Leucine metabolites do not induce changes in phase angle, bioimpedance vector analysis patterns, and strength in resistance trained men

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Head title: Effect of leucine supplementation in trained men

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Abstract

We aimed to assess the effects of off-the-shelf leucine metabolite supplements on phase angle (PhA), bioimpedance vector analysis (BIVA) patterns and strength during an 8-week resistance training protocol. Fifty-three male participants were allocated into 4 groups: α-hydroxyisocaproic acid ([α-HICA], n=12, age=30.9±9.3 yr), β-hydroxy-β-methylbutyrate free acid ([HMB-FA], n =12 , age=31.0±9.3 yr), calcium β-hydroxy-β-methylbutyrate ([HMB-Ca], n=15, age=32.1±5.2 yr) or placebo ([PLA]; n=14, age=28.9±6.6 yr). Bioimpedance parameters and 1 repetition maximum (1RM) for back squat and bench press were

assessed at baseline and at the end of weeks 4 and 8. Additionally, fat-free mass and fat mass were evaluated by dual-energy x-ray absorptiometry. No statistically group by time interactions were found, even adjusting for age. PhA and vector did not change over the training period, while time-dependent increases were observed for 1RM back squat and 1RM bench press. A direct association was observed between PhA and 1RM bench press changes (whole sample), while PhA and strength were correlated throughout the study, even when adjusting for fat-free mass and percentage of fat mass. Leucine metabolites have no effect on PhA, BIVA patterns or strength during an 8-week resistance training program, in resistance trained subjects.

The trial was registered at clincicaltrials.gov: NCT03511092.

Novelty:

- Supplementation with leucine metabolites is not a supplementation strategy that improves bioelectrical phase angle, cellular health, and strength after an 8-week resistance training program.

- When consuming a high protein diet, none of the α-hydroxyisocaproic acid, β-hydroxy-β-methylbutyrate free acid, and calcium β-hydroxy-β-methylbutyrate metabolites resulted in an ergogenic effect in resistance trained men

Key Words: BIVA, body composition, resistance training, R-Xc graph.

Introduction

The search for new strategies aiming to improve body composition and sports performance in athletes is one of the most discussed topics in sports science research. Not surprisingly, the focus has been shifted towards athletes' diet and nutritional supplements, which may support athletes to achieve optimal health and enhance physical performance.

Leucine is an essential branched-chain amino acid capable of stimulating protein synthesis via activation of the mammalian target of rapamycin (mTOR). More recently, leucine metabolites have also been thoroughly studied, with some research reporting extraordinary findings regarding body composition and performance. Conversely, previously studies have presented data not supporting a superior effect of these leucine derivatives (Teixeira et al. 2019; Sanchez-Martinez et al. 2017).

In recent years, research in the sports field has focused on the analysis of body composition and cellular health by monitoring the bioelectric phase angle (PhA) and bioimpedance vector analysis (BIVA) patterns, in order to monitor the improvement of

performance and achieve better sports results, as a team or individually (Campa et al. 2018a; Campa et al. 2020; Levi Micheli et al. 2014; Reis et al. 2020). PhA is calculated as an arctangent of the ratio between resistance (R) and reactance (Xc), therefore not depending on assumptions, and was found to accurately reflect the relationship between intra (ICW) and extra (ECW) cellular fluids (Campa et al. 2020a). Additionally, important relationships between PhA and the state of cellular health, muscle strength and therefore physical performance in different populations, including elite athletes, have been observed (Bourgeois et al. 2019; Campa et al. 2018b; Nabuco et al. 2019; Rodriguez-Rodriguez et al. 2016). Monitoring PhA and vector displacements into the R-Xc graph can be a practical way of monitoring body composition changes and therefore determining the evolution of an athlete during a training program (Campa et al. 2020; Reis et al. 2020). Recent research has shown that resistance training (RT) leads to an increase in PhA and a rise in cellular hydration and lean soft tissue (Ribeiro et al. 2017). Thus, BIVA provides more detailed information than the evaluation of PhA alone and is therefore now widely applied in the

sports field (Carrasco-Marginet et al. 2017; Reis et al. 2020). In fact, by monitoring BIVA patterns, direct bioimpedance parameters (R and Xc) may be considered as a vector within a graph whose displacement following intervention programs or during the competitive season indicates increases or decreases in fluids and muscle mass (Campa et al. 2020b; Melchiorri et al. 2018; Reis et al. 2020).

As previously stated, data pertaining leucine metabolites on body composition and performance is equivocal, however the importance of mTOR as a cellular regulator interface in health and disease, warrants more research towards cellular health in athletes. As mentioned above, changes in PhA and BIVA patterns identify changes in body composition after a training period; in particular, increases in muscle mass can be the result of increases in PhA and vector displacements in the R-Xc graph. However, to our knowledge, no research has explored the effects of leucine metabolites on bioelectric PhA and BIVA patterns, during an 8-week RT program and how these changes might relate with physical performance. For this reason, the goal of this study is to assess the effects of off-the-shelf

leucine metabolite supplements on PhA, BIVA patterns, and physical performance during an 8-week RT program.

Methods

Participants and study design

Fifty-three men were recruited according to the eligibility criteria stated elsewhere (Teixeira et al. 2019) with 40 completing the investigation. Participants were between the ages of 18 and 45 yr and were recruited from social networks and local gyms, being engaged in RT for at least 1 yr and training at least three times per week. Subjects were randomly assigned to groups, according to a randomly generated list and allocated in varying block sizes with participants matched for baseline grip strength, age and fat-free mass (FFM). As a result, no baseline statistically significant differences existed among groups for handgrip strength, age, fat mass (FM) or FFM as previously described (Teixeira et al. 2019). This investigation was approved by the Faculty of Human Kinetics Institutional Review Board

(approval number 15/2017) and conformed to all standards of human research set out in the declaration of Helsinki. Participants were informed about the objectives and the procedures of the study and signed the informed consent form. The trial was registered at clincicaltrials.gov as NCT03511092.

Supplementation and diet control

Participants were individually instructed by licensed and trained dieticians to consume sufficient energy and protein to allow for training-induced gains of lean mass. Additionally, each participant received a commercial form of either α-hica α-hydroxyisocaproic acid (α –HICA; HICA, Onsalesit, SA, Funchal, Portugal), β-hydroxy-β-methylbutyrate free acid (HMB-FA; Beta-TOR, Body Attack, Hamburg, Germany), β-hydroxy-β-methylbutyrate calcium (HMB-Ca; HMB Mega Caps 1250, Olimp Labs, Pustynia, Poland), or placebo (magnesium stearate; EightJuice, Seixal, Portugal), depending on the group they were randomly assigned. The bioactive components of the supplements were

tested for purity by an independent lab. Participants ingested supplements three times daily, alongside with meals or before the workout: 3 x 500 mg for α -HICA and 3 x 1 g for HMB-FA, HMB-Ca or placebo.

Body composition and performance assessments

Body composition and performance assessments were made at baseline, weeks 4 and 8, comprising of:

Body composition and Bioimpedance measurements

Height was recorded to the nearest 0.1 cm with a stadiometer, and weight was measured to the nearest 0.1 kg with a high-precision scale (Secca, Hamburg, Germany). Dual-energy x-ray absorptiometry (DXA) was performed according to standard procedures described elsewhere (Matias et al. 2013) on a Hologic Explorer-W, fan-beam densitometer (Hologic, Waltham, Massachusetts, USA) to obtain total whole-body FM and FFM.

The bioimpedance parameters (R and Xc) were directly measured with a multispectrometer analyzer (BIS model 4200, Xitron Technologies, San Diego, CA, USA) at a frequency of 50 kHz, as previously stated by our group (Matias et al. 2013). Whole-body R and Xc were analyzed according to the BIVA method (Campa et al. 2019a; Piccoli et al. 1994) and PhA was calculated as the arctangent of Xc/R × 180°/π. BIVA was carried out using the classic methods, e.g., normalizing R and Xc for height (H) in meters. The R-Xc graph was used to plot the BIVA data; in this approach, the mean bioimpedance vectors are considered in relation to their specific reference population (Campa et al. 2019b).

Muscle strength

Muscle strength was assessed by one repetition maximum (1RM) of the bench press and back squat exercises. The evaluation of 1RM was obtained on a Multipower machine (Model-M953; Technogym, Cesena, Italy), according to the National Strength and Conditioning Association (NSCA) guidelines, as described before (Teixeira et al. 2019).

Training protocols

The training protocol consisted of whole-body hypertrophy-type RT routine for intermediate-trained individuals and consisted of three training sessions per week, during an 8-wk period, with a minimum of 48-h interval between sessions, as previously described by our group (Teixeira et al. 2019) (Figure 1).

*** Please, insert Figure 1 near here ***

Statistical analysis

Descriptive statistics was applied to characterize the sample. All variables were assessed for normality, using Shapiro-Wilk test. The main hypothesis was interpreted using analysis of variance for repeated measures with time (baseline, weeks 4 and 8) as within-subject variables, and group (α-HICA, HMB-FA, HMB-Ca, and PLA) as the between-subject factor. When a significant F ratio was obtained, a Bonferroni post hoc test was used to evaluate the time or group effect. In addition, analysis of covariance for repeated measures

was applied for comparisons, using age as covariate. The paired, one-sample Hotelling T^2-test was performed to determine if the changes in the mean group vectors (measured at the baseline, weeks 4 and 8) were significantly different from zero (null vector). Multiple regression analysis was conducted to further test whether PhA was related with 1RM back squat and 1RM bench press, after adjusting for FFM and FM%. In all regression analyses, residuals were tested for normality. Statistical analysis was performed using the statistical software SPSS v.21, IBM® (IBM Corporation, Chicago, IL, USA) Significance was set at $P < 0.05$.

Results

No differences ($P > 0.05$) were found for dietary intake throughout the study. High compliance levels were reported among participants regarding both supplement intake and training sessions. Body composition of the participants is showed according to supplementation and time of the study in Table1.

*** Please, insert Table 1 near here ***

No time-group interactions were found ($P > 0.05$) for any of the bioimpedance or strength parameters (Table 2; Figure 2), even adjusting for chronological age. A significant effect of time ($P < 0.05$) was detected for 1RM bench press and back squat for all the groups, where an increase was measured during the training period (Table 2) (Figure 3).

*** Please, insert Table 2, Figure 2, and Figure 3 near here ***

The vector displacement was plotted on the R-Xc graph, and the results of the paired one-sample Hotelling T^2-test were not only not significantly different ($P > 0.05$) but also similar in all the groups (Figure 4). In particular, in the right panel of Figure 4 the mean vector displacements considering the bivariate variations in R/H and Xc/H are shown; a 95% confidence ellipse excluding the null vector indicates a significant vector shift over time.

*** Please, insert Figure 4 near here ***

As no interaction was found between supplementation groups and time in these relevant variables, an additional association analysis between PhA and strength was performed using the whole sample (excluding groups). Significant direct associations were detected in all assessment moments between PhA and 1RM back squat (baseline: $r = 0.301$, $P = 0.042$; week 4: $r = 0.433$, $P = 0.003$; week 8: $r = 0.525$, $P = 0.002$) and between PhA and 1RM bench press (baseline: $r = 0.545$, $P < 0.001$; week 4: $r = 0.595$, $P < 0.001$; week 8: $r = 0.729$, $P < 0.001$) (Figure 5). These associations remained significant even when corrected for FFM and FM% with variance inflation factors even below of 5. In this regard, by adding FFM and FM% as additional independent variables, all models increase their ability in 1 RM back squat (independent variables: PhA and FFM: baseline: $R^2 = 0.305$, $P = 0.001$; week 4: $R^2 = 0.426$, $P < 0.001$; week 8: $R^2 = 0.382$, $P = 0.002$; independent variables:

PhA, FFM, and FM%: baseline: $R^2 = 0.326$, $P = 0.001$; week 4: $R^2 = 0.447$, $P < 0.001$; week 8: $R^2 = 0.402$, $P = 0.004$) and 1RM bench press predictions (independent variables: PhA and FFM: baseline: $R^2 = 0.544$, $P < 0.001$; week 4: $R^2 = 0.527$, $P < 0.001$; week 8: $R^2 = 0.647$, $P < 0.001$; independent variables: PhA, FFM, and FM%: baseline: $R^2 = 0.554$, $P < 0.001$; week 4: $R^2 = 0.531$, $P < 0.001$; week 8: $R^2 = 0.648$, $P < 0.001$) . Regarding differences between baseline and week 4, a direct association ($r = 0.396$; $P = 0.014$) was observed between PhA and 1RM bench press variations, even when adjusted for FFM and FM%.

*** Please, insert Figure 5 near here ***

Discussion

The aim of this study was to analyse the effects of an 8-week supplementation protocol with leucine metabolites and RT program on PhA and BIVA patterns by further analysing the relationship of these patterns with strength parameters. Based on previous reports (Sanchez-Martinez et al. 2017), our hypothesis was that participants would not have

changes in bioelectrical proprieties, reflecting body composition, during a program of RT with supplementation of leucine metabolites. Our results show, for the first time, that leucine metabolites supplementation does not play a decisive role to improve PhA or BIVA patterns. In all the 4 groups the observed PhA and BIVA patterns are in line with the proposed references for the sport population, with a vector positioning within the 50th percentile of specific tolerance ellipses (Campa et al. 2019b). However, after 4 weeks and at the end of the training period (8-weeks), no change on the bioimpedance parameters was observed, thus not generating a significant displacement of the vector in the R-Xc graph. Therefore, independently of the group, no changes for these bioimpedance parameters were observed, but a significant increase of strength, assessed by one repetition maximum squat and bench tests, was verified.

Previous studies have shown that changes in PhA following a RT program can be attained after a significant time in athletes. It has been reported that increases in muscle mass can occur after 3-4 weeks of RT (DeFreitas et al. 2011). Using a study design of 16

weeks of progressive RT, Ribeiro et al. (2017) showed an increase in PhA, regardless of gender, contrarily Roberts et al. (2017) did not detect changes in PhA after a 10-day RT protocol, with the administration of a protein supplement during the recovery period, perhaps related with the aforementioned question regarding the training period.

In our study, the athletes performed an 8-week RT workout, although this time frame may be too short to observe bioimpedance adaptations, increases in PhA and vector shifts to the left side of the R-Xc graph reflect increases in ICW/ECW ratio, as well as increases in body cell mass and lean soft tissue. In an early study, Mascherini et al. (2015) evaluated vector changes in the sports field for the first time, suggested that displacements to the left on the R-Xc graph were associated with body cell mass increase, during a competitive season in soccer players. Subsequently, Campa et al. (2020) in a 6-month observational study showed that vector displacements were associated with TBW changes, using BIVA and dilution techniques in male and female athletes. Additionally, several studies involving athletes of different competitive levels have identified vector differences when comparing

elite athletes with other groups of lower performance level categories, showing higher R/H values for the same Xc/H, thus reflecting a greater fluid content with the same cell density (Campa and Toselli, 2018a; Levi Micheli et al. 2014).

Throughout the duration of this study no vector changes were observed, which is in line with the body composition findings. Currently, there is no rationale to supplement with leucine or any leucine metabolite, when ingesting sufficient protein (Holland et al. 2019). This has been shown by recent meta-analysis [23] in both young subjects and athletes. It should, however, be noted that the effect of leucine metabolites in clinical setting populations may be more promising, albeit a direct comparison with a leucine supplement is still lacking (Deutz et al. 2013; Teixeira et al. 2018).

A recent systematic review has highlighted that changes in performance following a RT program will occur after 4 weeks, making this method a valid approach to increase muscle strength (Schoenfeld et al. 2016). In our case, 8 weeks were not sufficient to induce changes in bioimpedance parameters and therefore representative of fluid or even body cell

mass changes. However, significant improvements were observed for 1RM squat and the bench press. This is in agreement with previous studies showing that 8 weeks of RT are sufficient to induce strength gains (Madruga-Parera et al. 2020).

Another finding of our study is the positive association between PhA and 1RM squat and bench press tests, at baseline and both week 4 and 8. This is in line with previous studies where PhA has been correlated with sprint performance and muscle strength increases (Reis et al. 2020; Rodriguez-Rodriguez et al. 2016). Despite these associations, PhA should not be considered as a standalone parameter but in addition to the graphical displacement of the vector, since athletes with a similar PhA and therefore ICW/ECW ratio may display different dislocations and a higher or lower TBW content, resulting in different body composition outcomes (i.e. quantity and quality of fat free mass) (Campa et al. 2019b).

Summarizing, despite some literature suggestion that leucine metabolites may increase muscle strength, by observing the PhA and BIVA patterns, we can state that the cellular health and quality did not change. The results of the performance tests suggest that

an increase of strength occur (Table 2), but only when considering the sample as a whole, not discriminating the different leucine metabolites or even placebo. Therefore, we are positive to state that leucine metabolites do not give an enhancement of the skeletal muscle and the observed increases of the 1RM can derive from a myriad of neural adaptations (Enoka, 1988) in addition to changes in muscle moment arms (Sugisaki et al. 2015) and a greater lateral muscle force transmission (Erskine et al. 2010).

Considering the innovative approach of BIVA patterns and PhA in the sports field, one should bear in mind that this study displays a noteworthy strong point in the experimental design, since it is comprised of 3 different types of leucine metabolites and a control group. In addition, the foot-to-hand measurement technique used in this study represents the most accurate approach in measuring bioimpedance parameters and its ability to detect bioelectrical changes over time has been shown in previous studies (Campa et al. 2020a; Silva et al. 2019). Still, some limitations need to be considered. First of all, our results are applicable and comparable only with data obtained from instruments that analyze

bioelectrical impedance at a frequency of 50 Hz, given that different results can be obtained based on the type of frequency used. Furthermore, as only male subjects have been included, our conclusions should not be extrapolated to the female population. Future studies are warranted to address the aforementioned limitations and also insofar to test different training protocols and populations.

Conclusions

Leucine metabolites supplementation during an 8-week resistance training program does not induce changes in phase angle and BIVA patterns in resistance trained individuals nor affect the changes detected for 1RM back squat and bench press. This study shows that despite the absence of changes in phase angle and BIVA patterns, they were significantly and directly associated with strength. Interpretation of PhA and vector shifts on the R-Xc graph may be used by nutritionist and coaches as a practical method to monitor body composition changes and to adapt training and nutrition in athletes. The supplementation

with leucine metabolites showed no effect on PhA, BIVA patterns or even physical performance, when comparing with placebo, thus suggesting no interest in this supplement when trying to improve these parameters.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this article.

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Figure caption

Figure 1. The 8-week resistance training program.

Figure 2. Box-plot figures of phase angle changes during the 8-week resistance training program.

Figure 3. 1 repetition maximum (1RM) for back squat and bench during the 8-week resistance training program. Data are reported as mean and standard deviation. * = Different from baseline, # = Different from baseline and week 4 ($P<0.01$).

Figure 4. On the left side mean impedance vectors, plotted on the 50%, 75%, and 95% tolerance ellipses of the male athlete's reference population (Campa et al. 2019b) are displayed for all the groups. On the right side, mean vector displacements and results of the Hotelling's T^2 test.

Figure 5. Correlation between phase angle and 1 repetition maximum (1RM) for back squat and bench at baseline, week 4, and week 8.

Table 1. Descriptive parameters throughout the resistance training program.

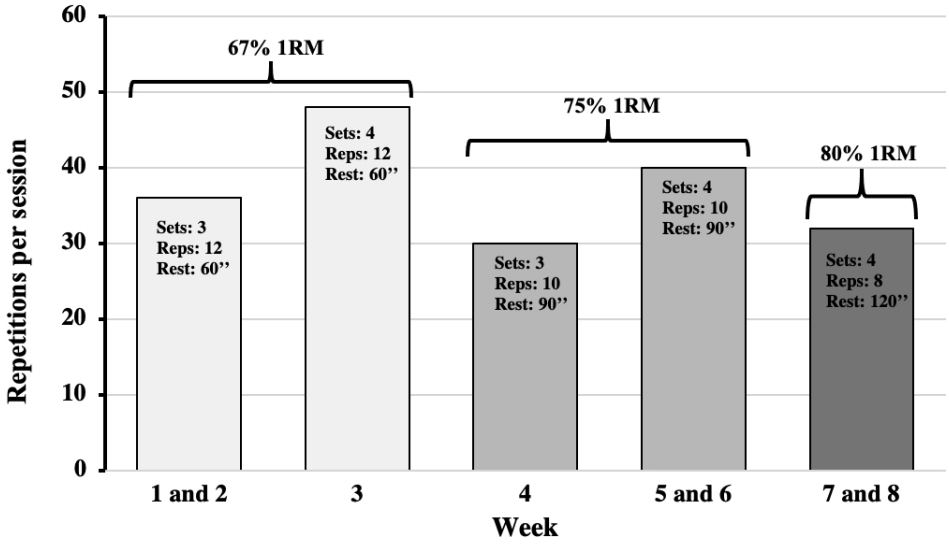
	Group	Baseline	4 week	8 week
		Mean (SD)	Mean (SD)	Mean (SD)
Fat-free mass (kg)	HMB-FA (n=12)	63.3 (9.4)	63.2 (1.0)	63.1 (10.6)
	PLA (n=14)	63.6 (5.7)	62.9 (5.4)	63.6 (5.3)
	α-HICA (n=12)	63.0 (7.6)	62.9 (8.3)	62.9 (8.2)
	HMB-CA (n=15)	67.1 (1.0)	67.9 (1.0)	67.6 (8.9)
Fat mass (kg)	HMB-FA	13.9 (3.9)	13.5 (3.8)	13.1 (3.9)
	PLA	10.7 (2.3)	11.2 (2.5)	10.5 (1.7)
	α-HICA	10.9 (4.6)	10.8 (4.3)	10.8 (4.1)
	HMB-CA	11.6 (3.6)	10.9 (1.4)	10.7 (3.3)

Note: HMB-FA = β-hydroxy-β-methylbutyrate free acid group, PLA = placebo group, α-HICA = α-hydroxyisocaproic acid group, HMB-Ca = calcium β-hydroxy-β-methylbutyrate group.

Table 2. Bioelectrical and performance parameters throughout the resistance training program.

		Baseline	4 week	8 week	ANOVA					
					Time effect			Time x group		
	Group	Mean (SD)	Mean (SD)	Mean (SD)	F	P	η^2p	F	P	η^2p
R/H (ohm/m)	HMB-FA (n=12)	235.1 (37.2)	232.7 (36.2)	229.0 (35.1)	0.45	0.63	0.19	0.68	0.66	0.79
	PLA (n=14)	221.7 (24.5)	220.1 (25.4)	219.9 (24.6)						
	α-HICA (n=12)	228.3 (32.7)	229.8 (32.9)	230.8 (39.8)						
	HMB-CA (n=15)	211.7 (28.8)	211.0 (28.7)	210.8 (32.5)						
Xc/H (ohm/m)	HMB-FA	34.9 (5.5)	33.3 (5.4)	33.3 (4.6)	2.49	0.94	0.94	1.38	0.24	0.14
	PLA	34.2 (5.4)	32.8 (5.1)	34.8 (4.3)						
	α-HICA	34.3 (3.6)	33.9 (4.5)	35.4 (4.7)						
	HMB-CA	32.0 (4.2)	31.3 (4.3)	30.8 (4.7)						
PhA (°)	HMB-FA	8.5 (0.8)	8.1 (0.5)	8.3 (0.5)	2.90	0.65	0.10	1.14	0.36	0.12
	PLA	8.7 (0.6)	8.5 (0.7)	9.0 (1.0)						
	α-HICA	8.6 (0.5)	8.4 (0.7)	8.8 (0.9)						
	HMB-CA	8.6 (0.7)	8.5 (0.6)	8.3 (0.5)						
1RM Bench Press (kg)	HMB-FA	90.4 (30.1)	97.7 (29.8) *	103.2 # (31.4)	34.07	<0.01	0.50	0.26	0.95	0.02
	PLA	92.7 (24.1)	101.3 (23.0) *	105.4 # (25.7)						
	α-HICA	95.1 (29.9)	100.6 (24.6) *	105.9 # (25.7)						
	HMB-CA	89.3 (18.6)	95.7 (23.9) *	98.4 # (23.9)						
1 RM Back Squat (kg)	HMB-FA	135.0 (30.4)	139.9 (29.7) *	155.3 # (34.4)	41.31	<0.01	0.55	1.05	0.40	0.08
	PLA	134.5 (23.7)	147.8 (22.6) *	156.3 # (25.3)						
	α-HICA	123.2 (28.7)	135.3 (30.3) *	145.9 # (33.4)						
	HMB-CA	133.0 (25.1)	138.9 (25.6) *	145.0 # (24.3)						

Note: R/H = resistance standardized for height, Xc/H = reactance standardized for height, PhA = phaase angle, RM = repetition maximum, HMB-FA = β-hydroxy-β-methylbutyrate free acid group, PLA = placebo group, α-HICA = α-hydroxyisocaproic acid group, HMB-Ca = calcium β-hydroxy-β-methylbutyrate group, * Different from baseline, # Different from baseline and week 4 (P<0.01).



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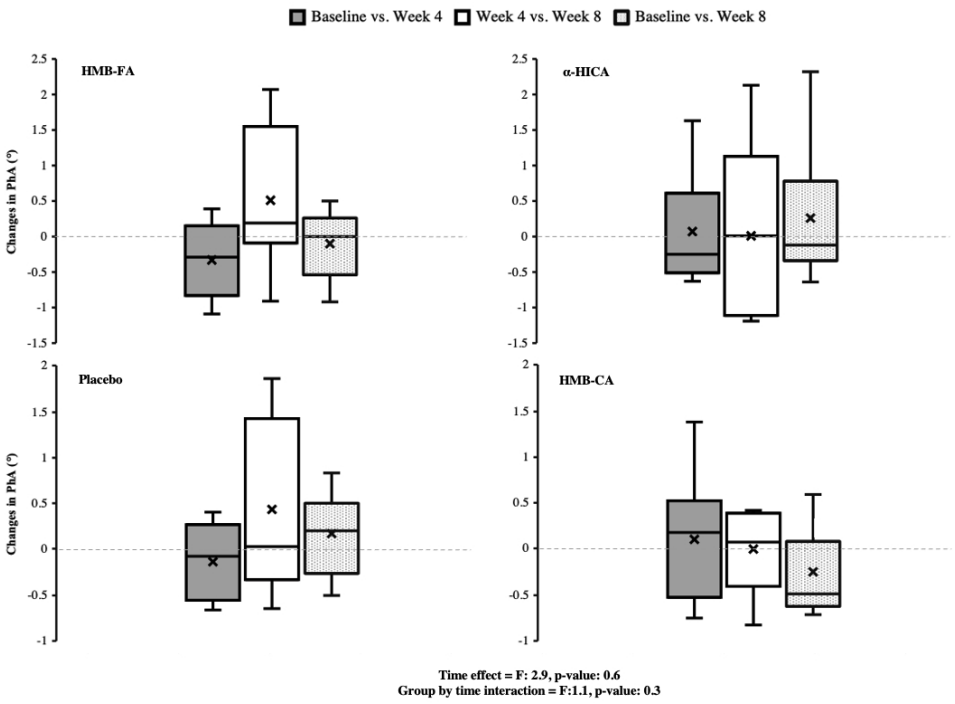

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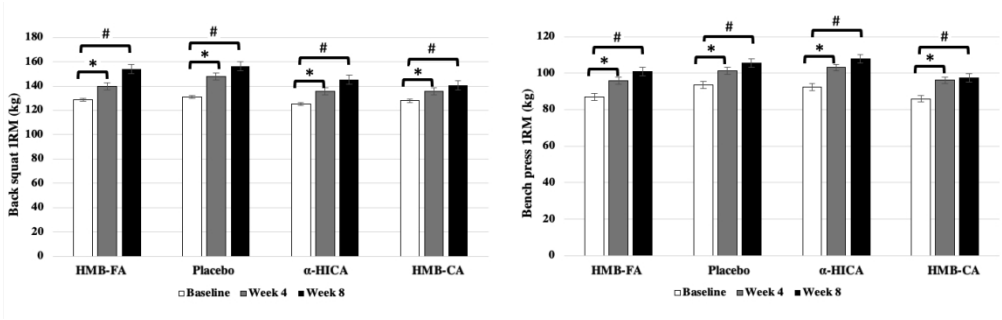

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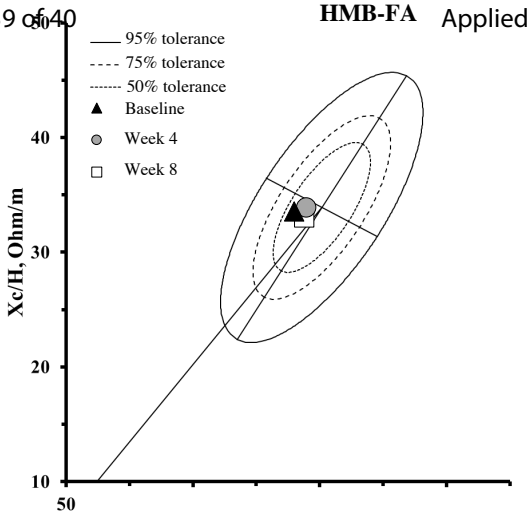
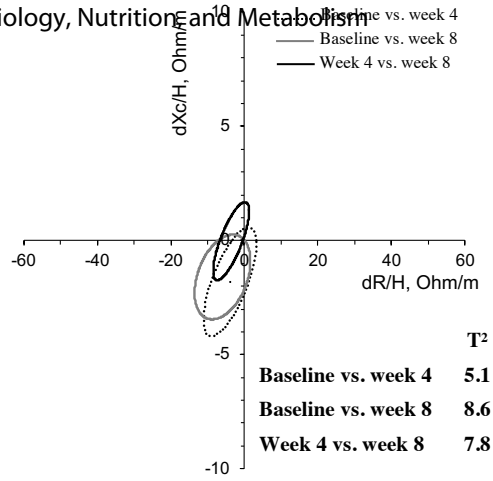
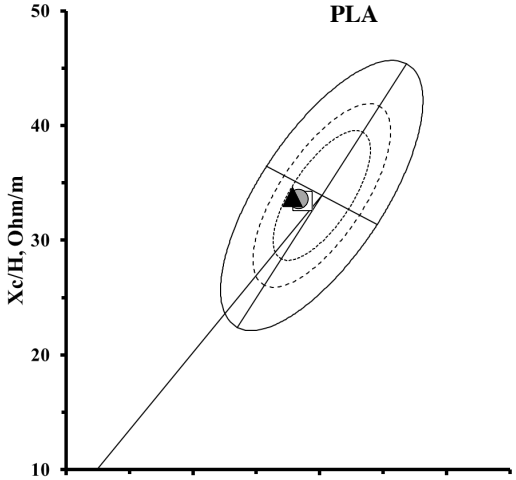
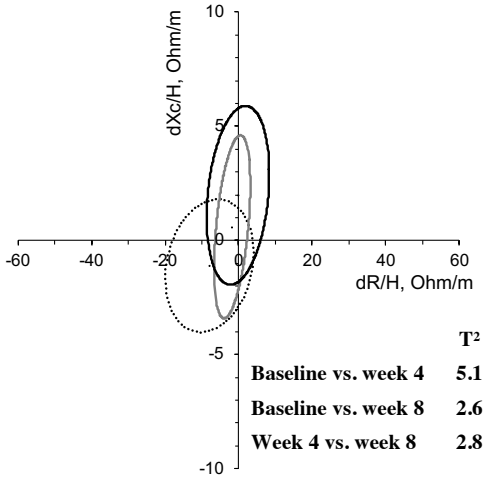


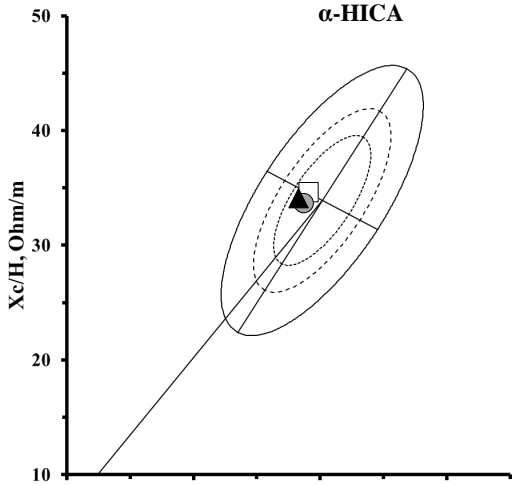


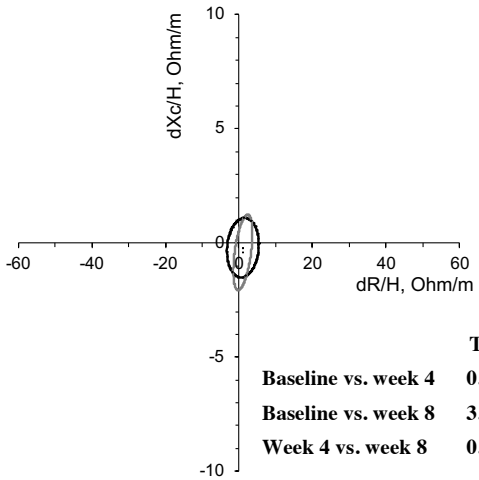
	T^2	P
Baseline vs. week 4	**5.1**	**0.18**
Baseline vs. week 8	**8.6**	**0.15**
Week 4 vs. week 8	**7.8**	**0.16**



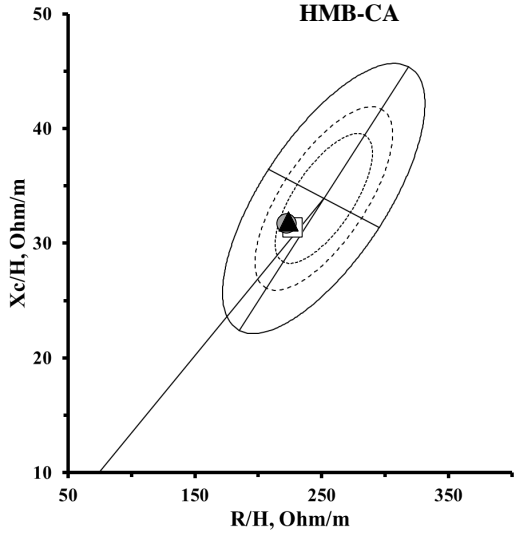


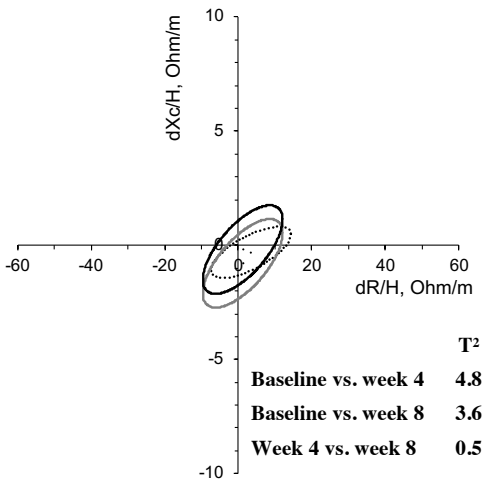
	T^2	P
Baseline vs. week 4	**5.1**	**0.21**
Baseline vs. week 8	**2.6**	**0.34**
Week 4 vs. week 8	**2.8**	**0.32**



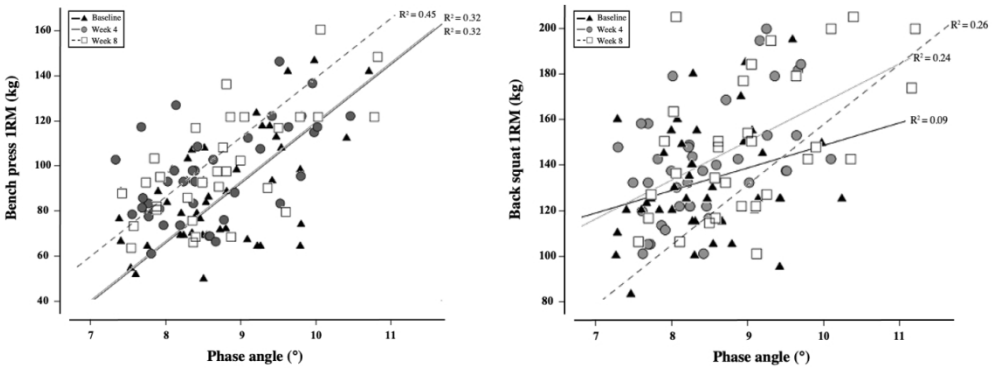


	T^2	P
Baseline vs. week 4	**0.8**	**0.78**
Baseline vs. week 8	**3.6**	**0.29**
Week 4 vs. week 8	**0.9**	**0.79**





	T^2	P
Baseline vs. week 4	**4.8**	**0.21**
Baseline vs. week 8	**3.6**	**0.29**
Week 4 vs. week 8	**0.5**	**0.89**



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