

# Effects of Benzylglucosinolate on Endurance Capacity in Mice

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The present study was designed to determine the effects of benzylglucosinolate on endurance capacity in mice. Mice were orally administered either vehicle or benzylglucosinolate (0.015 or 0.03 mg/kg) via stomach intubation for a 6-week period. Benzylglucosinolate-treated mice showed a significantly increased endurance exercise capacity. Benzylglucosinolate significantly decreased blood lactate concentrations and significantly elevated plasma non-esterified fatty acid (NEFA) during exercise. Lastly, benzylglucosinolate treatment significantly decreased fat accumulation. These data suggest that benzylglucosinolate enhanced swimming endurance due to increased fatty acid utilization as an energy source.

**Key words** — benzylglucosinolate, endurance capacity, *Lepidium meyenii* (Maca)

## INTRODUCTION

Exercise-induced fatigue has been attributed to several factors. First, cells and tissues are damaged by exercise resulting in leakage of myoglobin and metabolic coenzymes into the blood, as well as the destruction of red blood cells. Second, exercise requires consumption of energy sources such as glycogen, and maximally induces metabolism of internal energy stores thereby depleting this energy source. Third, through these processes, exercise causes production and accumulation of metabolism-related substances such as lactic acid in the body.<sup>1–3)</sup> Therefore recovery from exercise-induced fatigue requires repair of damage that has occurred in the body. Specifically, re-synthesis of the consumed energy sources and leaked cell and tissue components is needed, as are degradation and removal of internal reduction substances. Thus early-stage recovery from exercise-induced damage and minimizing damage can be considered anti-fatigue mechanisms; it can be said that anti-fatigue

functionality is the promotion of recovery from fatigue or minimizing fatigue development. We consider increased resistance to the development of fatigue to be synonymous with increased endurance capacity.

*Lepidium meyenii* (Maca) has become increasingly popular as a nutritional supplement, with its use spreading to medical practice.<sup>4)</sup> Maca has traditionally been used by Peruvians living at high altitudes as a nutrient, energizer, aphrodisiac, and/or fertility enhancer and physical endurance enhancer, as well as for male impotence and female hormonal imbalances.<sup>5–8)</sup> Maca is capable of attenuating stress responses by decreasing changes of corticosterone and glucose levels, as well as reducing or abolishing stress-induced ulcers and increases of adrenal gland weight.<sup>7)</sup>

Benzylglucosinolate is one of the constituents of Maca and is thought to be its active ingredient. However, few studies have examined benzylglucosinolate, and its effects on endurance capacity have not been previously quantified. The aim of this study was to determine the effects of oral administration of benzylglucosinolate on endurance capacity in male mice.

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## MATERIALS AND METHODS

**Benzylglucosinolate** — Benzylglucosinolate was purchased from C2.Bioengineering Glucosinolates.com, Copenhagen, Denmark (Purity, 98.9%). All chemicals used were of the highest analytical grade.

**Animals** — Four-week-old male ddY mice (SLC, Shizuoka, Japan) were housed in standard cages (21.5 × 32 × 14 cm, 5 mice/cage) under controlled conditions: temperature (24 ± 1°C), humidity (50 ± 2%), and lighting (lights on from 08:00 to 20:00). The mice were provided a normal diet (MR stock, NIHON NOUSAN, Kanagawa, Japan) and water *ad libitum*.

The animal studies were undertaken according to the regulations of our laboratory and were in line with the 1980 guideline titled Notification No. 6 of the Prime Minister's Office of Japan.

**Swimming Exercise Test Protocol** — The mice were habituated to laboratory housing for 1 week prior to the start of the experiments. Thirty mice were divided into three groups ( $n = 10$  each) that were given either vehicle (distilled water) or 0.015 or 0.03 mg/kg benzylglucosinolate via stomach intubations. Intubations were performed at 17:00, 5 days/week for 6 weeks. Swimming exercise was conducted with mice supporting constant loads (lead fish sinkers, attached to the tail) corresponding to 10% of their body weight. The animals were considered fatigued when they were unable to rise to the surface for 5 seconds.<sup>9)</sup> The mice performed swimming exercise tests once weekly for 4 weeks. Swimming exercise was carried out in a tank (28 × 46 × 29 cm), filled with water to a depth of 26 cm and maintained at 30 ± 1°C. To avoid the influence of circadian variations in physical activity,<sup>10)</sup> swimming exercise was done from 11:00 to 17:00, a period in which minimal variation of endurance capacity has been confirmed in rats.

After a period of 5 weeks, the mice were made to swim for 15 min supporting loads corresponding to 5% of their body weight. Blood samples for lactate, glucose, and non-esterified fatty acid (NEFA) determinations were collected from the tail vein at seven-time points: prior to exercise, during exercise (at 5, 10, and 15 min), and at 10, 30, and 60 min after exercise. Lactic acid concentration was determined by Kyowa Medex Kit (Determiner LA, Tokyo, Japan). NEFA was measured by acyl-CoA-synthetase and acyl-CoA-oxidase enzyme method (NEFA C-test Wako, Wako Pure Chemical Indus-

tries, Osaka, Japan). Glucose was assayed by a combination of mutase and glucose oxidase (Glucose CII test Wako, Wako Pure Chemical Industries, Osaka, Japan).

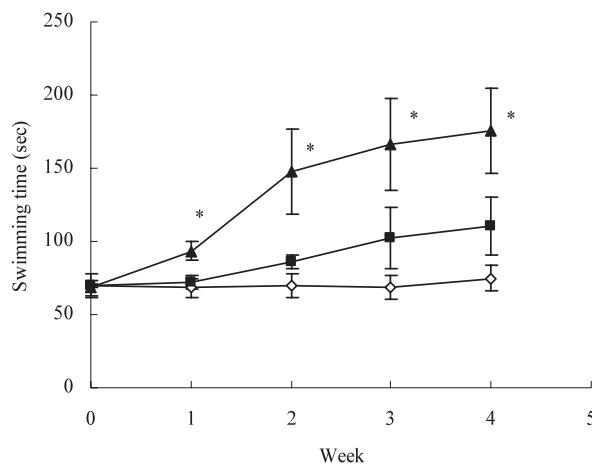
One week later, the groups were further subdivided into no-exercise and exercise groups. Exercise groups were made to swim for 15 min with a load of 5% body weight and immediately thereafter were killed by cervical dislocation. Liver samples were removed and stored at -20°C. Liver glycogen content was subsequently determined using the method of Lo *et al.*<sup>11)</sup>

**Statistical Analysis** — Data are expressed as mean ± S.E. Comparisons of swimming capacity between control and treated groups and data on metabolic parameters and tissue weight were assessed by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. Data on liver glycogen concentration were assessed by two-way ANOVA. A  $p$ -value < 0.05 was used as criterion for statistical significance.

## RESULTS

### Effect of Benzylglucosinolate on Exercise Capacity

Mice receiving benzylglucosinolate (0.03 mg/kg) showed a significant increase of exercise capacity from the first week onward (Fig. 1). However, 0.015 mg/kg benzylglucosinolate administration did not significantly increase exercise capacity.



**Fig. 1.** Effect of Benzylglucosinolate on Swimming Capacity in Mice

Swimming capacity was determined in mice administered vehicle (◇, control) or 0.015 (■) or 0.03 mg/kg benzylglucosinolate (▲). Swimming was performed with a 10% body weight load attached to the tails of the mice. Each value represents mean ± S.E. ( $n = 10$ ). \*Significantly different from control group ( $p < 0.05$ ).

### Effect of Benzylglucosinolate on Glycometabolism during Exercise

In control animals, 15 min of exercise decreased plasma glucose levels with plasma glucose levels recovering after the end of the exercise period (Fig. 2A). However, in mice administered 0.03 mg/kg benzylglucosinolate, plasma glucose levels were maintained throughout the exercise period (Fig. 2A). In all groups, exercise increased blood lactate concentrations. However, blood lactate concentrations were significantly lower in mice administered benzylglucosinolate (Fig. 2B). Although exercise significantly decreased liver glycogen content in all treatment groups, liver glycogen content following exercise was significantly higher in mice administered benzylglucosinolate (Fig. 3).

### Effect of Benzylglucosinolate on Lipid Metabolism during Exercise

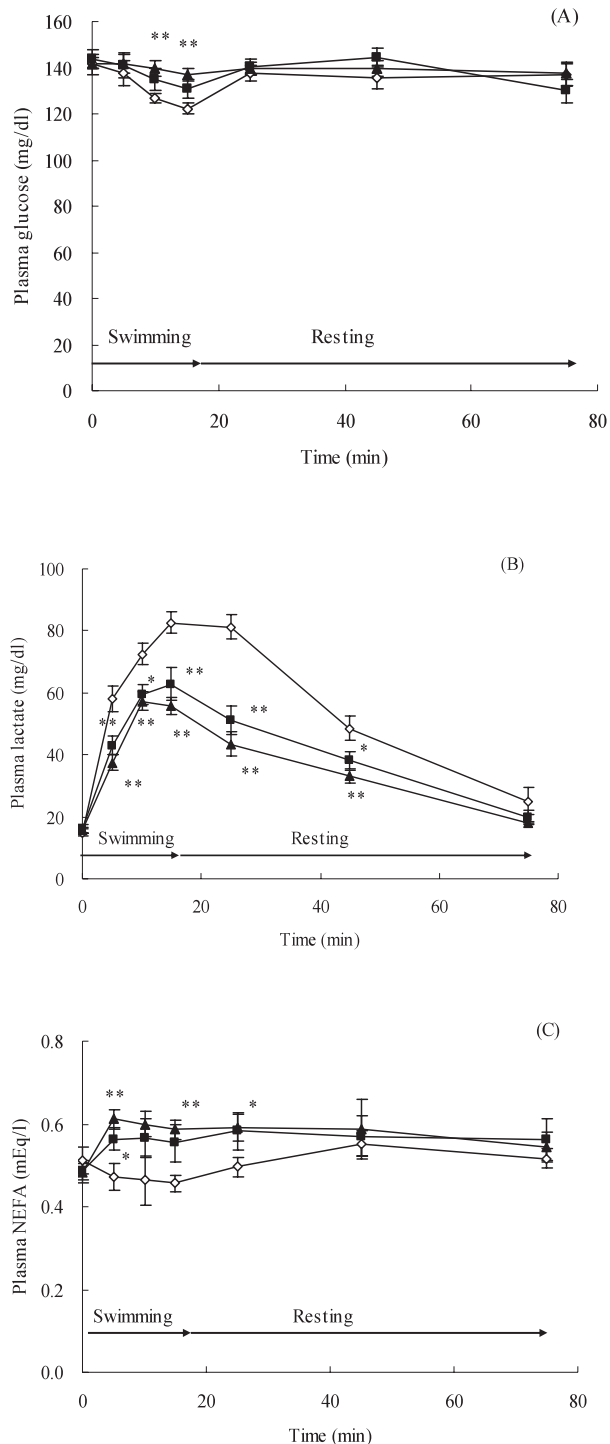
In the control mice, plasma NEFA concentrations were unaffected by 15 min of exercise. However, in mice administered 0.03 mg/kg benzylglucosinolate, exercise caused a significant increase of plasma NEFA levels (Fig. 2C).

No significant differences in body weight between the experimental groups were observed during the course of the experiment (control,  $42.1 \pm 1.0$  g; benzylglucosinolate 0.015 mg/kg,  $42.9 \pm 1.1$  g; benzylglucosinolate 0.03 mg/kg,  $42.3 \pm 1.2$  g). However, in mice administered 0.03 mg/kg benzylglucosinolate, epididymal adipose tissue weight was significantly ( $p < 0.05$ ) decreased (Fig. 4).

## DISCUSSION

In the present study, the effect of benzylglucosinolate supplementation on endurance capacity and fatigue was evaluated in male mice subjected to swimming exercise. Here, we report that administering benzylglucosinolate significantly prolonged swimming time. Additionally, we further sought to clarify the mechanism of this effect.

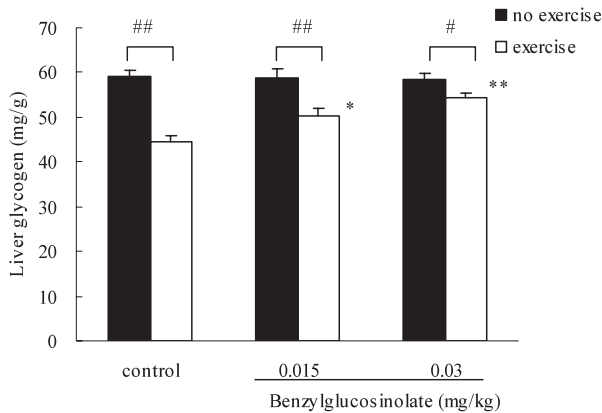
Exercise decreased plasma glucose in control animals, whereas in mice receiving benzylglucosinolate, plasma glucose levels were maintained. No difference of amount of glycogen was noted; no exercise groups. Hence benzylglucosinolate is considered not to participate in synthetic promotion of the glycogen in liver. Whereas, mice receiving benzylglucosinolate exhibited significantly higher liver



**Fig. 2.** Effect of Benzylglucosinolate on Plasma Glucose (A), Blood Lactate (B), and Non-esterified Fatty Acids (C) during Exercise and at Rest

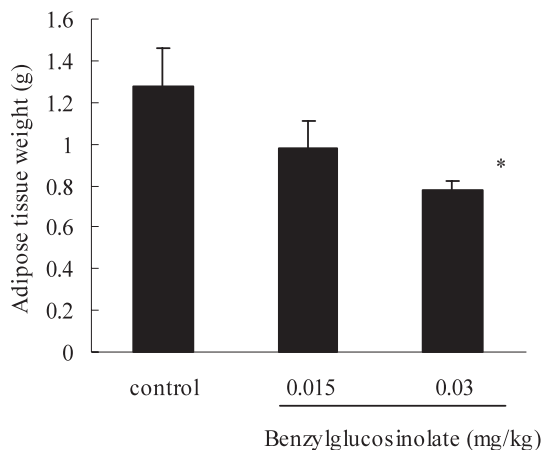
Mice were administered vehicle ( $\diamond$ , control) or 0.015 ( $\blacksquare$ ) or 0.03 mg/kg benzylglucosinolate ( $\blacktriangle$ ). Swimming (15 min) was performed with a 5% body weight load attached to the tails of the mice. Each value represents mean  $\pm$  S.E. ( $n = 10$ ); \* $p < 0.05$ ; \*\* $p < 0.01$  vs. control group.

glycogen content compared with control animals following swimming. These results indicate that benzylglucosinolate supplementation can moderate



**Fig. 3.** Effect of Benzylglucosinolate on Liver Glycogen after 15 min of Exercise

Mice were administered vehicle (control) or 0.015 or 0.03 mg/kg benzylglucosinolate. Half the animals in each group were subjected to 15-min exercise (swimming with a 5% body weight load attached to their tails) and liver glycogen was subsequently determined. ■: no exercise; □: exercise. Each value represents mean  $\pm$  S.E. ( $n = 5$ ). \* $p < 0.05$ ; \*\* $p < 0.01$  vs. post-exercise control group. # $p < 0.05$ , ## $p < 0.01$  vs. no-exercise group.



**Fig. 4.** Effect of Benzylglucosinolate on Epididymal Adipose Tissue Weight

Each value represents mean  $\pm$  S.E. ( $n = 10$ ). \* $p < 0.05$  vs. control.

the supply of glucose and/or decrease glucose utilization as an energy source during exercise. In addition, benzylglucosinolate supplementation significantly decreased exercise-induced blood lactate concentrations. Since lactic acid is produced as a result of carbohydrate metabolism, these results are consistent with the hypothesis that benzylglucosinolate decreases glucose utilization as an energy source during exercise.

Increasing fatty acid utilization during exercise reduces the glycogen depletion rate and improves endurance exercise performance.<sup>12)</sup> Therefore increased fatty acid utilization is thought important for endurance performance. Caffeine is a meaningful example of this because caffeine in-

creases plasma free fatty acid (FFA) concentrations and reduces glycogen depletion.<sup>13)</sup> Oral administration of capsaicin successfully improves endurance capacity during prolonged exercise.<sup>14,15)</sup> This increase was associated with enhanced lipolysis and sparing of stored glycogen, resulting in delayed glycogen depletion by increasing circulating catecholamines. The enhanced availability of NEFA is thought to cause greater fat metabolism in active muscles, which in turn decreases carbohydrate utilization and leads to increased exercise capacity.<sup>16)</sup> In the control animals, exercise decreased plasma NEFA concentrations, whereas in mice receiving benzylglucosinolate, exercise significantly increased plasma NEFA levels. These results indicate that it is likely that benzylglucosinolate favors lipid utilization more than glucose utilization as an energy source during exercise. While benzylglucosinolate supplementation had no significant effect on body weight, mice receiving 0.03 mg/kg benzylglucosinolate had significantly decreased adipose tissue weights. Benzylglucosinolate is thought to increase hormone-sensitive lipase activity and enhance fat mobilization from adipose tissues, resulting in increased plasma NEFA levels. We intend to investigate this mechanism in detail. The metabolic effects of benzylglucosinolate on increasing endurance performance are consistent with the hypothesis that increasing fatty acid utilization as an energy source leads to glycogen sparing. Thus, preserved glycogen would be available as an energy source during prolonged exercise, delaying the onset of fatigue.

The experimental dose used in this study was previously determined (during the swimming endurance test) as the effective quantity (Ikeuchi, *et al.*, unpublished results). Generally, in commonly used dietary supplement (extract of Maca), about 2 mg of benzylglucosinolate is contained. This quantity can sufficiently be taken in by human. We think that the same effect as observed in our animal experiment may be transferable to human.

In conclusion, our study suggests that benzylglucosinolate may have beneficial effects on endurance exercise capacity. Administration of benzylglucosinolate causes increases of fatty acid utilization as an energy source, leading to sparing of liver glycogen. However, comprehensive chemical and pharmacological research is required to determine the precise mechanism of benzylglucosinolate in enhancing endurance exercise capacity.

## REFERENCES

- 1) Grenhaff, P. L. and Timmonns, J. A. (1998) Interaction between aerobic and anaerobic metabolism during intense muscle exercise. *Exerc. Sport Sci. Rev.*, **26**, 1–30.
- 2) Pedersen, T. H., Nielsen, O. B., Lamb, G. D. and Stephenson, D. G. (2004) Intracellular acidosis enhances the excitability of working muscle. *Science*, **305**, 1144–1147.
- 3) Skinner, J. S. and McLellan, T. H. (1980) The transition from aerobic to anaerobic metabolism. *Res. Q. Exerc. Sport*, **51**, 243–248.
- 4) Muhamado, I., Zhao J., Dunbar, D. C. and Khan, I. A. (2002) Constituents of *Lepidium meyenii* “maca.” *Phytochemistry*, **59**, 105–110.
- 5) Cicero, A. F. G., Bandieri, E. and Arletti, R. (2001) *Lepidium meyenii* Walp. improves sexual behaviour in male rats independently from its action on spontaneous locomotor activity. *J. Ethnopharmacol.*, **75**, 225–229.
- 6) Oshima, M., Gu, Y. and Tsukada, S. (2003) Effects of *Lepidium meyenii* Walp. and *Jatropha macrantha* on blood levels of estradiol-17 $\beta$ , progesterone, testosterone and the rate of embryo implantation in mice. *Theriogenology*, **65**, 1145–1146.
- 7) Lopez-Fando, A., Gomez-Serranillos, M. P., Iglesias, I., Lock, O., Upamayta, U. P. and Carretero, M. E. (2004) *Lepidium peruvianum* chacon restores homeostasis impaired by restraint stress. *Phytother. Res.*, **18**, 471–474.
- 8) Ciero, A. F. G., Piacente, S., Plaza, A., Sala, E., Arletti, R. and Pizza, C. (2001) Hexanic maca extract improves rat sexual performance more effectively than methanolic and chloroformic maca extracts. *Andrologia*, **34**, 177–179.
- 9) Ikeuchi, M., Koyama, T., Takahashi, J. and Yazawa, K. (2006) Effects of astaxanthin supplementation on exercise-induced fatigue in mice. *Biol. Pharm. Bull.*, **29**, 2106–2110.
- 10) Matsumoto, K., Ishihara, K., Tanaka, K., Inoue, K. and Fushiki, T. (1996) An adjustable-current swimming pool for the evaluation of endurance capacity of mice. *J. Appl. Physiol.*, **81**, 1843–1849.
- 11) Lo, S. J., Russell, C. and Taylor, A. W. (1970) Determination of glycogen in small tissue samples. *J. Appl. Physiol.*, **28**, 234–236.
- 12) Azevedo, J. L., Jr., Linderman, J. K., Lehman, S. L. and Brooks, G. A. (1998) Training decreases muscle glycogen turnover during exercise. *Eur. J. Appl. Physiol.*, **78**, 479–486.
- 13) Tarnopolsky, M. A., Atkinson, S. A., Macdaugall, J. D., Sale, D. G. and Sutton, J. R. (1989) Physiological responses to caffeine during endurance running in habitual caffeine users. *Med. Sci. Sports Exerc.*, **21**, 418–424.
- 14) Tae-Woong, O. H. and Ohta, F. (2003) Capsaicin increases endurance capacity and spares tissue glycogen through lipolytic function in swimming rats. *J. Nutr. Sci. Vitaminol. (Tokyo)*, **49**, 107–111.
- 15) Kim, K. M., Kawada, T., Ishihara, K., Inoue, K. and Fushiki, T. (1997) Increase in swimming endurance capacity of mice by capsaicin-induced adrenal catecholamine secretion. *Biosci. Biotechnol. Biochem.*, **61**, 1718–1723.
- 16) Klein, S., Coyle, E. F. and Wolf, R. R. (1993) Fat metabolism during low-intensity exercise in endurance-trained and untrained men. *Am. J. Physiol.*, **267**, 934–940.