PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Elastic-band resistance exercise or vibration treatment in combination with hydroxymethylbutyrate (HMB) supplement for management of sarcopenia in older people: a study protocol for a single-blinded randomized controlled trial in Hong Kong
AUTHORS	Chim, Yu Ning; Chow, Simon Kwoon Ho; Cheng, Keith Yu Kin; Ho, Chung Yan; Ho, Wing Tung; Cheng, Kenneth Chik-Chee; Wong, Ronald Man Yeung; Cheung, Wing Hoi

VERSION 1 – REVIEW

REVIEWER	Michal Steffl Charles University, Czech Republic
REVIEW RETURNED	28-Nov-2019

GENERAL COMMENTS	Physical activities and nutrition are important in older people are definitely necessary for healthy aging. The study would be interesting because it is going to deal on these two important variables as they relate to sarcopenia. However, some clarifications, adjustments and inclusions have to be made. I am a little confused with the methods. Based on your title you are supposed to have two experimental groups and control group. However, you chose a wrong statistical tool. A repeated measure ANOVA will not inform you where the differences between groups lie, as it is an omnibus statistical test. The repeated measure ANOVA serves perfectly when measuring 3 times (and more) one group. You need something that allows you to see between-group as well as within-group effects. I suggest that you should use ANCOVA with Bonferroni correction as a post-hoc analysis. Than you may use the baseline as covariate, within-group effect size (e.g. Cohen d) as the dependent variable, and groups as fixed factor. I am not happy that you did not calculate a real power analysis. Just to say that we will use similar group sizes that we already used is not a right approach. I strongly recommend that you should calculate a power analysis for ANCOVA that is for example available in Excel using the XLSTAT statistical software. However, what are you going to do if the data do not come from a normal distribution? Are you going to test normality? Statistical significance should not be $p \leq 0.05$ it has to be $p < 0.05$ or even less! Why are you going to do the regression analysis? I do not see any reason for the regression analysis. What factors are supposed to be included? This exactly you may avoid by using ANCOVA. I understand what the interventions groups will do; nevertheless, what about the control group? Will be the participants from control group offered of any beneficial activities? Will be the control “active” or “passive” one? According to me, you should offer them

	some physical activities after finishing the trial. This would be an ethically correct approach.

REVIEWER	Javier Courel-Ibáñez University of Murcia, Spain
REVIEW RETURNED	29-Nov-2019

GENERAL COMMENTS	This is a very interesting and well-written protocol facing the aging-related sarcopenia with HMB and exercise combined. I just find some minor comments to address, in particular, about the need of further discussion on the potential benefits (or not) of HMB when combined with exercise in older adults, based on two recent reviews on the topic. Abstract: please define HMB in the text. Introduction: Whereas the exercise-based interventions are well justified, the combined nutritional strategy with HMB requires further literature. Certainly, HMB is a promising strategy as a nutritional supplement strategy when taken alone. Nonetheless, the current trial includes combined “HMB + exercise” interventions. There is a very recent meta-analysis on 10 RCT suggesting that HMB supplementation in addition to physical exercise may have no or fairly low impact in improving body composition, muscle strength, or physical performance in adults aged 50 to 80 years, compared to exercise alone (1). In this sense, the current protocol includes a “HMB-only” group as controls, therefore it will be possible to identify whereas the gains in strength and muscle mass came from the exercise or the HMB, and confirm the need of exercises (mainly resistance training) to manage sarcopenia. I find this is an important point that should be included in the introduction. 1. Courel-Ibáñez, Vetrovsky, Dadova, Pallarés, Steffl. Health Benefits of β-Hydroxy-β-Methylbutyrate (HMB) Supplementation in Addition to Physical Exercise in Older Adults: A Systematic Review with Meta-Analysis. Nutrients. 2019;11:2082. doi:10.3390/nu11092082. Sample size: Muscle strength, please clarify the test. After checking the paper cited, I would say this is the leg extension 1RM (kg). Discussion: At some point [P14, L18], the authors state the benefits of HMB in old people, but there is no information about the potential effects of HMB + exercise combined. I suggest reviewing the abovementioned meta-analysis on 10 RCT (1) and the recent review on the physical exercise in the oldest old (2) to include some lines about the potential anabolic, anticatabolic and anti-inflammatory effects of regular resistance training, which may be even more effective than HMB. 1. Courel-Ibáñez, Vetrovsky, Dadova, Pallarés, Steffl. Health Benefits of β-Hydroxy-β-Methylbutyrate (HMB) Supplementation in Addition to Physical Exercise in Older Adults: A Systematic Review with Meta-Analysis. Nutrients. 2019;11:2082. doi:10.3390/nu11092082. 2. Valenzuela PL, Castillo-García A, Morales JS, Izquierdo M, Serra-Rexach JA, Santos-Lozano A, et al. Physical exercise in the

	oldest old. Compr Physiol. 2019;9:1281–304.doi: 10.1002/cphy.c190002. Limitations: I agree this study has important strengths, but the authors should make and effort and detail the difficulties to be overcome in the future.

REVIEWER	Hiroyasu Mori Tokushima university, Japan
REVIEW RETURNED	05-Feb-2020

GENERAL COMMENTS	Comments to the Author This is a practically important research indicates that need to clarify from when elastic-band resistance exercise or vibration treatment in combination with hydroxyl methylbutyrate (HMB) supplement for management of sarcopenia in older people: a single-blinded randomized controlled trial. However, it is necessary to description the method etc. in several respects. After the first revision of this paper, there are still some points to description as below. Major Comments Title The title is misspelling. Hydroxy methylbutyrate Method, Inclusion and Exclusion Criteria Page 7, line 48-55: The subjects who cannot use a vibration machine (v-health), should be excluded from this study. Can subjects using the artificial cardiac pacemaker use a vibration machine? Method, HBM supplement Page 8, line 33-46: Please describe in detail how to take the supplement. 1. The name of the HMB supplement should be listed. 2. Do subjects intake 3g of HBM in divided doses? 3. When are the HMB intake: in the morning or in the afternoon, before breakfast or after breakfast, before lunch or after lunch? After exercise or vibration? The time of food intake may affect the results of this study. Method, Elastic-band Exercise Page 8, line 49- Page 9, line 26: When are the Elastic-band Exercise: in the morning or in the afternoon, before breakfast or after breakfast, before lunch or after lunch? Before intake HMB supplement? The time of food intake may affect the results of this study. In this context, the exercise regimen needs to be more detailed: chair-based resistance exercise? Method, Vibration Treatment Page 9, line 29-42: Can subjects using the artificial cardiac pacemaker use a vibration machine? Primary Outcome, Muscle Strength in the Lower Extremity Page 10, line 6-21: The name of the lower leg extension machine should be listed. Page 11, line 47-55: The manuscript should describe dietary management of total energy or protein intake during the intervention. In the pre- and post-intervention period, the total energy intake or protein intake amount may affect the results of this study.

REVIEWER	Dennis Görlich University of Münster, Germany
REVIEW RETURNED	04-Mar-2020

GENERAL COMMENTS	The authors present a clearly written study protocol. With respect to the statistical aspects of the study design and planned data analysis the protocol contains some points to discuss (see below). Overall, this is a very clearly written manuscript and a well planned trial. The following points need some clarification. Minor comments are: Sample size is clearly justified and the effect size (f-statistic) can be reproduced with 0.2487 (compared to 0.248 in the manuscript, which is potentially a rounded number). The reproduced sample size was N=120 (compared to N=123 in the manuscript). Adjusting for 15% dropout then leads to a final sample size of N=120/0.85= 141.2 (result as reported). But I would assume that the reported N=141 was calculated as (123 x 1.15) which ignores that additionally recruited patients might drop-out also. Finally, the planned recruiment size is the same, so the studies power is very likely not impaired, but I would suggest to recheck the numbers and correct if necessary. Additionally, the planned sample size is based on a correlation among repeated measures of 0.5 (checked by recalculation). Please add this (or the correct choice) to your justification of the sample size. Also, while this might be a reasonable choice, no prior information (e.g. from the literature) is mentioned to support it. In particular, stronger correlations would inflate the sample size, i.e. reduce power (to ca. 70% in the worst case) if detected during analysis. The planned two-factor repeated measure ANOVA (to cope with 3 groups at 2 visits) seems appropiate to analyze normally distributed outcomes. A strategy on data analysis if normality is not given is not described. Also a general strategy (choice of methods) for other explorative analyses might be helpful. The description of the strcture of primary confirmatory analysis could be improved in the statistical analysis paragraph. I assume that the global test of the ANOVA (Type III test on the randomized group factor) was planned as primary objective, since power calculations were planned on the ANOVA's effect size f. A Bonferroni-correction of the post-hoc tests (which then might be secondary or explorative), to not inflate the global confirmatory multiple significance level, could be reconsidered. Furthermore, the closed testing principle might be applicable for post-hoc tests. Additionally, I suggest the following minor changes: Title: Please add of the terms "study protocol"/"trial protocol"/"protocol" appropiately to the manuscript's title, otherwise it might be mistaken for a result paper.

	Figure (Flow diagram): Please add the "Randomization" Box before the group allocation to conform CONSORT flow charts for randomized trials. Also please move the Baseline assessment (as one box) before the randomization step, since this was the order in the manuscript (Baseline assessment => randomization => Intervention => 3months assessment). Please add a figure caption to your manuscript. The figure caption should also explain the abbreviations used in the figure.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Nº		Response	Changes and Location
1	I am a little confused with the methods. Based on your title you are supposed to have two experimental groups and control group. However, you chose a wrong statistical tool. A repeated measure ANOVA will not inform you where the differences between groups lie, as it is an omnibus statistical test. The repeated measure ANOVA serves perfectly when measuring 3 times (and more) one group. You need something that allows you to see between-group as well as within-group effects. I suggest that you should use ANCOVA with Bonferroni correction as a post-hoc analysis. Then you may use the baseline as covariate, within-group effect size (e.g. Cohen d) as the dependent variable, and groups as fixed factor. I am not happy that you did not calculate a real power analysis. Just to say that we will use similar group sizes that we already used is not a right approach. I strongly recommend that you should calculate a power analysis for ANCOVA that is for example available in Excel using the XLSTAT statistical software. However, what are you going to do if the data do not come from a	Your comments are well-taken. We agree that the suggested ANCOVA would be a more comprehensive strategy to control for the various other measurements collected in this study. We would include this analysis method as our statistical analysis plan. However, after checking the sample size calculation for this statistical method by GPower, n=160 is required. Thus, the final sample size would be n=188 with adjustment for 15% dropout, which is 44 more than our current sample size. This increase in sample size would be limited by the funding agency and therefore may not be feasible. Therefore, two-way repeated measure ANOVA would remain the choice for this study to investigate the within-subject and between-group changes in the outcomes; where ANCOVA would be utilized as a strategy to reveal potential confounders. Wilcoxon signed-rank test will be used when data do not follow normality.	Lines 285-297

	normal distribution? Are you going to test normality? Statistical significance should not be p≤0.05 it has to be p<0.05 or even less! Why are you going to do the regression analysis? I do not see any reason for the regression analysis. What factors are supposed to be included? This exactly you may avoid by using ANCOVA.	Statistical significance has been revised as p<0.05.	
2	I understand what the interventions groups will do; nevertheless, what about the control group? Will be the participants from control group offered of any beneficial activities. Will be the control "active" or "passive" one? According to me, you should offer them some physical activities after finishing the trial. This would be an ethically correct approach.	Thank you for the comment. No activities are provided to the control group because there is no well-accepted recommended clinical intervention for sarcopenia patients. Advice is usually given to patients in normal clinical practice. For the participants in the control group, we will follow the current clinical practice to offer them advice on exercise and diet based on their body conditions at their end-point assessment. A print-out of this information (attached as supplementary material) will be given to each participant as a reminder to reinforce our advice. Vibration treatment or elastic-band exercise will be arranged for them when necessary after they complete the end-point assessments.	Supplementary file 2; Lines 220-222

Reviewer 2

Nº		Response	Changes and Location
1	Abstract: please define HMB in the text.	Thank you for the comment. HMB has been defined in the abstract.	Line 31
2	Introduction: Whereas the exercise-based interventions are well justified, the combined nutritional strategy with HMB requires further literature. Certainly, HMB is a promising strategy as a nutritional supplement strategy	Your suggestion is well taken. We agree that HMB when taken alone is a promising strategy and therefore it was	Lines 104-119; Lines 350-352

	when taken alone. Nonetheless, the current trial includes combined “HMB + exercise” interventions. There is a very recent meta-analysis on 10 RCT suggesting that HMB supplementation in addition to physical exercise may have no or fairly low impact in improving body composition, muscle strength, or physical performance in adults aged 50 to 80 years, compared to exercise alone (1). In this sense, the current protocol includes a “HMB-only” group as controls, therefore it will be possible to identify whereas the gains in strength and muscle mass came from the exercise or the HMB, and confirm the need of exercises (mainly resistance training) to manage sarcopenia. I find this is an important point that should be included in the introduction. 1. Courel-Ibáñez, Vetrovsky, Dadova, Pallarés, Steffl. Health Benefits of β-Hydroxy-β-Methylbutyrate (HMB) Supplementation in Addition to Physical Exercise in Older Adults: A Systematic Review with Meta-Analysis. Nutrients. 2019;11:2082. doi:10.3390/nu11092082.	taken into consideration when this study was planned. However, our recently published pre-clinical study showed that 3-month of combined vibration treatment and HMB supplementation enhanced muscle strength and decreased percentage fat mass and intramuscular fat infiltration as compared with HMB alone in sarcopenic mice models. This revealed that HMB supplementation alone is not as effective as combined treatments in managing sarcopenia. Thus, in this study, we mainly focus on the effect of combined HMB and exercise on sarcopenic people, which are still not well investigated. Also, increase in sample size would be limited by funding agency and there would be difficulties to recruit more sarcopenic people, so adding HMB-only group is not feasible. Without the HMB-only group, we will not able to distinguish whether the changes in muscle mass or strength are contributed by HMB or vibration or elastic band exercise. We have added this in the limitation part. As for your suggested paper by Courel-Ibanez et al, the conclusions drawn from the available literature may be attributed to the individual study’s characteristics including the inclusion of healthy and younger subjects from 50 years of age (instead of targeted disease group). The suggestion is well-taken	

		and we have included this into our introduction to support our choice of intervention. In our current study, subject inclusion will be sarcopenic patient based on the AWGS definition and aged 65 or above.	
3	Sample size: Muscle strength, please clarify the test. After checking the paper cited, I would say this is the leg extension 1RM (kg).	Thank you for the comment. The test has been added accordingly.	Line 128
4	Discussion: At some point [P14, L18], the authors state the benefits of HMB in old people, but there is no information about the potential effects of HMB + exercise combined. I suggest reviewing the abovementioned meta-analysis on 10 RCT (1) and the recent review on the physical exercise in the oldest old (2) to include some lines about the potential anabolic, anticatabolic and anti-inflammatory effects of regular resistance training, which may be even more effective than HMB. 1. Courel-Ibáñez, Vetrovsky, Dadova, Pallarés, Steffl. Health Benefits of β-Hydroxy-β-Methylbutyrate (HMB) Supplementation in Addition to Physical Exercise in Older Adults: A Systematic Review with Meta-Analysis. Nutrients. 2019;11:2082. doi:10.3390/nu11092082. 2. Valenzuela PL, Castillo-García A, Morales JS, Izquierdo M, Serra-Rexach JA, Santos-Lozano A, et al. Physical exercise in the oldest old. Compr Physiol. 2019;9:1281–304.doi: 10.1002/cphy.c190002.	Thank you for the suggestions. We have reviewed these two publications and included them in our discussion. The potential benefits of combined HMB and exercise as well as the potential effects of resistance training have been further discussed in the discussion part.	Lines 324-327; Lines 332-337; References 11 and 18
5	Limitations: I agree this study has important strengths, but the authors should make an effort and detail the difficulties to be overcome in the future.	Thanks for the comment. Some limitations of this study have been listed in the discussion part.	Lines 343-352

Reviewer 3

Nº		Response	Changes and Location
1	*Title*: The title is misspelling. Hydroxy methylbutyrate *Method, Inclusion and Exclusion Criteria* Page 7, line 48-55: The subjects who cannot use a vibration machine (v-health), should be excluded from this study. Can subjects using the artificial cardiac pacemaker use a vibration machine?	Thank you for your reminder. The misspelled word in the title has been corrected. We do not recommend those with artificial cardiac pacemakers or cancers to use the vibration platform as the safety data is lacking. Also, chair-bound or bed-bound subjects are excluded as they cannot stand on the vibration platform. These have been added to the exclusion criteria.	Title; Lines 175-178
2	*Method, HMB supplement* Page 8, line 33-46: Please describe in detail how to take the supplement.	Subjects are provided HMB capsules to be taken three times daily with meals at 1g doses.	Lines 194-196
3	1. The name of the HMB supplement should be listed	The name of the HMB supplement used has been added.	Line 194
4	2. Do subjects intake 3g of HBM in divided doses?	The subjects are instructed to take the total 3g of HMB supplement three times a day (3 x 1.0 g), separately with meals.	Lines 194-196
5	3. When are the HMB intake: in the morning or in the afternoon, before breakfast or after breakfast, before lunch or after lunch? After exercise or vibration? The time of food intake may affect the results of this study.	HMB is taken one hour before exercise or vibration and with meals to aid compliance.	Lines 194-196
6	*Method, Elastic-band Exercise* Page 8, line 49- Page 9, line 26: When are the Elastic-band Exercise: in the morning or in the afternoon, before breakfast or after breakfast, before lunch or after lunch? Before intake HMB supplement? The time of food intake may affect the	It is a chair-based elastic band resistance exercise. Exercise is administered one hour after HMB intake.	Lines 194-196; Line 202; Line 205

	results of this study. In this context, the exercise regimen needs to be more detailed: chair-based resistance exercise?		
7	*Method, Vibration Treatment* Page 9, line 29-42: Can subjects using the artificial cardiac pacemaker use a vibration machine?	We do not recommend those with artificial cardiac pacemakers or cancers to use the vibration platform as the safety data is lacking. This has been added in Methodology in the revised manuscript.	Lines 175-177
8	Primary Outcome, Muscle Strength in the Lower Extremity Page 10, line 6-21: The name of the lower leg extension machine should be listed.	A digital dynamometer will be used. The name of digital dynamometer used to measure quadriceps muscle strength has been added.	Line 230
9	Page 11, line 47-55: The manuscript should describe dietary management of total energy or protein intake during the intervention. In the pre- and post-intervention period, the total energy intake or protein intake amount may affect the results of this study.	A food frequency questionnaire (FFQ) will be conducted at baseline and all subjects are advised to stick to their current dietary intake during the study period. Another FFQ at final assessment will be used to validate the consistency in their diet during study period.	Lines 271-272

Reviewer 4

Nº		Response	Changes and Location
1	Sample size is clearly justified and the effect size (f-statistic) can be reproduced with 0.2487 (compared to 0.248 in the manuscript, which is potentially a rounded number). The reproduced sample size was N=120 (compared to N=123 in the manuscript). Adjusting for 15% dropout then leads to a final sample size of N=120/0.85= 141.2 (result as reported). But I would assume that the reported N=141 was calculated as (123 x 1.15) which ignores that additionally recruited patients might drop-out also. Finally, the planned recruitment size is the same, so the studies power is very likely not impaired, but I would	Thank you for the comment. We have re-checked the sample size using GPower and cross-validated the numbers for accuracy. As pointed out, the statistical power is not impaired, we still opt for n=123 to boost the statistical power at 0.81 compared to 0.8 for n=120. Thus, the final adjusted sample size will be n=123/0.85=144. The corresponding numbers have been corrected.	Line 132; Line 136

	suggest to re-check the numbers and correct if necessary.		
2	Additionally, the planned sample size is based on a correlation among repeated measures of 0.5 (checked by recalculation). Please add this (or the correct choice) to your justification of the sample size. Also, while this might be a reasonable choice, no prior information (e.g. from the literature) is mentioned to support it. In particular, stronger correlations would inflate the sample size, i.e. reduce power (to ca. 70% in the worst case) if detected during analysis.	Thank you for the comment. The information about correlation among repeated measures has been added. Also, we have added literature support for this choice when justifying the sample size calculation.	Lines 138-139
3	The planned two-factor repeated measure ANOVA (to cope with 3 groups at 2 visits) seems appropriate to analyze normally distributed outcomes. A strategy on data analysis if normality is not given is not described. Also a general strategy (choice of methods) for other explorative analyses might be helpful.	Thank you for the comment. Normality will be assessed by visual inspection of Q-Q plots. Wilcoxon signed-rank test will be used when data do not follow normality. A strategy for other explorative analyses has been added as well.	Lines 285-297
4	The description of the structure of primary confirmatory analysis could be improved in the statistical analysis paragraph. I assume that the global test of the ANOVA (Type III test on the randomized group factor) was planned as primary objective, since power calculations were planned on the ANOVA's effect size f. A Bonferroni-correction of the post-hoc tests (which then might be secondary or explorative), to not inflate the global confirmatory multiple significance level, could be reconsidered. Furthermore, the closed testing principle might be applicable for post-hoc tests.	Thank you for the comment. The description of the structure of primary confirmatory analysis has been improved.	Lines 285-297
5	Title: Please add of the terms "study protocol"/"trial protocol"/"protocol" appropriately to the manuscript's title, otherwise it might be mistaken for a result paper.	Thank you for the comment. “A study protocol” has been added in the title.	Title
6	Figure (Flow diagram): Please add the "Randomization" Box before the group allocation to conform CONSORT flow charts for randomized trials. Also please move the Baseline assessment (as one box) before the randomization step, since	Thank you for the suggestion. The flow diagram has been revised accordingly and figure caption has been added as well.	Figure 1; Lines 457-458

	this was the order in the manuscript (Baseline assessment => randomization => Intervention => 3months assessment). Please add a figure caption to your manuscript. The figure caption should also explain the abbreviations used in the figure.		

VERSION 2 – REVIEW

REVIEWER	Javier Courel-Ibáñez University of Murcia, Spain
REVIEW RETURNED	07-Apr-2020

GENERAL COMMENTS	The authors have made a great job and correctly addressed all my previous suggestions. I must also congratulate the editor and reviewers for their valuable suggestions. This is an interesting intervention and I am looking forward to see the results.

REVIEWER	Hiroyasu Mori Institute of Advanced Medical Sciences, University of Tokushima, Japan
REVIEW RETURNED	07-Apr-2020

GENERAL COMMENTS	Comments to the Author This is a practically important research indicates that need to clarify from when elastic-band resistance exercise or vibration treatment in combination with hydroxylmethylbutyrate (HMB) supplement for management of sarcopenia in older people: a study protocol for a single-blinded randomized controlled trial. After the second revision of this paper, there are still points to description as below. Major Comments Method, HBM supplement Supplementary file 2; 預防骨質疏鬆及少肌症的健康小貼士 Is the HMB supplement used in this intervention study "Ensure NutriVigor", which contains enriched HMB made by Abbott, Co? The manuscript describes it as an HMB capsule made by Double Wood Supplements. Please describes in this manuscript the amount of energy, macro-nutrients (protein, fat, carbohydrate) and micro-nutrients contained in the "Ensure NutriVigor". For example, the "Ensure NutriVigor" per one serving.

REVIEWER	Dennis Görlich University of Münster, Germany
REVIEW RETURNED	14-Apr-2020

GENERAL COMMENTS	The authors answered the reviewers comments appropriately.

VERSION 2 – AUTHOR RESPONSE

Response to reviewer's comments
Comment: Method, HBM supplement
Supplementary file 2; 預防骨質疏鬆及少肌症的健康小貼士
Is the HMB supplement used in this intervention study "Ensure NutriVigor", which contains enriched HMB made by Abbott, Co?
The manuscript describes it as an HMB capsule made by Double Wood Supplements.
Response: The supplementary file 2 is a piece of educational material supplied to research subjects at THE END of the study containing some recommendations. The "Ensure NutriVigor" is an example of product that is known to contain HMB used in this study and is NOT used as an intervention. Therefore, the supplement product used in this study is accurately described in the manuscript, and is purchased from Double Wood Supplements of USA. No revision has been made to the manuscript.