

Applied nutritional investigation

Delayed-onset muscle injury and its modification by wheat gluten hydrolysate

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Abstract

Objective: We investigated the pattern of delayed-onset muscle injury in well-trained athletes after a competitive half-marathon and the effects of post-race intake of wheat gluten hydrolysate (WGH).

Methods: Thirty well-trained college runners with a best time of 14–15 min over 5000 m raced in a half-marathon. Thereafter, they were divided into three groups based on finish times and given 0 (control), 10, or 20 g of WGH. Blood biochemical parameters were monitored at –1 d, +1 h, +1 d, and +2 d after the race. Data selected according to finish times and biochemical parameters were then analyzed.

Results: Plasma creatine kinase activity peaked at 1 d after the race in the control group and correlated with post-race white blood cell counts. The post-race elevation of creatine kinase activity was dose-dependently suppressed by WGH.

Conclusion: Delayed-onset muscle injury peaked in well-trained distance runners at 1 d after a half-marathon and was dose-dependently suppressed by a post-race intake of WGH. © 2009 Published by Elsevier Inc.

Keywords:

Half-marathon; Creatine kinase; Inflammation; Glutamine; Peptide

Introduction

Muscular exercise causes injury in active muscle fibers, particularly when the exercise is relatively intense, eccentric, and/or of long duration. This initial injury results in a temporary reduction in force production and elevated circulating levels of intramuscular proteins such as creatine kinase (CK) and myoglobin [1]. Subsequently, muscular

injury is amplified after exercise, and this can be observed by microscopy and by measuring levels of circulating intramuscular proteins. Although the mechanism of delayed-onset muscle injury has not been fully elucidated, the immune response might be involved in the process [2].

Prolonged intensive exercise mobilizes white blood cells (WBCs), mainly neutrophils and monocytes, into the circulation. The recruited neutrophils then migrate to the injured tissue and become activated to cause inflammatory lesions, although this process does not always lead to injury [3]. Cytokines released from monocytes and other cells contribute to activation. Recent reviews have provided an excellent overview of exercise-induced muscle injury. Butterfield et al. [2] summarized the role of neutrophils and macrophages during this process, Peake et al. [4] interpreted the inflammatory response focusing on cytokines, and Falvo and Bloomer [5] analyzed related published data to clarify the issue in athletic populations.

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Despite extensive research, understanding delayed-onset muscle injury in athletes is complicated by the nature of the study subjects and exercise loads examined.

Most investigations have used untrained subjects, but the pattern of postexercise muscle injury differs according to training status. Plasma CK activity in untrained subjects after a 5000-m run increases three- to five-fold on the following day, but only slightly increases in habitual runners [6]. When leg resistance was used as the exercise load, serum CK activity peaked several days later and reached much higher values in untrained than in trained subjects [7,8]. The CK response differs even among individuals with a homogeneous training background according to their physical condition; Nosaka and Clarkson [9] showed that postexercise increases in plasma CK activity are diminished when the initial level is increased by eccentric exercise, which is known as the “repeated bout effect.”

In addition to subject selection, exercise load is another concern. Recent studies have tended to use simplified model exercise to induce initial muscle damage, e.g., isokinetic elbow flexion. This approach is necessary to investigate mechanisms, but whether the findings are applicable to the athletic population remains unclear. The postexercise transition of circulating CK activity differs according to the exercise: a single peak appears at 24–48 h after distance running [6], whereas the peak is biphasic after power training [8]. Therefore, the results obtained from a laboratory-based model exercise might not be directly applicable to the training programs associated with specific sports.

We previously found that wheat gluten hydrolysate (WGH) suppresses exercise-induced muscle injury [10] by comparing biochemical parameters before and after a half-marathon with or without a post-race intake of WGH in seven healthy male well-trained college distance runners. The increase in plasma CK activity was significantly suppressed in the WGH group when compared with controls at 90 min after intake (104.6 versus 110.0 adjusted at intake as 100). These findings suggested that WGH suppresses postexercise muscle injury. However, this observation was not definitive. First, the number of the subjects was too small to elicit decisive results, and second, the timing of this observation did not agree with other reports. Plasma CK activity often peaks at 24–48 h after eccentric exercise, whereas the parameters were monitored only up to 90 min after the race, because the main purpose of the study was to assess glutamine (Gln) absorption from WGH after distance running.

To determine the nature of exercise-induced muscle injury in well-trained college distance runners after a half-marathon and the effects of WGH on postexercise amplification of muscle lesions, we reproduced the previous experiment on a larger scale over a longer observation period. Therefore, the present study aimed to determine the nature of postexercise injury, the timing of peak CK, and its relation to inflammatory cells and muscle injury and to confirm the suppressive effect of WGH on muscle injury.

Materials and methods

Subjects

Thirty male college athletes 18–22 y of age provided written informed consent to participate in the present study. All were long-distance runners whose mean \pm SD (range) best time over a 5000-m race, height, and weight were, respectively, 14 min 38 s \pm 12 s (14 min 11 s–14 min 56 s), 170.5 \pm 4.1 cm (162–178 cm), and 55.9 \pm 4.7 kg (47.4–65.1 kg). Twenty-four of them had previously participated in a half-marathon, with mean times of 1 h 8 min 33 s \pm 2 min 8 s (1 h 3 min 39 s–1 h 10 min 37 s).

Wheat gluten hydrolysate

The granulated WGH prepared for this study contained WGH Glutamine Peptide GP-1N (Nisshin Pharma, Tokyo, Japan), 96.7% w/w; pullulans, which is an extracellular polysaccharide from *Aureobasidium pullulans*, 1.5% w/w; lemon flavor, 1.5% w/w; and extract of *Stevia rebaudiana*, 0.1% w/w. The Gln content of the granulated WGH estimated using Wilcox's method was 27.4% [11], assuming that all nitrogen in the amide form was derived from Gln.

Granulated WGH was provided as lots of 10 g in laminated polyethylene and aluminum packets and was consumed orally.

Study design

After racing in a half-marathon at Ageo City, Saitama, Japan, on November 21, 2005, the participants were randomly assigned to one of three groups according to their finish times to balance the race performance among groups. To assemble and group the participants required 1 h. That is, 1 h after completing the race, the participants consumed zero, one, or two packets of granulated WGH (hereafter referred to as control, WGH-10, and WGH-20 groups, respectively). Blood samples were collected at 1 d before the race, immediately before WGH administration, and in the morning 1 and 2 d after the race. The participants were instructed to refrain from hard training and to avoid taking antioxidant supplements such as vitamin C and β -carotene for 3 d before the race and for the duration of the study.

After completing all measurements, we selected data for further analyses according to preset criteria. Eleven of the participants were excluded according to these criteria (Table 1). The background characteristics of the remaining 19 athletes were proved homogeneous among the three experimental groups (Table 2).

The ethics committee of Juntendo University approved the study protocol.

Table 1
Excluded subjects with rationales according to exclusion criteria

#	Criteria	Indicator	No. of excluded subjects			
			Total	Group		
				Control	WGH-10	WGH-20
1	Race times >3 min slower than best time in half-marathon and >1 h 10 min	Poor physical condition or inability to compete at full potential	3	1	1	1
2	Biochemical parameters unrelated to exercise outside normal ranges at any point during experimental period	Possible physical difficulties unrelated to those induced by the race	1	0	0	1
3	Initial CK activity >500 U/L	Potential delayed-onset muscle injury or other injury	4	2	1	1
4	Peak CK activity >1000 U/L.	Potential hidden injury	3	1	1	1
Total			11	4	3	4

CK, creatine kinase; WGH-10, 10 g of wheat gluten hydrolysate; WGH-20, 20 g of wheat gluten hydrolysate

Analyses

Blood samples were collected from the cubital vein for the following analyses: total protein, albumin, urea nitrogen, creatinine, uric acid, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride, total lipid, non-esterified fatty acid, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactate dehydrogenase, γ -glutamyl transpeptidase, leucine amino peptidase, CK, glucose, lipid peroxide, iron, total iron-binding capacity, unsaturated iron-binding capacity, amino acids, WBC count, red blood cells, hemoglobin, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelets. All measurements were performed at HKK (Yokohama, Tokyo, Japan).

Statistical analyses

Data are presented as mean \pm SD (range), unless otherwise indicated. Differences among the three groups were assessed using one-way analysis of variance and Fisher's Protected Least Significant Difference (PLSD) post hoc test when appropriate. Trends in post-race CK elevation as-

sessed among the groups using incremental areas under the curve from after the race to 1 and 2 d were examined using Williams' test. Relations between post-race WBC and peak CK activity were analyzed using Pearson's correlation coefficient. The criteria for statistical significance was set at $P < 0.1$ for Fisher's PLSD and Pearson's correlation coefficient analysis and $P < 0.05$ for Williams' test. All data were statistically analyzed using the add-in software for Microsoft Excel, Statcel [12].

Results

Mean plasma CK activity

Mean plasma CK activity increased from 264.0 to 438.5 U/L immediately after the half-marathon, increased to 669.7 U/L at 1 d after the race, and then declined in the control group (Fig. 1). This profile was the same in all athletes in the control group and in most of those in the other groups.

Effects of WGH on CK activities

The mean CK activities at 1 d after the race in the control, WGH-10, and WGH-20 groups were 669.7, 583.6,

Table 2
Background characteristics of data selected for analyses*

Characteristic	Group		
	Control	WGH-10	WGH-20
No. of subjects	6	7	6
Age (y)	20.3 \pm 1.2	20.1 \pm 1.3	19.5 \pm 0.6
Height (cm)	171.1 \pm 3.6	170.4 \pm 3.8	171.6 \pm 2.6
Weight (kg)	54.2 \pm 4.7	55.2 \pm 2.0	56.5 \pm 2.0
Best time over 5000 m	14 min 32 s 50 \pm 14 s 53	14 min 38 s 51 \pm 10 s 53	14 min 30 s 10 \pm 10 s 17
Time of half-marathon	1 h 6 min 50 s \pm 2 min 1 s	1 h 6 min 18 s \pm 1 min 52 s	1 h 6 min 46 s \pm 1 min 27 s

WGH-10, 10 g of wheat gluten hydrolysate; WGH-20, 20 g of wheat gluten hydrolysate

* Data are presented as mean \pm SD.

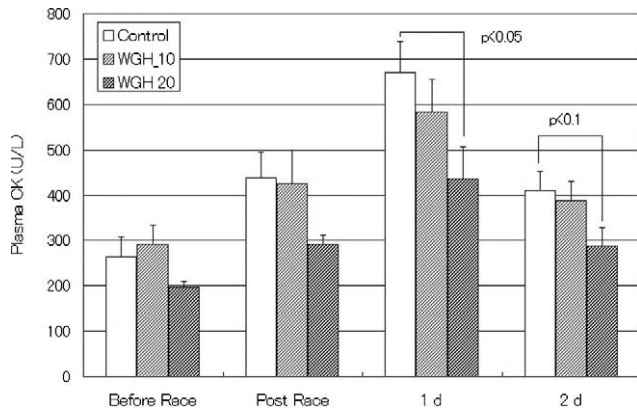


Fig. 1. Changes in plasma CK activity. Error bars indicate SD. CK, creatine kinase; WGH_10, 10 g of wheat gluten hydrolysate; WGH_20, 20 g of wheat gluten hydrolysate.

and 436.7 U/L, respectively. The peak CK activity of WGH-20 was lower than the control value at 1 d ($P < 0.05$) and 2 d ($P < 0.1$), whereas the value did not differ between -1 d and $+1$ h of the race among groups (Fig. 1). The results of Williams' test showed that WGH suppressed the incremental area under the CK curve after the race for 1 d ($P < 0.05$) and 2 d ($P < 0.05$).

Postexercise WBC and peak CK activity

Postexercise WBC counts and peak CK activities at 1 d after the race positively correlated in the control groups ($R = 0.871$, $P < 0.1$), when the WBC count was beyond the normal range ($3.9\text{--}9.8 \times 10^9/\text{L}$; Fig. 2a), but not in the WGH groups: the P values of the WGH-10 and WGH-20 groups were 0.91 and 0.82, respectively (Fig. 2b,c).

Other parameters

Plasma Gln concentration declined after the half-marathon and recovered 1 d later (Fig. 3), with no significant differ-

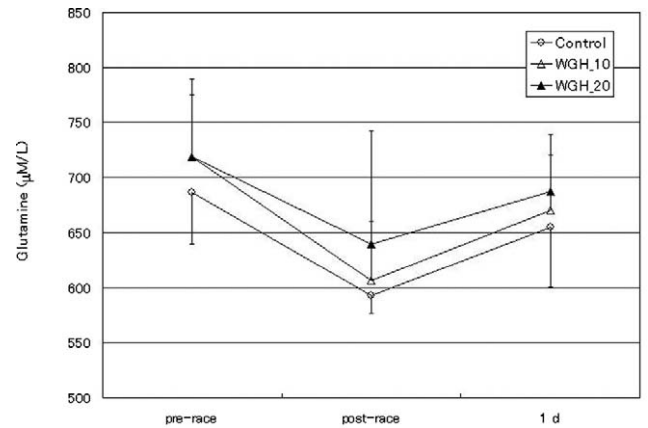


Fig. 3. Changes in plasma glutamine concentration. WGH_10, 10 g of wheat gluten hydrolysate; WGH_20, 20 g of wheat gluten hydrolysate.

ences among groups. Other data obtained will be published elsewhere.

Discussion

Delayed-onset muscle injury, observed as elevated plasma CK activity, is thought to peak at 24–48 h after distance running. However, large intersubject variability causes difficulties with predicting the timing and extent of injury. Ikawa et al. [6] observed that these parameters depend on the training status of the subjects. Plasma CK activity peaked at 1 d after running a 5000-m race in two of five untrained subjects and at 2 d or later in three more. After 6 mo of training, the peaks were much smaller and occurred mainly at 1 d ($n = 4$) and at 2 d ($n = 1$) after the run. Suzuki et al. [13] reported that plasma CK activity peaked at 24 h after a full-marathon in individuals with a running habit of 4–10 y. The CK values of master runners (>60 y of age) [14] and of well-trained runners ($n = 6$, 28–35 y of age) [15] also peaked at 1 d after a full-

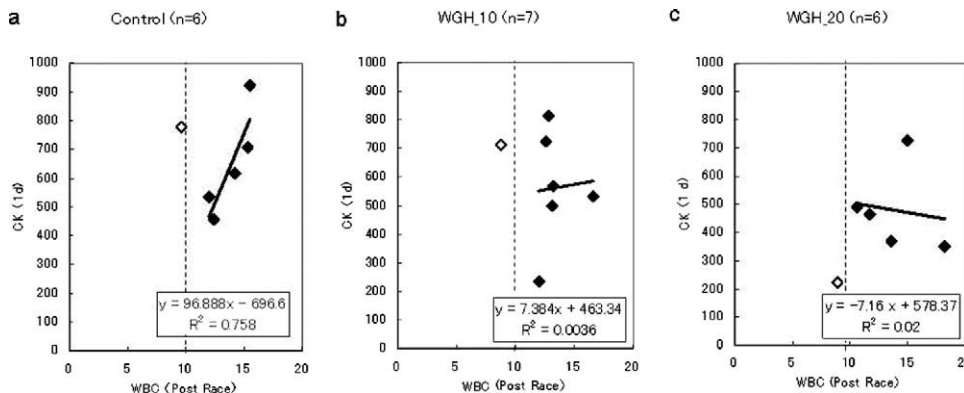


Fig. 2. Relation between post-race WBC count and peak plasma CK activities: (a) control, (b) WGH-10, (c) WGH-20. Small dots and thin lines, individual data; bold bars and lines, means. CK, creatine kinase; WBC, white blood cell; WGH_10, 10 g of wheat gluten hydrolysate; WGH_20, 20 g of wheat gluten hydrolysate.

marathon. Although these reports showed that plasma CK activity peaks in well-trained subjects at 1 d after a full marathon, little is understood about such activity after running half-marathons.

We recruited top-class college distance runners with similar running abilities who participated in a competitive half-marathon. The runners were expected to take the event seriously because it was an important qualifier for the Hakone Ekiden, which is the most popular national intercollegiate long-distance race in Japan. Ten athletes per team run approximately 20 km each during a round-trip race from Tokyo to Hakone. Therefore, to assess delayed-onset muscle injury after running 20 km or a half-marathon (21.0975 km) is a concern. After excluding inappropriate data according to the criteria established in advance, plasma CK peaked 1 d after the race in all control subjects (Fig. 1). Thus, we determined that the CK activity peaked in well-trained runners at 1 d after a competitive half-marathon.

In addition to timing, the intensity of peak CK activity is a concern because it represents the magnitude of muscle injury. Paulsen et al. [16] compared parameters using unilateral maximal isokinetic eccentric actions with quadriceps to induce initial injury, based on the hypothesis that initial muscle damage caused by eccentric exercise would initiate a weak local inflammatory response, which would then be amplified by recruited immune cells attracted by proinflammatory mediators. They found that acute reductions in force-generating capacity correlated with postexercise peak leukocyte count and peak CK activity [16]. Our data (Fig. 2a) showed a positive correlation ($R = 0.871$) between postexercise WBC count and peak CK activity in WBCs beyond the normal range ($P < 0.1$). Although the numbers of data are limited and data were truncated above the normal range, our results agreed with the findings of Paulsen et al. Therefore, postexercise WBC count would be a parameter with which to predict the magnitude of peak CK activity at 1 d after exercise.

The post-race intake of WGH dose-dependently suppressed peak CK activity (Fig. 1), which agreed with our previous findings [10]. Thus, postexercise WGH suppressed delayed-onset muscle injury after distance running. Postexercise WBC count did not correlate with peak CK activity in the WGH groups, unlike the control group. This indicated that postexercise WGH intake modified the postexercise inflammatory response and supported the suppressive effect observed as peak CK activity. However, the mechanism of the anti-inflammatory effect of WGH remains to be elucidated.

Wheat gluten hydrolysate comprises about 30% (w/w) Gln. The WGH groups thus consumed 2.7 g (WGH-10) and 5.4 g (WGH-20) of Gln after the race. Gln is the most abundant amino acid in the body, where it is produced and pooled in skeletal muscle and supplied to the circulation. Under conditions of prolonged exercise, the plasma Gln concentration initially increases due to increased release from skeletal muscle and then declines after consumption exceeds the supply [17]. Skeletal muscle is the main supplier

of Gln, and thus it must produce Gln during and after exercise. The Gln concentration after running a half-marathon decreased (Fig. 3), and therefore post-race Gln supplementation could ease the skeletal muscle load to produce Gln and help the muscle recover from initial injury. This process might reflect the suppression of peak CK activity.

Specific physiologic properties of WGH other than Gln have also been reported. Yoshikawa et al. [18] established opioidergic peptide “exorphins,” and Motoi and Kodama [19] described an anti-angiotensin I-converting enzyme peptide. In addition, accumulating data indicate that specific food-derived peptides have physiologic activity [20]. Therefore, a specific peptide might induce an anti-inflammatory response.

Some hypothetical mechanisms could be posited to explain the suppressive effect of WGH on delayed-onset muscle injury. However, a definitive explanation remains elusive due to the absence of supporting data. Therefore, extensive research on this subject is required.

Conclusion

Plasma CK activity peaked at 1 d after a competitive half-marathon in well-trained distance runners, and its magnitude correlated with post-race WBC counts. Post-race administration of WGH dose-dependently suppressed peak CK activity, but the mechanism remains unknown.

Acknowledgments

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