

## Note

# Effect of Wheat Gluten Hydrolysate on the Immune System in Healthy Human Subjects

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**Nine healthy volunteers were divided into a test group (n = 5) and a control group (n = 4). The test group consumed 3 grams per d of wheat gluten hydrolysate for 6 d, and their NK cell activity and hematological parameters were measured: The same assessments were performed in the control group, which did not receive wheat gluten hydrolysate.**

**In the test group, NK cell activity increased significantly ( $P = 0.018$ ) after wheat gluten hydrolysate intake. No adverse effects were observed in either group.**

**Key words:** wheat gluten hydrolysate; immune system; natural killer cell activity; NK

Wheat gluten has a unique amino acid composition: glutamyl residues account for about 40% of the amino acids.<sup>1)</sup> Due to its amino acid composition, wheat gluten has been considered a natural glutamine source.<sup>2,3)</sup>

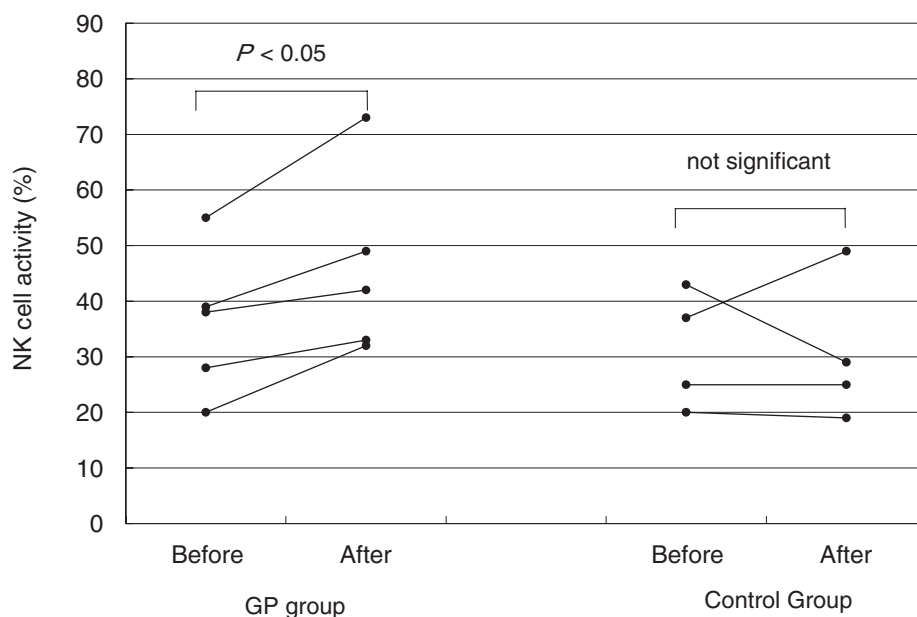
Glutamine, the most abundant amino acid in the body, has many roles in the physiological system.<sup>4)</sup> It is the preferred substrate for rapidly dividing cells, such as those in the gut and the immune system. It has been reported to modulate the functions of immune cells, *e.g.*, lymphocytes,<sup>5,6)</sup> monocytes,<sup>7)</sup> and granulocytes.<sup>8)</sup> A meta-analysis revealed that glutamine supplementation reduced the incidence of infectious complications in surgical patients.<sup>9)</sup>

In addition, studies have been conducted on the specific physiological functions of wheat gluten hydrolysate apart from the effect of glutamine. In 1980, Schusdziarra *et al.* reported opioidergic activity in wheat gluten hydrolysate,<sup>10–12)</sup> and in the early 1990s Fukudome *et al.* identified the active peptides.<sup>13,14)</sup> Motoi and Kodama identified an inhibitory peptide against angiotensin-I converting enzyme.<sup>15)</sup> In addition, we encountered some cases with mild hepatitis that exhibited significant improvement in response to the administration of wheat gluten hydrolysate.<sup>16)</sup>

We examined the effects of the administration of wheat gluten hydrolysate on the immune function of

healthy human volunteers. All experiments were performed in accordance with the guidelines of the Helsinki Declaration and the ethical committee of the clinic. Nine volunteers (ages 22–59) were divided into two groups, a test group (n = 5; 3 male and 2 female; mean age, 41.6, range, 23–58) and a control group (n = 4; female; mean age, 32.8, range, 22–59). The test group (GP group) took one gram of wheat gluten hydrolysate after every meal (3 grams per d) for 6 d. The wheat gluten hydrolysate used was “Glutamine Peptide” (GP) of Amino Health (Sakaide, Japan), a product consisting of granulated wheat gluten hydrolysate “Glutamine Peptide GP-1” (Nisshin Pharma, Tokyo), slightly sweetened. The wheat gluten hydrolysate was the same hydrolysate as that used by Sawaki *et al.*,<sup>3)</sup> and by us in our previous report.<sup>16)</sup> The control group did not receive a placebo. Although no instructions were given to subjects concerning food intake, they did not eat or drink immoderately during the experimental period. Blood samples were collected at a time between 9:00 and 11:00 AM before and after the administration period. The blood count and differential white blood count were measured at our institute. NK cell activity was determined using K562 target cells in a <sup>51</sup>Cr-release assay<sup>17)</sup> at SRL Laboratories (Tokyo). Peripheral blood mononuclear cells (PMBC) were separated from heparinized blood by Ficoll-Conray density gradient centrifugation. After two washings with PBS, they were suspended at  $1 \times 10^6$  cell/ml with RPMI 1640 with 10% FBS and used as a source of NK cells. The target cells were labeled with <sup>51</sup>Cr and suspended at a concentration of  $1 \times 10^6$  cell/ml. Then triplicates of 10  $\mu$ l of the target cells and 200  $\mu$ l of PMBC were mixed together and incubated in microtiter plates for 3.5 h. The plates were centrifuged for 10 min, 150  $\mu$ l of the supernatant was transferred to new tubes, and radioactivity was determined. Spontaneous release was determined by incubation of 10  $\mu$ l target cells with 200  $\mu$ l of medium. Maximum release was determined by incubation of 10  $\mu$ l target cells plus 200  $\mu$ l of 1 N hydrochloric acid. NK cell activity (<sup>51</sup>Cr release) was calculated as

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**Fig. 1.** NK Cell Activity before and after the Experimental Period.

Changes in values were analyzed using the paired *t* test. Statistical difference was considered significant at  $P < 0.05$ .

$$\text{NK cell activity (\%)} = \frac{[\text{experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}]}{[\text{maximum } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}]} \times 100.$$

Figure 1 shows the change in NK cell activity in the GP group and the control group. In the GP group, an increase in NK cell activity was observed in all subjects ( $P = 0.018$ , before vs. after intake), whereas the control group did not show any significant change during the same period ( $P = 0.897$ ). In this experiment, there were apparently some biases in the grouping, such as gender and age, due to the limited number of subjects. Such differences should affect NK activity. Therefore, it is invalid to compare the two groups directly. However, changes within the subjects are worth discussing. As shown in Fig. 1, an increase in NK activity was observed in all, five out of five, subjects in the GP group, whereas the subjects in the control group showed one increase, one decrease, and two unchanged. This result suggests that intake of wheat gluten hydrolysate is effective in augmenting NK cell activity in healthy people.

NK cells are known to play a critical role in immune surveillance against tumor development and viral functions.<sup>18,19)</sup> Activation of NK cells is regarded as effective in patients with autoimmune disease or cancer<sup>20)</sup> and in elderly people,<sup>21)</sup> who usually have low levels of NK cell activity. Therefore, the ability of various materials that affect NK cell activity in human subjects has been investigated.

Morphine<sup>22)</sup> and simvastatin<sup>23)</sup> have been reported to decrease NK cell activity, while ubenimex,<sup>24)</sup> sizofiran,<sup>25)</sup> refecoxib,<sup>26)</sup> low-molecular-weight-heparin,<sup>27)</sup> and

hochu-ekki-to<sup>28)</sup> enhanced it. Many functional food materials, *e.g.*, glutamine,<sup>29)</sup> tomato extract,<sup>30)</sup> conjugated linoleic acid,<sup>31)</sup> and bitter melon,<sup>32)</sup> have been investigated, but were not found to influence NK cell activity. Among food components, only probiotics<sup>33–35)</sup> and polysaccharides<sup>36,37)</sup> have been consistently reported to increase NK cell activity.

Independent groups have reported that continuous intake of fermented milk containing *Lactobacillus casei*,<sup>34,35)</sup> *L. rhamnosus*,<sup>35)</sup> or *Bifidobacterium lactis*<sup>35)</sup> augments NK cell activity. Polysaccharides from the mushrooms *Ganoderma lucidum* (450 mg/d, 12 weeks)<sup>36)</sup> and *Sparassis crispa* (300 mg/d, 8 weeks)<sup>37)</sup> have been reported to elevate NK cell activity. The polysaccharides from lactobacilli and mushrooms are known to contain  $\beta(1-6)$  and  $\beta(1-3)$  glucans in large quantities. Therefore, the reported immunomodulatory effects might result from indigestible polysaccharides.

The granulated wheat gluten hydrolysate we used in this study contained 0.1% of an extract of *Stevia rebaudiana* (stevia), an intense sweetening agent widely used in Japan. Since the stevia content is very limited, the immunoenhancing effect appears to result from the wheat gluten hydrolysate itself. The hydrolysate was manufactured by hydrolyzing wheat gluten by protease and amylase; it contained 70% protein (Kjeldahl method,  $F = 5.70$ ), 0.1% fat, and 6% water. The hull of the wheat kernel is known to contain indigestible polysaccharides such as hemicellulose<sup>38)</sup> and arabinoxylan,<sup>39)</sup> and some reports suggest that these kinds of

**Table 1.** Changes in Blood Test and Differential White Blood Counts

		GP group						Control group						Normal Range	
	Sex	Pre			Post			Pre			Post			Unit	
<i>Blood test</i>		mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n		
WBC		56 ± 13		5	49 ± 8		5	67 ± 8		4	66 ± 23		4	40~80	× 10 <sup>2</sup> /μL
RBC	M	510 ± 7		3	503 ± 8		3						4	400~550	× 10 <sup>4</sup> /μL
	F	436 ± 43		2	432 ± 52		2	440 ± 30		4	444 ± 28		4	350~550	× 10 <sup>4</sup> /μL
Hb	M	15.5 ± 0.4		3	15.2 ± 0.4		3							13.0~17.0	g/dL
	F	12.3 ± 0.4		2	12.1 ± 0.2		2	12.5 ± 1.3		4	12.5 ± 1.1		4	11.0~15.0	g/dL
HCT	M	44.9 ± 1.1		3	44.4 ± 1.2		3							40~52	%
	F	36.6 ± 1.0		2	36.5 ± 0.6		2	35.8 ± 3.3		4	36.0 ± 2.9		4	35~45	%
MCV	M	88 ± 3		3	89 ± 2		3							90~105	fL
	F	85 ± 11		2	85 ± 11		2	82 ± 10		4	82 ± 10		4	85~100	fL
MCH		29.6 ± 2.2		5	29.5 ± 2.3		5	28.5 ± 3.6		4	28.3 ± 3.9		4	30~35	pg
MCHC		34.1 ± 0.5		5	33.9 ± 0.7		5	34.8 ± 0.4		4	34.7 ± 0.6		4	30~35	%
PLT	M	23 ± 4		3	23 ± 4		3							17.6~32.0	× 10 <sup>4</sup> /μL
	F	32 ± 9		2	31 ± 6		2	31.8 ± 6.7		4	31.5 ± 8.3		4	17.2~32.8	× 10 <sup>4</sup> /μL
<i>Differential white blood count</i>															
Neutrophil		56.8 ± 5.4		5	58.4 ± 7.9		5	58.8 ± 4.4		4	58.4 ± 7.8		4	37 ~ 70	%
Eosinophil		2.2 ± 0.6		5	2.6 ± 1.2		5	3.4 ± 1.3		4	3.8 ± 2.9		4	0 ~ 5	%
Basophil		0.7 ± 0.2		5	0.4 ± 0.1		5	0.6 ± 0.3		4	0.6 ± 0.2		4	0 ~ 1	%
Monocyte		7.7 ± 1.3		5	6.8 ± 1.4		5	6.7 ± 1.4		4	6.8 ± 0.9		4	2 ~ 8	%
Lymph		32.6 ± 4.5		5	31.8 ± 6.2		5	30.6 ± 4.8		4	30.4 ± 8.3		4	24 ~ 53	%

Abbreviations are as follows: WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet.

polysaccharides have immunomodulatory activity.<sup>40,41)</sup> However, dietary fiber was not detectable (Prosky method<sup>42,43)</sup>) in the wheat gluten hydrolysate. Since only protease and amylase were used in the preparation, this means that the content of indigestible polysaccharides was not more than the detectable limit of 1 g/kg, in other words, 3 mg per d at most. Hence it does not appear likely that the small amount of polysaccharide provoked the increase in NK cell activity.

Changes in blood count and differential white blood count parameters are summarized in Table 1. These parameters stayed within the normal range and did not show significant changes during the study. There were no clinical incidents indicative of adverse effects in either the GP group or the control group during the study. Therefore, the intake of wheat gluten hydrolysate is considered to be safe under the conditions used.

In conclusion, intake of wheat gluten hydrolysate might possibly augment NK cell activity without severe side effects. Although the mechanism of the immuno-enhancing effect observed in this study remains to be elucidated, it is meaningful to note the phenomenon that such proteinous material might possess immuno-enhancing activity in humans.

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