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Effects of a chickpea protein hydrolysate on exercise-induced biomarkers of muscle damage and inflammation in federated road cyclists

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ABSTRACT

Background: This randomized, double-blind, placebo-controlled trial examined the effects of a chickpea protein hydrolysate (CPH) on biomarkers associated with exercise-induced muscle damage and inflammation in federated male road cyclists.

Methods: Twenty-four participants completed a 14-day intervention receiving either 12 g/day of chickpea protein hydrolysate (EXP) or a placebo (PLA) following training sessions. The selected dose was based on previous studies demonstrating antioxidant and immunomodulatory bioactive properties of the hydrolysate. Nutritional intake and training were registered. Body composition and biochemical markers of muscle damage and inflammation were assessed before and after the study.

Results: No significant differences were observed in body composition, training load, or dietary intake between groups, indicating controlled experimental conditions. However, between-group analyses of change scores revealed significant differences for lactate dehydrogenase (LDH; $p = 0.04$) and C-reactive protein (CRP; $p = 0.02$), favoring the experimental group. LDH decreased in the EXP group while increasing slightly in the placebo group, whereas CRP decreased in EXP and increased in PLA. Although creatine kinase (CK) showed a favorable pattern in the EXP group, the between-group comparison did not reach statistical significance ($p = 0.062$). Myoglobin followed a similar downward trend. The high baseline protein intake (>2 g/kg/day) and short intervention duration may have limited changes in body composition, nevertheless, the observed biomarker responses suggest that CPH supplementation may influence selected physiological processes associated with exercise-induced inflammation and cellular stress.

Conclusions: In trained cyclists with high habitual protein intakes, 14 days of CPH supplementation did not alter body composition but was associated with favorable changes in selected biomarkers related to exercise-induced inflammation and cellular stress compared with placebo. Given the short intervention period and the absence of direct assessments of recovery or performance, these findings should be interpreted as short-term biochemical responses. Further studies are required to determine their physiological relevance and whether these biomarker changes translate into meaningful improvements in recovery, training adaptation, or athletic performance.

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1. Introduction

Protein is a fundamental nutrient for exercise recovery and adaptation, providing the amino acid substrates necessary for muscle repair and remodelling after physical stress. It can be obtained from both animal and plant sources or as supplements derived from them [1]. The nutritional quality of a protein source depends primarily on its amino acid composition and digestibility, which together determine its capacity to support muscle protein synthesis (MPS) and recovery [2]. High-quality proteins supply adequate quantities of all essential amino acids, particularly leucine, and are efficiently digested and absorbed to stimulate anabolic

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signalling pathways [1]. Numerous studies have shown that protein ingestion in proximity to exercise reduces markers of muscle damage, attenuates performance decrements, and accelerates recovery [3–6]. When consumed before and immediately after exercise, protein has been shown to increase mRNA expression of anabolic regulators and prevent post-exercise declines in myogenic markers [5], thereby promoting faster recovery and tissue remodelling.

Although most research on protein supplementation has focused on resistance-trained individuals, endurance athletes may also benefit from increased protein availability, especially during periods of high training loads and elevated energy demands [7]. Protein supplementation in endurance athletes has been associated with attenuated muscle damage, improved remodelling of skeletal muscle tissue, and enhanced tolerance to training stress [8–11]. However, the literature is not unanimous; several studies have reported no significant improvements in performance or recovery when protein is added during periods of heavy endurance training [12–14]. Nonetheless, the consistent mechanistic evidence linking protein intake to enhanced MPS and reduced muscle damage underlines its importance in recovery-oriented sports nutrition [1].

Protein supplements rank among the most commonly consumed nutritional products worldwide, with their market value expected to reach approximately USD 21.5 billion by 2025. Within this context, sports nutrition represents their primary area of application, and plant-based proteins constitute the most rapidly expanding segment of this industry [15]. Whey protein, a by-product of cheese production, remains the most widely consumed and studied supplement due to its high biological value, rapid digestibility, and favourable amino acid profile rich in branched-chain amino acids (BCAA) [16,17]. Numerous investigations have demonstrated the ergogenic effects of whey protein on muscle recovery, strength development, and body composition [18,19]. Beyond its anabolic effects, whey exhibits additional bioactive properties, including antioxidant, cardioprotective, anti-obesity, and antidiabetic activities [20]. However, the growing global population, finite natural resources, and increasing environmental awareness have stimulated interest in alternative, more sustainable protein sources to meet future nutritional needs [21].

In recent years, plant-based proteins have gained prominence as viable alternatives to animal-derived supplements, driven by environmental, ethical, and health considerations [17,22]. Plant proteins are generally characterised by lower digestibility and incomplete amino acid profiles, particularly limited in lysine, methionine, or leucine, which may reduce their anabolic potential compared to animal proteins [16,23,24]. Nevertheless, advances in food technology have led to the development of plant protein isolates, blends, and hydrolysates with improved nutritional and functional characteristics. For example, combinations such as pea–rice or canola–potato can match whey's efficacy when consumed in appropriate doses (≥30 g providing ~2.5 g leucine) [25–27]. These developments have contributed to a paradigm shift in sports nutrition, emphasising sustainability without compromising performance outcomes.

Beyond their amino acid contribution, plant proteins and their hydrolysates contain bioactive compounds that may confer additional physiological benefits. Several studies indicate that plant-rich diets reduce oxidative stress and systemic inflammation [28,29]. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, leading to cellular damage and impaired recovery [30–32]. Exercise, particularly of high intensity or long duration, transiently increases ROS generation, which can activate proinflammatory cytokines, impair muscle protein synthesis, and contribute to chronic fatigue or overtraining if unmitigated [33]. In this context, antioxidant and anti-inflammatory strategies—whether through polyphenol-rich foods or plant protein supplementation—may play a valuable role in recovery and adaptation [34–36]. Plant proteins such as soy and oat, which contain bioactive components like β-conglycinin, avenanthramides, and β-glucans, have demonstrated beneficial effects on glucose regulation, inflammation, and oxidative balance [36–39]. These properties support the rationale for exploring plant proteins not only as anabolic substrates but also as functional compounds with recovery-enhancing potential.

Among the available plant sources, legumes have drawn considerable attention due to their favourable nutritional composition, high protein content, and presence of peptides with antioxidant and anti-inflammatory activities [40–42]. In particular, chickpea (Cicer arietinum) is an underutilized but promising source, providing a balanced amino acid profile, complex carbohydrates, dietary fibre, and a wide range of bioactive molecules [42]. Previous studies conducted with the same chickpea protein hydrolysate (CPH) used in the present investigation demonstrated a high content of low-molecular-weight bioactive peptides (<2.88 kDa), high digestibility, excellent solubility, and marked antioxidant activity measured through trolox

equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) assays. Furthermore, cellular studies showed its ability to modulate inflammatory signalling pathways, including nuclear factor kappa B (NF-κB) and NOD-like receptor family pyrin domain-containing 3 (NLRP3)-related responses. These characteristics provided the biological rationale for evaluating whether the hydrolysate could influence biomarkers associated with exercise-induced muscle damage and inflammation in trained athletes [43,44].

Despite the growing body of literature on plant-based proteins, evidence regarding the effects of CPH on post-exercise recovery in athletes remains scarce [7,45]. Given the physiological demands of endurance sports such as road cycling—characterised by prolonged exercise bouts, frequent intensity fluctuations, and substantial oxidative stress [46,47]—developing sustainable nutritional strategies to enhance recovery is of particular relevance [48–50]. Therefore, the present study aimed to analyse the effects of CPH supplementation on biomarkers of muscle damage and inflammation and body composition in male road cyclists. By providing new insights into the efficacy of a novel plant-based protein hydrolysate, this research contributes to the scientific understanding of sustainable sports nutrition and its role in optimising recovery in high-demand endurance contexts.

2. Materials and methods

2.1. Experimental design

This study was structured as a randomised, double-blind, placebo-controlled trial intervention. The total duration was 14 days, during which participants were assigned to two randomised groups: experimental (EXP) and placebo (PLA), each consisting of 12 cyclists. The allocation of the two supplements (placebo/protein hydrolysate) was coded by personnel external to the research team, ensuring neutrality in administration. The supplements were consumed in single-dose format immediately after each training session and at the same time on rest days.

2.2. Sample

Twenty-four male road cyclists residing in the province of Seville (Spain) participated in the study, all holding a valid federation license from the Andalusian Cycling Federation (FAC). Initially, 40 volunteers were recruited, of whom 25 met the inclusion criteria. Ultimately, 24 completed the study, with one dropout due to reasons unrelated to the intervention.

The inclusion criteria were: no consumption of nutritional supplements or anti-inflammatory/antioxidant drugs in the 30 days prior; no legume allergies; federation membership during the previous year; consistent training; no recent participation in similar studies; commitment to protocol adherence (≥95%); and full understanding of the informed consent. Exclusion criteria included failure to meet the above requirements, lack of cooperation, health risks, or metabolic disorders. The following table presents the descriptive data of the sample (Table 1).

2.3. Supplementation

The active supplement consisted of a chickpea protein hydrolysate (CPH) developed by the Plant Protein Group of the Institute of Fat (Instituto de la Grasa) at the Spanish National Research Council (CSIC; https://www.ig.csic.es/es/), following the methodology described by Rodríguez-Martín et al. (2024) [44]. The final formulation and packaging of the supplement were carried out by Aurora Intelligent Nutrition S.L. The formulation of the supplement is protected under an industrial patent owned by Aurora Intelligent Nutrition S.L.

Table 1. Age, height and body mass of the sample, placebo and experimental group.

Group	Age (years)	Height (cm)	Body Mass (kg)
ALL ($n = 24$)	42.9 ± 6.4	179.3 ± 6.8	78.1 ± 2.4
PLA ($n = 12$)	42.0 ± 7.2	179.0 ± 7.4	80.5 ± 12.2
EXP ($n = 12$)	43.8 ± 5.7	179.0 ± 6.6	75.7 ± 8.8

Both supplements were provided as powdered formulations in identical coded sachets and dissolved in 550 mL of water prior to consumption. The composition of the experimental and placebo supplements is presented in Table 2. The experimental supplement contained chickpea protein hydrolysate (H30BIO) as the main ingredient, whereas the placebo formulation contained maltodextrin in its place. Both products contained the same excipients, including vitamin C, acidity regulators, sweeteners, and natural flavourings. Product coding and allocation were performed by personnel external to the research team, and both participants and investigators remained blinded to treatment assignment throughout the study.

Supplements were consumed once daily immediately following training sessions and at the same time of day on rest days. Although the formulations were designed to be visually similar, complete taste matching could not be guaranteed due to the intrinsic sensory characteristics of the protein hydrolysate. The supplements were not strictly isocaloric; however, the small difference in energy content was considered unlikely to influence the measured outcomes.

The 12 g/day dose of chickpea protein hydrolysate was selected based on previous physicochemical and biological characterisation studies of the same hydrolysate [43,44], which demonstrated that this hydrolysate contains bioactive peptides with antioxidant and immunomodulatory potential. Given that the participants already reported a high habitual protein intake (>2 $g \cdot kg^{-1} \cdot day^{-1}$), the purpose of the intervention was not to substantially increase total protein intake or maximise muscle protein synthesis, but rather to evaluate whether a practical and realistic daily dose of CPH could modulate biomarkers associated with exercise-induced muscle damage and inflammation. Furthermore, the selected dose was considered appropriate for daily use by athletes and representative of a feasible supplementation strategy under real-world conditions.

2.4. Data custody and protection

Data and biological samples were stored at the CIRFD (Physical Performance & Sports Research Centre) of Pablo de Olavide University, with restricted access to the research team. The study was approved by the Andalusian Biomedical Research Ethics Portal (PEIBA), internal code: 2824-*N*-21, and all participants signed informed consent in accordance with Organic Law 3/2018 on Personal Data Protection. Ethical principles of the Declaration of Helsinki and international bioethics agreements were respected. This study was retrospectively registered at ClinicalTrials.gov (Identifier: PID2019-111368RB-100 - provisional registration identifier).

2.5. Data collection

Variables related to training, nutrition, body composition, and blood biomarkers were recorded.

2.5.1. Training data

Cyclists recorded their sessions using the Strava© and Garmin Connect© applications. The weekly training plan included four cycling sessions, two strength sessions, and one rest day. Intensity was monitored using heart rate (HR) and the rating of perceived exertion (RPE) scale. HR zones were individually estimated, prioritising work in zones II and III, except on the day prior to blood sampling, when a high-intensity session (zone V) was performed [51]. The high-intensity cycling session performed on the day prior to blood sampling lasted 120 min and included a 25-30 min warm-up, 10×1 km intervals at 90-95% of maximal heart rate (HRmax) with 1 min recovery periods, and low-intensity cycling until session completion.

Table 2. Composition of experimental and placebo supplements.

Component	PLA (%)	EXP (%)
Chickpea protein hydrolysate (H30BIO)	0	84
Maltodextrin	93	9
Excipients*	7	7

*Excipients included vitamin C, acidity regulators, sweeteners, and natural flavourings.

Strength training was conducted twice per week, following a muscular endurance protocol: five sets of 20 repetitions per exercise, with a load of 50–60% of one-repetition maximum (1RM), slow execution speed, and 1.5 minutes of rest between sets. Exercises included: squats, lat pulldown, leg press, chest press, barbell row, leg curl and dumbbell row.

2.5.2. Nutritional data

Nutritional intake over six days was recorded using 24-hour dietary recalls via the FatSecret app, three days per week: one rest day, one cycling training day, and one strength training day. Macronutrients (carbohydrates, proteins, and fats) were analysed, and prior nutritional guidelines were provided to homogenise the data. These guidelines were based on a balanced diet, adjusted to individual energy demands, prioritising minimally processed foods and high-quality sources for each macronutrient.

2.5.3. Anthropometric measurements

Body composition was assessed using segmental bioelectrical impedance analysis (Tanita BC-418 MA), recording body mass, fat mass, and lean mass. Height was measured with a digital stadiometer (AD-6227), and body mass index (BMI) was calculated. Basal metabolism was estimated automatically by the Tanita BC-418 MA using the manufacturer's proprietary predictive algorithm derived from the anthropometric and body composition variables measured during the assessment.

2.5.4. Blood samples

Blood samples were collected in a fasting state, following a high-intensity cycling session and 12 hours of rest. Biochemical and haematological parameters were analysed, including glucose, insulin, creatine kinase (CK), myoglobin, C-reactive protein (CRP), and lactate dehydrogenase (LDH).

2.6. Statistical analysis

Descriptive analysis included the calculation of mean and standard deviation as measures of central tendency and dispersion, respectively. For reliability analysis, 95% confidence intervals of the means were calculated. Before inferential analyses, the assumptions of normality and homoscedasticity were assessed using the Shapiro-Wilk and Levene's tests, respectively. Within-group comparisons of pretest vs posttest changes were performed using either paired-samples t-tests or Wilcoxon signed-rank tests, depending on the normality. Between-group comparisons were conducted using a Generalised Linear Model (GLM), with baseline (pre-test) values included as a covariate when the assumptions of normality and/or homoscedasticity were not met. Effect sizes were calculated using Cohen's d. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics® version 29.

3. Results

3.1. Body composition and basal metabolism

Table 3 presents body composition and basal metabolism data before and after the intervention period for the total sample, the PLA group, and the EXP group. No statistically significant pre-post differences were found in any variable across any group.

Table 3. Pre and post-intervention data on body composition and basal metabolism.

	ALL ($n = 24$)		PLA ($n = 12$)		EXP ($n = 12$)	
	Pre	Post	Pre	Post	Pre	Post
Total Mass (kg)	78.1 ± 10.7	77.6 ± 10.5	80.5 ± 12.2	79.6 ± 11.9	75.7 ± 8.8	75.5 ± 9.0
BMI (kg/m^2)	24.2 ± 2.2	24.1 ± 1.9	24.9 ± 2.5	24.6 ± 2.3	23.4 ± 1.6	23.6 ± 1.5
BM (kcal)	1852.8 ± 199.1	1853.5 ± 199.7	1900.8 ± 233.5	1900.8 ± 233.5	1804.7 ± 152.7	1806.1 ± 154.7
Fat Mass (%)	13.5 ± 4	13.6 ± 3.9	14.9 ± 4.2	15.2 ± 4.3	12.2 ± 3.6	12.1 ± 2.9
Fat Mass (kg)	10.8 ± 4.5	10.9 ± 4.4	12.3 ± 5.0	12.5 ± 5.1	9.3 ± 3.6	9.3 ± 3.0
Lean Mass (kg)	67.3 ± 7.1	66.7 ± 6.9	68.2 ± 8.1	67.2 ± 7.4	66.5 ± 6.1	66.2 ± 6.5

Values are presented as mean ± standard deviation. BMI: Body Mass Index; BM: Basal Metabolism.

3.2. Cycling and strength training sessions

Detailed data on cycling and resistance training sessions for the total sample, the placebo group, and the EXP group are presented in Tables 4 and 5, respectively. No statistically significant differences were found between the PLA and EXP groups across any of the cycling-related variables. Likewise, no statistically significant differences were found between the PLA and EXP groups for any of the resistance training session variables. This indicates that, within the limits of the available data and variability, both groups performed similarly across all measured metrics.

3.3. Nutritional information recording

Nutritional intake was recorded by the cyclists using 24-hour dietary recalls on three different days of the week, covering one rest day, one cycling training day, and one strength training day. Table 6 details the recorded data for the total sample, the placebo group, and the EXP group. Figure 1 displays the reported intake data for total calories and each macronutrient across the studied groups and the total sample.

Table 4. Cycling training data.

	ALL ($n = 24$)	PLA ($n = 12$)	EXP ($n = 12$)
Average Km/day	72.3 ± 6.6	72.5 ± 7.3	72.1 ± 6.2
Total Km/Week 1º	250.1 ± 22.0	262.6 ± 20.9	237.7 ± 15.3
Total Km/Week 2º	243.3 ± 16.4	247.1 ± 21.6	239.5 ± 7.8
Average Weekly Km	246.7 ± 14.7	254.8 ± 15.5	238.6 ± 8.2
Total Km/14 Days	493.4 ± 27.3	509.7 ± 30.9	477.2 ± 23.1
Total Hours/Week 1º	8.6 ± 0.7	8.6 ± 0.7	8.7 ± 0.7
Total Hours/Week 2º	8.5 ± 0.7	8.3 ± 0.8	8.7 ± 0.6
Total Cycling Hours/14 Days	17.2 ± 1.3	17.0 ± 1.3	17.4 ± 1.2
Average Total Cycling Sessions	7.9 ± 0.3	7.9 ± 0.3	7.9 ± 0.3

Values are presented as mean ± standard deviation.

Table 5. Resistance training session data.

	Total ($n = 24$)	PLA ($n = 12$)	EXP ($n = 12$)
Number of Sessions	48	48	48
Total Training Time (min)	604	600	607
Session Duration (min)	50 ± 6	50 ± 7	51 ± 5
Maximum Repetition (% of 1RM)	59.0 ± 3.5	60.0 ± 1.4	58.0 ± 4.5
RPE (0–10)	7.3 ± 0.6	7.2 ± 0.7	7.5 ± 0.5

Values are presented as mean ± standard deviation.

Table 6. Nutritional intake data.

	ALL ($n = 24$)	PLA ($n = 12$)	EXP ($n = 12$)
Calories—Rest Day	2022 ± 171	1961 ± 182	2043 ± 152
Calories—Cycling Session	3003 ± 353	3011 ± 295	2995 ± 409
Calories—Strength Session	2721 ± 310	2792 ± 246	2650 ± 354
Average Daily Calories	2582 ± 278	2588 ± 240	2653 ± 305
Carbohydrates—Rest Day (g)	269 ± 59	255 ± 71	282 ± 42
Carbohydrates—Cycling Session (g)	330 ± 49	325 ± 47	336 ± 51
Carbohydrates—Strength Session (g)	294 ± 38	297 ± 36	290 ± 41
Average Daily Carbohydrates (g)	298 ± 49	292 ± 51	303 ± 47
Protein—Rest Day (g)	143 ± 31	139 ± 35	147 ± 27
Protein—Cycling Session (g)	165 ± 23	160 ± 23	170 ± 22
Protein—Strength Session (g)	145 ± 23	146 ± 23	144 ± 24
Average Daily Protein (g)	151 ± 26	148 ± 27	154 ± 25
Fat—Rest Day (g)	73 ± 17	74 ± 21	71 ± 11
Fat—Cycling Session (g)	83 ± 12	83 ± 13	83 ± 10
Fat—Strength Session (g)	78 ± 13	78 ± 14	77 ± 13
Average Daily Fat (g)	78 ± 14	78 ± 17	77 ± 11

Values are presented as mean ± standard deviation.

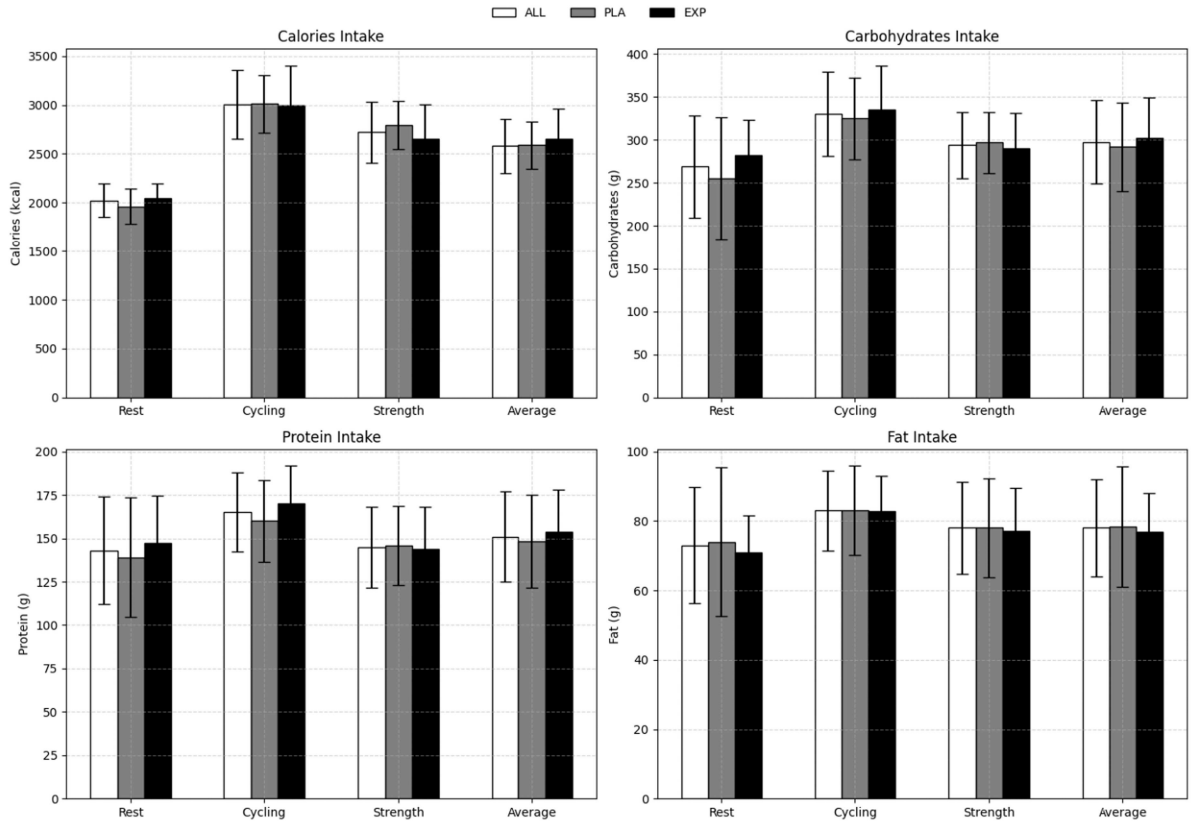


Figure 1. Average distribution of total energy and macronutrient intake.

3.4. Biochemical variables

Table 7 presents the pre- and post-intervention values of biochemical markers for the total sample (ALL), the placebo group (PLA), and the experimental group (EXP). No statistically significant within-group pre-post changes ($p > 0.05$) were observed in any biochemical variable in either the PLA or EXP groups when analysed separately (Table 7). However, between-group analyses of change scores using a Generalised Linear Model revealed significant differences for LDH ($p = 0.04$) and CRP ($p = 0.02$), with a trend toward significance for CK ($p = 0.062$) (Figure 2). On the other hand, some trends and effect sizes are worth noting. In the PLA group, the largest mean increase was observed in CK ($\Delta = 112.92 \pm 94.37$ UI/L; ES = 1.5), although the change was not statistically significant ($p = 0.814$). CRP also showed a moderate increase ($\Delta = 0.03 \pm 0.03$ mg/dL; ES = 1.43), suggesting a potential inflammatory response. The average effect size in this group was moderate (mean ES = 0.89).

In contrast, the EXP group showed a decrease in LDH ($\Delta = -55.58 \pm 51.54$ UI/L; ES = 1.5) and CK ($\Delta = -32.67 \pm 47.03$ UI/L; ES = 0.74), with large effect sizes despite non-significant *p*-values. These reductions may suggest a potentially favourable biomarker response to CPH supplementation; however, the absence of statistical significance precludes definitive conclusions regarding efficacy.

Figure 2 illustrates the between-group comparison of post-intervention changes. Glucose levels increased slightly in all groups, with the EXP group showing the highest mean change (+2.75 mg/dL; +3.21%). LDH levels decreased in both the ALL and EXP groups (−7.13% and −15.93%, respectively), while a modest increase was observed in the PLA group (+2.31%). Creatine kinase (CK) showed a marked increase in PLA (+112.92 UI/L; +39.99%), whereas it decreased in EXP (−32.67 UI/L; −14.83%), suggesting a potential protective effect of the intervention. Myoglobin levels rose in PLA (+11.75%) but declined in EXP (−7.34%), further supporting a possible attenuation of muscle damage in the experimental group. Insulin levels increased across all groups, with the EXP group exhibiting the greatest relative change (+16.80%). C-reactive protein (CRP) increased in PLA (+24.29%) and ALL (+9.27%), while it decreased in EXP (−8.40%), indicating a potential anti-inflammatory effect of the intervention.

Table 7. Within-group pre-post comparisons of biochemical markers in the total sample (ALL), placebo group (PLA), and experimental group (EXP).

	PRE-TEST		POST-TEST		DIFFERENCES			
	M ± SD	CI (95%)	M ± SD	CI (95%)	M ± SD	CI (95%)	*p value*	Effect Size
				ALL				
Glucose (mg/dl)	86.42 ± 6.29	83.76–89.07	88.58 ± 7.72	85.32–91.84	2.17 ± 6.71	−0.67–5.00	0.115	0.31
LDH (UI/L)	337.00 ± 125.50	284.00–390.00	312.96 ± 50.80	291.51–334.41	−24.04 ± 133.55	−80.43–32.35	0.710	−0.25
CK (UI/L)	251.29 ± 156.27	185.30–317.28	291.42 ± 268.03	178.24–404.60	40.13 ± 263.32	−71.07–151.32	0.855	0.18
Myoglobin (ng/ml)	53.08 ± 25.40	42.36–63.81	54.33 ± 21.78	45.14–63.53	1.25 ± 23.93	−8.85–11.35	0.511	0.05
Insulin (mcU/mL)	3.55 ± 1.71	2.82–4.27	3.98 ± 1.56	3.32–4.64	0.44 ± 1.14	−0.04–0.92	0.067	0.27
CRP (mg/dl)	0.11 ± 0.05	0.09–0.13	0.12 ± 0.06	0.09–0.14	0.01 ± 0.08	−0.02–0.04	0.398	0.18
				PLA				
Glucose (mg/dl)	87.25 ± 1.58	83.77–90.73	88.83 ± 1.96	84.53–93.14	1.58 ± 1.60	−1.94–5.11	0.266	0.89
LDH (UI/L)	325.08 ± 10.51	301.94–348.22	332.58 ± 15.62	298.21–366.95	7.50 ± 16.44	−28.69–43.69	0.666	0.56
CK (UI/L)	282.33 ± 34.29	206.87–357.80	395.25 ± 98.06	179.43–611.07	112.92 ± 94.37	−94.79–320.63	0.814	1.54
Myoglobin (ng/ml)	53.92 ± 7.08	38.34–69.50	60.25 ± 8.10	42.43–78.07	6.33 ± 4.79	−4.22–16.88	0.272	0.83
Insulin (mcU/mL)	4.02 ± 0.57	2.77–5.27	4.38 ± 0.50	3.28–5.47	0.36 ± 0.33	−0.36–1.08	0.239	0.67
CRP (mg/dl)	0.12 ± 0.02	0.07–0.16	0.15 ± 0.02	0.10–0.19	0.03 ± 0.03	−0.04–0.10	0.350	1.43
				EXP				
Glucose (mg/dl)	85.58 ± 2.07	81.04–90.13	88.33 ± 2.56	82.70–93.97	2.75 ± 2.28	−2.28–7.78	0.264	1.18
LDH (UI/L)	348.92 ± 51.07	236.51–461.32	293.33 ± 11.65	267.68–318.98	−55.58 ± 51.54	−169.02–57.86	0.666	−1.50
CK (UI/L)	220.25 ± 53.89	101.63–338.87	187.58 ± 30.70	120.02–255.15	−32.67 ± 47.03	−136.19–70.85	0.790	−0.74
Myoglobin (ng/ml)	52.25 ± 7.88	34.90–69.60	48.42 ± 3.28	41.21–55.63	−3.83 ± 8.49	−22.52–14.86	0.937	−0.63
Insulin (mcU/mL)	3.08 ± 0.38	2.24–3.91	3.59 ± 0.39	2.74–4.44	0.52 ± 0.34	−0.24–1.27	0.136	1.35
CRP (mg/dl)	0.10 ± 0.009	0.08–0.12	0.09 ± 0.008	0.09–0.09	−0.01 ± 0.01	−0.03–0.01	0.317	−0.97

4. Discussion

The aim of the present study was to analyse the effects of CPH supplementation on biomarkers of muscle damage and inflammation and body composition in male road cyclists during a 14-day intervention. Although no significant differences were found in body composition, the biochemical analysis revealed physiologically meaningful trends and significant changes in markers of muscle damage and inflammation. Specifically, the experimental group receiving CPH supplementation demonstrated a significant reduction in LDH and CRP levels, along with a non-significant but moderate effect size reduction in CK, compared with an increase in these biomarkers in the placebo group. These findings suggest that, despite the absence of observable changes in body composition, CPH supplementation may influence selected biomarkers associated with inflammation and muscle damage.

The absence of measurable changes in body composition can be explained by two key factors: the short duration of the intervention and the already high habitual protein intake of the participants. Both groups consumed more than 2 g/kg/day of protein, well above the threshold of ~1.6 g/kg/day identified as sufficient to maximise muscle protein synthesis (MPS) and recovery processes [52,53]. Previous work has shown that, under conditions of high protein availability, the benefits of additional supplementation tend to plateau [54]. Moreover, structural adaptations in lean body mass variables typically require longer interventions—often 6 to 8 weeks or more—before they can be detected [25,27]. Consequently, the lack of significant changes in anthropometrics in this study should not be interpreted as evidence of inefficacy, but rather as a reflection of the study design and population characteristics.

In contrast, the biochemical markers provide valuable insights into the potential of CPH supplementation to influence physiological responses associated with exercise-induced muscle damage and inflammation. The significant between-group difference favoring the EXP group in LDH reduction may suggest a lower degree of exercise-induced cellular stress and membrane disruption [55]. LDH release into circulation is frequently used as an indicator of exercise-induced cellular stress, tissue damage, and oxidative stress [56]. However, LDH is not specific to skeletal muscle, as it is widely distributed across several tissues, including cardiac muscle, liver, and erythrocytes. Therefore, changes in circulating LDH should be interpreted as a general marker of exercise-induced tissue disruption rather than direct evidence of skeletal muscle damage. The reduction observed in the present study may nevertheless suggest a lower degree of

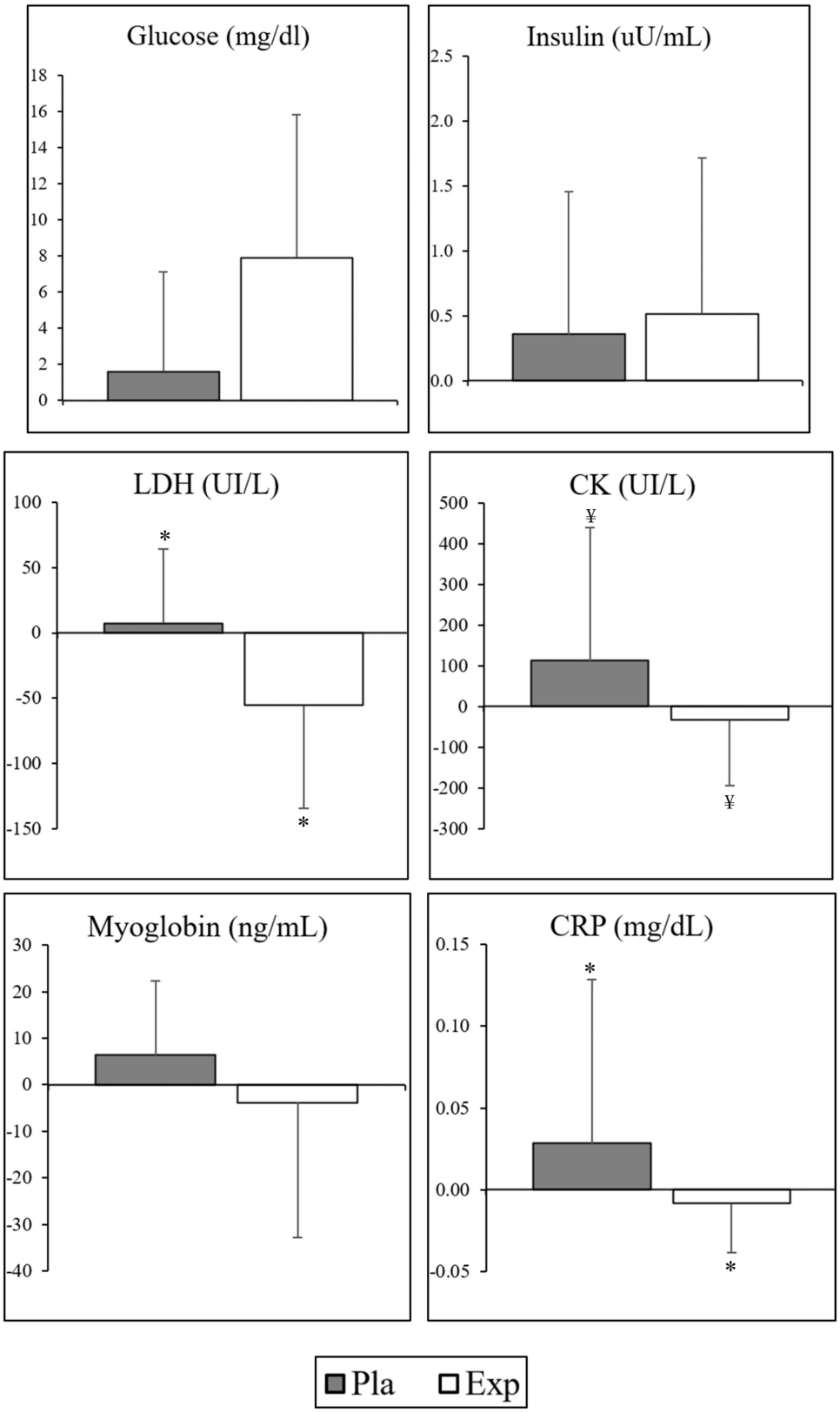


Figure 2. Between-group comparison of post-intervention differences (posttest—pretest). (*) $p < 0.05$, Generalised Linear Model; (¥) $p = 0.062$, Generalised Linear Model.

exercise-related cellular stress and membrane disruption. This finding aligns with previous evidence showing that hydrolysates from legumes, including chickpeas, can yield bioactive peptides with antioxidant activity capable of neutralising reactive oxygen species [42,44]. Although the physiological magnitude of the observed changes should be interpreted cautiously, the direction of the LDH response is consistent with previous studies conducted with the same hydrolysate, which demonstrated significant antioxidant activity and biological effects on pathways associated with oxidative stress regulation. Therefore, the present findings may provide preliminary in vivo support for mechanisms previously identified under experimental conditions [43,44].

Likewise, the reduction in CRP levels observed in the EXP group, in contrast to the increase in the placebo group, may suggest a modulation of the inflammatory response following exercise. CRP is a sensitive acute-phase protein, and its modulation by plant-derived proteins has been observed in prior studies emphasising anti-inflammatory properties of legume hydrolysates [41,43]. However, these findings should be interpreted with caution. CRP concentrations remained within normal physiological ranges throughout the study in both groups, and the absolute magnitude of the observed changes was small. Consequently, while the between-group differences reached statistical significance, their clinical or practical relevance for trained athletes remains uncertain. These results may therefore be more indicative of subtle changes in the inflammatory response rather than evidence of a meaningful anti-inflammatory effect. Nevertheless, the observed pattern remains biologically plausible considering that the same chickpea protein hydrolysate has previously demonstrated the capacity to modulate inflammatory signalling pathways, including NF-κB and NLRP3-related responses, under controlled experimental conditions [44]. Therefore, although the practical significance of the CRP changes remains uncertain, the findings are mechanistically consistent with previous evidence.

Creatine kinase (CK) and myoglobin, two of the most widely recognised markers of structural muscle damage, also followed a pattern supporting this interpretation. Although the reduction in CK did not reach statistical significance, the moderate effect size and contrasting increases in placebo suggest that CPH supplementation may attenuate muscle fibre disruption. On the other hand, myoglobin, a smaller molecule, peaks shortly after muscle damage, whereas CK peaks 24–48 hours later [57,58]. Given that samples were taken 12 hours post-exercise approximately, the trends observed in both CK and myoglobin are consistent with early modulation of sarcolemmal integrity. This suggests that the benefits of CPH supplementation may be more evident in recovery windows directly relevant to athletes facing consecutive days of competition or training, although more robust data are needed.

A notable feature of the present results was the considerable inter-individual variability observed across several biomarkers, particularly CK and LDH. Such variability is commonly reported in exercise recovery studies and may reflect differences in training status, recovery capacity, habitual dietary intake, genetic factors, and individual physiological responses to exercise. Consequently, the response to supplementation may not be uniform across all athletes, and the present findings should be interpreted considering this biological variability.

These results should also be interpreted in the broader context of the debate over plant versus animal protein sources. Whey protein is widely regarded as the “gold standard” due to its rapid digestibility, high leucine content, and complete amino acid profile, all of which optimise the stimulation of MPS. In contrast, isolated plant proteins such as soy or pea have been criticised for lower digestibility and suboptimal amino acid composition [16,23,24,59]. Recent systematic reviews confirm that while plant proteins are superior to no supplementation, they remain less effective than animal proteins for promoting muscle hypertrophy and strength [17,59,60]. However, these conclusions are nuanced by emerging evidence showing that well-formulated blends of plant proteins (e.g. pea–rice–potato) can elicit anabolic responses comparable to whey, provided that leucine thresholds (~2.5 g/serving) are achieved [25–27]. Importantly, processing techniques such as hydrolysis—applied to CPH supplementation—further enhance digestibility and release bioactive peptides, offering antioxidant and anti-inflammatory benefits that extend beyond anabolic stimulation. In this regard, CPH supplementation may represent a promising plant-based protein strategy with potential bioactive properties. However, the present study was not designed to compare CPH with whey protein, and therefore no conclusions can be drawn regarding their relative efficacy.

The interpretation of the present findings must also take into account modulating factors such as dosage, duration, and population characteristics. The 12 g/day dose of CPH supplementation used here is relatively modest compared to studies that have employed larger amounts (e.g. 50 g/day soy protein in Shenoy et al., 2016) [61] and observed more robust effects [7]. Furthermore, the short 14-day intervention

likely limited the capacity to detect changes in body composition. Nevertheless, the significant reductions in LDH and CRP, alongside moderate CK effects, despite a short timeframe and modest dose, may be consistent with the previously described bioactive properties of CPH [43,44].

Equally important, the high baseline protein intake (>2 g/kg/day) in this sample of trained cyclists may have masked any additional anabolic benefit of the supplement [54]. Consequently, the ability to detect treatment effects attributable to additional protein provision may have been substantially reduced. This consideration should be taken into account when interpreting both the positive and null findings of the study. Interestingly, between-group differences in selected biomarkers were observed despite this already high protein intake; however, whether these responses reflect specific bioactive effects of the hydrolysate cannot be determined from the present study design and requires further investigation. Nevertheless, the observed findings support the rationale for further research examining the potential contribution of the hydrolysate's previously described antioxidant and immunomodulatory properties in athletic populations.

Beyond effects, these findings align with broader considerations of sustainability in nutrition [62]. Plant-based proteins are increasingly promoted not only for health but also for environmental and ethical reasons, with international organisations such as UN Nutrition calling for a transition from animal to plant-based sources [63–65]. Hydrolysates such as chickpea protein represent a valuable step in this direction, offering a sustainable alternative that combines acceptable anabolic potential with unique bioactive properties [66,67]. From a practical perspective, CPH supplementation could serve as a complementary option within athletes' recovery strategies, particularly when integrated into diets emphasising sustainability without compromising performance.

Several limitations should be considered when interpreting the present findings. The modest sample size limited statistical power, likely contributing to non-significant results for CK despite clinically meaningful trends. Likewise, the short intervention duration restricted the interpretation of the results to acute biochemical responses and prevented extrapolation to longer-term adaptations in body composition or training-induced physiological outcomes. Functional and perceptual measures such as exercise performance, delayed-onset muscle soreness (DOMS), perceived recovery, and fatigue were not assessed, which limits the practical interpretation of the observed biomarker responses. Similarly, direct measures of muscle protein synthesis using isotopic tracer methodologies were not included, preventing definitive conclusions regarding anabolic responses.

Although dietary intake was monitored throughout the intervention and training loads were comparable between groups, other factors known to influence post-exercise recovery and biomarker responses, including sleep quality, psychological stress, hydration status, perceived fatigue, and individual recovery practices, were not directly assessed. Moreover, while blood samples were collected following a standardised 12-hour recovery period after the final high-intensity training session, complete control of all recovery-related factors was not feasible under real-world training conditions. Consequently, these variables may have contributed to the inter-individual variability observed in the biochemical responses.

Another limitation is that nutritional intake was evaluated at the daily level rather than specifically within the immediate post-exercise recovery window. Therefore, potential differences in the timing and composition of post-exercise meals, particularly regarding carbohydrate and protein intake, may have influenced the measured biomarkers and recovery-related physiological responses. In addition, reliance on self-reported dietary intake records may have introduced some degree of reporting bias.

The study design also presents limitations regarding the interpretation of the supplementation effects. First, no protein-matched control group was included, limiting the ability to determine whether the observed responses were attributable to the bioactive properties of the chickpea protein hydrolysate or simply to the additional protein provided by the supplement. Second, only a single supplementation dose (12 g/day) was examined, preventing the evaluation of potential dose-response relationships. Finally, although the experimental supplement was designed to isolate the effect of the hydrolysate, the intervention evaluated a complete formulation rather than the isolated ingredient itself.

Despite these limitations, the present study contributes novel evidence regarding the potential physiological effects of CPH supplementation in trained cyclists. The attenuation of CRP and LDH levels, together with favourable trends observed for CK, should be interpreted cautiously given the modest sample size and the lack of statistical significance for some outcomes. This suggests that CPH supplementation may be particularly relevant for athletes exposed to repeated high-intensity sessions where efficient recovery is

critical. A notable strength of the present study is that the intervention was based on a chickpea protein hydrolysate that had previously undergone extensive physicochemical and biological characterisation. Earlier studies demonstrated high digestibility, excellent solubility, a favourable peptide profile characterised by low-molecular-weight peptides, strong antioxidant activity, and immunomodulatory effects in cellular models [43,44]. Consequently, the present intervention was built upon a product with established biological potential rather than a generic plant protein supplement, providing a stronger mechanistic basis for interpreting the observed responses.

Future research should build upon these findings through longer intervention periods (≥8 weeks), larger and more diverse cohorts, and study designs incorporating protein-matched comparator groups, multiple supplementation doses, and direct comparisons with established protein sources such as whey protein. Particular attention should also be given to populations with lower habitual protein intake or greater susceptibility to exercise-induced muscle damage, including vegan athletes and older adults.

In addition, future investigations should incorporate tighter control of post-exercise nutritional intake and recovery-related behaviours, together with mechanistic assessments such as muscle protein synthesis, redox balance, and cytokine responses. The inclusion of functional and perceptual outcomes, including exercise performance, delayed-onset muscle soreness (DOMS), perceived recovery, fatigue, and subsequent training capacity, as well as sex-specific analyses, would provide a more comprehensive understanding of the efficacy and mechanisms of chickpea protein hydrolysate supplementation. Such approaches will help determine whether the biomarker responses observed in the present study translate into meaningful benefits for recovery, adaptation, and athletic performance.

5. Conclusions

In conclusion, 14 days of chickpea protein hydrolysate supplementation did not alter body composition in trained cyclists with high habitual protein intakes. However, the intervention was associated with significant between-group differences in selected biomarkers related to exercise-induced inflammation and cellular stress, as well as favourable trends in markers of muscle damage. These findings suggest a potential modulatory effect of chickpea protein hydrolysate on acute physiological responses to exercise, although the practical significance of these changes remains to be established.

When interpreted alongside previous evidence demonstrating antioxidant and immunomodulatory properties of the same hydrolysate, the present results provide preliminary support for its biological activity in athletes. Nevertheless, the short intervention period precludes conclusions regarding long-term adaptations, recovery outcomes, or performance benefits. Further studies with larger samples, longer durations, and direct assessments of recovery and performance are required before definitive conclusions can be drawn.

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A.J.S.-O. and J.A.G.-J. designed the research study. J.G.-D., N.M.R.-M., M.-S.F.-P., and J.P. performed the research. J.G.-D. and J.A.G.-J. analysed the data. N.M.R.-M., M.-S.F.-P., J.P., and F.M. contributed to the technical methodology. A.J.S.-O., J.A.G.-J. and J.G.-D. wrote the manuscript. All authors contributed to the editorial changes in the manuscript. All authors have read and approved the final manuscript.

Disclosure statement

Several authors (N.M.R.-M., M.-S.F.-P., J.P., F.M., and J.A.G.-J.) participated in previous publicly funded research projects involving the development, production, and characterisation of the chickpea protein hydrolysate evaluated in this study. The results of these investigations, including the physicochemical characterisation and biological evaluation of the hydrolysate, have been previously published in peer-reviewed journals. The authors declare that they hold no patents, ownership interests, licensing agreements, royalty rights, or direct financial benefits related to the commercialisation of the product. The funding bodies and industrial collaborators had no role in the study design, data collection, statistical analyses, interpretation of results, manuscript preparation, or the decision to publish. All research

activities related to the present trial were conducted independently by the authors. The authors declare no other competing interests.

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Data availability statement

In alignment with Research Data Policies promoting open science, the dataset from this study is accessible to interested researchers via the Figshare repository: https://figshare.com/s/a46a8d32ba739611dc96 (provisional private link).

Ethics approval statement

The study was approved by the Andalusian Biomedical Research Ethics Portal (PEIBA), internal code: 2824-*N*-21, and all participants signed informed consent in accordance with Organic Law 3/2018 on Personal Data Protection. Ethical principles of the Declaration of Helsinki and international bioethics agreements were respected.

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