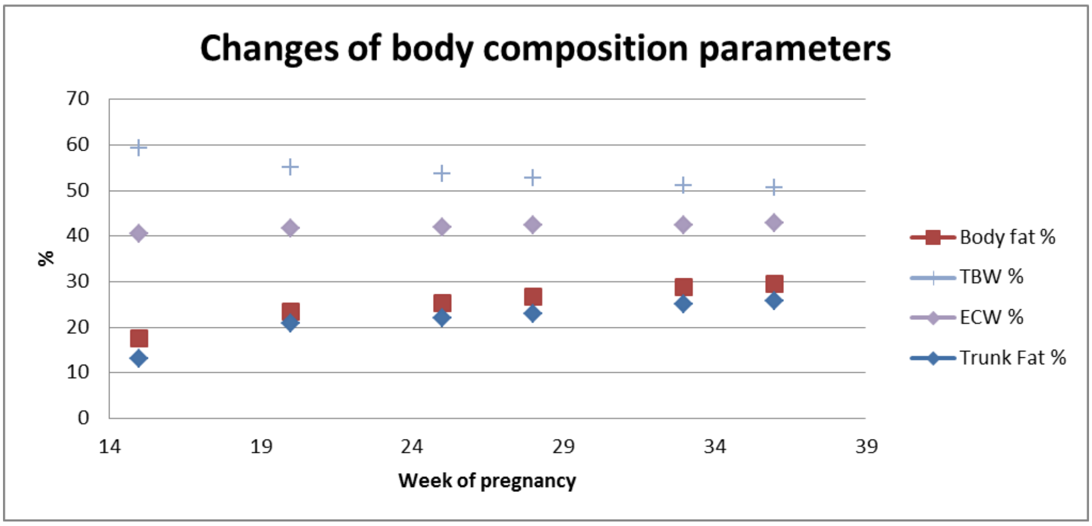


Figure 39: Changes of body composition parameters during gestation patient id. 60

Gain of body weight during pregnancy shouldn`t be caused by such body fat increase as was observed in patient id 60. The increase in patient`s weight should be due to overall body composition parameters increase and not because of such big fat mass increase. This increase in body fat can result to problematic results of pregnancy.

This case of fat mass increase was specific because in general increase in body fat % was not statistically significant. Other body composition values were increased within range of 16%.

Displayed courses of resistance and calculated/obtained body composition also show that the biggest changes are between second and third trimester. This corresponds with previously mentioned findings. All these outcomes result to important finding that most important period for measurements and evaluation is between 25th and 30th week of pregnancy.

Correlation of five basic calculated/obtained values (weight, TBW kg, TBW %, body fat kg, body fat %) and resistances (on frequencies 5, 50, 250, 500 kHz) was calculated to found out if there are some changes between second and third trimester and if values correlate together.

Weight vs 5 kHz		**Weight vs 50 kHz**		**Weight vs 250 kHz**		**Weight vs 500 kHz**	
2. trim	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*
-0,55	-0,76	-0,54	-0,73	-0,50	-0,67	-0,48	-0,65
Increase	**YES**	Increase	**YES**	Increase	**YES**	Increase	**YES**
TBW kg vs 5 kHz		**TBW kg vs 50 kHz**		**TBW kg vs 250 kHz**		**TBW kg vs 500 kHz**	
2. trim	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*
-0,76	-0,82	-0,75	-0,79	-0,72	-0,74	-0,71	-0,72
Increase	**YES**	Increase	**YES**	Increase	**YES**	Increase	**YES**
Body fat % vs 5 kHz		**Body fat % vs 50 kHz**		**Body fat % vs 250 kHz**		**Body fat % vs 500 kHz**	
2. trim	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*
-0,15	-0,43	-0,14	-0,38	-0,10	-0,32	-0,08	-0,31
Increase	**YES**	Increase	**YES**	Increase	**YES**	Increase	**YES**
Body fat kg vs 5 kHz		**Body fat kg vs 50 kHz**		**Body fat kg vs 250 kHz**		**Body fat kg vs 500 kHz**	
2. trim	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*
-0,37	-0,65	-0,36	-0,61	-0,32	-0,56	-0,30	-0,54
Increase	**YES**	Increase	**YES**	Increase	**YES**	Increase	**YES**
TBW % vs 5 kHz		**TBW % vs 50 kHz**		**TBW % vs 250 kHz**		**TBW % vs 500 kHz**	
2. trim	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*	*2. trim*	*3. trim*
0,12	0,45	0,11	0,40	0,06	0,34	0,04	0,33
Increase	**YES**	Increase	**YES**	Increase	**YES**	Incerese	**YES**

Table 31: Correlation increase between parameters

Table number 31 describes correlation of all calculated/obtained values of body composition and resistance on all frequencies in first and second trimester with marking of increase/decrease in correlation. The first row shows correlation in second trimester between selected calculated/obtained value and resistance on specific frequency. The second row describes correlation in third trimester between selected calculated/obtained value and resistance on specific frequency. Increase row indicates if there was an increase in correlation from second trimester to third trimester.

The statistically significant (P=0.05) negative correlations were found for TBW kg and weight in third trimester for all used frequencies and for body fat kg in frequency 5 kHz. This can point to good relationship between TBW kg and resistance. The highest correlation was discovered for 5 kHz frequency in all three parameters which can point 5 kHz to be set frequency for measurements in second and third trimester of pregnancy. Second highest negative correlation coefficient for weight and TBW was found for frequency of 50 kHz.

Both of these two frequencies had best correlation along whole set of measurements. Which was expected to be other equation because measurement done on 5 kHz frequency for TBW is disputable (at this frequency electrical current shouldn`t penetrate cell membranes).

After calculation of correlation in second and third trimester, increase of correlation in all parameters was found. Increase of the correlation between second and third trimester was very high in some cases. This can indicate increase in dependence of observed values and that third trimester`s obtained/calculated values are more related to resistance than second trimester.

These correlations (Table 31) show that it would be probably better to use reactance and resistances to track gestation process from body composition view. This should also eliminate biases in TBW kg and fat kg values that are represented by errors of variables in equations used for calculation of these parameters.

There were only few research studies published in a field of body composition and bioimpedance evaluation during pregnancy on European women [66, 68, 138] and USA women [139]. The studies and guidelines that are observing the pregnancy are mainly focused on weight gain IOM [128] and BMI. In clinical praxis there are mainly observed basic anthropometrical parameters – changes of body weight and they are compared to IOM standards. It has to be also considered that measurements of body composition are race specific. There are studies that are describing and setting reference ranges in Italian women [68, 138] gestation in terms of body composition. However, no evidence based research on Czech population in this field was done before. Because of this and the fact that body composition can be race specific and the fact that my research was focused on Czech pregnant women to discover exact changes of body composition during pregnancy and to describe whole gestation from all possible points of view.

In pregnancy, increase in vascular bed is reported, this occurs because of blood volume increases. Hence increase in cardiac output of pregnant women is needed. Observation of pregnancy progress in my dissertation shows that most important (from body composition view) are changes between second and third trimester. The overall decrease between all trimesters was statistically significant (P=0.05) for all electrical values of body composition and increase for all calculated/obtained values (excluding values recorded in percentage). Nevertheless, in some cases even percentage values differed dramatically, which is shown in subject number 60.

The decrease in directly measured parameters was bigger than increase in calculated/obtained parameters. This leads to conclusion that directly measured parameters have better descriptive capabilities than calculated/obtained. Also directly measured values do not introduce an error of other parameters that are used for calculation of calculated/obtained. In a case of directly measured values examiner has to calculate only with device measurement error whereas in case of calculated/obtained values examiner has to calculate with errors caused by equations.

The significant differences in bioimpedance (5 kHz, 50 kHz, 250 kHz and 500 kHz) during the 3 trimesters represented known increase in body-fluid and mass volume in the observed group. The overall decrease of both reactance and resistance values were probably because of increase of whole body conductivity during gestation. This is because of majority of mass gain is amniotic water and also fetus that is composed mainly from water [133]. Although it was expected that decrease of reactance would be smaller than decrease of resistance because reactance stands for cell membranes whereas resistance stands for a pure resistive property of a tissue.

When resistance and reactance (at measuring frequency 50 kHz) that were obtained in second and third trimesters were compared to reference ranges that were reported in previous study [68, 138] it was discovered that these ranges did not apply to my measured values. This can conclude that reference ranges as reported by Ghezzi et al. need to be revised for specific population. This leads to conclusion that not just equations that are used for calculation of body composition parameters are race specific but also a reactance and resistance of pregnant women can be race specific.

In all calculated/obtained measurements increases that are typical for gestation were reported. Weight, body fat kg and TBW kg values were for measured obese (in terms of BMI evaluation) group of women within range reported by IOM [128]. Measurements also showed good agreement that have reached BIA method in comparison with classical anthropometrical measurements reported by A. Paxton et al. [126].

TBW kg, evaluated by multi-frequency bioimpedance, increased significantly during the second trimesters in the control group. The same results were reported in some previous studies although they were using different frequencies [122]. It has also been reported that a change in TBW kg is strongly due to plasma volume that is a major contributor of TBW kg which correlated with birth weight of an infant [123]. This also confirms importance of TBW kg and other body composition parameters observation.

Nevertheless, calculated/obtained values are showing problematic representation in some devices. Because in manuals of some machines it is mentioned that gestation can have influence on calculated/obtained values. This can be due to equations that are used for calculation of body composition values. Because variables in these equations do not count that the measured patient can be pregnant.

It is known that equations for calculation of specific body composition are different depending on race, gender and age. This can lead to biases in values and misinterpretation. Hence resistance and reactance values should be more suitable as a standard estimator of gestation process than calculated/obtained because electrical values are measured every time exactly the same and do not include variables as equations for calculation of body composition values.

My dissertation discusses ranges of resistance in specific trimesters and concludes that measurements should be taken in all trimesters although first trimester is used only as a starting value in progress observation. Most important measurements should be done in second trimester to obtain information whether gestation process is physiological.

Gestation is specific period in women’s live. During this period physical and psychical change, levels of glycaemia, hormones are observed. Body composition is dramatically changing during all three trimester of gestation, increase of body weight, waist circumference is observed.

In this period all changes should be physiological. However, there is a risk of pathological situation that is even more dangerous than in non-gestation state of women.

In some cases there are risks of hypertension (caused by increase in TBW), polyhydramnion (caused by increase of amniotic water), post-gestation obesity (caused by increase of body fat during pregnancy), changes of newborn`s body weight.

Hence pregnant women are very closely observed to prevent pathological event and fatal result of gestation.

Measuring of all body composition parameters by bioimpedance method can give the examiner precise information about the process of gestation. This can help to identify some of mentioned pathologies early.

Bioimpedance proved to be important tool in gestation process evaluation. Hence this method is fast and repeatable [129] and is reporting very good information about gestation process. This method is also very suitable for pregnant women because there is no need for specific preparation of a woman. Nevertheless, new approaches should be considered in a case of pregnancy.

4.3.2. Conclusion

I have proposed experimental pilot measurements for obtaining calculated and directly measured electrical bioimpedance data of Czech pregnant women. The experimental observation was designed to monitor body composition values during whole gestation process.

I have observed statistically significant changes in all calculated (excluding BF% and TBW %) and directly measured bioimpedance values. Statistical significance was proved between all trimesters. From my measurements can be concluded that directly measured electrical values have better descriptive power than calculated values. In directly measured bioimpedance values is no need for specific equations for calculation of body composition parameters.

These measurements were done for the first time on women living in Czech Republic to obtain specific data of Czech population.

5. *Proposed methodology*

This chapter deals with the important parts of bioimpedance measurements – methodology and evaluation/interpretation. Methodology of proposed measurements is an important issue because correct final results depend on correct measurements and data acquisition. During discussion with doctors and examiners it was found that there is no specific knowledge of correct bioimpedance measurement methodology. Even if an examiner knows the measurement protocol it is often not considered as important and thus it is not followed. These facts motivated me to propose and specify a new methodology that should result in obtaining systematic and comparable results each time when applied. By using this methodology, results from all laboratories should be comparable and long-term observation should be without biases.

The chapter is divided into three parts. Each part is describing one topic that I encountered during experimental measurements, and theoretical assumptions that were proposed during development of the whole PhD thesis.

The first part deals with measurements, methods and technical aspects that are connected and can be encountered in BIA evaluation.

The second part discuss possibilities of standardization for the whole bioimpedance measurement process and points out possible problems that can be encountered during handling of devices, measurements and evaluation of data in the bioimpedance process.

In the third part evaluations and new interpretations of values obtained by measurements of bioimpedance during various experimental setups are mentioned. New models to calculate specific body composition values from directly measured anthropometric and electrical values are proposed.

5.1. Measurement

Measurement part is divided into three sections as various topics were described in this PhD thesis. Every section is describing and discussing obtained data and methodology is proposed to avoid problems and errors during measurements.

a) Standard conditions – to prove and propose methodology if conditions (in this case medium between electrodes and connecting body parts) do have an influence on obtained values. Experimental verification of influence described in Chapter 4.1 showed that there were only few statistically significant differences between measurements with different moistures. All results are explained in Chapter 4.1.2 and discussed in Chapter 6. The final conclusion is that different tested media have no influence on obtained measurements. However, it is concluded and strictly recommended that conditions should be the same during continuous measurements. This means that no matter the starting conditions (for example hands greased by cream), following measurements have to be done under the same initial conditions. Moreover, it cannot be easily estimated the influence of any possible medium that was not tested.

b) Frequencies – it was discussed and evaluated if there is specific frequency that can be used for all measurements. However, there was proved that more frequencies had high correlation in dependence on the observed composition value. In Chapter 4.2 was shown that the best correlation was obtained in dependence of followed parameters. The best frequency was determined to be 5 kHz. However, from electrical and physiological theory can be concluded that two frequencies (one high and one low) are needed to obtain separate body composition parameters. According to my results, it is recommended to use two frequencies that had the best results which are 5 and 250 kHz.

c) Devices and measurement methods (differences) – It was also shown in Section 4.2 that BIA method is comparable to DXA method by means of precision and that in case of the Czech population BIA method can be used instead of DXA with the same precision.

   Another important result of Section 4.2 is that equations of BMI and body fat obtained from BIA method were calculated. Mathematical relations between DXA and BIA were proposed and discussed.

5.2. Evaluation and interpretation

It was shown in all sections of chapter 4 and is discussed in chapter 6 and 7 that the interpretation of obtained body composition values can be sometimes a bit difficult. Also the fact that all basic body composition values such as body fat, muscle mass, TBW, etc. are calculated results lead us to important conclusion. That the more precise and nonbiased values are directly measured electrical (reactance and resistance) values of a body. By using reactance, resistance or impedance of specific body regions examiner can only expect measurement error and no additional errors that are presented in calculated values (each term in an equation is obtained with error this leads to overall equation bias increase). This implicates that directly measured values should have better outcomes. The experimentally evaluated directly measured values during pregnancy showed that reactance and resistance values can be very good estimators in body composition evaluating and in future can be used as method to track physiological process of gestation.

5.3. Recommendation for minimalizing human factor influence

Whole process of body composition estimation by BIA method is demanding for precise measurements and good handling with bioimpedance devices. It is important to have the same measurement conditions during (4.1) the whole monitoring. This is even more important in cases of long-term observation. Only by usage of the same conditions during the whole observation process valid unbiased data can be obtained.

It is also recommended to take two sets of measurements and then use means (in case of that difference is no bigger than 5%) or repeat measurements (if the difference is bigger than 5%) to overcome possible bias in evaluated data.

Overall it is strictly recommended to remove grease and dirt from patient`s hands and feet to set the same starting conditions before every measurement. This precaution should be followed by cleaning or exchanging (in case of disposable electrodes) electrodes to finalize the whole preparation process.

Patient should be measured in normal hydration state (no dramatically changes of hydration) and in room with humidity around 60% and temperature from 18 to 25°C with only underwear on.

For better understanding and visualization methodology and exact measurement process is displayed in Figure 40.

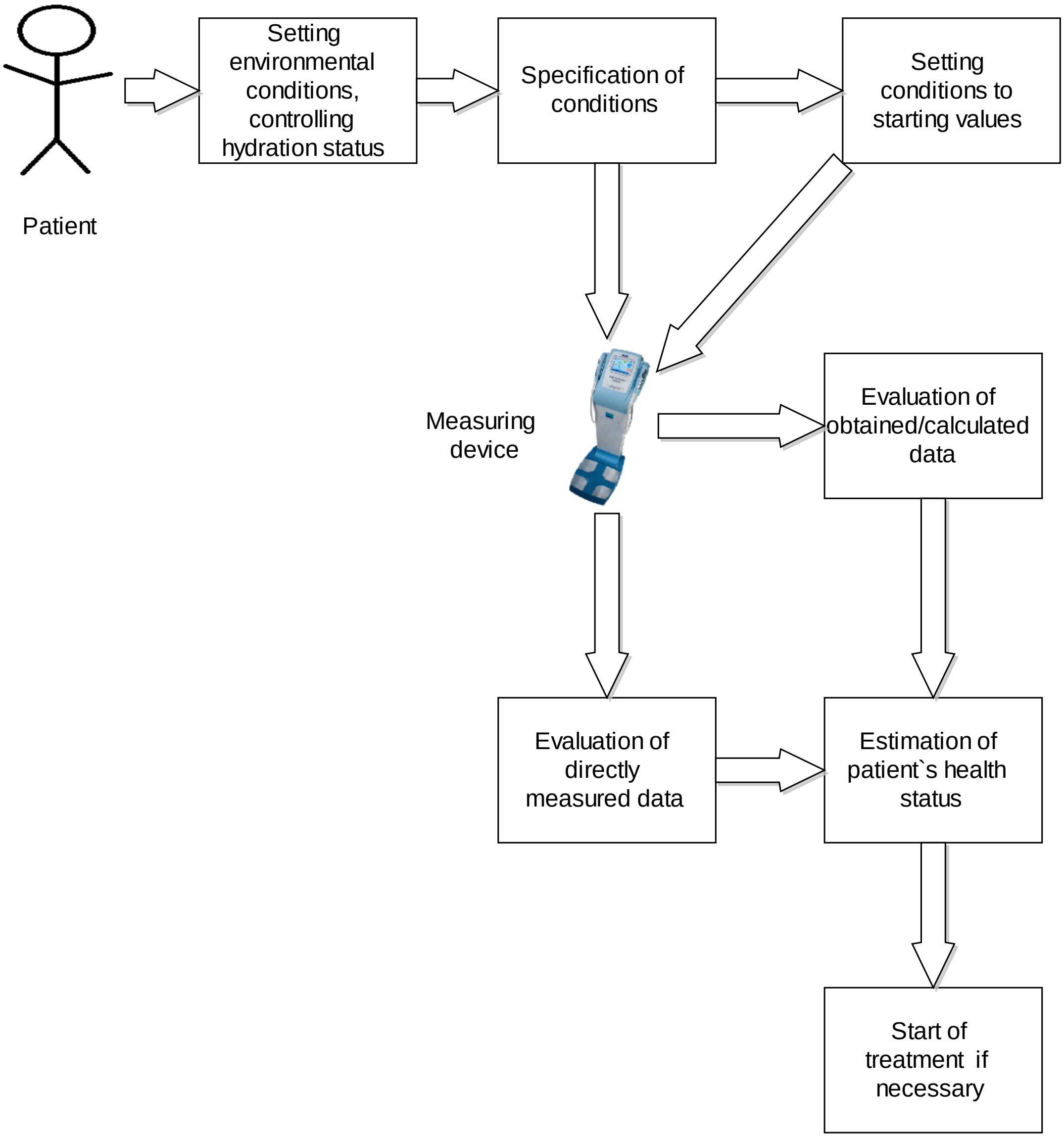


Figure 40: Methodology diagram, calculated/obtained

By complying all mentioned recommendation and following proposed methodology obtained results will be the same and errors caused by service staff and external conditions should be reduced to minimum. This new methodology considerate majority of possible factors that can influence results and is minimalizing biases caused by these factors.

5.4. New model equations for estimation of body fat percentage from anthropometric values – Czech population

The process of estimation of patient`s health status can be really difficult. Sometimes a lot of examinations are needed in terms of obesity or overweight. Previously the BMI index, anthropometric measurements with observation were used to classify patient`s health status and start treatment.

However, these days one of the major obesity markers is percentage of body fat. There are lots of methods how to estimate body fat percentage in the human body such as calipers, hydrostatic weighing, WHR. Not all of these methods are convenient for a patient and a doctor. Some of these methods can be painful or very expensive. All these methods estimate %BF from anthropometric measurements and then calculate %BF using equation.

The method of %BF estimation directly from easily measurable values is very interesting for all patients and doctors. This approach combines easily measurable values and a good result of %BF. There were proposed several equations during the last decades [142,143] however, none of them was proposed for the Czech population. When I used the model [142], I discovered that values obtained from this model overestimated %BF by more than 50% in the measured population.

Because of this I proposed a new model and equations, especially for the Czech and Eastern European populations. The model and equations were consulted with obesity physicians and variables in the model were used according to the results of this discussion.

The new equation takes into account measured anthropometric values and calculates %BF directly.

$$\boxed{\%\mathrm{BF} = -21.91 + \left(1.569 * \frac{weight}{height^2}\right) + (10.62 * \mathrm{gender})}$$

Results from the new model were tested against directly measured InBody 720 values and DXA values. Obtained values of %BF gave valid estimates for males and females aged from 22 to 60 years and normal %BF ranges. However, in athletic and fitness subjects the prediction formulas overestimated results of %BF. Nevertheless, it was only in subjects that had body fat under 16.5 %, which is athletic or fitness range in male population and has to be always processed with caution.

This new model gives the doctors a new possibility to estimate Czech patient`s %BF with very good results without using expensive, uncomfortable ionization methods. These results can be compared with BF ranges and thus conclusion can be done.

Physicians get a new additional marker for estimation of subject`s health status and can start overall treatment. This allows more detailed analysis of body composition and estimation of correct treatment. This new approach to obtain %BF can be used overall in the Czech and Eastern European populations.

5.5. Use of directly measured electrical values – new approach

Majority of recent body composition estimations are done by using calculated values such as body fat, muscle mass, TBW, BMI etc. This approach has, however, one disadvantage that all values are calculated by using specific equations (often derived by comparison with reference methods). These equations are composed of different variables that can present an error in the final result. That is why it was discussed in the PhD thesis that it would be better to use directly measured electrical values such as impedance, reactance and resistance that do not include such variables.

Use of directly measured values should also overcome problems of extreme cases such as athletes (very small percentage of body fat), obese persons (high percentage of body fat) and population diversification. For example athletes (people who do 12 or more hours of training (exercise) a week, members of gymnastics or sports organizations aiming to participate competitively, people such as bodybuilders who undergo training to build up muscles, sports professionals – Tanita specification [137]) have specific sets of equations that should be used before starting the measurement (Tanita, InBody devices). Using normal equations for athletes can result in over- or underestimation of body composition results and setting up a wrong dietary plan.

However, a bigger problem occurs in obese persons where correct estimation is even more important. New equations were reported for obese patients [140,141] but these equations are used very rarely in practice. In fact most of the examiners do not use these specific equations for obese patients. This means a high risk of misclassification of patient`s health status by BIA method and a wrong start of treatment.

Next problem of body composition estimation by composition equations is population diversification (big differences can occur between American, European, Asian and Japanese populations by using only one equation). This means that results of specific populations can be different. Hence an examiner should use equation that is the most suitable for the measured population. However, in most of the currently used devices (in some devices it is possible to choose an Asian type) this fact is not considered. This fact results in using equations for causation population in case of black, Asian and other populations. From Mott [114] it is clear that for example body fat of the black is different from body fat of causation population. By using race specific equations for the wrong population results can be biased and obtained values can misclassify patient´s health state.

All these previously mentioned disadvantages can be overcome by using directly measured electrical values. These values incorporate only exact properties of a tissue and do not consider any other variables. By setting standard resistance and reactance values new measurements that are more precise, race specific and unbiased can be proposed.

Directly measured bioimpedance values together with calculated values can increase the strength of body composition estimation and can lead to a better estimation of treatment.

5.6. Model of resistance process during pregnancy – new approach to monitoring of gestation – pilot study

Monitoring of physiological values during gestation process is an important procedure in the Czech Republic. This monitoring allows obstetricians to have detailed information about the whole pregnancy process and gives opportunity for a fast reaction (if necessary). Majority of the obtained information is from physiological and anthropometric measurements, blood samples and ultrasound examination. However, the information about body composition is frequently missing. These data can give a doctor more precise view of health status of a female and can help evaluate correctly a healthy gestation process.

There are lots of various parameters that can be used for estimation of body composition. However, one of the most important is percentage of body fat (that is directly connected with the weight of a female). The second important value is body water of a pregnant woman. This parameter describes if amniotic fluid increases correctly and if there are no problems in gestation process.

Both these results are calculated values from obtained data. Using specific equations for calculation of these values can result in biases and misclassification of composition parameters in gestation process.

The disadvantage of calculated values of body composition is used equations. There are a lot of equations for calculation of %BF and TBW [38, 112, 10]. Every equation consists of various variables in dependence on population that was used for estimation of these equations (for example blacks and whites have different composition parameters). These differences in used variables can result into over- or underestimation of calculated parameters. Moreover, bias in every variable can result into increase of overall equation bias.

Another problem is that majority of equations does not include pregnancy which can also result in biased results of body composition.

Because of these facts I proposed a new model of body resistance that describes the gestation process in dependence on gestation week and weight. The model does not include empirical equations for calculation and uses only directly measured values.

This will give obstetricians a new body composition parameter to evaluate patient`s health and progress status. The advantage of resistance is that it is a directly measured method that includes only measurement errors and does not include errors from other variables used in equations.

Another advantage of resistance is that this value is directly connected with body fat (increase in body fat results in increases in resistance) and body water (increase in BW results in decreases in resistance). This means that if the resistance is at certain levels both values are processed correctly.

This proposal of a new approach for a resistance parameter was included as a predictor of a healthy gestation process and can serve as a guideline of a healthy pregnancy in future.

There was proposed a pilot study to estimate a new model that will be describing resistance process in a healthy gestation and can set correct values of resistance during the whole gestation process. This proposed model will provide new additional information to obstetricians and help them to get more precise information about a woman´s body composition.

The model was derived directly from measured data of seven healthy women with normal gestation process. The model considers directly measured values such as weight, resistance and week of gestation.

Resistance that was used in this model is obtained by using the whole-body approach, often 50 kHz. Frequency was chosen according to the fact that majority of BIA devices are using this frequency for estimation of body composition values. This can increase the use of this model in more devices in future.

The whole-body measurement was chosen to cover majority of possible changes that are located mainly in the abdominal region. The whole-body resistance should give more precise results than a compartment approach because limbs in some women can bloat during pregnancy and this can cause bias in results.

The first part of the new approach is a weight model that describes process of a woman´s weight change during whole pregnancy. The weight model defines dependence of a woman´s weight on week of gestation and body/weight type of woman. The weight model is defined as a function

$$f_M(T, M_T)$$

where T – week of gestation,

M_T – body/weight type of woman,

The weight model was obtained by polynomial interpolation through the measured data in a least squares sense. The best fit for dependence $f_M(T)$ with minimal error, was gained by polynomials of the 1st order.

$$f_M(T) = a_0(M_T) + a_1(M_T)T$$

First order polynomials were also identified as the best fit for dependence.

$$f_M(M_T) = b_0(T) + b_1(T)M_t$$

Moreover, coefficient $a_1(M_T)$ was almost the same for each value of M_T and coefficient $b_1(T)$ was almost the same for each value of T. Dependences $a_0\ (M_T)$ and $b_0(T)$ are almost linear. Above mentioned findings brought me to 2-D interpolation of the measured data. Hence, the weight model was finally identified as a 3-D plane that includes time, weight and type of woman. The plane was defined as

$$ax + by + cz + d = 0$$

where a, b, c and d are coefficients and a, b, and c are not all zero, then the graph of the equation is a plane having the vector **n** = (a,b,c) as a normal.

The final plane is described in Figure 41. The 3-D plot shows dependence of a woman´s type on the weight progress during whole pregnancy.

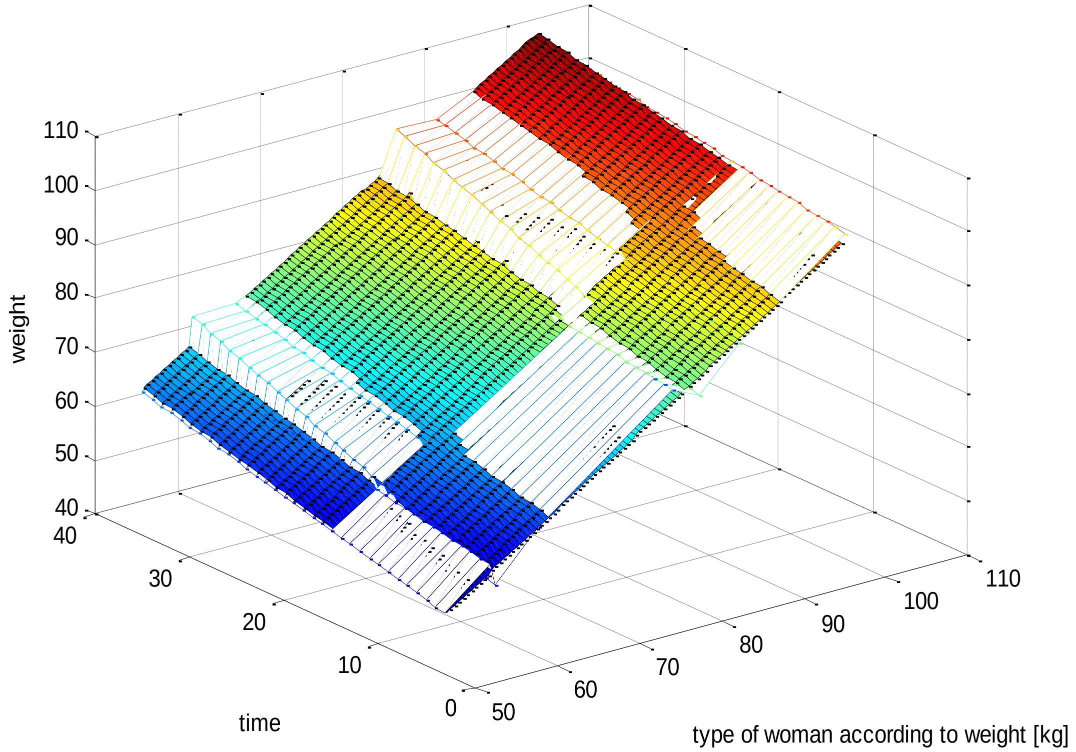


Figure 41: Dependence of women´s types on weight

The type of woman which is derived from the weight model is used in a model of resistance as an input parameter.

The second part of the new approach is a resistance model that was obtained from resistance data in dependence on time and weight type of woman. Resistance data were interpolated to get progress throughout whole gestation. The dependence of resistance on weight type of woman was approximated by a degree 3 polynomial. The coefficients of a polynomial change during the progress of gestation. The resistance model is obtained as

$$R = c_0(T) + c_1(T)M_T^1 + c_2(T)M_T^2 + c_3(T)M_T^3$$

The degree 3 polynomial was chosen because the results obtained by this polynomial were the best to approximate obtained values.

The coefficients of the polynomial were calculated by using the least squares method as

$$\boldsymbol{A}\mathbf{u} = \mathbf{b} + \mathbf{e}$$

where A is overdetermined matrix, b is right-side vector, u is unknown vector, e is vector of errors.

$$A = \begin{bmatrix} x_1^1 & x_2^1 & \cdots & x_n^1 \\ x_1^2 & x_2^2 & & \\ \vdots & & \ddots & \vdots \\ x_1^k & x_2^k & \cdots & x_n^k \end{bmatrix}, b = \begin{bmatrix} y^1 \\ y^2 \\ \vdots \\ y^k \end{bmatrix}, \mathrm{u} = \begin{bmatrix} c_0 \\ c_1 \\ \vdots \\ c_{n-1} \end{bmatrix}$$

$$\mathbf{u}^* = \arg\min_{\mathrm{u}}\{e^T e\} = \arg\min_{\mathrm{u}}\{(\mathbf{Au}-\mathbf{b})^T(\mathbf{Au}-\mathbf{b})\} = \arg\min_{\mathrm{u}}\|\mathbf{Au}-\mathbf{b}\|_2^2$$

where u* is solution of the least squares method.

Dependence of polynomial coefficients on time was interpolated by a linear function using the least squares method. Final dependence of coefficients on time is

$$\begin{bmatrix} c_0(T) \\ \vdots \\ c_3(T) \end{bmatrix} = \begin{bmatrix} P_1^1 \\ \vdots \\ P_1^4 \end{bmatrix} .\boldsymbol{T} + \begin{bmatrix} P_2^1 \\ \vdots \\ P_2^4 \end{bmatrix}$$

The resistance model calculates then the exact resistance in a given week in dependence on weight type of woman. The model is displayed in Figure 42.

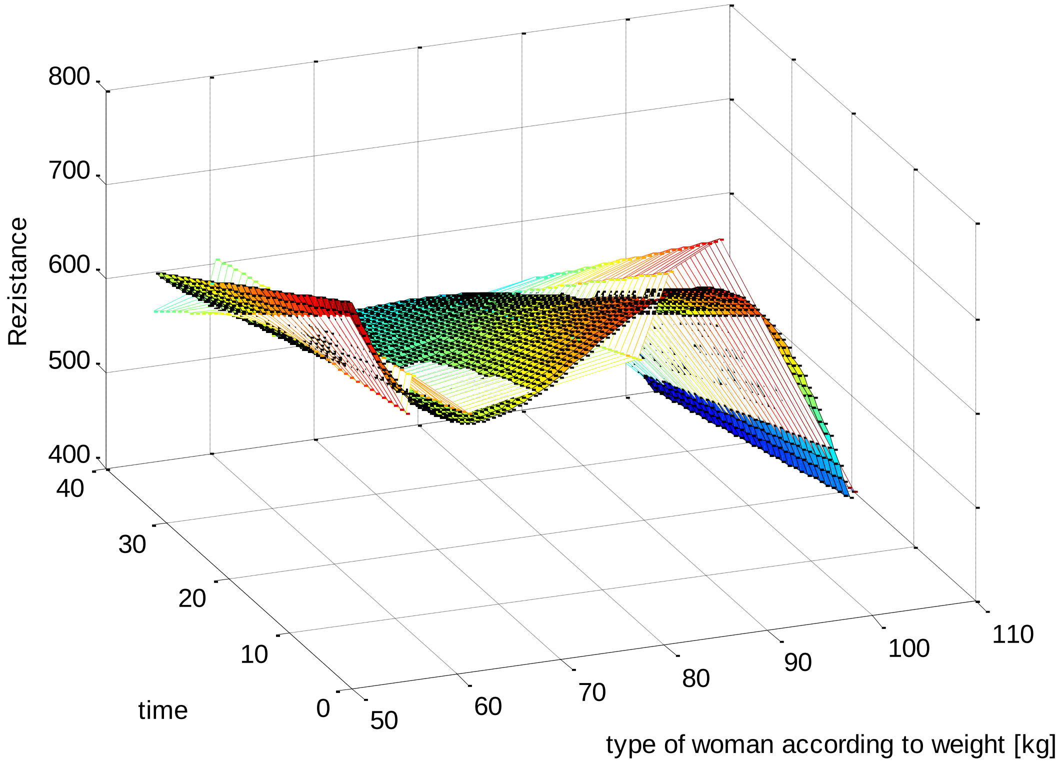


Figure 42: 3-D plane model for resistance, weight type and week of gestation spaced by measured data

The model was obtained directly from the measured weight and resistance data in dependence on week of gestation. Standard deviation of my model was calculated to obtain margins that the resistance should fit to correspond to my model of a healthy resistance process during pregnancy.

The value of standard deviation (sigma ϭ) of resistance data from the fitted 3D plane was 25, 27 **Ω**. Because of this fact boundary of a healthy gestation was set to 2ϭ. Boundaries of a healthy gestation process are displayed in Figure 43.

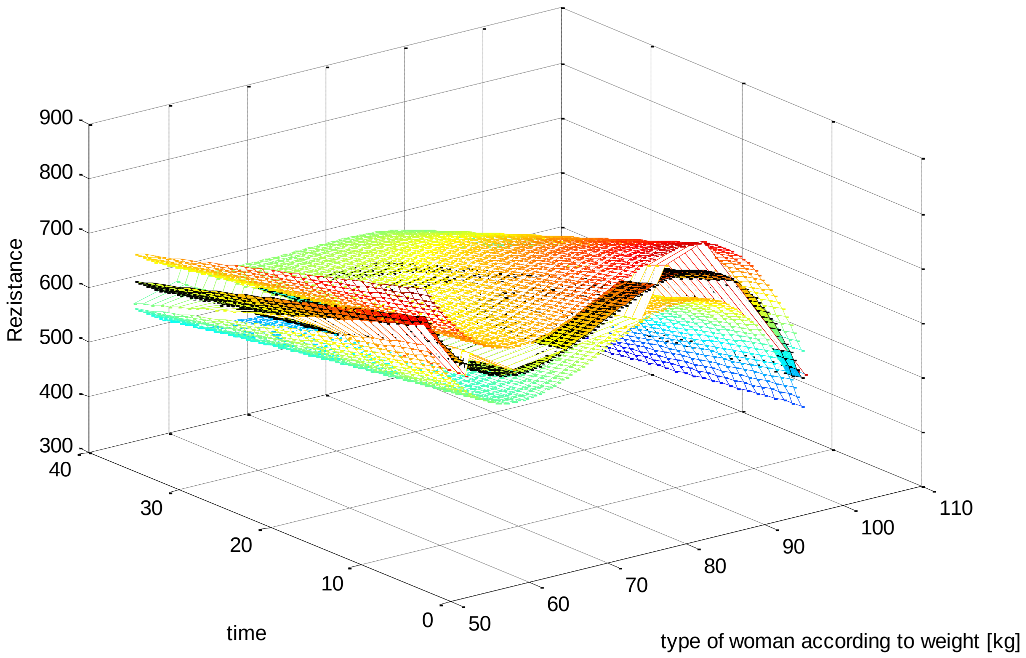


Figure 43: 3-D plane model for resistance, weight type and week of gestation with display of 2ϭ margin

The new pilot study is describing the process of resistance during gestation in healthy women living in Czech Republic. The model is suitable for calculating resistance values during the whole gestation process in a given week.

The model was evaluated in four gestation progresses. Three women had good health results during gestation. In these three cases measured resistance values corresponded to the model situation describing the process within the selected boundaries of 2ϭ.

In the case number four (woman with a high increase in weight and a statistically significant increase in %BF) my new model showed that the measured resistance values differed from the predicted values.

My model underestimated this woman`s resistance in average by/at 200 **Ω**. In a weight of 58kg that this woman had I would expect smaller resistance than it was measured (850 **Ω** - starting conditions in 15th week of gestation). The resistance profile was not decreasing optimally, and in some measurements an increase instead of a constant decrease was reported.

If the starting resistance conditions were in normal ranges it was expected that the measured resistance would be closer to the model. The increase in body weight was constant during whole gestation which could result in higher or lower resistance (it depends on the tissue that caused an increase in weight).

However, it was found out that the increase in body weight was mainly due to the increase in body fat. This increase in body fat which is composed mainly of poorly conductive lipids resulted in higher resistance than it would be expected. In this case the advantage of directly measured resistance is obvious.

Resistance is referring to the increase in fat mass and can indicate a non-optimal process of gestation. In this case my new model would warn doctors that the women do not have a standard body composition process and this can result in a closer observation.

This example shows the strength of my new model and resistance observation during whole pregnancy. So then, obstetricians have the opportunity to compare measured values with values of the model and decide if another observation, laboratory of ultrasound examination is needed. The resistance is a directly measured parameter. This fact concludes that only an error in measurement should occur in obtained data and no other variable biases.

The new model is based on a pilot study of pregnant women´s measurements. It takes into consideration only directly measured values and is capable of modeling progress during whole pregnancy.

The advantage of the model is that it uses directly measured data and adapts coefficients of polynomials according to the provided dataset. This allows more accurate fitting of planes to the obtained data and increase in prediction power.

The results in this pilot study are very promising. Further measurements and evaluation are needed to improve the whole model.

6. *Discussion on outcomes of the PhD thesis*

This chapter discusses results achieved through practical implementation of proposed methodology. In this PhD thesis problems of body composition obtained by BIA method were mapped and described. The whole process of obtaining body composition data was described and data were processed. This dissertation describes basic problems that can be encountered by using bioimpedance analysis as a method for estimation of body composition parameters.

In the first part of this dissertation there was discussed and experimentally tested if current procedures done before measurements are necessary and if testing standard protocols [127] are mandatory to be done before every measurement. Although there are lots of parameters that should be taken into account in BIA measurements, some of these environmental parameters are affected with difficulty such as humidity and temperature. Some studies discussed the influence of temperature although Garby et al. [80] showed that a change of temperature from 24 to 35°C does not affect impedance values.

Other parameters are easier to affect such as pressure (no pressure on electrodes to prevent a change in outcome values [78]), food intake (influence was discussed in [79]) and moistures as a medium for current between electrodes and body parts (body part should be treated by alcohol before starting every measurement [127]).

Overall standard conditions that are considered for patient status (hydration, temperature or feasting) [119,120,121] should be monitored to obtain the most accurate and repeatable measurements.

It was shown that various lotions and moistures have influence only in minority of values and only by using specific highly conductive lotions such as EEG gels, water and by application of oil free hand cream and water based foot cream statistically significant changes can be reported.

In obtained/measured values changes were observed only in the experiment setup number nine for TBW%, fat mass kg, body fat percentage. Values of muscle mass, ECW, ICW were not influenced and no change was reported.

In measured values influence occurred in more conditions (EEG gel and application of water). Nevertheless, influence did not occur across the whole frequency spectrum and for example in frequency of 500 kHz no statistical change was observed.

These results lead to the conclusion that special treatment for contact areas of a body and bioimpedance device is not needed before starting every measurement. They also lead to a question if alcohol treatment cannot have opposite rather than positive influence stated in the standard protocol. This fact should be discussed and experimentally approved in next research targeted on influence of medium between electrodes and body.

In the next part of this dissertation comparison between InBody 720 device and DXA method was done to prove that bioimpedance-based method has a comparable outcome with DXA method that is considered to be one of gold standards of body composition evaluation.

A statistically significant positive correlation was proved at the significance level of $P<0.01$. The correlation coefficient reached the following values: in body fat % - 0.9452, in adipose tissue kg - 0.9588 and in lean body mass the value of 0.9820. In assessing the amount of adipose tissue by using BIA method a lower amount of adipose tissue was proved in comparison with DXA method – 35.7 % versus 35.3 %, 30.6 kg versus 30.9 kg. Other authors also show analogous outcomes in the literature. [130, 131].

These outcomes show a very good agreement of values obtained by DXA and bioimpedance. Linear regression equations for body fat percentage, body fat kg and fat free mass kg were calculated to prove that values obtained by DXA and bioimpedance are identical in terms of body composition estimation.

Proving the relevance of outcomes leads to the conclusion that it is possible to use the method of body impedance measurement in practice on a larger scale and both methods can be interchanged. The advantage of using the bioimpedance BIA method is a significantly smaller load in the monitored person, from the mobility of examination even outside the clinical workplace which is specially adapted and at least but not last from a significantly lower purchase cost of the equipment for measuring the bio-impedance in comparison with the equipment for measuring the dual absorptiometry.

Also the need of ionizing radiation in case of DXA means that bioimpedance body composition evaluation can be used in cases where the use of X-ray is strictly forbidden. This gives bioimpedance new possibilities where this method can be used with obtaining similarly precise data as DXA method.

The new model was proposed to calculate %BF from directly measured anthropometric values of patients. The new model has very good results and in comparison with [142] results overestimates body fat values only in case of extreme body composition types.

Overestimation was reported only for %BF ranges under 16.5. This new model can be used for calculation of %BF directly from anthropometric data. This new approach can be used for calculation of body fat in white adults in normal ranges of body composition in the Czech and Eastern Europe populations.

The final part of the dissertation is describing the new approach of body composition evaluation during gestation. The topic for the final part of the dissertation that used all previous parts was chosen because body composition during gestation plays an important role in gestation process evaluation.

Changes in maternal body composition can mean a poor outcome or problems during delivery (hypertension, post-gestation obesity, changes in newborn`s body weight). Another motivation was the problem obtaining body composition for pregnant women because it is difficult, unpleasant (for a measured woman) or strictly forbidden (by some techniques) to obtain body composition values.

All values that are obtained by bioimpedance method are directly measured electrical parameters of a body or calculated values using directly measured electrical properties of a body. Hence, this method is very suitable for measurements of gestation because during this period female`s hydration status is changing dramatically. Also an increase in cells (fat free mass and body fat) mass during gestation is very notable. This fact allows bioimpedance to be used as a good estimator of the gestation progress.

In this dissertation body composition values such as: Weight, Body fat %, Fat mass, Fat free mass, Muscle mass, TBW (kg), TBW%, Intracellular water (kg), Extracellular water (kg), ECW % were monitored and more importantly, body electrical values (reactance and resistance) obtained by bioimpedance measurements were monitored as well. Changes of electrical values during gestation were used to discover if proposed standard values of bioimpedane by Ghezzi et al. [68] are valid even for the Czech population. Thus bioimpedance and body composition values are important markers and may be used as an additional examination for gestation progress and outcome. From experiments it was discovered that these electrical parameters may be better estimators of gestation process than body composition parameters and weight increase.

However, body electrical parameters during pregnancy in women living in Czech Republic have not been described yet, that is why it is necessary to propose a new experimental approach to standardize electrical parameters of pregnant women during the whole gestation cycle as done by Ghezzi for American women because with exact guidelines and boundaries of reactance and resistance of specific time periods of gestation body composition can be described more precisely than by using conventional parameters.

During examinations and experiments it was discovered that all values of body composition, excluding values indicated in percentage, were statistically changing during gestation. However, in some cases increase in values recorded in kg was so notable that even values recorded in percentage were statistically significantly changed.

It was described that the best period for measurements is from second to third trimester where the increase in all values is bigger than increase between first and second trimester. Nevertheless, first measurements to obtain reference values for further comparison should be taken in first trimester (14 weeks of gestation) rather than in second trimester (24 weeks of gestation) and then before delivery (36 to 38 weeks of gestation).

After comparison of my results to Ghezzi et al. and Larciprete et al. [68, 137] it was found that my values obtained at frequency of 50 kHz are different. It was also discussed if 50 kHz is a correct frequency to be used for measurements. Hence, used frequency sweep (5, 50, 250 and 500 kHz) should give examiners better overall information.

Proving all described results leads to the conclusion that my obtained impedance values are nation/race-specific. This can explain the difference between values obtained in this work and Ghezzi`s. It was also concluded that more frequencies should be used to obtain a better description of all body composition parameters. Hence, a spectrum of 5 to 500 kHz should be used to get impedances at low and high frequencies because of intracellular and extracellular water differentiation.

A new pilot study for estimation or resistance model during pregnancy was created. This model was evaluated in three women with good results. In the fourth case it was discovered that the model did not fit to the measured data and selected boundaries.

However, this case of pregnancy was not normal because of a big increase in fat mass. This could result into unexpected resistance. This was also proved by the measurements that reported unusual resistance ranges.

The new model will be helpful to obstetricians and it will give a new parameter that can be used as a marker of gestation process. Resistance as a directly measured parameter connected with fat mass and hydration can be used in future as an estimator of a healthy body composition process during gestation.

Overall bioimpedance is one of the possible methods for observation of gestation process, because there are no known threats for pregnant women and fetus. This method gives satisfactory outcome values. The biggest advantage of this method is speed and flexibility. Bioimpedance device can be used nearly everywhere with very easy and fast result delivery. Also results obtained by impedance are repeatable [124] and easy to use with the possibility of comparison with values obtained by other devices.

7. Conclusion

The thesis is describing the whole process of obtaining, processing and evaluating body composition data. One of the goals of this work was also to define a new methodology for bioimpedance measurements that will minimize errors caused by operating examiners and possible environmental influences. With regard to these facts and assumptions from literature and experience the goals of this PhD thesis were proposed as:

1) Measurements of specifically designed external conditions (creams and moistures) excluding highly conductive moistures (EEG gel and water) did not prove any statistically significant changes of measured values. This concludes that no specific conditions are required. Just in case that highly conductive gel or moisture was applied directly before measurement, standard conditions should be applied.

2) Comparison of two body composition approaches DXA and bioimpedance did not show any statistically significant changes and showed high correlation of results. This concludes that both approaches can be interchanged. Bioimpedance approach that is faster, cheaper and does not use X-Ray can be fully exchanged for DXA.

   A new and more precise equation for estimation of body fat % in Czech population was designed. New design was discussed and improved after discussion with obesity specialists. My new equation increased precision of body fat % calculation in Czech population.
3) New experimental measurements of Czech pregnant women were proposed. I was observing and evaluating calculated and electrical values of women during gestation. Both value sets (excluding calculated values in percentage) showed statistically significant changes. Because of no statistically significant change of calculated values in percentage I advise to use directly measured values (electrical). This also excludes error of equations used for calculation of body composition parameters.

4) In final part a new pilot resistance model of pregnancy was created. This new model can be used as a new methodology for observing of pregnancy process.

Usage of directly measured electrical values excludes biases caused by equations.

All goals were successfully fulfilled and the whole bioimpedance process was described. New experimental setups were designed to describe and evaluate specific data of Czech patients and possible obstacles that can be encountered by the examiner. Parts one to three are describing the process of evaluation of body composition data and were used as a core material for the part four where observation of gestation was performed.

In parts one to three a detailed analysis and experiments of the problem were done and new results were found in all three parts. The new findings helped in the final experimental setup.

It was concluded that the whole body composition evaluation process can be done in many subjects and in many conditions and on different devices obtaining similarly accurate data. It was also concluded that the data from bioimpedance method are comparable with values that are obtained by one of the gold standard methods. This conclusion points out that in the Czech population bioimpedance method is as accurate as DXA. The results of high precision of bioimpedance in comparison with DXA method can conclude the usage of bioimpedance as an interchangeable method in cases when DXA cannot be used,

This dissertation is a complex material describing bioimpedance methods from basic principles to a design of new experimental setups. New findings were proved (no need of preparations before measurements, equality of measuring machines and follow up of body composition and bioelectrical values during pregnancy) in this work and new experimental designs were proposed for a specific Czech population. Work of this scale has never been done before. Hence, the facts of this dissertation can be used as a basic material for further work in the field of bioimpedance.

7.1. Body composition analysis: future work

My future plans in a field of bioimpedance and body composition evaluation are to perform new sets of measurements to minimize possible measurement errors and incorporate a wider range of possible factors that can influence obtained measurements to overcome all possible errors caused by these conditions.

Next step in my plans is better description of European population from the point of view of body composition by incorporating measurements in wide groups (men, women, ethnical and physiological differences) of individuals to describe all possible differences between different nations, genders and body types.

From these previously mentioned goals my next future goal to set standard values of directly measured parameters is postulated. By setting these optimal values it would be possible to estimate body composition directly from the measured result which should result in more precise composition estimation and exclusion of errors presented by terms of equations for body composition calculation.

I also plan to cooperate with doctors in a field of obesity and obesity-related diseases and to discover body composition markers that will be the best for classification, determination and start of treatment of obesity in early stages. This includes mathematical classification and evaluation of collected data and feedback from doctors.

My last but not least project is gestation observation. In this project I am cooperating with doctors in gynecology and obstetrics clinics. We are trying to discover influence of specific body composition values on pregnancy and delivery. Further cooperation will include measurements to improve the new resistance model.

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