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1 2	Bioelectrical Impedance Vector Analysis (BIVA) for the monitoring of body composition in pregnancy						
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33 Abstract

Background/Objectives: during pregnancy, body composition alterations can be considered as markers of complications and in this context, a non-invasive and low-cost method such as Bioelectrical Impedance Vector Analysis (BIVA), can be employed to monitor such changes. This study aimed at identifying body compartments trend during physiological pregnancy. Subjects/Methods: classic and specific BIVA variables have been measured in a sample of 37 pregnant women approximately every 4 weeks of gestation and once postpartum, Researchers used both longitudinal and cross-sectional approach. The first case included data of women from the 11th to the 15th week along with data from the 28th to the 32nd week of gestation. The cross-sectional approach regarded two more specific moments (11th-12th weeks and 30th -31st weeks) and data within two months postpartum. Results: the longitudinal approach showed a significant decrease in classic BIVA variables (R/H, Xc/H, Z/H p<0.001) and a shortening of the vector, pointing out that TBW and hydration increased significantly. Specific vector length increased significantly, indicating a physiological gain of FM% (p<0.01). The cross-sectional approach showed lower values of R/H, Xc/H, Z/H between 12th-13th and 30th-31st weeks (p<0.01), while in the postpartum period values tended to those registered at the beginning of pregnancy. No changes have been found for the phase angle in both approaches, indicating that ECW/ICW ratio remained constant. Conclusions: among physiological pregnancies, bioelectric values showed a coherent trend and these results represent a first contribution to support routine exams, leading to an early detection of anomalous values potentially correlated to pathologies.

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64 1. Introduction

65 Pregnancy is a particular period in a woman's life and is characterised by many changes, being for most 66 women a physiological event. In several cases, complications may arise in the course of pregnancies, 67 ranging from minor discomforts to severe diseases (1). For example, in Piedmont (Italian North-West region), 68 the percentage of pregnancies considered as low risk, and thus defined physiological, fluctuated from 70.2% 69 up to 87.7% during the period 2006-2016 (2). However, in Italy, almost 30% of pregnancies starting without 70 any risk can eventually show complications such as hypertension, with an incidence of 3-5% (3) and 71 gestational diabetes mellitus (GDM) with a prevalence of 4.7% (4). The whole duration of pregnancy should 72 indeed be monitored carefully, ensuring the early identification of risk factors (5). Regularly monitoring of 73 pregnancy can be quite valuable to identify potential complications at their inception: at this purpose, national 74 and international clinic protocols (1,6) include routine exams such as blood count, ultrasounds, blood 75 pressure, glucose checks and Gestational Weight Gain (GWG) controls, which are checked through the 76 Body Mass Index (BMI, kg/m²) according to the guidelines of the Institute of Medicine (6). Besides these 77 controls, previous studies have shown the usefulness of evaluating body composition, in terms of body 78 fluids, fat mass and fat-free mass: this yields additional information regarding the status of mother and foetus 79 and the advancement of pregnancy (7). A fast, cheap and reliable technique to assess body composition is 80 Bioelectrical Impedance Analysis (BIA), that is applied either to monitoring physiological conditions or to 81 identifying body composition changes potentially related to metabolic complications or pathologies (8). BIA 82 measures the bioelectrical variables of resistance and reactance (9). The data processing can be carried out 83 using different approaches depending on the targeted outcomes. In particular, conventional BIA analysis, 84 which has been frequently employed to estimate body liquids during pregnancy, results in an important 85 method as reported by many authors who highlighted differences between physiological pregnancies and 86 those showing complications, especially regarding Total Body Water (TBW) (10-17). Excessive expansion of 87 the volume of TBW could be caused by oedema and lead to hypertension (18), while a progressive reduction 88 in TBW has been linked to gestational hypertension and preeclampsia (12,15) as well as to low birth weight 89 and adverse outcomes of the pregnancy (16,19,20). Moreover, an excessive gaining in adipose tissue (and 90 thus FM) could be related to GDM, preterm birth, foetal macrosomia and increase of number of Caesarean 91 section (21). As pregnancy is a particular condition in which the balance of body liquids is very different from 92 the physiological state of the general population (TBW increases as reported by Lukaski et al. (22)), the 93 quantitative estimation of FFM and other compartments through conventional BIA cannot be quantified 94 through population-specific equations which assume a physiological condition of 73% body hydration (19).

95 Besides that, a qualitative analysis is offered by Bioelectrical Impedance Vector Analysis (BIVA) (23) . The 96 classic BIVA approach standardises resistance and reactance for height (23) and has proved to be a useful 97 method to monitor TBW (8,24). A further approach, defined "specific BIVA", adjusts resistance and reactance 98 for height and for cross-sectional areas, and has been found to be more accurate in the evaluation of FM% 99 (24–26). Both classic and specific BIVA have demonstrated to correctly detect variations of ECW/ICW (Extra 100 Cellular Water/ Intra Cellular Water) compared to dilution techniques (21). To our knowledge, few studies 101 have used classic BIVA to assess body composition during pregnancy, whereas specific BIVA has never 102 been used. In 2007, Lukaski and collaborators validated the use of classic BIVA for the assessment of 103 hydration during gestation in a cohort of 15 women, using isotope dilution as a reference (22). They identified 104 classic BIVA as a valid method to monitor body hydration during pregnancy: thus, the vector progressively 105 reduces its length during the last two trimesters of gestation, indicating physiological liquid increase during 106 pregnancy. Rodríguez Atristain et al. (21), highlighted an increase in body fat deposition and in TBW amount 107 in each pregnancy trimester, in a sample of Mexican women. Massari et al. (27) outlined the case of a 108 woman who experienced paroxysmal supraventricular tachycardia (SVT) that was intercepted and monitored 109 also thanks to the use of BIVA, which had shown liquid depletion.

The present study aimed at identifying body composition changes during pregnancy through both longitudinal and cross - sectional approaches, applying either classic or specific BIVA in a sample of Caucasian pregnant women. The expected findings could be used as a reference for the monitoring of physiological changes and for the early identification of anomalies potentially related to pathologies.

114 2. Materials and methods

115 Data were collected in the Ambulatory of Obstetrics and Gynaecology of the Hospital "Ospedale Civile E. 116 Agnelli" (Pinerolo, Italy) from March 2018 to January 2019. The study was approved by the Bioethics 117 Committee of the University of Turin in February 2018 (Protocol n. 127246/06-03-2018), it was conducted in 118 accordance with the Declaration of Helsinki for human studies of the World Medical Association (28) and the 119 recruitment of women was on a voluntary basis. Participation involved signing an informed consent, following 120 a detailed verbal explanation and a written memorandum of the guidelines regarding measurements was 121 offered to each participant. The data presented in this study are available on request from the corresponding 122 author.

123 2.1 Study design

124 Inclusion criteria involved European women with a physiological pregnancy at the end of the first trimester. 125 Exclusion criteria were the presence of previous pathologies or those that occurred during the early weeks of 126 gestation. Assessments started at the end of the first trimester, on the occasion of routine clinical exams in 127 the ambulatory. Women were measured approximately every 4 weeks of gestation, for a minimum of 5 128 appointments during pregnancy and once postpartum. During every appointment, clinical, personal and 129 socio-demographic information was collected for each woman, and anthropometric and bioelectrical 130 measurements were taken.

The research was developed as follows: (i) monitoring the body composition during the advancement of pregnancy; (ii) excluding all the cases of pathologies occurring during the development of pregnancy; (iii) identifying bioelectrical variables differences and thus physiological body composition changes through a longitudinal and a cross-sectional approach.

The longitudinal approach included data related to two distinct periods of gestation: from the 11th to the 15th week, which represents the stage in which the risk of an abortion occurring is considered overcome and the foetus is healthy and growing (1), along with data from the 28th to the 32nd week, the phase in which the pathologies caused by pregnancy start manifesting and are consequently diagnosed and treated, while the foetus is completing its most crucial moment of development (1).

The cross-sectional approach included data of the central two weeks of the abovementioned periods (weeks 12th-13th and weeks 30th-31st respectively), and data within two months postpartum, in order to analyse body composition in three key moments of the course of pregnancy. The choice of these specific periods of gestation has been taken in accordance to the gynaecological suggestions (author: CV) (1).

144 **2.2** Anthropometric, bioelectrical measurements and data interpretation

145 Anthropometric measurements were collected according to the international standard and literature 146 guidelines: weight and stature in addition to body circumferences such as waist, calf (29), and arm (30). 147 Bioelectrical variables were measured using the phase sensitive BIA 101 analyser (50 kHz, 400µA) and 148 mono-use electrodes Biatrodes (by Akern S.r.I., Pontassieve, Florence, Italy). Every woman was asked to 149 remove any metal object, and subsequently measured wearing only underwear, making sure to have an 150 empty bladder and skin free of oils or body lotions, according to the standard procedures introduced by the 151 National Institutes of Health (NIH) (9). Participants lay on their back in a medical non-conductive surface 152 (bed), and bioelectrical tissue values were measured on the right hemisoma between the ipsilateral wrist and 153 anklebone prominences (metatarsus – metacarpus region). Distance between electrodes pair was 5 cm. The

154 analysis of bioelectrical variable was performed according to classic BIVA (23,31), correcting variables for 155 stature (R/H, Xc/H and Z/H) and according to specific BIVA (25,26) which corrects for both stature and cross-156 sectional areas (A/L), where A is estimated as 0.45 mid-arm area + 0.10 waist area + 0.45 calf area, yielding 157 specific resistance, reactance and impedance (Rsp, Xcsp and Zsp). Arm, waist and calf areas were 158 estimated by the formula $C^2/4\pi$, where C (m) is the circumference of the respective segment. Phase angle 159 was calculated as follows: φ = arctan Xc/R·(180/ π) and is not affected by the correction shown for classic and 160 specific BIVA. In both BIVA approaches, as first shown by Piccoli et al. (23) for classic BIVA, impedance can 161 be considered as a vector, whose interindividual variability is plotted in a Cartesian plane, defined by a 162 bivariate distribution of its component (R/H and Xc/H or Rsp and Zsp). Three tolerance ellipses are 163 considered, respectively the areas including 50%, 75% and 95% of the whole reference population. Inter-164 group comparisons can be realised using confidence ellipses. For classic BIVA, vectors moving parallel to 165 the major axes indicate progressive changes in tissue hydration (dehydration corresponds to long vectors 166 and hyperhydration to short vectors), while vectors moving parallel to the minor axes indicate alterations in 167 the phase angle (greater values on the left), which means changes in the cellular mass (24). For specific 168 BIVA, vectors moving parallel to the major axes indicate progressive changes in Fat Mass percentage (FM%) 169 (more FM% corresponds to long vectors) and vectors moving parallel to the minor axes give the same 170 information as classic BIVA.

171 2.3 Data Analysis

172 The normality of the data was verified using the Shapiro-Wilk test. Statistical analysis was carried using IBM 173 SPSS Statistics (version 25). Paired and independent samples Student's t-tests regarding bioelectrical and 174 anthropometric variables were performed in order to analyse differences according to the longitudinal and 175 cross-sectional approach, respectively. BIVA Software (31) and specific BIVA software 176 (www.specificbiva.unica.it) were used to draw tolerance ellipses and confidence ellipses and performing 177 independent and paired T^2 Hotelling tests. Statistical significance was accepted for p<0.05.

178 3. Results

Of the 37 women involved in the study, 20 carried out a healthy and physiological pregnancy and were thus considered for data analysis. The mean age of the sample was 31.95±4.02 years. Measurements suitable for the longitudinal approach, according to the study design's criteria, were 24 (12 women were assessed twice: in the period 11th-15th weeks and in the period 28th-32nd weeks of gestation, supplementary table 1 and supplementary table 2). For the cross-sectional study, we considered 27 measurements (9 measurements in

the weeks 12th-13th, supplementary table 3; 8 measurements in the weeks 30th-31st, supplementary table 4;
10 measurements postpartum, supplementary table 5) referable to the entire sample of 20 healthy women.
Postpartum data analysis was possible only in the context of cross-sectional study as not every woman
considered in the longitudinal approach respected the last appointment.

188 **3.1 Longitudinal approach**

189 Differences between the two periods (11th to the 15th week and 28th to the 32nd week) regarding 190 anthropometric and bioelectrical variables were calculated (Table 1). Weight significantly increased by 191 approximatively 10 Kg, corresponding to a significant increment of BMI. Classic BIVA variables (R/H, Xc/H, 192 Z/H) decreased significantly, despite the great dispersion of data shown by the SD values. The lowering of 193 classic BIVA variables refers, according to Piccoli et al. (23), to a significant increase in TBW. Specific BIVA 194 variables, conversely, increased significantly during gestation for Xcsp (p<0.05) and even more significantly 195 for Zsp and Rsp (p<0.01), pointing out a physiological increase of FM% (24) with a particularly high SD. 196 Phase angle did not change, indicating that the ECW/ICW ratio did not change between the two moments of 197 pregnancy.

The classic BIVA ellipse did not overlap with the origin of the axis, indicating a significant change in body composition (p<0.001). In particular, the vector was shortening, thus confirming that TBW and hydration increase during the two periods of gestation considered (Figure 1A-1B). The specific BIVA vector significantly increased its length (p<0.001), confirming the increase of FM%.

202 **3.2 Cross-sectional approach**

The comparison between different groups of women in three gestational moments $(12^{th}-13^{th} \text{ weeks}, 30^{th}-31^{st})$ weeks, and postpartum) showed that classic BIVA variables (R/H, Xc/H, Z/H) were lower (p<0.01) in the week $30^{th}-31^{st}$ with respect to the week $12^{th}-13^{th}$ of pregnancy (Table 2).

This result highlighted an increase in TBW and hydration during pregnancy (for R/H, Xc/H, Z/H p<0.01 between 12th-13th and 30th-31st weeks) as detected in the longitudinal study as well. The cross-sectional study also highlighted the tendency to the normalisation of body hydration in the postpartum period whose data tended to reach values close to 12th-13th weeks (none of classic BIVA values showed statistical significance), as also emerged in the confidence ellipses analysis (Figure 2).

211 4. Discussion

Pregnancy is a significant and particular moment in women's life. It involves physiological modifications inthe function of organs, tissues and mechanism as well as in body composition and bioelectrical properties of

214 tissues. In order to monitor anomalies that could potentially lead to complications, BIVA represents a valid, 215 non-invasive method to assess body composition status and its changing. However, the literature presents 216 few reference information concerning the trend of bioelectrical variables during pregnancy. In the stretch of 217 time between the end of the first and third trimester, as shown by both the longitudinal and the cross-218 sectional approach, TBW and body hydration increased significantly, as indicated by classic BIVA (lower 219 values of R/H, Xc/H and Z/H). These results are consistent with those of Lukaski et al. (22) and Rodríguez 220 Atristain et al. (21). In the cross-sectional study, postpartum classic bioelectrical values resulted higher, 221 reaching conditions close to the beginning of pregnancy (12th-13th weeks) as already shown in the study of 222 Lukaski et al. (22), although considering different gestation weeks and periods. Thus, the increase in 223 hydration during pregnancy appears to be easily observable with BIVA approach, whose transferability and 224 replicability in a context of routine monitoring are relatively easy to guarantee. Consequently, it is interesting 225 to amplify these observations by assessing the ECW/ICW ratio, documented by the phase angle. Literature 226 results concerning the phase angle in physiological pregnancies did not highlight significant changes 227 between the end of the first and the third trimester either in the longitudinal (according with Lukaski et al., 228 2007 (22) and with Berlit et al., 2013 (14)) or in the cross-sectional approach (according to Rodriguez-229 Atristain et al., 2016 (21)). In the same way, in our study an unchanged phase angle was confirmed in the 230 data from the pregnancy and postpartum measurement. Hence, during the advancement of pregnancy, even 231 though TBW and body hydration increase, the ECW/ICW ratio can remain constant. Indeed, the literature 232 shows an increase of ICW in the third trimester of pregnancy, along with the increase of ECW, that could be 233 associated with the lack of ICW/ECW changes (17). This finding suggests how PA can be used as a potential 234 marker to monitor the displacement of TBW in compartments occurring for example in preeclampsia or 235 hypertension (7). Thus, the early identification of ECW/ICW displacement through phase angle may be 236 adopted as a fast, non-invasive and practical support tool for the early diagnosis and potential treatment of 237 those pathologies. Regarding the choice of methods to interpret bioelectrical data, the value of classic BIVA 238 must be underlined, mainly for the assessment of TBW and hydration, while specific BIVA was particularly 239 useful to monitor FM% alterations. Indeed, the longitudinal approach showed a significant increase of FM% 240 during physiological pregnancy (Rsp and Zsp p<0.01; Xcsp p<0.05). This result demonstrates that it is 241 possible to monitor if the weight gain is excessive or insufficient in terms of FM% and thus potentially leading 242 to complications and/or pathologies such as gestational diabetes mellitus (7). Concerning both TBW and 243 FM%, such health prevention-oriented interpretations were made possible by a qualitative approach (BIVA), 244 which seems to be more suitable for the body composition monitoring in this kind of context. To our

knowledge, this is the first study to use the complementarity of phase angle, classic BIVA, and specific BIVA in order to monitor body composition changes during pregnancy. An analogous approach has already been applied for the body composition assessment in other fields such as sport (24,32) the identification of sarcopenia and sarcopenic obesity (33) as well as in paediatrics (34).

249 **4.1 Strengths and limits**

Through our approach, which for the first time employs the analysis of bioelectrical changes in terms of classic, specific BIVA and phase angle during pregnancy, it is possible to expect its application to routine monitoring of pregnancies thanks to its replicability, non-invasivity, rapidity of use and moderate price, that allows the early identification of anomalies and complications. The limits, on the other hand, concern the lack of availability of postpartum measurement. Moreover, the number of physiological pregnancies could be increased. With a broader sample, it could be possible to estimate possible differences by age and primigravidae/multigravidas in relation to a distinctive balance in body composition.

257 4.2 Conclusions and future perspectives

According to our results, women tend to increase their TBW and FM% during pregnancy but tend to recover their characteristics during postpartum. The combination of the analysis of phase angle, of classic BIVA and specific BIVA is an effective, fast, low-cost and non-invasive method to monitor body composition changes during pregnancy. As pregnancy-correlated pathologies/complications are linked to inadequate modifications of body composition, especially in term of body fluids, the constant monitoring through BIVA, together with routine exams, could lead to an early diagnosis and treatment.

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268 6. Conflict of Interest

269 The authors declare no conflict of interest.

270 7. Author Contributions

- 271 AM contributed to the conceptualisation of the study design, revised the literature, conducted the research,
- 272 was responsible for data analysis and interpretation and wrote the original article draft.
 - 9

- 273 CV contributed to the conceptualization of the study design, provided resources, helped in the medical
- 274 interpretation of results and supervised the development of the entire research.
- AG contributed to the data analysis and curation.
- 276 SS contributed to the data analysis, curation and helped in data interpretation.
- 277 EM contributed to the validation of the methodology, helped in data interpretation and reviewed the entire
- 278 manuscript.
- 279 MMC: contributed to the conceptualization of the study design, provided resources, helped in data analysis
- and interpretation of results, supervised the development of the entire research and reviewed the manuscript
- from the original draft.
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284 9. References

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		Δ betwe 32^{nd} v	en 28 th - veeks	Paired Student's		
		and 11 th -15 th weeks		t-test results		
		Mean	SD	t	Significance	
Weight (k	g)	9.88	2.53	13.51	p<0.001	
BMI (kg/m ²)	3.76	0.87	14.94	p<0.001	
	R/H (Ohm/m)	-47.45	25.21	-6.52	p<0.001	
Classic BIVA	Xc/H (Ohm/m)	-4.94	2.98	-5.75	p<0.001	
	Z/H (Ohm/cm)	-47.70	25.32	-6.53	p<0.001	
	Rsp (Ohm∙ cm)	22.07	20.19	3.79	p<0.01	
Specific BIVA	Xcsp (Ohm· cm)	1.80	2.51	2.49	p<0.05	
	Zsp (Ohm∙ cm)	22.14	20.23	3.79	p<0.01	
Phase A	ngle (°)	-0.06	0.33	-0.59	0.57	

Table 1. Results of the longitudinal study on 12 healthy pregnant women: difference (Δ) between weeks 28th- 32nd and 11th-15th of anthropometric and bioelectrical variables (mean and SD) and significance in bold.

Table 2. Results of cross-sectional study on anthropometric and bioelectrical variables (mean and SD) of healthy pregnant women during weeks 12th-13th, weeks-30th-31st, and within two months postpartum. Significance in bold.

Independent Student's t-test results										
	12 th -13 th and 30 th - 31 st weeks		30 th -31 st weeks and postpartum		12 th -13 th weeks and postpartum					
	t	p value	t	p value	t	p value				
R/H (Ohm/m)	3.18	p<0.01	-1.60	0.13	1.94	0.07				
Xc/H (Ohm/m)	3.42	p<0.01	-2.05	0.06	0.80	0.44				
Z/H (Ohm/cm)	3.20	p<0.01	-1.62	0.13	1.94	0.07				
Rsp (Ohm⋅ cm)	0.15	0.88	-0.68	0.51	-0.52	0.61				
Xcsp (Ohm⋅ cm)	0.64	0.53	-1.27	0.22	-0.91	0.38				
Zsp (Ohm⋅ cm)	0.16	0.88	-0.69	0.50	-0.52	0.61				
Phase Angle (°)	0.81	0.43	-1.22	0.24	-0.63	0.54				