

# Female distance runners show a different response to post-workout consumption of wheat gluten hydrolysate compared to their male counterparts

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**Abstract.** Wheat gluten hydrolysate (WGH) is rich in glutamyl residue; glutamine is considered a conditionally essential amino acid under physical stress. WGH has been reported to suppress post-exercise rises in serum creatine kinase in male distance runners. This study aimed to reproduce the effects in female distance runners under similar conditions. The study was conducted in a double-blinded crossover manner. Six female collegiate distance runners ingested WGH or a placebo after a 2-h run at an intensity estimated as 60-70% of their maximum oxygen uptake. Blood was sampled before, immediately after, and at 10 and 24 h after the run. Unlike those in male runners, serum creatine kinase (CK) increased slightly, with a peak at 10 h after the run, while plasma glutamine kept declining. The anti-inflammatory effect of WGH was not evident since the post-exercise elevation of CK was ambiguous. Plasma glutamine concentrations also showed a different kinetics from that in men.

## Introduction

Glutamine, a conditionally essential amino acid, comprises approximately 60% of the free amino acids found in skeletal muscle and is closely associated with the synthesis and breakdown of muscle protein (1,2). Chronic fatigue, underperformance and decreased plasma glutamine concentrations are features of athletes with overtraining syndrome (3). Therefore, glutamine is supplemented in exercise and sports training (1).

As wheat gluten is a protein rich in glutamine, its enzymatic hydrolysate is used for fortification of glutamine in clinical

enteral nutrition, especially in immunonutrition. Recently, wheat gluten hydrolysate (WGH) was used for glutamine supplementation in athletes as well. When consumed after a half marathon, WGH suppressed increases in serum creatine kinase (CK) (4,5), suggesting the mitigation of muscle inflammation and promotion of recovery. Sawaki *et al* investigated changes in biochemical parameters among male runners with or without WGH intake after completing a half-marathon and a 45-km run. Plasma Gln was elevated after the ingestion, representing that the glutamyl residue contained in WGH was absorbed and contributed to the plasma level. The increase in serum CK was suppressed at 90 min after WGH consumption, indicating that WGH inhibits post-exercise muscle inflammation (4). Koikawa *et al* validated these results through an additional investigation (5).

This anti-inflammatory effect of WGH has been investigated in male distance runners, while its effect in females has not been characterized. We, therefore, investigated the effects of WGH consumption on distance running in female runners.

## Materials and methods

**Subjects.** Six female distance runners belonging to a university track team provided written, informed consent to participate in this study. Their age, height, weight, body fat ratio (%) and personal best for 5,000-m races (mean  $\pm$  SD) were, respectively, 19.8 $\pm$ 1.6 years, 157.3 $\pm$ 5.2 cm, 48.2 $\pm$ 5.8 kg, 13.6 $\pm$ 0.8% and 16 min 34.61 $\pm$ 19.31 sec. Their maximum oxygen uptake (VO<sub>2</sub> max) was estimated from their best time in a 12-min run (Table I).

**Wheat gluten hydrolysate.** One 4.5-g packet contained 3 g of WGH as a granulated powder supplement, citric acid, sugars and thickeners. The placebo supplement contained water-soluble dietary fiber replaced for WGH. Nisshin Pharma (Tokyo, Japan) prepared and provided the study supplements (Table II).

**Study protocol.** This randomized crossover, double-blind investigation comprised two 3-day sessions at an interval of 2 weeks between them. The runners were divided into two

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Table I. Baseline characteristics of the subjects.

Subject (n=6)	Age (years)	Height (cm)	Weight (kg)	Body fat (%)	Personal best at 5,000 m	VO <sub>2</sub> max (ml/kg/min)
A	21.0	162.5	52.5	14.4	16 min 08.76 sec	62.5
B	18.0	155.0	40.6	12.8	16 min 55.57 sec	66.8
C	20.0	149.0	43.0	14.2	16 min 19.72 sec	59.4
D	22.0	155.0	46.0	12.8	16 min 38.00 sec	61.1
E	20.0	162.1	54.3	13.0	16 min 56.77 sec	65.8
F	18.0	160.0	53.0	14.4	16 min 28.87 sec	65.8
Mean	19.8	157.3	48.2	13.6	16 min 34.61 sec	63.6
SD	1.6	5.2	5.8	0.8	19.31 sec	3.0

Table II. Composition of the wheat gluten hydrolysate (WGH) study supplement and placebo (per six-packet dose).

Study supplement	Composition	Content (g)
WGH	Wheat gluten hydrolysate	18.0
	Anhydrous glucose	6.0
	Anhydrous citric acid	2.7
	Sucralose (as sweetener)	0.048
	Lemon flavor	0.252
	Pullulan (as thickener)	0.405
Placebo	Polydextrose	18.0
	Anhydrous glucose	6.0
	Anhydrous citric acid	2.7
	Sucralose (as sweetener)	0.048
	Lemon flavor	0.252
	Pullulan (as thickener)	0.405

groups, three each, and performed a 2-h run at a pace of 190–200 m/min. One group was given WGH, while the other group received the placebo at the first session, and then the supplement was switched at the second session.

The runners refrained from training on the day before the run. On the day of the experiment, a blood sample was drawn from the median cubital vein before and immediately after the 2-h run. The runners then ingested six packets of WGH or placebo supplements, and blood was collected at 10 and 24 h later.

White blood cell count (WBC) and serum CK activities were determined in the blood samples, and plasma glutamine concentrations were measured in the samples collected from before, immediately and 10 h after the run. SRL (Tokyo, Japan) conducted the analyses according to the methods standardized by the Japan Society of Clinical Chemistry.

Nutritional intake of the runners was determined based on the 'Run! x Shoku-net Athlete Dietary Advice' program (Quality Life Service, Kanagawa, Japan). The program requires detailed documentation of the type and amount of food that has been consumed on a survey form and photographs of

described meals before and after consumption. Energy and nutrient intake were calculated from the data on the survey forms and from the photographs. The survey proceeded during the study from breakfast on the pre-run day to breakfast on the day after the run.

All the runners participated in the same run and were not permitted to participate in any strength conditioning or other intense training. Fig. 1 shows the schedule and measurements taken during the study.

The protocol of this study was reviewed and approved by the Ethics Committee of Juntendo University.

*Statistical analysis.* All data are expressed as the means  $\pm$  SD. Serum CK is expressed relative to the post-workout level, which was defined as 100. Differences between the groups were assessed using a paired t-test. The criterion for statistical significance was set at  $p < 0.05$ .

## Results

The subjects ran  $\sim 23$  km at a mean speed of 191.7 m/min during the 2-h run. The workout intensity, estimated by their maximal oxygen uptake of  $63.6 \pm 3.0$  ml/kg/min, was 63.8% of VO<sub>2</sub> max.

Serum CK increased, peaked at 10 h after the run, and then declined (Table III). When post-run activity was normalized at a value of 100 due to large individual differences, the decline from 10 to 24 h after the run was significant ( $p < 0.05$ ) in the WGH, but not in the placebo group. However serum CK levels did not significantly differ between the two groups at either 10 or 24 h after the run.

The WBC counts in the WGH group increased from  $5.3 \pm 1.2 \times 10^3/\mu\text{l}$  before, to a peak of  $5.8 \pm 1.9 \times 10^3/\mu\text{l}$  immediately after the run, and then gradually decreased to the level before the run. On the other hand WBC counts in the placebo group were elevated by the 2-h run to a peak at 10 h thereafter, and then declined. The decline significantly differed between 10 and 24 h after the race ( $p < 0.05$ ; Table III).

Mean plasma glutamine concentrations consistently declined from before to 10 h after the run in both groups with no significant differences between them (Table III).

No difference was observed in macronutrient intake between the placebo and WGH groups (Table IV).

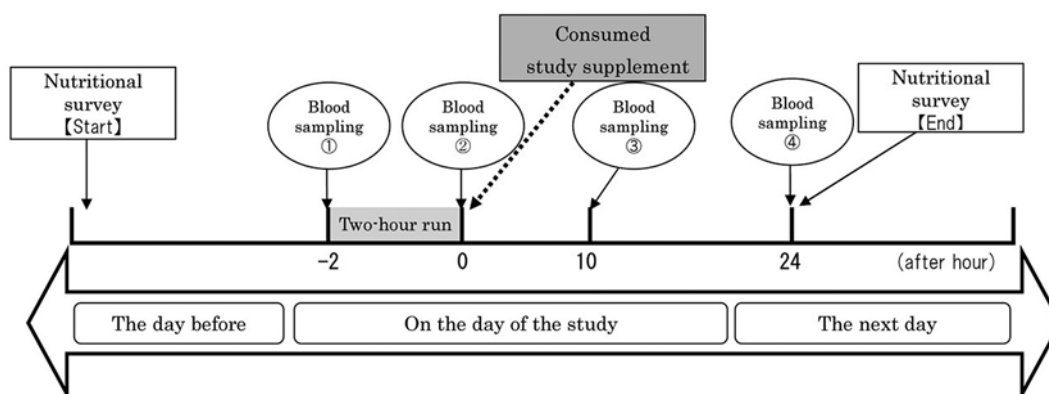


Figure 1. Study protocol.

Table III. Transition of the biomarkers before and after a 2-h run (mean  $\pm$  SD).

Parameter (unit) <normal range>	Group	Before	Immediately after run	10 h after run	24 h after run
CK (U/l) <45-210>	WGH	234 $\pm$ 99.3	319 $\pm$ 119.8	338 $\pm$ 113.8	312 $\pm$ 118.5
	Placebo	199 $\pm$ 55.3	295 $\pm$ 76.3	330 $\pm$ 139.1	306 $\pm$ 120.7
WBC ( $10^3/\mu$ l) <3.5-9.1>	WGH	5.3 $\pm$ 1.2	5.8 $\pm$ 1.9	5.5 $\pm$ 1.2	5.3 $\pm$ 1.7
	Placebo	5.7 $\pm$ 1.9	5.9 $\pm$ 1.5	6.7 $\pm$ 2.2	5.5 $\pm$ 2.1
Gln (nmol/ml) <478.3-658.5>	WGH	691 $\pm$ 40.9	656 $\pm$ 53.2	633 $\pm$ 26.8	-
	Placebo	702 $\pm$ 73.3	648 $\pm$ 83.9	629 $\pm$ 74.4	-

CK, creatine kinase; WBC, white blood cell count; Gln, glutamine.

Table IV. Macronutrient intake during the study (mean  $\pm$  SD).

Group	Energy (kcal/day)	Protein (g/day)	Lipids (g/day)	Carbohydrates (g/day)
WGH group	1,976 $\pm$ 467.4	75.1 $\pm$ 12.1	63.5 $\pm$ 14.7	272.6 $\pm$ 74.1
Placebo group	1,678 $\pm$ 336.8	65.8 $\pm$ 16.7	53.5 $\pm$ 9.0	239.4 $\pm$ 51.8

## Discussion

Sustained running, defined as running for 1-3 h at a pace of 40-75% of  $VO_2$  max, is regarded as the fundamental training for long distance runners to build aerobic capacity. Glycogen in the skeletal muscles is exhausted within 2 h of exercising at 60-70% of  $VO_2$  max (6). Therefore, the exercise load was set as a 2-h sustained running at a pace of 190-200 m/min, and intensity was estimated as 60-70% of  $VO_2$  max.

Extensive exercise results in delayed-onset muscle soreness (DOMS), with an increase in serum CK activity and a decrease in muscular strength (7). Serum CK is thus considered an indicator of muscular damage generated by training. Post-run levels of serum CK peak at 24 h after completing a full marathon at 410 $\pm$ 164 U/l (8) and a 100-km run at 7,012 $\pm$ 2,262 U/l (9). By contrast, mean serum CK in the present study peaked

at 10 h after the run and then declined. These findings indicate that the run in this study was less intensive than a full marathon or a 100-km run. Indeed, the pace was moderate and the duration was shorter. Swaminathan *et al* (10) identified a significant correlation between lean body mass and serum CK activity. Therefore, the absence of the expected increase in serum CK may be attributed to the gender of the subjects, since female runners have less lean body mass than male runners.

The serum CK activities significantly decreased from 10 to 24 h after the run in the WGH ( $p<0.05$ ), but not in the placebo group, when adjusted by the value obtained immediately thereafter that was designated as 100. However, the difference between the two groups was not significant. The rise in serum CK activity up to 24 h after the run was approximately 10% of that immediately after the run. The response of CK could

be too small to detect the anti-inflammatory effect of WGH in these female runners.

The increase in the WBC count after an endurance exercise is mainly due to the neutrophils mobilized into circulation (11). The WBC counts in the WGH group peaked immediately after the 2-h run and subsequently declined to the pre-run level, whereas those in the placebo group remained elevated for up to 10 h after the run. This may reflect prolonged excitation in the placebo group, but the changes were within the normal range.

Glutamine is the most abundant amino acid in the body, and skeletal muscle is the primary source of plasma glutamine. Plasma concentrations of glutamine fall after intensive exercise, and also in athletes with overtraining syndrome. Since the glutamine supply cannot meet its demand under physical stress condition, it is considered to be a conditionally essential amino acid (12).

Plasma glutamine concentrations markedly decline at 1 h after completing a 3-h workout at 55% of  $\text{VO}_2$  max (13). Four hours of sustained exercise at 50% of  $\text{VO}_2$  max cause a 60% decrease in glutamine concentrations from baseline that recovers to 88% of baseline after 5.5 h (14). According to these data, we hypothesized that plasma glutamine concentration should decrease after a 2-h run and then recover within 10 h. Therefore plasma glutamine was monitored up to 10 h after a 2-h run. However, it was even lower at 10 h than immediately after the run. Since plasma glutamine is supplied by skeletal muscle, the lower lean body mass in female runners may have contributed to these findings.

This study aimed to confirm the anti-inflammatory effect of WGH intake on changes in biomarkers associated with running in female distance runners. However, we could not achieve this goal because the kinetics of the biomarker were different from that hypothesized based on the male runners; changes in serum CK were insufficient and plasma glutamine remained decreased for up to 10 h after a 2-h run. The insignificant changes in serum CK may have been due to the moderate intensity of the workout, although changes in plasma glutamine levels seemed to contradict this notion as they continued to decrease for up to 10 h after the run. As skeletal muscle is the source of both CK and glutamine, the smaller skeletal muscle mass in female runners may have affected the results.

Since human trials with extensive exercise load tend to be conducted with male subjects, studies investigating female cases are limited. Thus, responses induced by intense physical stress in females must often be estimated extrapolating from male cases assuming the same kinetics. However, as described in this study, there could be gender differences in the kinetics of post-exercise biomarkers. It should be worthwhile to examine the response to exercise in female subjects contrasting their male counterparts.

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