

No effect of increasing protein intake during military exercise with severe energy deficit on body composition and performance

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In this study, we compare the effects of isocaloric high- (HIGH: 2 g kg⁻¹ d⁻¹, n = 19) and low-protein diet (LOW: 1 g kg⁻¹ d⁻¹, n = 19) on changes in body composition, muscle strength, and endocrine variables in response to a 10-day military field exercise with energy deficit, followed by 7 days of recovery. Body composition (DXA), one repetition maximum (1RM) bench and leg press, counter-movement jump height (CMJ) and blood variables were assessed before and after the exercise. Performance and blood variables were reassessed after 7 days of recovery. The 10-day exercise resulted in severe energy deficit in both LOW and HIGH (−4373 ± 1250, −4271 ± 1075 kcal d⁻¹) and led to decreased body mass (−6.1%, −5.2%), fat mass (−40.5%, −33.4%), 1RM bench press (−9.5%, −9.7%), 1RM leg press (−7.8%, −8.3%), and CMJ (−14.7%, −14.6%), with no differences between groups. No change was seen for fat-free mass. In both groups, the exercise led to a switch toward a catabolic physiological milieu, evident as reduced levels of anabolic hormones (testosterone, IGF-1) and increased levels of cortisol (more pronounced in HIGH, *P* < .05). Both groups also displayed substantial increases in creatine kinase. After 7 days of recovery, most variables had returned to close-to pre-exercise levels, except for CMJ, which remained at reduced levels. In conclusion, increased protein intake during 10-day military field exercise with severe energy deficiency did not mitigate loss of body mass or impairment of physical performance.

KEYWORDS

cortisol, energy deficiency, nutrient supplement, soldiers, strength, testosterone

1 | INTRODUCTION

Soldiers participating in military field exercises or warfare often operate in a state of energy deficit in a demanding environment, with little opportunity for rest, recovery, and nutritional intake.¹⁻⁴ The energy imbalance typically leads to a catabolic physiological state, accompanied by alterations in

body composition, including loss of overall body mass, lean body mass (LBM), and fat mass.^{1,2,5,6} The catabolic state is characterized by disturbances in endocrine functions, including reduced circulating levels of anabolic hormones such as testosterone (TESTO) and insulin-like growth factor 1 (IGF-1), and increased levels of catabolic hormones such as cortisol (COR),³ which correlate well with observed losses in muscle mass

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during military exercises of both short⁷ and long^{1,3,8} duration. This is in turn associated with impaired physical performance, especially strength and power performance, measured as maximal dynamic strength and vertical jump.^{1,2,7,9} For military personnel, it is essential to identify strategies to avoid or minimize the loss of muscle mass and performance during periods of energy deficit and psychological and physiological stress.

Dietary intervention with increased protein intake stands out as an interesting approach for maintaining muscle mass,¹⁰⁻¹² ensuring amino acids availability and a sustained anabolic stimuli for muscle protein metabolism.^{10,13} Indeed, intake of protein amounting 2-3 times the prevailing recommendation (0.8 g protein kg⁻¹ day⁻¹, RDA) leads to preservation of lean mass and muscle strength in diet-controlled weight-loss programs.^{11,14-16} Protein supplementation may thus be a potent action for sustaining muscle functions also in soldiers participating in military field exercises.

In line with this, selected studies suggest that increased protein intake attenuates LBM loss during military exercises (2.0-2.3 g kg⁻¹ d⁻¹ vs 1.5-1.6 g kg⁻¹ d⁻¹).^{5,6} In these studies, surplus protein was ingested as an addition to the regular diet, essentially meaning that the total energy intake was higher in protein-ingesting subjects than in control subjects.^{5,6} Hence, they did not investigate the effect of protein supplement on muscle mass and performance per se, as energy availability is a potent modulator of these variables.^{5,6} Indeed, overall energy intake and corresponding degrees of energy deficiency may be decisive for whole-body homeostasis rather than the nature of the energy source ingested (eg, protein vs carbohydrate content).¹⁷ For example, increased protein intake (1 vs 2 g kg⁻¹ d⁻¹) does not seem to hinder loss of muscle mass during 21 days of concomitant severe energy deficit (~-70%) and high altitude exposure in recreationally active men.¹⁸ However, there is evidence to the contrary, as increased protein intake mitigates loss of muscle mass within an isocaloric diet in both resistance-trained subjects and military personnel undergoing 40% energy deficit, respectively.^{11,14} The heterogeneity of available studies, with regard to aspects such as the degree of energy deficit, protein supplementation protocols, duration of the intervention and the human subpopulation of interest, thus prohibits consensus around the benefits of protein intake for maintenance of LBM and preservation of muscle performance during military exercises with severe energy deficiency. Despite these issues, a recent review concluded that the energy deficit threshold for benefiting from excessive protein ingestion on preservation of LBM resides around -40%.¹⁹ In addition, we know little about the immediate physiological recovery from such military exercises, though 2-6 weeks seems to be sufficient to reestablish important factors for soldier readiness such as physical performance levels and endocrine variables.^{2,7} Nor do we know if higher protein intake during the

exercise exerts beneficial effects on these variables within such short recovery period.²⁰

The aim of this study was to investigate the effect of 10-day military field exercise with severe energy deficit on changes in body composition, endocrine responses, and physical performance in soldiers. We aimed to investigate whether these variables were affected by ingestion of isocaloric diets containing either LOW (1 g kg⁻¹ d⁻¹) or HIGH protein amounts (2 g kg⁻¹ d⁻¹), combined with low carbohydrate intake (1.9 and 0.6 g kg⁻¹ d⁻¹, respectively). We also aimed to investigate the effect of seven days of refeeding and recovery on these variables.

2 | METHOD

2.1 | Participants

Thirty-eight soldiers (age; 21.6 ± 0.8 years, height; 182 ± 9 cm, males/females ratio; 4.4) from the 2nd year at the Norwegian Defence Cyber Academy volunteered for the study. The study was approved by the local Ethics Committee at Inland Norway University of Applied Sciences and the Norwegian Centre for Research Data (ref 43901/3). Written informed consent was obtained from all participants prior to inclusion, and the study was carried out in accordance with the Declaration of Helsinki. Participants were randomly assigned into LOW (1 g kg⁻¹ d⁻¹, male = 15, female = 4) or HIGH protein intake (2 g kg⁻¹ d⁻¹, male = 16, female = 3) prior to the 10-day military exercise. There was no difference between the two groups for any of the characteristics or variables prior to onset of the study (Table 1).

2.2 | Experimental design

The soldiers performed a 10-day strenuous military exercise in a state of energy deficit, followed by 7 days of recovery (Figure 1). During the exercise, soldiers performed physically and cognitively demanding military tasks in a challenging outdoor environment. The exercise consisted of cyber-specific tasks, as well as marching, patrolling and physical combat conditioning training lasting for several hours. Throughout the entire exercise, the soldiers carried their personal military combat equipment (~20 kg). Most days contained activities lasting from 06.00 to 24.00 h, some days even longer. The exercise aimed to condition the participants for military combat situations, with gradual decreases in sleep and rest and gradual increases in physical and mental demands. The recovery phase (lasting for 7 days after finalization of the military exercise) was performed without restrictions in energy intake or physical activity. Pre-exercise testing was conducted 2 days prior to the exercise, which commenced toward the end of April. Post-exercise testing was conducted immediately after the exercise. Post-recovery testing was performed 7 days after finalization

TABLE 1 Absolute changes in body composition and endocrine biomarkers in LOW (1 g kg⁻¹ d⁻¹) and HIGH (2 g kg⁻¹ d⁻¹) supplementation groups before (pre), after 10-day military exercise (post-exercise) and following seven days of recovery (post-recovery)

	LOW			HIGH			ES (95% CI)
	Pre	Post-exercise	Post-recovery	Pre	Post-exercise	Post-recovery	
Body composition							
Body mass, scale (kg, n = 18, 19)	76.2 ± 12.2	71.6 ± 11.6*	75.3 ± 11.2 [§]	75.9 ± 12.2	71.9 ± 11.9*	76.2 ± 11.2 [§]	-0.38 (-1.06, 0.28)
Body mass, scale (kg, n = 12, 11) ^a	75.2 ± 11.9	70.5 ± 11.7*		77.1 ± 14.2	73.2 ± 13.3*		
Body mass, DXA (kg, n = 12, 11)	75.3 ± 11.4	71.4 ± 10.6*		78.2 ± 12.8	74.4 ± 13.2*		
Mean difference scale - DXA	0.15 ± 2.1	0.86 ± 2.0		1.06 ± 2.8	1.23 ± 0.5		
Fat mass (kg from scale)	13.8 ± 8.1	8.9 ± 7.3*		16.5 ± 6.1	11.7 ± 6.7*		-0.55 (-1.43, 0.32)
Fat-free mass, arms (kg from scale)	7.8 ± 1.5	7.7 ± 1.3		7.3 ± 1.5	7.3 ± 1.3		
Fat-free mass, legs (kg from scale)	20.8 ± 3.1	21.2 ± 2.9		20.9 ± 4.5	21.8 ± 3.9		
Fat-free mass, total body (kg from scale)	61.4 ± 8.5	61.5 ± 7.9		60.6 ± 11.7	61.5 ± 10.3		-0.37 (-1.24, 0.50)
Fat mass (kg from DXA)	13.2 ± 7.7	8.6 ± 7.0*		16.0 ± 5.6	11.3 ± 6.5*		-0.50 (-1.39, 0.37)
Fat-free mass, arms (kg from DXA)	7.4 ± 1.0.31	7.4 ± 1.1		7.1 ± 1.7	7.1 ± 1.2		
Fat-free mass, legs (kg from DXA)	19.8 ± 2.9	20.4 ± 2.6		20.3 ± 4.1	21.1 ± 3.7*		
Fat-free mass, total body (kg from DXA)	58.8 ± 7.8	59.5 ± 6.9		58.8 ± 10.5	59.7 ± 9.8		-0.12 (-0.99, 0.74)
Blood biomarkers							
TESTO (nmol L ⁻¹)	14.15 ± 2.63	4.44 ± 2.03*	16.21 ± 3.48 [§]	13.58 ± 2.38	4.14 ± 2.14*	15.17 ± 4.19 [§]	-0.06 (-0.67, 0.80)
Free TESTO	5.50 ± 1.70	0.99 ± 0.44*	4.63 ± 1.34 [§]	5.20 ± 1.50	1.05 ± 0.50*	4.53 ± 1.12 [§]	0.42 (-0.33, 1.17)
SHBG (nmol L ⁻¹)	30.30 ± 12.8	54.2 ± 20.2*	40.6 ± 12.8 [§]	29.0 ± 8.99	41.0 ± 11.3*	37.4 ± 13.4*	1.65 (0.89, 2.42)
IGF-1 (nmol L ⁻¹)	23.00 ± 5.36	9.50 ± 2.49*	20.90 ± 4.51 [§]	21.7 ± 5.53	8.98 ± 2.60*	19.1 ± 4.20 [§]	-0.02 (-0.68, 0.63)
COR (nmol L ⁻¹)	397.57 ± 81.7	435.52 ± 111.32	380.55 ± 102.21	389.15 ± 148.61	529.21 ± 111.84* [#]	362.78 ± 76.82 [§]	-0.80 (-1.49, -0.12)
T3 (pmol L ⁻¹)	5.84 ± 0.45	4.04 ± 0.84*	5.17 ± 0.48 [§]	6.00 ± 0.47	3.56 ± 0.86* [#]	5.03 ± 0.53 [§]	-0.67 (-0.01, 1.35)
T4 (pmol L ⁻¹)	17.36 ± 2.26	14.77 ± 3.10*	14.83 ± 2.03*	17.42 ± 2.14	13.70 ± 3.03*	14.21 ± 2.32*	-0.37 (-0.29, 1.03)
TSH (mIE L ⁻¹)	2.03 ± 0.72	2.04 ± 0.96	3.56 ± 1.24 [§]	2.18 ± 0.92	2.06 ± 1.12	3.18 ± 1.90 [§]	-0.24 (-0.41, 0.90)
CK (U L ⁻¹)	324.89 ± 205.72	3161.73 ± 1992.42*	129.16 ± 56.54 [§]	421.84 ± 565.33	3876.63 ± 3786.72*	190.78 ± 144.62 [§]	-0.40 (-1.1, 0.26)
TESTO/COR ratio	0.036 ± 0.01	0.010 ± 0.008*	0.050 ± 0.02 [§]	0.043 ± 0.015	0.008 ± 0.004*	0.046 ± 0.016 [§]	-0.43 (-0.30, 1.18)
Blood lactate							
Lactate (mmol L ⁻¹)	11.3 ± 1.5	7.7 ± 1.5*	10.0 ± 1.0 [§]	11.8 ± 1.2	7.8 ± 1.7*	10.8 ± 1.0 [§]	0.19 (-0.47 - 0.86)

Note: Lactate following Wingate 30-s sprint cycling. Effect size change score at post-exercise with confidence interval (ES (95% CI)).

Abbreviations: CK, creatine kinase; COR, cortisol; Free TESTO, free testosterone; IGF-1, insulin-like growth factor 1; SHBG, sex hormone-binding globulin; T3, Free T3; T4, Free T4; TESTO, Total testosterone; TSH, thyroid-stimulating hormone.

^aData from subjects with DXA measurement.

**P* < .05 significantly different from pre.

[§]*P* < .05 significantly different from post-exercise.

[#]*P* < .05 significant change between groups

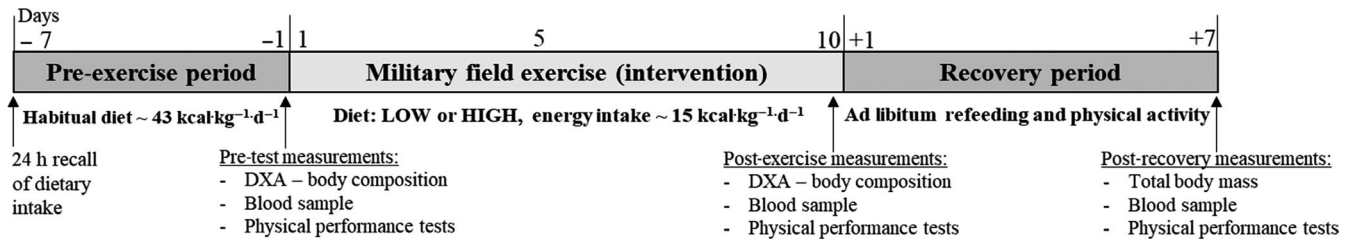


FIGURE 1 Overview of the intervention, including time points for collection of data on energy intake (24 h recall: 24 h recall of dietary intake), body mass composition (DXA; dual-energy X-ray absorptiometry), blood samples, and physical performance. During the intervention, participants were allocated to two different dietary programs, consisting of either HIGH (2 g kg⁻¹ d⁻¹) or LOW (1 g kg⁻¹ d⁻¹) amounts of protein, both providing 15 kcal kg⁻¹ d⁻¹

of the exercise. At each time point, all tests were conducted within one test day and were supervised by trained personnel. All physical and biological tests were performed at all test days, except for measurement of body composition, fat mass, and fat-free mass (FFM), which was only performed pre- and post-exercise.

2.3 | Diet

Prior to the intervention, data on dietary intake were collected using 24 hours recall. These data were analyzed by a nutritionist using the international food database program “Dietitian Net Pro version.” The reported macronutrient composition and energy intake (LOW, 3196 ± 996 kcal d⁻¹; HIGH, 3338 ± 1313 kcal d⁻¹; $P = .72$) provided the soldiers with a balanced diet with adequate levels of protein¹³ (see Table 2). These estimates of energy intake corresponds well with predicted energy requirements in the two groups (LOW, 3425 ± 278 kcal d⁻¹; HIGH, 3394 ± 404 kcal d⁻¹; $P = .65$), calculated from age, sex, height, and total body mass (floor scale),²¹ showing no difference from 24 hours recall data ($P = .69$). During the 10 days of exercise, the diet was restricted to ~15 kcal kg⁻¹ d⁻¹ (equivalent to a ~60% reduction in energy intake), which corresponds to the energy content of field rations utilized during prolonged military field exercise¹ and in weight-loss programs for athletes¹⁴ (Table 2). In HIGH, the

relative content of protein constituted a larger proportion and carbohydrate a lower proportion of the total energy intake than in LOW (Table 2). The daily energy intake for individuals were as follows: 900 kcal d⁻¹ for individuals with pre-intervention weight of 56–65 kg, 1050 kcal d⁻¹ for 66–75 kg, 1200 kcal d⁻¹ for 76–85 kg, 1350 kcal d⁻¹ for 86–95 kg, 1500 kcal d⁻¹ for 96–105 kg, and 1650 kcal d⁻¹ for 106–115 kg. Food was pre-packed in rations to be ingested for breakfast (consumed between 08.00–10.00 h), lunch (15.00–17.00 h), and dinner (22.00–24.00 h), providing similar amounts of protein intake in every meal throughout the day. The modified rations typically contained white bread, egg, ham and 100% whey protein powder (35 g, chocolate, Proteinfabrikken, Norway). Participants were instructed to refrain from eating anything else. Rations were distributed to the soldiers every 2.5 days. The soldiers had free access to water throughout the exercise. Adherence to the provided rations was controlled through daily contact with the soldiers. Both soldiers and test personnel were blinded to supplementation group affiliation.

2.4 | Body composition and estimation of energy deficit

Lean body mass and fat mass were measured using DXA Lunar Prodigy densitometer (Prodigy Advance PA + 302 047, Lunar), using the standard scanning mode

TABLE 2 Mean and standard deviation for energy and macronutrient composition of 24 h recall conducted prior to intervention compared to the diet during the 10-day military field exercise for LOW and HIGH

Time	LOW (n = 18)		HIGH (n = 19)	
	24h recall prior intervention	Intervention diet	24h recall prior intervention	Intervention diet
Energy kcal d ⁻¹ (kcal kg ⁻¹ d ⁻¹)	3196 ± 996 (41.5 ± 13.7)	1183 ± 168 (15.2 ± 0.6)	3338 ± 1313 (43.4 ± 16.6)	1174 ± 170 (15.1 ± 0.6)
Carbohydrate g (g kg ⁻¹ d ⁻¹)	370.4 ± 122.9 (4.9 ± 1.8)	146.1 ± 16.1 (1.9 ± 0.1)	395.8 ± 179.6 (5.1 ± 2.2)	50.1 ± 10.6 (0.6 ± 0.0) [#]
Protein g (g kg ⁻¹ d ⁻¹)	159.2 ± 61.1 (2.1 ± 0.9)	79.2 ± 11.4 (1.0 ± 0.0)	144.8 ± 44.1 (2.0 ± 0.6)	156.5 ± 22.6 (2.0 ± 0.1) [#]
Fat g (g kg ⁻¹ d ⁻¹)	101.4 ± 48.8 (1.3 ± 0.6)	27.8 ± 6.9 (0.4 ± 0.0)	123 ± 61.3 (1.6 ± 0.8)	37.8 ± 4.4 (0.5 ± 0.0)

[#] $P < .05$ significantly different from LOW.

(13–25 cm). Analysis was performed using GE enCORE version 17.0 software (GE Healthcare). The soldiers were positioned supine within the marked lines on the scanning bed and a strap secured around the ankles to ensure standardized body position in each of the two scans, in accordance with the manufacturer. During the pre-test, soldiers were scanned in a fasted state between 07.00 and 09.00 AM, wearing limited clothing (boxer-short and sports top) and no jewelry. The post-exercise scan was performed 1–2 hours after finalization of the exercise. Before onset of each scanning session, a phantom scanning was conducted to prevent baseline drifting from affecting analyses. The same technician was used at both time points. As suggested by Nindl et al.,¹ measurement of LBM should be carefully interpreted due to risk of overestimating soft tissue FFM in soldiers during extended periods of caloric deficit. Therefore, measures of FFM were calculated using a floor scale (SECA 770 Scale, Vogel & Halke) and DXA-derived percent body fat.²² Accordingly, estimation of arm, legs, and truncus were calculated using regional mass relative to total body mass by subtracting equivalent regional percent body fat. Unfortunately, it was not possible to perform DXA scanning post-recovery, due to limited access to the equipment.

Energy deficit during the 10-day exercise was calculated based on DXA-estimated changes in fat mass and FFM using the equation from Westerterp et al.²³:

$$\text{Energy deficit (kcal d}^{-1}\text{)} = ((\Delta\text{fat mass} \times 38) * 238.846 + (\Delta\text{FFM} \times 6) * 238.846) / 10 \quad (1)$$

where Δ is the change in fat mass or FFM in kg, the energy densities of fat mass and FFM are assumed to be 38 and 6 MJ d⁻¹, respectively. The factor 238 846 was used to convert megajoule into kilocalories and 10 represents the duration of the energy restriction period in days. Resting metabolic rate was calculated as described by Cunningham.²⁴

2.5 | Blood samples

Fasting blood samples for hormonal analyses were obtained from an antecubital vein using serum-separating tubes, with soldiers resting in a supine position. At all three time points (pre-, post-exercise, and post-recovery), samples were taken at the same time of the day (between 08.00 and 10.00 AM). Blood samples were incubated for 30 minutes at room temperature before they were centrifuged at 1500 g for 10 minutes. Serum was aliquoted into Eppendorf tubes and immediately transferred to -80°C for storage until analyses. Serum concentrations of total testosterone (TESTO), cortisol (COR), insulin-like growth factor 1 (IGF-1), and sex hormone-binding globulin (SHBG) were measured using an Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics), using kits from the Immulite Immunoassay System menu

(Siemens Medical Solutions Diagnostics), performed according to manufacturer's protocols. Free testosterone (Free TESTO) was calculated from testosterone and SHBG data as follows: free testosterone = 10 × testosterone/SHBG. Free triiodothyronine (T3), free thyroxine (T4), thyroid-stimulating hormone (TSH), and creatine kinase (CK) were measured using a Cobas 6000 (Roche Diagnostics/Hitachi SYSTEMS, Roche Diagnostics Norge AS). Reference intervals were as follows: TESTO (8.0–35.0 nmol L⁻¹), COR (138–690 nmol L⁻¹), IGF-1 (17–63 nmol L⁻¹), SHBG (8–100 nmol L⁻¹), T3 (3.1–6.8 pmol L⁻¹), T4 (8–20 pmol L⁻¹), TSH (0.27–4.20 mIE L⁻¹), and CK (35–400 U L⁻¹). Coefficient of variation (analytic) for the analyses were TESTO 14%, IGF-1 9%, SHBG 9%, COR 14%, T3 7%, T4 5%, TSH 4%, and CK 5%.

2.6 | Physical performance tests

Physical performance was measured using four functional tests, performed in the following order: counter-movement jump (CMJ), 1RM (one repetition maximum) leg press, 1RM bench press, and Wingate 30-second sprint power test. Each test session started with 10 minutes of general warm-up on a cycle ergometer, with intensities equivalent to 10–12 on the 6–20 Borg Rating of Perceived Exertion Scale.

Counter-movement jump height was performed on a force plate (SG-9, Advanced Mechanical Technologies, sampling frequency of 1 kHz). Throughout jumps, hands were placed on the hips and legs were placed with their individual hip width on the platform. The soldiers descended to a squat position of self-selected depth and immediately jumped upward as high as possible. If the third attempt resulted in the highest jump, an additional jump was performed. There were 30 seconds of rest between each jump. Participants were blinded to the results, and the best jump was used in data analyses.

Muscle strength of the lower and upper body was measured using 1RM. The 1RM test started with a specific warm-up, consisting of two sets with gradually increasing load (40% and 75% of expected 1RM) and decreasing number of repetitions (10 and 6). The first attempt was performed with a load approximately 5% below the expected 1RM. If a lift was successful, the load was increased by approximately 5%. For muscle strength of the lower body, a pneumatic bilateral seated leg press machine (Keiser A420, Keiser Sport Health Equipment Inc) was used. Briefly, the pneumatic equipment utilizes cylinders pressurized with air to provide different resistance. Soldiers were seated with knee and hip flexed at approximately 90°–96° and

45°, respectively. Approved 1RM efforts were defined as the maximal resistance that could be moved through the full range of motion with proper form one time. For upper-body strength, 1RM in bench press was performed. Soldiers were lying supine with their shoulders and hips kept in contact with the bench throughout the test and with their feet touching the floor. Efforts were accepted when the barbell smoothly touched the chest during the eccentric phase and the elbows were fully extended at the end of the concentric phase. Soldiers had 3-4 attempts with 2 minutes of rest between each lift for leg and bench press and the best attempt was used in data analyses. For each soldier, the same seating adjustment (leg press), body position, vocal encouragement, and supervisor were used during all tests.

Wingate 30-second sprint was performed on a cycle ergometer (Lode Excalibur Sport, Lode BV). The soldiers started pedaling at 100 W and 60 revolutions per minute for 30 seconds. Then, following a 3-second countdown, braking resistance was applied to the flywheel with a torque factor of 0.67 for females and 0.70 for males, which remained constant throughout the 30-second all-out test. Mean power output (W_{mean}) was defined as the average power output sustained throughout the 30 seconds, and peak power output was defined as the peak power (W_{peak}). Cyclists remained seated throughout the test and were given strong verbal encouragement. Cyclists were instructed to pedal as fast as possible from the start of the test and to avoid conserving energy for the last part of the test. Cyclists remained seated for one minute following the test, before blood was sampled from a fingertip and analyzed for whole blood $[la^-]$ using Biosen C-line lactate analyzer (EKF Diagnostic BmbH, Barlebe, Germany). The seating position was adjusted according to each soldier's preference for seat height, horizontal distance between tip of seat and bottom bracket, and handlebar position. For each soldier, identical seating positions were used at all test time points.

2.7 | Physical activity and sleep

Soldier recorded minutes spent on physical activity and sleeping on a daily basis. During the ten days of the military exercise, an average of $459 \pm 273 \text{ min d}^{-1}$ and $210 \pm 111 \text{ min d}^{-1}$ were spent on physical activity and sleep, respectively.

2.8 | Statistics

Data in text and figures are presented as mean \pm standard deviation. The energy requirements from 24 hours recall were analyzed using a linear mixed-effect model,²⁵ with energy intake and macronutrient data acting as dependent variables and protein grouping and sex acting as main effects (fixed). To

evaluate the effect of protein supplementation on body composition, physical performance, and blood markers (dependent variables), a linear mixed-effect model was utilized.²⁵ Interactions between groups and time points, as well as the interaction between fraction (arm, leg, truncus) for different segments of FFM, groups, sex, and time points were included as fixed effects in the model. The model included the maximal random effect structure justified by the data. Random by-subject slopes for the fraction effect were added to the model, thereby allowing fraction effect to vary by subjects. All models contained random intercept by subject. When there was an effect of time, a pairwise comparison was conducted with Satterthwaite correction. Effect size of protein supplementation was calculated with the following formula: $([\text{HIGH mean} - \text{LOW mean}]/\text{LOW SD})$. The scale proposed by Rhea²⁶ for highly trained subjects was used to interpret the magnitude of the treatment effect; 0.0-0.24 trivial, 0.25-0.49 small, 0.5-1.0 moderate, >1.0 large. These analyses were run in R.²⁷ Significance level was set at $P = .05$ for all analyses.

In Wingate data, two significant outliers were detected by calculating z scores.²⁸ The two samples deviated by more than >3.0 standard deviations from the mean for both mean and peak power (z score -3.04 , $\text{chisq } P = .0023$ and z score 3.21 , $P = .001$, respectively, Figure 2). Models were therefore fitted with and without these outliers for mean power (fitted with outliers, estimate -28.08 , standard error 12.94 , $P = .04$; fitted without outliers, estimate -20.65 , standard error 11.74 , $P = .08$) and peak power (fitted with outliers; estimate -85.81 , standard error 42.55 , $P = .04$; fitted without outliers, estimate -75.20 , standard error 42.65 , $P = .08$), which resulted in a significant and non-significant interaction between the groups. Removal of the outliers were justified based on the observation exceeded the cutoff of $z \geq \pm 3.0$ standard deviation around the mean.²⁸

3 | RESULTS

3.1 | Calculated energy expenditure and body composition

LOW and HIGH displayed similar total daily energy expenditure during the exercise, corresponding to $5536 \pm 1305 \text{ kcal d}^{-1}$ and $5427 \pm 1029 \text{ kcal d}^{-1}$ ($P = .86$), respectively, calculated from changes in fat mass/FFM and daily resting metabolic rates of $1699 \pm 172 \text{ kcal d}^{-1}$ and $1698 \pm 222 \text{ kcal d}^{-1}$, respectively. With an energy intake corresponding to $1183 \pm 168 \text{ kcal d}^{-1}$ and $1174 \pm 170 \text{ kcal d}^{-1}$, the daily energy deficit corresponded to $-4373 \pm 1250 \text{ kcal d}^{-1}$ (LOW, $-77.0 \pm 1.8\%$) and $-4271 \pm 1075 \text{ kcal d}^{-1}$ (HIGH, $-77.7 \pm 6.8\%$). After subtracting RMR from the total daily energy expenditure, this gives a field exercise-induced energy expenditure of $3836 \pm 1290 \text{ kcal d}^{-1}$ and

3769 ± 1106 kcal d⁻¹ for LOW and HIGH, respectively ($P = .82$). This fits well with the estimated energy expenditure of the reported levels of physical activity during the exercise, amounting to 3800 kcal d⁻¹ (7.6 h d⁻¹ of low to moderate intensity, based on values from Tharion et al²⁹).

Ten days of military field exercise led to decreased total body mass (using floor scale) and fat mass in LOW ($-6.1 \pm 2.4\%$, $P < .001$ and $-40.5 \pm 12.4\%$, $P < .001$, respectively) and HIGH ($-5.2 \pm 1.9\%$, $P < .001$ and $-33.4 \pm 13.3\%$, $P < .001$, respectively, Table 1), with no difference between groups. No changes were observed for FFM in either LOW or HIGH ($0.5 \pm 4.2\%$, $P = .79$, $1.9 \pm 3.2\%$, $P = .20$, respectively). After 7 days of recovery, total body mass (floor scale) returned to pre-values in both LOW ($-1.0 \pm 3.2\%$, $P = .13$) and HIGH ($0.6 \pm 2.4\%$, $P = .75$, Table 1), with no difference between groups. Notably, similar estimates of body mass composition and changes thereof were seen when using DXA-based total body mass to calculate fat mass and FFM (rather than using floor scale, Table 1). The only exception was FFM of the legs, for which a significant increase was seen in HIGH only ($P < .05$, Table 1).

At baseline, male participants displayed higher body mass ($P = .02$) and higher FFM ($P < .001$) than females (independent of supplementation grouping), with FM being similar between sexes ($P = .58$, data not shown). There was no effect of sex on loss of body mass, FFM, and fat mass from pre- to post-exercise ($P = .17$, $P = .64$, $P = .15$, respectively, data not shown). Sex did not affect total daily energy expenditure ($P = .93$), exercise-induced energy expenditure ($P = .71$), or energy deficit ($P = .83$) at any time point. Female participants had significantly lower RMR (pre and post) than male ($P < .001$, data not shown).

3.2 | Blood markers

In both LOW and HIGH, 10 days of military field exercise led to decreased serum concentrations of TESTO ($-68.2 \pm 14.2\%$, $P < .001$ and $-69.1 \pm 15.3\%$, $P < .001$, respectively), free TESTO ($-82.0 \pm 6.42\%$, $P < .001$ and $-78.3 \pm 11.1\%$, $P < .001$, respectively), IGF-1 ($-58.3 \pm 8.7\%$, $P < .001$ and $-58.1 \pm 8.4\%$, $P < .001$, respectively), T3 ($-30.4 \pm 15.3\%$, $P < .001$ and $-40.3 \pm 13.9\%$, $P < .001$, respectively), T4 ($-14.0 \pm 18.1\%$, $P < .001$ and $-20.0 \pm 17.5\%$, $P < .001$, respectively), and TESTO/COR ratio ($-69.6 \pm 21.3\%$, $P < .001$ and $-77.6 \pm 15.2\%$, $P < .001$, respectively, Table 1). Similarly, both groups displayed increased concentrations of SHBG ($82.2 \pm 23.7\%$, $P < .001$ and $44.4 \pm 21.9\%$, $P < .001$, respectively), COR ($12.1 \pm 33.1\%$, $P = .39$ and $55.6 \pm 68.7\%$, $P < .001$, respectively), and CK ($991 \pm 617\%$, $P < .001$ and $1443 \pm 1364\%$, $P < .001$, respectively, Table 1), with no changes for TSH ($4.7 \pm 43.2\%$, $P = .99$ and $-4.9 \pm 34.9\%$, $P = .90$, respectively, Table 1). For most variables, LOW and

HIGH displayed similar changes. However, for T3 and COR, HIGH displayed a more pronounced decrease ($P = .02$) and increase ($P = .01$), respectively, compared to LOW (Table 1).

In general, after seven days of recovery, TESTO, free TESTO, SHBG, IGF-1, T3, COR, CK, and TESTO/COR ratio returned toward pre-values (or beyond) in LOW and HIGH ($P < .05$, Table 1). Only T4 remained at reduced levels compared to pre- ($-14.2 \pm 9.7\%$, $P = .99$ and $-18.5 \pm 7.2\%$, $P = .69$, respectively), resembling post-exercise levels. A couple of anomalies were detected in the post-recovery data set in both LOW and HIGH: CK was reduced to below pre-values ($-49.9 \pm 22.3\%$ $P < .001$ and $-23.2 \pm 47.7\%$, $P = .006$, respectively), while TSH and the TESTO/COR ratio were increased to above pre-values ($91.3 \pm 76.4\%$, $P < .001$ and $50.3 \pm 61.4\%$, $P = .002$, respectively; $40.3 \pm 45.2\%$, $P = .008$ and $13.9 \pm 42.6\%$, $P = .80$, respectively). There was no difference between LOW and HIGH for any of the blood variables at post-recovery.

3.3 | Physical performance

In both LOW and HIGH, the military field exercise led to decreased 1RM bench press ($-9.5 \pm 3.9\%$, $P < .001$ and $-9.7 \pm 5.4\%$, $P < .001$, respectively, ES = 0.04 (CI -0.62 to 0.72), Figure 3A), 1RM leg press ($-7.8 \pm 3.8\%$, $P < .001$ and $-8.3 \pm 4.7\%$, $P < .001$, respectively, ES = 0.13 (CI -0.52 to 0.79), Figure 3B), CMJ ($-14.7 \pm 6.7\%$, $P < .001$ and $-14.6 \pm 8.8\%$, $P < .001$, respectively, ES = -0.01 (CI -0.67 to 0.63), Figure 3C), Wingate mean power ($-16.5 \pm 5.4\%$, $P < .001$ and $-18.8 \pm 6.3\%$, $P < .001$, respectively, ES = 0.49 (CI -0.18 to 1.17), Figure 2A), Wingate peak power ($-19.6 \pm 9.5\%$, $P < .001$ and $-25.1 \pm 11.7\%$, $P < .001$, respectively, ES = 0.50, (CI -0.18 to 1.19), Figure 2B), and blood lactate levels after the Wingate 30-second sprint ($-31.0 \pm 11.6\%$, $P < .001$ and $-33.5 \pm 13.2\%$, $P < .001$, respectively, ES = 0.19 (CI -0.47 to 0.86), Table 1). There was no difference between LOW and HIGH for any of these variables.

After 7 days of recovery, both LOW and HIGH significantly increased strength and cycling power measurements toward pre-values, while CMJ remained at reduced level. (Figures 2 and 3). Compared to pre-exercise values, performance was still reduced in bench press ($-5.4 \pm 4.3\%$, $P < .001$ and $-5.5 \pm 5.6\%$, $P < .001$, respectively), leg press ($-4.3 \pm 4.6\%$, $P < .001$ and $-4.8 \pm 4.6\%$, $P < .001$, respectively), Wingate mean power ($-5.2 \pm 2.9\%$, $P = .002$ and $-5.6 \pm 4.1\%$, $P < .001$, respectively), and Wingate peak power ($-8.0 \pm 9.3\%$, $P < .01$ and $-11.9 \pm 7.3\%$, $P < .01$, respectively). This was also the case for blood lactate levels measured after Wingate 30-second sprint ($-10.4 \pm 11.4\%$, $P < .001$ and $-8.2 \pm 10.7\%$, $P < .001$, respectively). However, compared to post-exercise, performance was improved for all

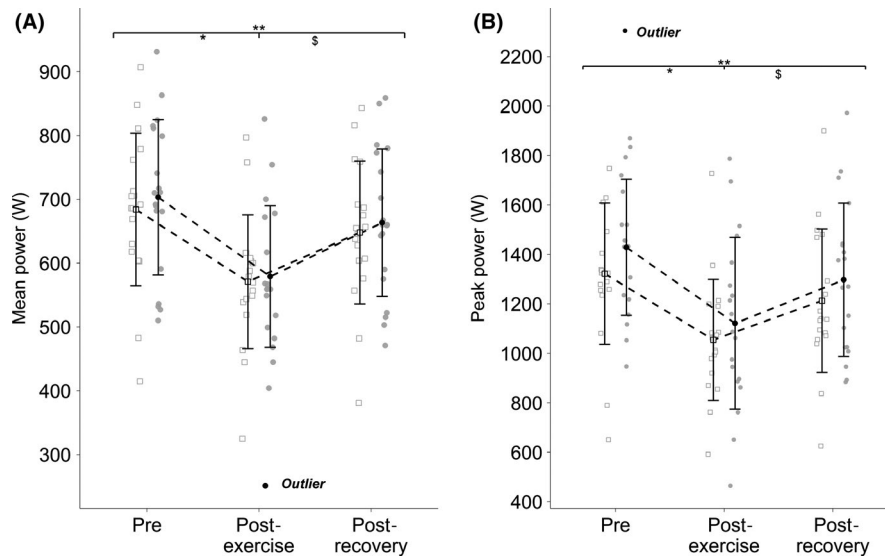


FIGURE 2 Absolute changes in Wingate 30-s sprint mean power (panel A) and Wingate 30-s sprint peak power (panel B) from before exercise (pre), after 10-day of exercise (post-exercise) and seven days of recovery (post-recovery) for LOW (white squares) and HIGH (black circles) protein supplementation groups. Mean \pm SD. Additional are each participant individual data points visualized for LOW (white squares) and HIGH (black circles) at the three time points whereas the two outliers are highlighted. $P < .05$ * significantly different from pre- to post-exercise. $P < .05$ \$ significantly different from post-exercise to post-recovery. $P < .05$ ** significantly different from pre

these variables ($P < .05$). In contrast, seven days of recovery had no effect on CMJ (LOW $-16.8 \pm 7.0\%$, $P = .43$; HIGH, $-13.0 \pm 6.4\%$, $P = .75$). There was no difference between LOW and HIGH for any of the performance variables at post-recovery.

At baseline, male participants displayed higher baseline levels for all performance variables than females ($P < .05$, independent of supplementation grouping, data not shown). In males, 10 days of military field exercise led to greater decline in 1RM bench press ($P < .001$), Wingate mean power ($P < .001$), Wingate peak power ($P < .001$), and CMJ ($P = .02$), and a tendency toward greater decline in 1RM leg press ($P = .06$) compared to females (data not shown). After seven days of recovery, male participants displayed less pronounced recovery in 1RM bench press ($P < .001$, normalized to pre-values) and CMJ ($P = .01$, normalized to pre-values).

4 | DISCUSSION

In this study, 10 days of military exercise with HIGH intake of protein and low intake of carbohydrate led to similar decreases in physical performance as LOW intake of protein and low intake of carbohydrate, measured as counter-movement jump height, maximal strength, and cycling sprint power. There was no benefit of ingesting more protein on muscle functionality in a setting with severe energy deficit and physical activity, supporting findings from a previous study.¹⁴ Surprisingly, FFM remained unchanged from pre- to post-exercise in both groups. This contradicts

most previous studies,^{1,2,6,7,9} though it is supported by others,^{5,6} a discrepancy that may be related to considerable variations in study design, including varying degrees of energy deficiency.^{5,6,11,14,18} It is important to note that in the present study, FFM measurements were associated with methodological uncertainty connected to the timing of post-exercise scanning as discussed in a later paragraph. In general, HIGH and LOW led to similar declines in blood concentrations of anabolic and pro-metabolic hormones (eg, testosterone and IGF-1) and markers of muscle damage (creatine kinase), with only T3 and COR showing differential responses between groups. The relatively marked changes in blood variables are in accordance with previous studies on the physiological effects of intense military exercise.^{1,3,6,7,30} Seven days of recovery led to improved performance toward pre-exercise values (eg, leg press, LOW -4.3% , HIGH -4.8% ; Wingate mean power, LOW -5.2% , HIGH -5.6%), except for CMJ, which remained at reduced levels (LOW -16% , HIGH -13%). Similarly, concentrations of hormones generally returned toward or beyond resting physiological levels (eg, COR, LOW -2.8% , HIGH 3.2% ; TESTO, LOW 19.2% , HIGH 11.8%), resembling pre-exercise values.

The severe level of energy deficiency experienced by the soldiers may explain the lack of beneficial effects of higher protein ingestion on performance, giving support to some studies,^{14,31} but contrasting other studies in soldiers^{5,6} and athletes undergoing weight loss.^{15,16,32} The resulting catabolic physiological environment may have counteracted anabolic signaling events, which arguably was more pronounced in HIGH, caused by the likely higher

amino acid availability from exogenous protein intake. Importantly, HIGH experienced severe carbohydrate deficit in addition to the general energy deficit (habitual intake $5.1 \text{ g kg}^{-1} \text{ d}^{-1}$ vs diet intervention $0.6 \text{ g kg}^{-1} \text{ d}^{-1}$). This may have impaired any positive effects of higher protein intake by further increasing the need for gluconeogenesis (ie, through amino acids) in order to sustain energy homeostasis.^{31,33,34} This being said, relative levels of energy deficit seems to be more decisive for whole-body protein loss^{17,18} and performance³⁵ than macronutrient composition in a state of severe energy deficit. However, this generalized perspective may not always be true. For example, in overweight subjects undergoing a four-day intervention with severe energy deficit ($\sim -94\%$), ingestion of a sucrose-only solution (dissolved in water) led to greater preservation of leg-pedaling performance than ingestion of protein only.³¹ Notably, even in LOW, carbohydrate intake was in the lower range of what is recommended (habitual intake $4.9 \text{ g kg}^{-1} \text{ d}^{-1}$ vs diet intervention $1.9 \text{ g kg}^{-1} \text{ d}^{-1}$), suggesting that any carbohydrate-specific effects on performance and body mass should have been present also in this group. The design of the present study demanded pre-fixed protein intake (HIGH or LOW) combined with a low-energy diet (eg, 900 kcal). As we had limited access to high-protein foods, we were unable to produce food packages that contained different amounts of protein while at the same time sustaining similar amounts of carbohydrates (see Table 2).

Surprisingly, neither HIGH nor LOW displayed changes in FFM in response to the military exercise, despite substantial impairment in muscle performance. This suggests that the amount of muscle mass was unaffected by the intervention, which contrasts findings in most previous studies,^{1,2,6,7,9,36,37} some of which even involved similar^{36,37} or less severe energy deficit and shorter duration compared to the present study.^{7,9,36,37} Conversely, our perspective data are supported by Tanskanen et al.,⁵ wherein 8 days of military exercise did not lead to decreases in FFM, though also in that study the intervention involved less severe energy deficit ($<50\%$) and higher energy intake and had shorter duration. It thus seems inappropriate to draw firm conclusions based on the sustained levels of FFM in the present study. Rather, it may have resulted from methodological artefacts, such as the timing of the post-exercise DXA analysis, which was conducted immediately after finalization of the exercise. Indeed, it seems plausible that levels of physical activity toward the end of the exercise led to redistribution of body fluids to working muscle and changes in hydration status (eg, blood volume/swelling), which in turn may have violated the soft tissue coefficient, and thus the estimation of FFM obtained during DXA scanning.³⁸ Notably, DXA-based FFM measurements are also sensitive to depletion of carbohydrate stores in skeletal muscle, which is typically accompanied by tissue dehydration. Because our participants likely displayed severe

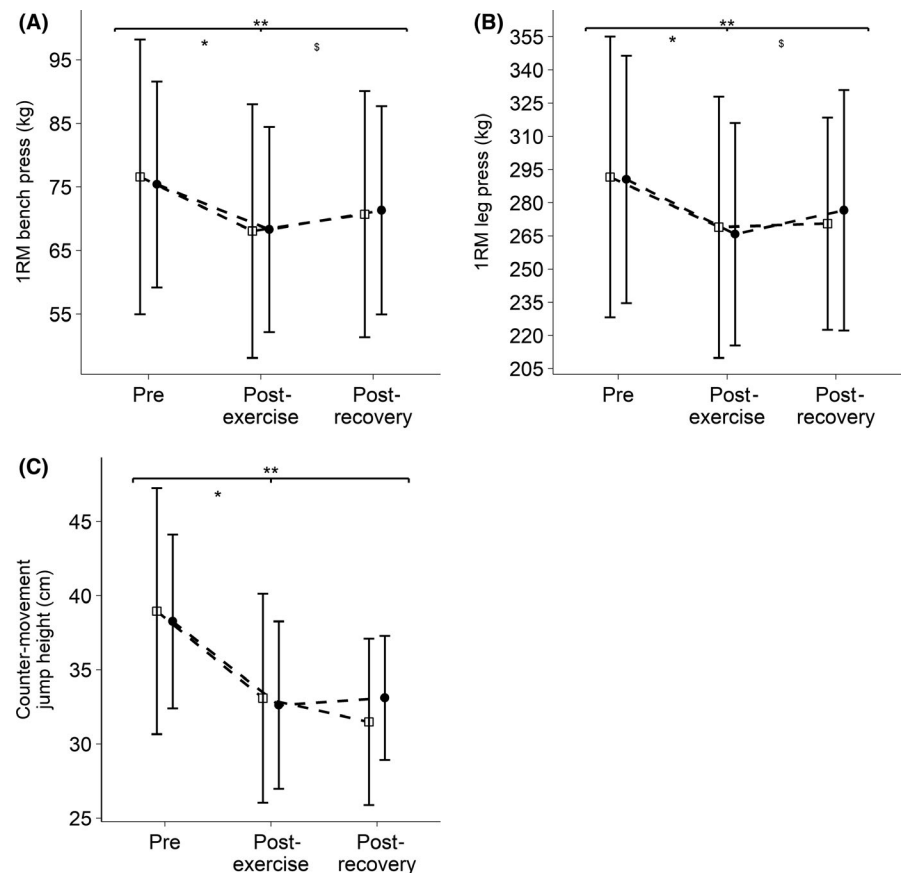


FIGURE 3 Absolute changes in 1RM bench press (panel A), 1RM leg press (panel B), and jump height (CMJ, panel C), from before exercise (pre), after 10-day of exercise (post-exercise) and 7 days of recovery (post-recovery) for LOW (white squares) and HIGH (black circles) protein supplementation groups. Mean \pm SD. $P < .05$ * significantly different from pre to post-exercise. $P < .05$ \$ significantly different from post-exercise to post-recovery. $P < .05$ ** significantly different from pre from post-recovery

depletion of carbohydrate stores at the time point of the DXA scan this may have affected FFM data. However, this should have led to underestimation of FFM level, opposing the potential overestimation caused by the timing of DXA scanning,³⁹ warranting further caution upon interpretation of FFM estimates. Importantly, however, DXA-derived estimates of total body mass post-exercise did not differ from floor scale based measurements (Table 1).

Regardless of these potential pitfalls in FFM estimates, our data did not reveal a beneficial effect of increased ingestion of protein on changes in FFM (though there was a low effect size of HIGH compared to LOW). This lack of an effect may also be related to the study design, as HIGH and LOW were on equally energy-restricted diets throughout the 10-day exercise, exposing soldiers in the two groups to similar energy-dependent catabolic signaling. This perspective is supported by a recent study,¹⁸ wherein an intervention with similar dietary groups (1 g kg⁻¹ d⁻¹ protein vs 2 g kg⁻¹ d⁻¹ protein, isocaloric) and similar levels of energy deficiency (-70%) disclosed no effect of additional protein intake on FFM. The authors thus concluded that increased protein intake during prolonged periods of negative energy balance seems to be used for energy metabolic purposes,¹⁸ which is also supported from findings in another protein supplementation study using isocaloric energy-restricted diet.⁴⁰ Indeed, most studies that reveal beneficial effects of increased protein ingestion on FFM during prolonged periods of energy restriction intake,^{5,6} involve intake of surplus protein as an additive to the regular diet, with a concomitant increase in overall energy intake.

Observed changes in endocrine variables in response to the military exercise, such as decreased levels of androgen hormones (TESTO, free TESTO, IGF-1, TESTO/COR ratio) and pro-metabolic hormones (T3 and T4) and increased levels of COR, suggest development of a catabolic physiological milieu, resembling observations made in previous studies.^{1,3,6-8} This may halt cellular growth and proliferation, while allocating available energy resources toward basal metabolic demands.^{3,17,41} Accordingly, during the exercise the soldiers were in a maladaptive state, with reduced ability to repair muscle tissue and sustain adequate tissue functions, providing a potential explanation for the observed impairment in physical performance. For most of the endocrine variables, there was no beneficial effect of higher protein ingestion, strengthening the notion that the severe energy deficiency was more decisive for responses to the exercise than amino acid and carbohydrate availability, as carbohydrate has a protein-sparing effect and vice versa.⁴² Two observations provide further insight into this; the elevated levels of COR in HIGH and the reduced levels of T3 in HIGH (both compared to LOW). These adaptations seem counterintuitive given the potential benefit of increased protein intake for anabolic metabolism. However, these adaptations may have been

necessary responses to the lowered availability of exogenous carbohydrates in HIGH (see Table 2), leading to cortisol-induced increases in gluconeogenesis through exploitation of endogenous fat stores,³ while simultaneously lowering whole-body metabolic rate.⁸

The observed impairment in physical performance in response to 10 days of military exercise is in line with results from other studies assessing the effect of periods of near-continuous physical activity, sleep deprivation and underfeeding on muscle strength and power.^{1,2,6,7,9,30} In these studies, the extent of the impaired performance co-varies with the severity of the intervention, including its length^{1,2,9,30} and its degrees of energy deficiency,^{5,6} as well as with differences in the timing of post-exercise testing, varying from 2 to 24 hours.^{5,6,9,30} This makes it difficult to evaluate and compare results across studies. Data from the present study are in the outer-most part of the specter, despite a relatively low level of physical exhausting activities during the intervention and a relatively short duration compared to other studies.^{1,2,6,7} It is thus reasonable to assume that the pronounced impairment in muscular performance was due to the substantial energy deficit, which was estimated to -4320 kcal d⁻¹ (overall energy deficit: -43203 kcal, ~-77%), calculated from changes in fat mass (-4.9 ± 1.4 kg) and FFM (0.5 ± 2.2 kg), resulting in more pronounced loss of fat mass than in many other studies.^{2,5,6,8,9,18,30} This is in agreement with a recent meta-analysis of data from nine military field exercise studies, which observed a decline in lower-body power and strength as an overall effect of daily energy deficit combined with exercise duration.⁴³ The authors concluded that the total energy deficit of military exercises/operations should not exceed -5000 to -19 000 kcal in order to limit negative effects on physical performance.⁴³ When energy deficit exceeds 40 000-60 000 kcal, moderate to large declines can be expected in physical performance, corroborating well with data from the present study.⁴³

The association between performance and energy status is evident as two observations. First, the decreased performance during 30-second cycling sprint at post-exercise was accompanied by decreased levels of blood lactate, suggesting lowered availability of glucose.⁴⁴ It thus seems likely that muscle glycogen stores in skeletal muscle were heavily depleted. This assumption is reasonable, as military field exercise has been shown to lead to 50% reduction in CHO content of muscle tissue after only 4 days,⁴⁵ in an experimental setting involving higher energy intake than the present study (3×) and higher relative intake of CHO (65% vs 17% in HIGH and 49% in LOW). This would attenuate the ability to generate muscle tension and reduce the number of physiological contractile muscle fibers at any given time point, effectively reducing the amount of metabolic end-products and reducing the ability to generate maximal force during anaerobic performance tests.^{34,35} Second, the increased rested-state

CK values post-exercise suggests an inability to sustain and repair muscle functions and may explain the overall reduction in performance. The increase in CK levels may also have affected performance in a more direct manner by inhibiting afferent neural feedback from muscle spindles, thereby reducing neuromuscular efficiency and maximal force-generation capacity.^{3,9,46} As a side note, male participants displayed larger declines in performance during the military exercise than did female participants (independent of protein grouping) and also displayed a slower rate of recovery. In previous studies, this phenomenon has been associated with a larger loss of FFM in men,⁴⁷ potentially driven by a smaller metabolic contribution from fat,^{47,48} and hence a larger contribution from other sources such as proteins. While this remains a potential explanation also in this study, we did not disclose sex-dependent differences in FFM changes, potentially related to methodological issues with our FFM estimates. Nor did we disclose sex-dependent differences in fat mass changes ($P = .15$), which should have been present if energy metabolism in female participants were indeed more reliant on fat. The small sample size of females in the present study ($n = 7$) and our selection of outcome measures and methods makes it difficult to conclude on this perspective.

After 7 days of recovery, body mass and most of the performance and endocrine variables had returned toward pre-exercise values. Increased protein intake during the field exercise did not affect recovery of any of the variables,²⁰ supporting the notion that protein dosage did not affect physiological responses to the exercise. The effectiveness of the recovery period was probably due to restoration of energy intake and rest, resembling observations made in previous studies on military exercises.^{2,3,7,49} As an example, after the recovery period, the TESTO/COR ratio were actually higher than at pre-exercise, suggesting increased need for, and occurrence of, cellular growth and repair.³ CMJ was the only variable that did not recover effectively, remaining at reduced post-exercise levels. This resembles the finding in Hamarland et al,⁷ wherein CMJ remained at reduced levels even after two weeks of recovery from an intense military hell week in military personnel. In another study, CMJ fully recovered after 5 weeks.² The prolonged recovery of CMJ may be due to reduced functions of muscle spindles, possibly linked to elevated CK concentrations and/or muscle fiber damage.^{50,51} This may impair the stretch reflex, which is an important contributor during CMJ,⁵¹ leading to delayed maximal shortening velocity and power.⁴⁶

4.1 | Limitations

The present study comes with a few limitations. For example, we used dietary recall (24 hours) to provide data on dietary intake and steady-state energy requirements. Such

self-report energy intake can lead to underestimation of the true energy requirement, as caused by underreporting.⁵² This being said, the reported energy intake was similar to the energy requirement calculated from anthropometric data (diff: -21 ± 1033 kcal, $P = .69$). The validity of our measure of energy intake also gains support from the relationship between basal energy deficiency/physical activity levels during the exercise and the accompanying loss of fat mass, with both perspectives giving similar measures of energy deficiency. During the exercise, adherence to the diet plan was facilitated by providing the soldiers with ready-to-eat food packages. Arguably, this mitigated the need for dietary recall measurement during the intervention itself (other than whether or not they had eaten the meal), while at the same time providing a feasible manner of blinding participants (and project staff) to supplement grouping. Unfortunately, we were not able to obtain dietary data during the 7 days recovery period due to a tight school schedule. However, the substantial restoration of performance level, endocrine markers, and body mass from post-exercise to post-recovery suggests adequate levels of energy intake during this period.

Information about physical activity levels during the field exercise was also collected in a self-reported manner, as opposed to other alternatives such as using accelerometers, in turn providing suboptimal measures of energy expenditure. Again, the validity of these data gains supported from their seeming ability to explain the observed loss of fat mass during the intervention (together with the overall energy intake). Moreover, as all participants took part in the same activities, only small differences would have been present between participants, with no likely significance for comparisons between LOW and HIGH, which were the main objective of the study.

As previously discussed, the timing of DXA measurements may have compromised the validity of FFM data. Unfortunately, it was not possible to perform DXA measurements at any other time points (or at surplus time points), as we had limited access to the apparatus. However, once again, these uncertainties should not have affected LOW vs HIGH analyses. Finally, this study was conducted on a relatively small population of Norwegian soldiers. This reduces the external validity in terms of predicting future responses in other groups of military personnel, particularly for the observed differences in responses between sexes, as we only had seven female participants. There is need for more studies to elaborate on the differences in responses to military field exercises with severe energy deficit between males and females.⁴⁷

In conclusion, 10-day of military field exercise in a state of energy deficiency led to loss of body mass, impaired physical performance and a switch toward a catabolic physiological milieu in soldiers. Increased intake of protein did not counteract these changes. Rather, the increased protein likely entered the overall energy metabolism, acting to compensate

for the substantial energy deficit, elevated energy needs and low carbohydrate availability. After seven days of recovery, most variables had returned to close-to pre-exercise levels, except for CMJ, which remained at reduced levels, suggesting impaired stretch-reflex functionality.

5 | PERSPECTIVES

This study provides novel insight into nutritional strategies for optimizing performance during strenuous military exercises. In face of substantial energy deficit, increased protein intake does not seem to counteract impairments in performance or alterations in body mass composition, at least not within the investigated timeframe. If the purpose is to maintain muscle performance, it therefore seems more pertinent to increase the total energy intake than to tweak the relative macronutrient composition of the diet (within the context of an appropriately balanced diet), ensuring the combat readiness of soldiers during prolonged military field exercises with substantial energy deficit.

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

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