STATISTICAL ANALYSIS PLAN AND RESULTS REPORT

ProMuscle Study

Clinical Trial on the use of a protein module enriched with Hydroxymethylbutyrate (HMB) in patients with deficient protein intake and/or increased protein requirements.

Official Title	ProMuscle Study. Clinical Trial on the use of a protein module enriched with Hydroxymethylbutyrate (HMB) in patients with deficient protein intake and/or increased protein requirements.
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Prepared for submission as supporting results documentation to ClinicalTrials.gov. This report pertains to the study record identified above.

1. Study identification and purpose

This document consolidates the prespecified statistical approach described in the study protocol with the final statistical analyses performed for the ProMuscle2025 clinical trial. It is intended to provide a clear, study-specific statistical record and a concise, clinically interpretable summary of the main results.

The trial was designed as a prospective, randomized, open-label, two-parallel-group clinical study in adults with deficient protein intake and/or increased protein requirements. Participants were allocated to either standard nutritional and exercise care (Control) or the same program plus an HMB-enriched whey-protein module (ProSup Protein®). Follow-up was 3 months.

2. Analysis populations and statistical principles

A total of 120 participants were included in the statistical dataset, with 60 participants in each randomized group. Analyses used all available observations for each endpoint. Continuous variables were summarized using mean, standard deviation, median and range as appropriate; categorical variables were summarized as counts and percentages.

Baseline comparability between groups was assessed using Student’s t test or the Mann-Whitney U test for continuous variables and chi-square tests for categorical variables. Within-group change was explored using paired t tests or Wilcoxon signed-rank tests according to distributional assumptions.

The principal between-group analyses of final outcomes were performed using analysis of covariance (ANCOVA), adjusting for the corresponding baseline value and relevant covariates. Depending on the endpoint, models included age, sex and GLIM category, as well as prespecified interaction terms. Two-sided p values <0.05 were considered statistically significant. Bonferroni-adjusted post-hoc comparisons were used where applicable. Effect size was summarized using partial eta squared (η^2) and Cohen’s d.

Categorical outcomes were explored with binomial or multinomial logistic regression. These models are reported as adjusted associations because the original statistical output used coded category levels; therefore, category-specific odds ratios should be interpreted together with the database coding dictionary.

3. Baseline comparability

The randomized groups were well balanced at baseline. No statistically significant between-group differences were observed for demographic, nutritional, functional or muscle-ultrasound variables included in the baseline comparison.

Variable	Control	ProSup	p value
Age, years	62.15 ± 14.08	63.87 ± 13.22	0.493
Weight, kg	65.68 ± 15.21	65.71 ± 13.92	0.990
BMI, kg/m²	24.72 ± 5.27	24.38 ± 4.53	0.709
Phase angle, degrees	4.88 ± 0.91	4.64 ± 0.93	0.147
Handgrip strength, kg	27.17 ± 9.50	28.23 ± 11.35	0.581
Timed Up and Go, s	7.36 ± 3.22	7.22 ± 2.77	0.793
30-s sit-to-stand, repetitions	14.69 ± 5.58	14.79 ± 6.66	0.928

5-times sit-to-stand, s	11.09 ± 4.32	10.73 ± 4.34	0.663
20RM	2.66 ± 0.99	2.80 ± 1.14	0.493
Rectus femoris CSA, cm²	3.57 ± 1.38	3.57 ± 1.44	0.978
Rectus femoris AP axis, cm	1.13 ± 0.33	1.06 ± 0.33	0.242

Values are mean ± SD. p values correspond to the baseline between-group comparison in the statistical output. CSA: cross-sectional area; AP: anteroposterior; 20RM: 20-repetition maximum test.

4. Functional outcomes

Functional performance improved in the ProSup group and deteriorated or remained less favorable in the Control group. The adjusted between-group effects were statistically significant for all principal functional endpoints.

Outcome	Control baseline → final	ProSup baseline → final	Adjusted ProSup benefit	F	p	η²	Cohen d
Handgrip strength, kg	27.17 → 24.39	28.23 → 31.35	+6.14 kg	139.21	<0.001	0.159	2.18
Timed Up and Go, s	7.36 → 8.25	7.24 → 6.42	-1.74 s	29.50	<0.001	0.072	1.06
30-s sit-to-stand, repetitions	14.86 → 12.95	14.96 → 17.50	+4.54 reps	64.15	<0.001	0.131	1.65
5-times sit-to-stand, s	10.56 → 13.12	10.56 → 8.87	-4.45 s	42.22	<0.001	0.190	1.43
20RM	—	—	+0.655	42.64	<0.001	0.164	1.28

For timed tests, a negative adjusted difference indicates faster performance in the ProSup group. Adjusted differences are expressed in the direction of benefit for ProSup and are derived from the post-hoc estimated marginal mean comparison.

5. Body composition and muscle ultrasound outcomes

The intervention was also associated with favorable changes in bioimpedance-derived cellular/muscle parameters and rectus femoris ultrasound measurements. The largest adjusted standardized effects were observed for rectus femoris anteroposterior axis, cross-sectional area, body cell mass and phase angle.

Outcome	Control baseline → final	ProSup baseline → final	Adjusted ProSup benefit	F	p	η²	Cohen d
Phase angle, degrees	4.88 → 4.59	4.64 → 5.06	+0.660°	41.56	<0.001	0.168	1.26

Fat-free mass index, kg/m²	—	—	+0.606	11.89	<0.001	0.027	0.67
Body cell mass, kg	22.65 → 21.16	22.20 → 23.74	+3.00 kg	47.22	<0.001	0.135	1.33
Rectus femoris CSA, cm²	3.57 → 3.23	3.57 → 3.99	+0.796 cm²	53.38	<0.001	0.120	1.42
Rectus femoris AP axis, cm	1.13 → 1.02	1.06 → 1.20	+0.250 cm	70.90	<0.001	0.162	1.64

6. Nutritional and categorical outcomes

Baseline nutritional severity was comparable between groups. At final follow-up, adjusted regression models showed a significant association between treatment group and both sarcopenia status and GLIM nutritional category. In the binomial model for final sarcopenia status, the treatment-group term was significant ($p=0.003$; odds ratio for Control versus ProSup=0.021, 95% CI 0.0017–0.261), after adjustment for baseline sarcopenia status, sex and age.

For final GLIM category, the multinomial model was significant overall (likelihood-ratio $\chi^2=121$, df=15, $p<0.001$; McFadden $R^2=0.425$). Treatment group contributed significantly to the model (omnibus $\chi^2=39.62$, df=3, $p<0.001$), as did age and baseline GLIM category, whereas sex was not significant in the omnibus test ($p=0.176$). Because category labels were numerically coded in the source output, the overall treatment association is emphasized rather than assigning a clinical direction to each category-specific odds ratio without the coding dictionary.

7. Safety, adherence and dietary intake

The available statistical output included dietary intake and supplement-intake summaries and did not identify significant baseline between-group differences in energy, carbohydrate, protein or fat intake. The source protocol prespecified adherence and tolerance as secondary outcomes. A formal adverse-event frequency table was not present in the statistical output supplied for this report; therefore, no unsupported safety estimate has been added.

8. Overall interpretation

Across the principal functional and morphofunctional endpoints, the 3-month HMB-enriched protein intervention was associated with consistently more favorable outcomes than standard nutritional and exercise care alone. Adjusted analyses demonstrated clinically and statistically relevant benefits in muscle strength, mobility, chair-rise performance, phase angle, body cell mass, fat-free mass index and rectus femoris ultrasound measurements.

The consistency of the direction of effect across independent domains—function, bioimpedance and nutritional ultrasound—supports a coherent treatment effect. The findings should nevertheless be interpreted in the context of the open-label design, endpoint-specific missing data and departures from normality/homogeneity assumptions observed for some models. The adjusted analyses and effect-size estimates therefore provide the most informative summary of the between-group differences.

9. Abbreviations

Abbreviation	Definition
ANCOVA	Analysis of covariance
AP	Anteroposterior
BCM	Body cell mass
BIA/BIVA	Bioelectrical impedance analysis / bioelectrical impedance vector analysis
BMI	Body mass index
CSA	Cross-sectional area
FFMI	Fat-free mass index
GLIM	Global Leadership Initiative on Malnutrition
HMB	β-hydroxy-β-methylbutyrate
ITT	Intention-to-treat
PP	Per-protocol
RM	Repetition maximum
STS	Sit-to-stand
TUG	Timed Up and Go

10. Source documentation

Study identification, design, objectives and prespecified analysis principles were taken from Protocol ProMuscle2025, Version 1.0. Numerical results were transcribed and reformatted from the final statistical output supplied for the study. No numerical result was generated or imputed for this report.