ORIGINAL ARTICLE

Mediation effect of the duration of diabetes mellitus on the decrease in bioimpedance phase angles in ethnically Korean people: A multicenter clinical study

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ABSTRACT

Aims/Introduction: We carried out a multicenter clinical study to investigate whether the decrease in segmental phase angles (PhA values) observed using bioelectrical impedance is useful in screening for diabetes mellitus and monitoring disease progression.

Materials and Methods: The segmental PhA values of the four limbs were acquired using multifrequency bioimpedance at 5, 50 and 250 kHz in three clinics. Differences in PhA values between the diabetes and control groups were analyzed using the two-sample *t*-test and analysis of variance (ANOVA). Changes in PhA values with increasing durations of diabetes were analyzed using a moderated mediation model and multivariate linear regression analysis. We recruited 217 participants aged ≥40 years (diabetes 158, controls 59, men 106, women 111, A-clinic 71, B-clinic 70 and C-clinic 76).

Results: PhA values at 50 kHz were significantly decreased in people with diabetes (PhA of the right arm in men: *t*-value −4.0, $P < 0.001$; PhA of the right leg in women: *t*-value −4.6 $P < 0.001$), and the difference was partially attributable to the duration of diabetes, as well as aging. Specifically, the mediation effect of the duration of diabetes on the decrease in PhA values was 29.8% in the left arm of men, 53.3% in the right arm of women, and 36.3% in the left arm of both sexes.

Conclusions: Phase angle values at 50 kHz decreased in people with diabetes, and the changes were exacerbated as the disease duration increased. Thus, bioimpedance PhA values represent a non-invasive tool for monitoring the progression of diabetes mellitus.

INTRODUCTION

The bioelectrical impedance analysis technique was shown to be appropriate for estimating total body water (TBW) in the early 1960s, and it has been popularized as a tool for estimating body composition. Bioelectrical impedance analysis is recognized as a safe, rapid, reliable, easy, portable, non-invasive and cost-effective technique. For decades, researchers have analyzed bioimpedance phase angle (PhA)[1,2] as an indicator of cell membrane properties and intra- and extracellular water content[3]. PhA is an indicator of the amount of electrical charge that cell membranes can hold[4]; thus, it could be a helpful index of cellular health and function. PhA is dependent on the capacitive behavior of tissues, which is associated with the cellularity, cell size and integrity of the cell membrane, and on its pure resistive behavior, which is mainly dependent on tissue hydration[1]. In summary, PhA has been widely investigated as an important index for monitoring and discerning various diseases and physiological conditions, such as mortality, nutrition status, hemodialysis, cancer and kidney disease[5–9]. In particular, PhA was reported to be decreased in adults[10], as well as children and adolescents[11] with diabetes mellitus, and PhA values were not explicitly dependent on short-term changes in blood glucose levels[12].

According to several studies, people with diabetes show decreased PhA compared with healthy people. However, the mechanism underlying the decreased PhA in people with diabetes is not yet clearly understood. The decrease in PhA reflects the decrease in physiological functions, presumably down to

the cellular level. Previous studies reported that PhA was dependent on age, sex, race and body mass index (BMI)[1,7,13]. Therefore, researchers should exercise caution when carrying out statistical analyses to avoid exaggerated interpretations when patients with diabetes and normal controls are not matched for age, sex, race or BMI; this is especially important in children. In the present multicenter clinical study, we aimed to investigate whether the decrease in PhA values observed using bioelectrical impedance analysis is useful for screening Korean people with diabetes and monitoring disease progression. Therefore, we tested whether the PhA values decreased due to diabetes, and whether the PhA values showed an association with the disease duration in Korean people with diabetes by carrying out a mediation analysis after stratification by age. Briefly, the reduction in PhA values is an indicator of aging, or physiological or metabolic degradation. The present study provides further evidence of the utility of PhA values as a biomarker of the degradation of physiological function as a result of diabetes and its progression, even if primary biomarkers, such as blood glucose or glycated hemoglobin levels, are well controlled by antidiabetic drugs.

METHODS

Participants

From June 2016 to October 2018, a total of 286 participants were recruited from three clinics: Dunsan Korean Medicine Hospital of Daejeon University (DJ), Woosuk University Medical Center at Jeonju (WS) and Jeonju Hospital of Oriental Medicine of Wonkwang University (WK). The study was approved by the institutional review board (IRB) of DJ (IRB number: DJDSKH-16-BM-04), WS (IRB number: WSOH IRB M1709-01-01) and WK (IRB number: WUJKMH-IRB-2017-0005). These observational studies were registered with the Clinical Research Information Service under the registration numbers KCT0002132 (DJ) and KCT0004358 (WS and WK). For the group with diabetes, participants with mild or moderate type 1 or type 2 diabetes whose blood glucose levels were controlled through diet or drugs without serious complications according to the guidelines of the World Health Organization criteria were included. The control group included individuals without a history of type 2 diabetes who presented a fasting plasma glucose level <126 mg/dL and an glycated hemoglobin (HbA1c) level <42 mmol/mol (6%), and individuals who had not been diagnosed with diabetes could also be included at the doctor's discretion. All participants were informed of the objectives and methods of the research, and they provided written informed consent. The study was carried out in accordance with the Declaration of Helsinki guidelines.

Data acquisition

Bioelectrical impedance was measured using a direct segmental multifrequency bioelectrical impedance analyzer with tetrapolar eight-point electrodes (InBody S10; InBody, Seoul, Korea). We measured the impedance at six frequencies (1, 5, 50, 250, 500

and 1,000 kHz), and reactance and PhA values at three frequencies (5, 50 and 250 kHz). Eight electrodes were used to measure five segmental impedances of the body. The participants in this clinical trial fasted for >9 h overnight, abstained from alcohol and avoided intense physical activity for 24 h before the measurements. After the readings sufficiently stabilized, bioimpedance measurements were carried out on the limbs of the body with the participants in a sitting position, which is one of the standard measurement positions for the InBody S10 device. Using the segmental PhA values of the right arm, trunk and right leg, we calculated the whole-body PhA at three frequencies (5, 50 and 250 kHz). Whole-body PhA values were obtained using a serial connection model with the impedance values of the right arm, trunk, and right leg[14], as follows:

$$PhA_{f,WB} = \sin^{-1}\big((X_{f,RA} + X_{f,TR} + X_{f,RL}) / (Z_{f,RA} + Z_{f,TR} + Z_{f,RL}) \big)$$

where X is reactance, Z is impedance, f is frequency (5, 50, 250 kHz), WB is the whole body, RA is the right arm, TR is the trunk and RL is the right leg.

Data selection

The inclusion criteria differed among the three clinics due to the different study purposes. Before data analysis, we adopted the inclusion criteria of the DJ site to select samples for analysis[12]; that set of inclusion criteria was the most stringent.

Exclusion criteria:

1. *Diabetes group*: Participants who failed to meet the diabetes criteria; outliers, defined as more than 3*IQR (interquartile range) below the first quartile or above the third quartile; and participants aged <40 years (participants aged >40 years were recruited at the DJ site).
2. *Control group*: Participants with fasting glucose levels >140 mg/dL and/or HbA1c of >47.5 mmol/mol (6.5%); outliers in measurement values, defined the same way as in the diabetes group, and participants aged <40 years.

When these exclusion criteria were applied, 69 participants were excluded from the entire sample: 43 participants were aged <40 years, 21 participants did not meet the criteria for glucose levels and HbA1c, and five participants had outlier values. As a result, a total of 217 participants were included in the final analysis.

Statistical analysis

The statistical analyses in the present study are divided into two parts: group comparisons and mediation analyses. Unless otherwise noted, the significance threshold for all statistical tests was set to $\alpha = 0.05$. All statistical analyses were carried out using R statistical software (version 3.6.0, released 26 April 2019; R Foundation for Statistical Computing, Vienna, Austria)[15]. Data from 217 participants were summarized as frequencies after stratification by sex and site (see Table S1). A two-sample independent *t*-test or one-way analysis of variance

(ANOVA) was carried out to assess the mean differences in variables between the diabetes and the control groups or between multiple sites. We investigated the mediation effect of diabetes duration on the relationship between age and 50 kHz PhA values at the measured locations (arms and legs on both sides). Mediation analyses were carried out using the mediation package[16,17] implemented in the statistical software R. The mediation analysis aimed to evaluate the statistical causal effect of the mediator on the relationship between the independent and dependent variables[18]. In other words, the mediation analysis decomposes the total effect of the independent variable on the response variable into an indirect effect (mediated effect) and direct effect (the effect of the independent variable when a fixed value is used for the mediator variable)[19]. Figure S1 shows the mediation models used in this study.

For illustrative purposes, we briefly introduce the mediation analysis based on a study by Pearl[20,21]. Let X be an independent variable, Y be a response variable and M be a mediator variable. The mediation model can be described with the following three regression models:

$$
\begin{aligned}
x &= a_0 + u_X \\
m &= b_0 + \gamma x + u_M \\
y &= c_0 + \beta x + \alpha m + u_Y
\end{aligned}
\tag{1}
$$

where $u_X \sim \mathcal{N}\left(0, \sigma_X^2\right)$; $u_M \sim \mathcal{N}\left(0, \sigma_M^2\right)$; $u_Y \sim \mathcal{N}\left(0, \sigma_Y^2\right)$; a_0, b_0, and c_0 correspond to regression intercepts; and γ, β, α correspond to regression coefficients. Then, the indirect (mediated) effect (IE), direct effect (DE) and total effect (TE) are given as:

$$
\begin{aligned}
\mathrm{IE}_{x,x'}(Y) &= \alpha\gamma(x' - x) = (\tau - \beta)(x' - x) \\
\mathrm{DE}_{x,x'}(Y) &= \beta(x' - x) \\
\mathrm{TE}_{x,x'}(Y) &= \beta + \alpha\gamma
\end{aligned}
\tag{2}
$$

where x and x' are any values from X with $x < x'$ and τ is the regression slope of the total effect.

The effect of disease duration on the relationship between age and 50 kHz PhA was explored by exploratory data analyses. We generated scatterplots, and calculated Pearson's correlation coefficients and univariate linear regression coefficients corresponding to the age tertiles of 40 years ≤ age < 55 years, 55 years ≤ age < 65 years and age ≥ 65 years, and diabetes duration tertiles of 0 years < duration < 5 years, 5 years ≤ duration < 12 years and duration ≥ 12 years, where controls were assigned to the group of duration = 0 (see Figures 2 and 3). In the present study, we carried out moderated mediation analyses[22] (see Figure S1c) corresponding to each estimated effect obtained with the percentile method based on 1,000 bootstrap samples.

Basic characteristics of the participants

The demographics and measurement data, along with results from the corresponding *t*-tests, are shown in Table 1. The ages of both men and women, and the weight and BMI of women were different between the diabetes and control groups, despite the effort to obtain age- and BMI-matched control groups. The duration of diabetes was 10.20 ± 8.83 years in men ($n = 84$) and 8.80 ± 7.08 years in women ($n = 74$). The glucose concentration in men with diabetes ($n = 84$) was 208.71 ± 42.26 mg/dL, and the concentration in men without diabetes was 115.58 ± 18.70 mg/dL. The glucose level in women with diabetes ($n = 74$) was 197.71 ± 47.68 mg/dL, and the concentration in women without diabetes ($n = 37$) was 94.87 ± 16.04 mg/dL. The HbA1c levels of men in the diabetes and control groups were $6.96 \pm 0.82\%$ and $5.40 \pm 0.37\%$, respectively, and those of women were $6.59 \pm 0.79\%$ and $5.56 \pm 0.28\%$, respectively. The differences in fat mass (FM) and visceral fat area between the diabetes and control groups in women were due to the difference in BMI. Other indicators, such as skeletal muscle mass, fat-free mass (FFM), TBW and intracellular water, did not show significant differences between the diabetes and control groups. The extracellular water (ECW)/TBW ratio is strongly associated with PhA, and showed differences in both sexes. Most patients with diabetes were maintaining their blood glucose levels with oral medications and had no diabetic complications; 79 men and 70 women were taking oral medications, and five men and four women were not medicated.

RESULTS

Site variations

Segmental PhA values in women are compared among the three data collection sites in Table 2. The male group is not shown because of the insufficient number of participants in the control groups at the two clinical sites. A large difference in age and BMI was observed at the WK site; therefore, special care is required in data interpretation, as both age and BMI greatly influence PhA variations[1,13,23–25]. Despite some variations between clinical sites, the data in DJ and WS showed lower segmental PhA values at all frequencies (5, 50 and 250 kHz) in the diabetes group than in the control group. In the WK data, the segmental PhA values of the legs were lower in the diabetes group than in the control group. Next, we carried out an ANOVA to test the mean differences in data among the three clinical sites in individuals with diabetes, as summarized in Table 3. No differences in segmental PhA at 5 or 50 kHz were observed between clinical sites, but significant differences were identified in segmental PhA at 250 kHz. According to Jun *et al.*,[12] the segmental PhA at 250 kHz showed larger differences than the PhA at 5 kHz or 50 kHz. However, the large site variations in PhA at 250 kHz, as shown in Table 3, make further analyses unclear. When the PhA values were compared among the three sites, only the diabetes groups were included to avoid the effect of unbalanced contributions of normal controls among sites. In addition, the PhA values of bioimpedance were affected by sex and disease, in accordance with previous reports[2,7,26]. However, there was no interaction between sex and the disease on PhA values of 50 kHz.

Table 1 | Demographics and measured data for the diabetes and control groups

Variables	Male (n = 106)			Female (n = 111)		
	Diabetes (n = 84)	Control (n = 22)	t-values (P-values)	Diabetes (n = 74)	Control (n = 37)	t-values (P-values)
Age (years)	60.82 ± 8.90	55.91 ± 10.37	2.225 (0.0282)	62.51 ± 7.86	52.81 ± 7.62	6.195 (<0.0001)
Height (mm)	168.41 ± 6.30	168.16 ± 3.79	0.174 (0.8623)	154.93 ± 5.62	155.85 ± 5.85	−0.807 (0.4214)
Weight (kg)	70.98 ± 11.24	71.45 ± 10.92	−0.174 (0.8619)	61.58 ± 9.99	56.14 ± 8.46	2.843 (0.0053)
BMI (kg/m^2)	24.97 ± 3.22	25.23 ± 3.58	−0.331 (0.7414)	25.64 ± 3.85	23.06 ± 2.83	3.625 (0.0004)
Glucose levels (mg/dL)	208.71 ± 42.26	115.58 ± 18.70	–	197.71 ± 47.68	94.87 ± 16.04	–
HbA1c (%)	6.96 ± 0.82	5.40 ± 0.37	–	6.59 ± 0.79	5.56 ± 0.28	–
SMM (kg)	32.58 ± 4.80	32.69 ± 3.92	−0.103 (0.9183)	23.32 ± 2.83	22.88 ± 3.21	0.736 (0.4634)
FM (kg)	13.14 ± 6.00	13.74 ± 6.72	−0.407 (0.6848)	18.75 ± 6.98	14.35 ± 5.35	3.376 (0.0010)
FFM (kg)	57.84 ± 7.87	57.71 ± 6.45	0.073 (0.9420)	42.83 ± 4.79	41.79 ± 5.28	1.041 (0.3003)
TBW (l)	42.36 ± 4.79	42.22 ± 4.65	0.105 (0.9166)	31.45 ± 3.51	30.55 ± 3.83	1.232 (0.2205)
BCM (kg)	37.97 ± 5.27	38.11 ± 4.30	−0.115 (0.9085)	27.80 ± 3.11	27.32 ± 3.54	0.726 (0.4693)
VFA (cm^2)	43.17 ± 22.94	42.97 ± 25.46	0.035 (0.9719)	70.16 ± 32.87	45.67 ± 21.97	4.091 (<0.0001)
ICW (l)	26.51 ± 3.68	26.61 ± 3.00	−0.114 (0.9092)	19.41 ± 2.17	19.07 ± 2.47	0.733 (0.4652)
ECW (l)	15.84 ± 1.99	15.61 ± 1.69	0.507 (0.6134)	12.04 ± 1.37	11.48 ± 1.37	2.037 (0.0441)
ECW/TBW	0.375 ± 0.000	0.370 ± 0.000	2.629 (0.0099)	0.383 ± 0.000	0.376 ± 0.000	4.921 (<0.0001)
SBP (mmHg)	130.95 ± 12.79	123.09 ± 13.11	2.424 (0.0177)	127.72 ± 15.15	127.90 ± 15.91	−0.043 (0.9659)
DBP (mmHg)	77.73 ± 9.54	75.82 ± 10.34	0.779 (0.4384)	75.77 ± 19.08	75.75 ± 9.80	0.004 (0.9969)
Duration (years)	10.20 ± 8.83	–	–	8.80 ± 7.08	–	–

Data are shown as the mean ± standard deviation. BCM, body cell mass; BMI, body mass index; DBP, diastolic blood pressure; ECW, extracellular water; FFM, fat-free mass; FM, fat mass; HbA1c, glycated hemoglobin; ICW, intracellular water; l, liter; SBP, systolic blood pressure; SD, standard deviation; SMM, skeletal muscle mass; TBW, total body water; VFA, visceral fat area.

t-test for the merged data from the multicenter study

A t-test analysis was carried out to test the differences in segmental PhA values at 5 and 50 kHz between the diabetes and the control groups with the merged data from the three clinical sites. The results are shown in Table 3. PhA values at 250 kHz were excluded here and in further analyses due to variations between different sites. The PhA values of both arms between the control and diabetes groups showed large mean differences among men, and the PhA values of both legs showed large mean differences among women. In particular, segmental PhA values at 50 kHz consistently showed significant differences between the control and diabetes groups after the multicenter data were merged. The box plots of segmental PhA at 50 kHz are shown in Figure S2.

Phase angle with aging

Through the above basic analysis, we excluded PhA data at 250 kHz due to site variations. Hereafter, we focused exclusively on the PhA at 50 kHz, as the 50 kHz data were the most consistent, and showed significant differences between the diabetes and control groups. Accordingly, Figure 1 shows a decrease in segmental PhA with age in both the control and diabetes groups. In men, the PhA decreased rapidly with age in the diabetes group. The mean values of PhA in women with diabetes were generally lower than in controls in each age group (only one participant in the control group was in her 70s; thus, a statistical analysis of that age group was not feasible). As shown in Figure 1, PhA exhibited a more substantial decrease with age in people with diabetes than in normal controls, and the former group also had lower absolute values than the latter. The data show that both diabetes and aging induce some pathophysiological changes toward the reduction in PhA. Pearson's correlation coefficients between age and segmental PhA were between 0.26 and 0.44 for our data. The correlation coefficients of age and PhA according to the prevalence of diabetes were between 0.12 and 0.40 in the control group, and between 0.29 and 0.51 in the diabetes group. In addition, correlation coefficients were slightly higher in men and the diabetes group (ranging between 0.24 and 0.51) than in women and the control group (between 0.11 and 0.44).

Phase angle and the duration of diabetes mellitus

The segmental PhA values were statistically divided into tertiles according to age and disease duration, and presented as a scatter plot matrix to observe the effect of the duration of diabetes on the reduction in PhA, as shown in Figure 2. "DM = 0" indicates controls, "0 < DM < 5" indicates a diabetes duration >0 years and <5 years, "5 ≤ DM < 12" indicates a diabetes duration ≥5 years and <12 years, and "DM ≥ 12" indicates a diabetes duration ≥12 years. The segmental PhA values according to age and disease duration are shown in the female group on the left side and in the male group on the right side. The figure also shows the PhA values of the right arm, left arm, right leg and left leg in order from top to bottom. In the female

Table 2 | Mean and standard deviation of age, body mass index and segmental phase angles in women collected at three sites

Variables	DJ		WK		WS		F-values (P-values)
	Diabetes (n = 17)	Control (n = 11)	Diabetes (n = 31)	Control (n = 17)	Diabetes (n = 25)	Control (n = 9)	Site (DM: n = 158)
Age (years)	62.18 ± 6.93	60.82 ± 4.84	63.48 ± 8.23	48.12 ± 2.11	61.58 ± 7.68	51.89 ± 8.63	2.73 (0.0686)
Height (mm)	152.69 ± 6.06	151.79 ± 6.22	154.68 ± 5.15	158.41 ± 4.33	156.69 ± 5.15	156.00 ± 4.64	0.54 (0.5819)
Weight (kg)	60.86 ± 8.76	54.74 ± 6.33	65.21 ± 10.86	58.24 ± 10.10	57.73 ± 7.66	53.89 ± 5.38	3.13 (0.0464)
BMI (kg/m^2)	26.05 ± 3.07	23.72 ± 1.99	27.23 ± 4.08	23.15 ± 3.52	23.49 ± 2.79	22.09 ± 1.48	8.69 (0.0003)
PhA5RA (°)	2.20 ± 0.23	2.37 ± 0.13	2.15 ± 0.33	1.92 ± 0.27	1.99 ± 0.34	2.12 ± 0.22	2.74 (0.0679)
PhA5LA (°)	2.04 ± 0.26	2.16 ± 0.15	2.05 ± 0.36	1.85 ± 0.30	1.84 ± 0.26	1.91 ± 0.14	0.71 (0.4956)
PhA5TR (°)	3.17 ± 0.70	3.33 ± 0.36	2.91 ± 0.51	2.32 ± 0.87	3.00 ± 0.98	2.62 ± 0.87	1.32 (0.2701)
PhA5RL (°)	2.21 ± 0.35	2.57 ± 0.37	2.50 ± 0.59	2.54 ± 0.52	2.23 ± 0.38	2.84 ± 0.61	0.06 (0.9405)
PhA5LL (°)	2.27 ± 0.35	2.54 ± 0.38	2.37 ± 0.51	2.65 ± 0.78	2.34 ± 0.71	2.66 ± 0.43	0.28 (0.7538)
PhA5WB (°)	2.24 ± 0.23	2.48 ± 0.19	2.29 ± 0.37	2.12 ± 0.28	2.11 ± 0.31	2.38 ± 0.29	0.80 (0.4518)
PhA50RA (°)	5.24 ± 0.41	5.69 ± 0.25	5.32 ± 0.68	5.13 ± 0.48	4.98 ± 0.62	5.34 ± 0.58	0.26 (0.7716)
PhA50LA (°)	4.82 ± 0.41	5.26 ± 0.32	5.01 ± 0.70	4.89 ± 0.51	4.76 ± 0.58	5.21 ± 0.59	0.44 (0.6442)
PhA50TR (°)	5.74 ± 1.29	6.55 ± 0.95	4.98 ± 0.83	4.52 ± 1.31	6.18 ± 1.18	6.18 ± 1.46	16.42 (<0.0001)
PhA50RL (°)	5.47 ± 0.77	6.49 ± 0.94	5.96 ± 1.05	6.64 ± 0.88	5.47 ± 0.95	6.74 ± 0.79	0.46 (0.6302)
PhA50LL (°)	5.48 ± 0.82	6.34 ± 0.93	5.94 ± 1.12	6.66 ± 0.86	5.62 ± 0.99	6.86 ± 0.79	1.50 (0.2267)
PhA50WB (°)	5.32 ± 0.47	5.98 ± 0.48	5.51 ± 0.75	5.55 ± 0.50	5.18 ± 0.69	5.82 ± 0.57	0.07 (0.9362)
PhA250RA (°)	5.35 ± 0.47	5.95 ± 0.29	6.26 ± 0.64	6.25 ± 0.42	5.86 ± 0.68	6.62 ± 1.44	20.25 (<0.0001)
PhA250LA (°)	5.06 ± 0.39	5.61 ± 0.38	6.06 ± 0.61	6.10 ± 0.49	5.84 ± 0.67	6.90 ± 1.85	27.42 (<0.0001)
PhA250TR (°)	6.77 ± 2.12	7.92 ± 1.31	2.13 ± 1.69	1.24 ± 2.04	6.98 ± 2.61	7.44 ± 3.42	62.18 (<0.0001)
PhA250RL (°)	3.21 ± 0.48	3.96 ± 0.44	4.48 ± 0.76	4.95 ± 0.58	3.55 ± 0.81	4.42 ± 1.11	24.77 (<0.0001)
PhA250LL (°)	3.25 ± 0.65	3.82 ± 0.56	4.76 ± 0.70	5.22 ± 0.68	4.38 ± 0.74	5.14 ± 0.86	55.41 (<0.0001)
PhA250WB (°)	4.71 ± 0.44	5.39 ± 0.31	5.53 ± 0.60	5.65 ± 0.32	5.18 ± 0.67	5.97 ± 1.31	14.65 (<0.0001)

DJ, WK and WS are the three sites where clinical studies were carried out. DM, diabetes mellitus; LA, left arm; LL, left leg; PhA250, 250 kHz phase angle; PhA5, 5 kHz phase angle; PhA50, 50 kHz phase angle; RA, right arm; RL, right leg; TR, trunk; WB, whole body.

Table 3 | Results of t-tests of segmental phase angles measured at 5 and 50 kHz in the diabetes and control groups

Variables	Men				Women			
	Control mean (95% CI)	Diabetes mean (95% CI)	Mean_diff mean (95% CI)	t-values (P-values)	Control mean (95% CI)	Diabetes mean (95% CI)	Mean_diff mean (95% CI)	t-values (P-values)
PhA5RA	2.82 (2.68, 2.96)	2.46 (2.39, 2.54)	−0.357 (−0.517, −0.196)	−4.385 (<0.0001)	2.10 (1.99, 2.21)	2.11 (2.03, 2.18)	0.005 (−0.129, 0.139)	0.071 (0.9431)
PhA5LA	2.59 (2.45, 2.73)	2.35 (2.28, 2.42)	−0.238 (−0.393, −0.084)	−3.036 (0.0027)	1.96 (1.85, 2.06)	1.97 (1.90, 2.05)	0.015 (−0.115, 0.145)	0.224 (0.8234)
PhA5TR	3.19 (2.76, 3.63)	3.52 (3.30, 3.74)	0.324 (−0.166, 0.815)	1.303 (0.1939)	2.70 (2.36, 3.03)	3.00 (2.76, 3.24)	0.304 (−0.107, 0.715)	1.459 (0.1461)
PhA5RL	3.39 (3.13, 3.66)	3.03 (2.86, 3.61)	−0.366 (−0.663, −0.069)	−2.432 (0.0159)	2.62 (2.42, 2.82)	2.34 (2.20, 2.48)	−0.283 (−0.531, −0.035)	−2.247 (0.0256)
PhA5LL	3.25 (2.99, 3.50)	2.99 (2.86, 3.12)	−0.263 (−0.549, 0.024)	−1.807 (0.0721)	2.62 (2.42, 2.81)	2.34 (2.20, 2.48)	−0.279 (−0.520, −0.039)	−2.292 (0.0229)
PhA5WB	3.03 (2.90, 3.16)	2.69 (2.48, 2.89)	−0.342 (−0.533, −0.151)	−3.269 (0.0015)	2.29 (2.20, 2.38)	2.22 (2.11, 2.33)	−0.073 (−0.178, 0.032)	−1.120 (0.2652)
PhA50RA	6.51 (6.25, 6.76)	5.93 (5.80, 6.06)	−0.578 (−0.863, −0.293)	−4.000 (<0.0001)	5.35 (5.15, 5.54)	5.18 (5.05, 5.32)	−0.164 (−0.402, 0.075)	−1.351 (0.1783)
PhA50LA	6.11 (5.86, 6.35)	5.68 (5.56, 5.81)	−0.426 (−0.700, −0.151)	−3.059 (0.0025)	5.08 (4.89, 5.26)	4.88 (4.75, 5.01)	−0.196 (−0.426, 0.035)	−1.675 (0.0953)
PhA50TR	7.34 (6.71, 7.96)	6.86 (6.54, 7.18)	−0.472 (−1.176, 0.233)	−1.319 (0.1886)	5.53 (5.04, 6.01)	5.58 (5.23, 5.92)	0.049 (−0.545, 0.643)	0.161 (0.8719)
PhA50RL	7.69 (7.26, 8.12)	6.98 (6.76, 7.20)	−0.712 (−1.192, −0.231)	−2.917 (0.0039)	6.62 (6.29, 6.95)	5.68 (5.44, 5.91)	−0.946 (−1.350, −0.542)	−4.611 (<0.0001)
PhA50LL	7.42 (6.98, 7.87)	6.99 (6.76, 7.22)	−0.434 (−0.935, 0.066)	−1.711 (0.0886)	6.61 (6.27, 6.96)	5.72 (5.48, 5.97)	−0.891 (−1.312, −0.470)	−4.174 (<0.0001)
PhA50WB	6.92 (6.64, 7.21)	6.30 (5.75, 6.86)	−0.621 (−1.122, −0.119)	−3.658 (0.0004)	5.74 (5.44, 6.05)	5.35 (4.87, 5.83)	−0.393 (−0.814, 0.027)	−3.015 (0.0032)

CI, confidence interval; Mean_diff, mean difference.

group, the PhA values of both arms were no lower than those of the control group according to age, but in the group with a disease duration <12 years, the slope of the PhA decreased dramatically with age. In addition, the PhA values of both legs decreased sharply with age in the group with a diabetes duration of 5 years compared with those of the controls, and the average PhA values of individuals with durations corresponding to >5 years were lower than those of the controls. In the male group, as the duration of diabetes increased, the slope of segmental PhA according to age decreased steeply compared with that of the controls. To express the effect of decreasing segmental PhA values according to the duration of diabetes, the diabetes duration and segmental PhA are shown graphically by dividing the age tertiles, as shown in Figure 3. In the same age group, segmental PhA values were lower when the duration of diabetes was longer. It seemed that the duration of diabetes affected the reduction in PhA.

Moderated mediation effect analysis in multiple linear regression was applied to investigate the effect of the duration of diabetes on the segmental PhA values. The results of the mediation effect on duration are shown in Table 4. The total effect includes all effects on the reduction in PhA, the direct effect is an age effect known to directly affect the reduction in PhA, the mediation effect is the duration of diabetes that is expected to have an indirect effect on PhA values, and the proportion of mediation is the ratio of the mediation effect to the total effect. The coefficients for the direct effects on the right and left arms, and on the right and left legs were estimated to be −0.006 (95% confidence interval [CI] −0.020, 0.006), −0.008 (95% CI −0.022, 0.004), −0.052 (95% CI −0.073, −0.034) and −0.058 (95% CI −0.079, −0.041) for women, and −0.025 (95% CI −0.038, −0.001), −0.019 (95% CI −0.030, −0.009), −0.046 (95% CI −0.066, −0.026) and −0.045 (95% CI −0.066, −0.023) for men, respectively. The mediation effects (indirect effects) in the same segments were −0.007 (95% CI −0.012, −0.003), −0.007 (95% CI −0.012, −0.004), −0.010 (95% CI −0.017, −0.004) and −0.007 (95% CI −0.014, −0.001) for women, and −0.008 (95% CI −0.013, −0.003), −0.008 (95% CI −0.013, −0.004), −0.011 (95% CI −0.020, −0.004) and −0.008 (95% CI −0.017, −0.001) for men, respectively. The total effects in the same segments were −0.013 (95% CI −0.027, −0.000), −0.016 (95% CI −0.029, −0.002), −0.062 (95% CI −0.083, −0.044) and −0.065 (95% CI −0.084, −0.048) for women, and −0.032 (95% CI −0.045, −0.021), −0.027 (95% CI −0.039, −0.016), −0.057 (95% CI −0.077, −0.038) and −0.053 (95% CI −0.073, −0.031) for men, respectively. In addition, sex differences in the mediation effects of each segment were not significant. The proportion of the mediation effect of the duration of diabetes on the reduction in PhA was 32.9% and 36.3% in the right and left arms, respectively, and 17.9% and 12.7% in the right and left legs, respectively. In particular, the reduction in PhA values caused by the duration of diabetes in both arms in women and men occupied a larger proportion, which were 53.3% and
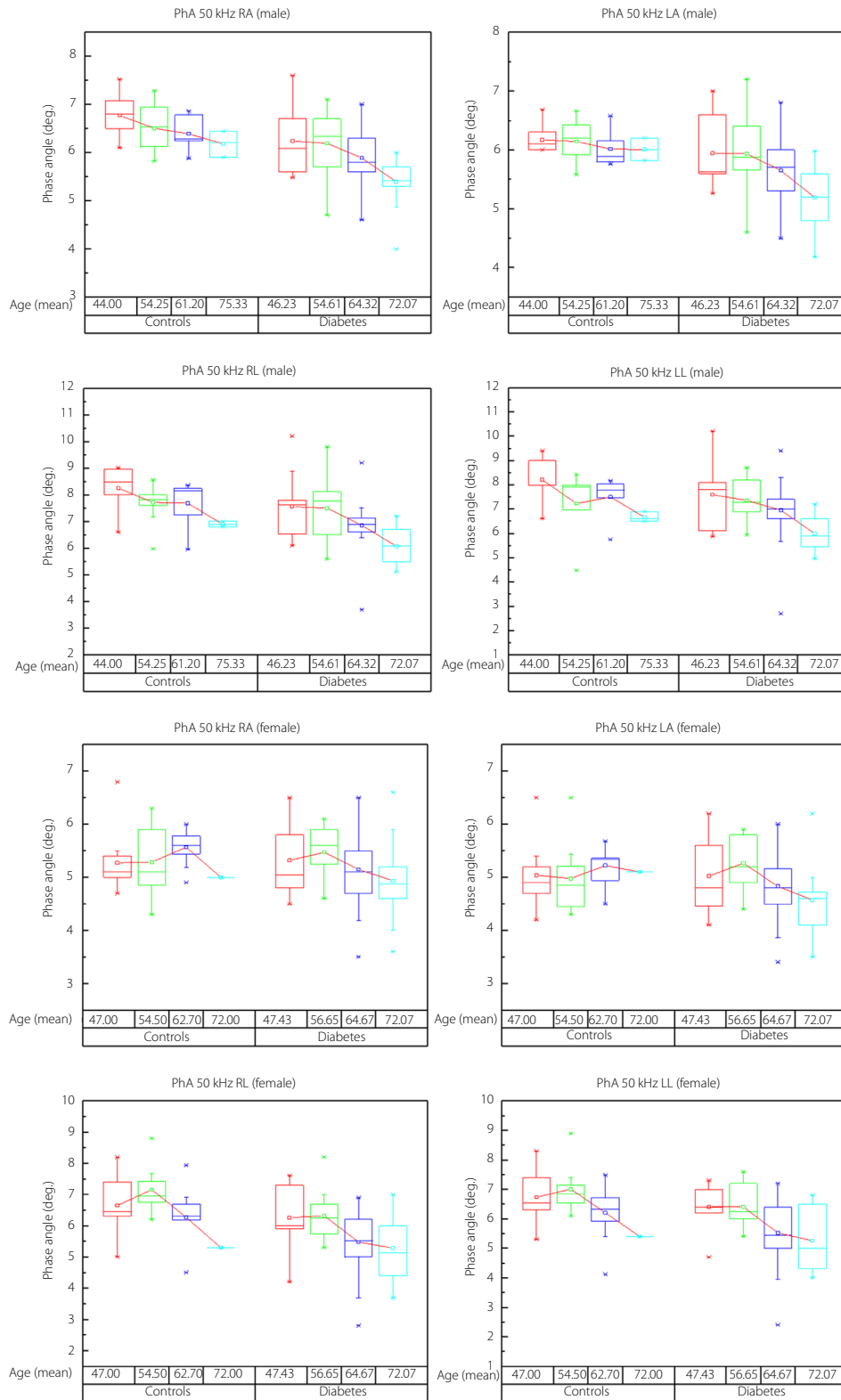
Figure 1 | Box plots of segmental phase angles (PhA) for controls and people with diabetes stratified according to age (the numbers of participants in the control and diabetes groups, respectively, are as follows: six and 13 aged in their 40s, eight and 18 aged in their 50s, five and 38 aged in their 60s, and three and 15 aged in their 70s for men; 18 and seven aged in their 40s, eight and 14 aged in their 50s, 10 and 39 aged in their 60s, and one and 14 aged in their 70s for women). deg., degree; LA, left arm; LL, left leg; RA, right arm; RL, right leg.
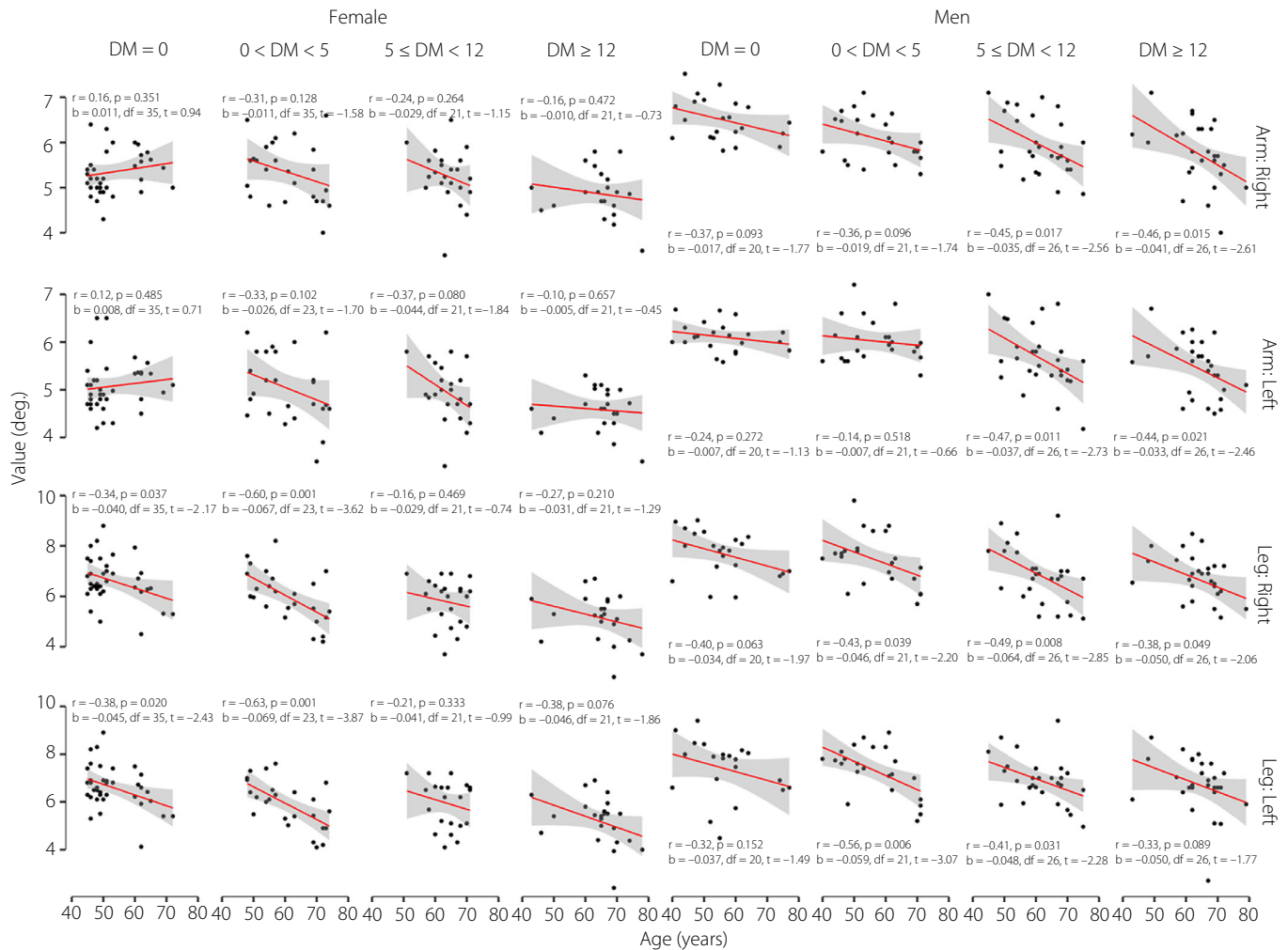
Figure 2 | Scatter plot matrix between age and segmental phase angles according to the subgroups of diabetes duration. Each red line represents the regression curve, and the shaded area indicates the 95% confidence interval for the slope of the regression curve. Pearson's correlation coefficient and its related statistics (correlation coefficient, regression slope, degrees of freedom, *t*-value and *P*-value) are denoted as *r*, *b*, df, *t* and *P*, respectively, on the top or bottom of each panel. deg., degree ; DM = 0, diabetes duration of 0 years (controls); 0 < DM < 5, diabetes duration >0 years and <5 years; 5 ≤ DM < 12, diabetes duration >5 years and <12 years; DM ≥ 12, diabetes duration >12 years.

46.9% in the right and left arms of women, and 23.9% and 29.8% in the right and left arms of men, respectively.

DISCUSSION

Many studies have reported that PhA decreases with advancing age[1,2,13,24]. The pathophysiological mechanism by which PhA decreases with aging is not yet well understood. In contrast, the PhA values of people with diabetes were reported to be lower than those of normal controls[3,10,12]. Using a multicenter study design, we found that PhA values at 50 kHz were decreased in people with diabetes, and the downward slope of PhA with age was steeper than that of the normal controls in the same age group. This finding implies that the decrease in PhA was caused by both physical aging and the duration of diabetes.

The onset of diabetes itself decreased the value of PhA compared with the levels observed in control participants without diabetes, and the PhA became even lower as the duration of diabetes increased; the PhA decreased, although the blood glucose and glycated hemoglobin levels were controlled by oral antidiabetic drugs. Therefore, PhA values are potentially useful not only in screening for diabetes, but also in monitoring the severity of diabetes. This result provides indirect evidence that controlling blood glucose and glycated hemoglobin levels is not sufficient to maintain the health of individuals with diabetes; thus, methods designed to improve the bioimpedance PhA of patients with diabetes should be developed.

According to several studies, a common factor contributing to organ damage in patients with type 2 diabetes mellitus is
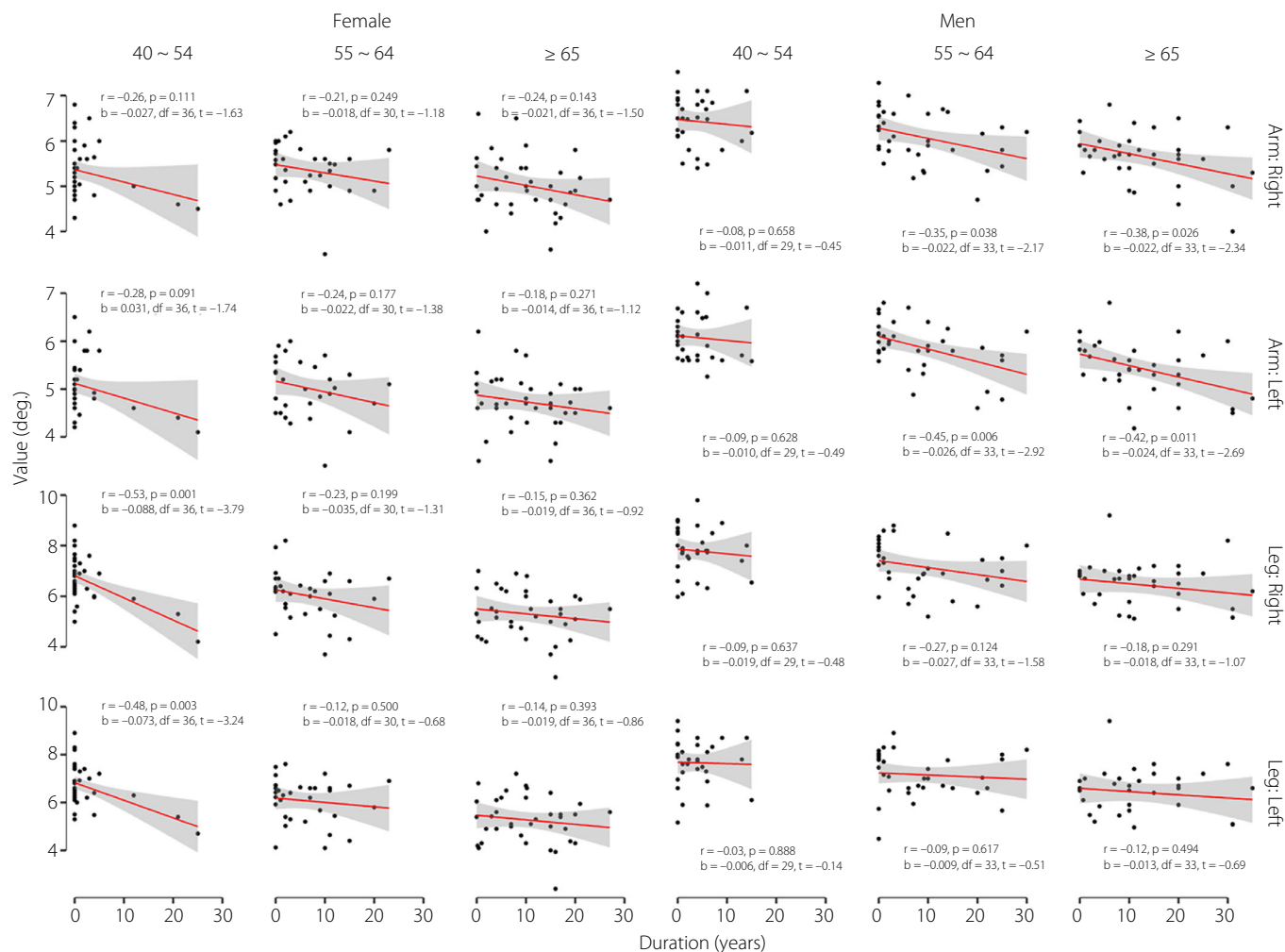
Figure 3 | Scatterplot matrix between disease duration and segmental phase angles according to age tertiles. Each red line represents the regression curve, and the shaded area indicates the 95% confidence interval for the slope of the regression curve. Pearson's correlation coefficient and its related statistics (correlation coefficient, regression slope, degrees of freedom, *t*-value and *P*-value) are denoted as *r*, *b*, df, *t* and *P*, respectively, on the top or bottom of each panel; 40–54, 55–64 and ≥65 years were the age tertiles for all participants. deg., degree.

impaired tissue blood flow caused by damage to vascular endothelial cells[27], which might be caused by a high average blood glucose concentration and/or large daily spikes in blood glucose levels[28]. In addition, the lack of a typical circulatory response to stress not only damages organs, but also affects functions throughout the body, such as balance, gait and the ability to respond to heat stress[29]. Glycemic variability was recently shown to be associated with mortality, cardiovascular diseases and diabetes-related complications[30–32]. Therefore, many articles have focused on continuous glucose monitoring and its importance in preventing complications in people with diabetes. The decrease in PhA observed using bioimpedance might reflect impairments in tissues caused by hyperglycemia or glycemic variability. Instead of the constant efforts associated with continuous blood glucose monitoring to prevent

complications, PhA values measured using bioimpedance are useful to simply determine the extent of tissue or organ damage.

In the moderated mediation effect analysis, we found that the PhA values of the arms were more sensitive than those of the legs to the duration of diabetes in both sexes. A prior study showed that muscle strength was inversely correlated with the duration of diabetes[33], and another study reported that PhA was correlated with age, FFM, height and the ECW/intracellular water ratio[34]. The loss of muscle mass, a component of FFM, has been reported as one of the major complications of diabetes mellitus[35–38]. Despite those supporting reports, no concrete evidence has confirmed pathophysiological changes in relation to the reduction in PhA with an increasing duration of diabetes.

Table 4 | Estimated mediation effect of the duration of diabetes on the association between age and segmental phase angles at 50 kHz

	Arm: right		Arm: left		Leg: right		Leg: left	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Total effect								
All	−0.023 (−0.031, −0.014)	<2e-16	−0.021 (−0.030, −0.012)	<2e-16	−0.06 (−0.074, −0.046)	<2e-16	−0.059 (−0.073, −0.045)	<2e-16
Female	−0.013 (−0.027, −0.000)	0.048	−0.016 (−0.029, −0.002)	0.022	−0.062 (−0.083, −0.044)	<2e-16	−0.065 (−0.084, −0.048)	<2e-16
Male	−0.032 (−0.045, −0.021)	<2e-16	−0.027 (−0.039, −0.016)	<2e-16	−0.057 (−0.077, −0.038)	<2e-16	−0.053 (−0.073, −0.031)	<2e-16
Direct effect								
All	−0.015 (−0.024, −0.006)	0.002	−0.013 (−0.023, −0.005)	0.004	−0.049 (−0.064, −0.034)	<2e-16	−0.051 (−0.067, −0.036)	<2e-16
Female	−0.006 (−0.020, 0.006)	0.34	−0.008 (−0.022, 0.004)	0.178	−0.052 (−0.073, −0.034)	<2e-16	−0.058 (−0.079, −0.041)	<2e-16
Male	−0.025 (−0.036, −0.014)	<2e-16	−0.019 (−0.030, −0.009)	<2e-16	−0.046 (−0.066, −0.026)	<2e-16	−0.045 (−0.066, −0.023)	<2e-16
Male–Female	−0.018 (−0.036, −0.001)	0.032	−0.011 (−0.028, 0.007)	0.206	0.006 (−0.020, 0.033)	0.656	0.013 (−0.015, 0.041)	0.4
Mediation effect								
All	−0.007 (−0.012, −0.004)	<2e-16	−0.008 (−0.012, −0.004)	<2e-16	−0.011 (−0.017, −0.005)	0.002	−0.007 (−0.014, −0.001)	0.026
Female	−0.007 (−0.012, −0.003)	<2e-16	−0.007 (−0.012, −0.004)	<2e-16	−0.01 (−0.017, −0.004)	<2e-16	−0.007 (−0.014, −0.001)	0.016
Male	−0.008 (−0.013, −0.003)	<2e-16	−0.008 (−0.013, −0.004)	<2e-16	−0.011 (−0.020, −0.004)	<2e-16	−0.008 (−0.017, −0.001)	0.032
Male–Female	−0.001 (−0.008, 0.006)	0.804	−0.001 (−0.008, 0.006)	0.798	−0.001 (−0.012, 0.009)	0.8	−0.001 (−0.012, 0.009)	0.874
Proportion of mediation								
All	0.329 (0.159, 0.586)	<2e-16	0.363 (0.186, 0.670)	<2e-16	0.179 (0.080, 0.299)	0.002	0.127 (0.010, 0.255)	0.026
Female	0.533 (0.114, 2.778)	0.048	0.469 (0.163, 1.605)	0.022	0.165 (0.068, 0.287)	<2e-16	0.11 (0.020, 0.226)	0.016
Male	0.239 (0.113, 0.406)	<2e-16	0.298 (0.140, 0.519)	<2e-16	0.196 (0.076, 0.364)	<2e-16	0.149 (0.014, 0.333)	0.032

Each effect is estimated using the following models: (1) $x_{dur} = \beta_{01} + \beta_{age}x_{age} + \beta_{sex}x_{sex} + \beta_{agexsex}x_{age}x_{sex} + \epsilon_{1}$; (2) $y_{k} = \beta_{02} + \beta_{age}x_{age} + \beta_{sex}x_{sex} + \beta_{agexsex}x_{age}x_{sex} + \beta_{dur}x_{dur} + \beta_{dur}x_{dur} + \epsilon_{2}$, where k indicates each limb. The non-parametric bootstrap 95% confidence intervals and P-values corresponding to each estimated effect for the limbs were obtained with the percentile method based on 1,000 bootstrap samples.

Some authors reported that people with diabetes showed declines in limb muscle mass and strength compared with controls[39,40]. However, we found no difference in SMM or FFM between the diabetes and control groups of both sexes in the present study. Therefore, the decrease in limb muscle mass might not be an essential feature in people with diabetes. According to a recent study, hyperglycemia in people with diabetes might induce a moderate osmotic effect, followed by a shift in water from the intracellular space to the extracellular space. The reduced PhA values in people with diabetes might result from a higher resistance induced by a moderate osmotic effect of hyperglycemia associated with diabetes[3]. The present results support this claim only weakly; there was no difference in TBW, intracellular water or ECW between the diabetes and control groups, except that women with diabetes had higher ECW content than women without diabetes. The differences in body composition indices were not significant between the groups with and without diabetes. In contrast, segmental PhA values showed significant differences. Therefore, PhA values might provide a relatively direct and straightforward reflection of pathophysiological changes due to diabetes and the gradual progression of the disease. Additional fundamental studies at the cellular level are required for further discussion.

The multicenter study design was an advantage of the present study. However, the present study had some limitations as well. One limitation was that the number of participants was not sufficient to provide disease and control groups of adequate size, and the control group had a smaller sample size than the disease group. Most participants with diabetes were type 2 diabetes patients who had mild symptoms and no complications as a result of diabetes, and were not prescribed insulin injections. As little is known about the characteristics of bioimpedance in type 1 diabetes, further study is required to establish whether the PhA features found in the present study can be applied to both type 1 and type 2 diabetes (we did not exclude patients with type 1 diabetes from study participation).

In addition, we failed to match age and BMI between the diabetes and control groups, and these factors are known to influence PhA[25]; nevertheless, the differences in mean segmental PhA values had the same tendency at all three measurement sites. Furthermore, the inclusion criteria for the control group included a fasting glucose level <140 mg/dL and/or HbA1c <47.5 mmol/mol (6.5%), values that are higher than the World Health Organization recommendations. If we selected non-diabetic controls according to the stricter World Health Organization criteria of fasting plasma glucose levels <126 mg/dL and HbA1c levels <42 mmol/mol (6%), better PhA outcomes would be expected, as the differences in fasting plasma glucose and HbA1c levels would be larger. All but nine of the 149 participants with diabetes were taking oral antidiabetic drugs. However, we were unable to provide detailed information on the medications used by the diabetes group, as the data from two clinics (WS and WK) did not include this information.

The present study was carried out entirely in ethnically Korean participants. We speculate that other ethnic groups would show the same tendency, but with shifted mean segmental PhA values. In addition, our study is the first to report a reduction in PhA according to the duration of diabetes; this finding should be confirmed through experiments in other ethnic groups.

DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Number of participants analyzed from each of the three sites.

Figure S1 | Schematic diagram of mediation and moderated mediation models: (a) total effect model; (b) mediation model, where a and b indicate the indirect effects of X on Y, whereas c' represents the direct effect of X on Y at a fixed value of M; and (c) moderated mediation model.

Figure S2 | Box plots and *t*-test results of segmental PhA in the diabetes and control groups.