

# Effect of *Momordica charantia* on Adenosine Monophosphate-activated Protein Kinase in Genetically Type 2 Diabetic Mice Muscle

Toshihiro Miura,<sup>\*,a</sup> Takanori Kawata,<sup>a</sup>  
Satoshi Takagi,<sup>b</sup> Mai Nanpei,<sup>a</sup>  
Haruka Nakao,<sup>a</sup> Eriko Ishihara,<sup>a</sup>  
and Torao Ishida<sup>c</sup>

<sup>a</sup>Department of Clinical Nutrition, <sup>b</sup>Graduate School of Health Science and <sup>c</sup>Hi-tech Research Center, Suzuka University of Medical Science, 1001-1 Kishioka, Suzuka, Mie 510-0293, Japan

(Received December 17, 2008; Accepted June 25, 2009;

Published online July 1, 2009)

**The hypoglycemic activity of *Momordica charantia* L. (Cucurbitaceae) (MC) was investigated in KK-Ay mice, an animal model of type 2 diabetes. The water extract of the fruit of MC (100 mg/kg) reduced the blood glucose of KK-Ay mice 2 and 4 hr after oral administration ( $p < 0.05$ ). In addition, the muscle adenosine monophosphate-activated protein kinase (AMPK) was significantly activated in the orally MC-treated mice when compared to that of the controls ( $p < 0.01$ ). These results suggest that the antidiabetic effect of MC is derived, at least in part, from an activation of AMPK in the muscle.**

**Key words**—adenosine monophosphate-activated protein kinase, hypoglycemic effect, KK-Ay, *Momordica charantia*, Cucurbitaceae

## INTRODUCTION

Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for indigenous natural antidiabetic agents is ongoing. The plant kingdom is a wide field in which to look for effective oral hypoglycemics. More than 400 species have been reported to display hypoglycemic effects, but only a few of them have been

investigated.<sup>1–3)</sup>

In Okinawa, where many people live to an advanced age, the inhabitants eat fruits of the *Momordica charantia* L. (MC). The fruits of the MC (Cucurbitaceae) (named Nigauri in Japanese), a plant widely used in traditional medicine as an antidiabetic agent, has been shown to lower blood glucose in laboratory animals.<sup>4–10)</sup> Previously, we reported that the water extract of MC induced muscle glucose transporter isoform4 (GLUT4) translocation in KK-Ay mice.<sup>11)</sup> However the detail mechanism of GLUT4 translocation is not clear.

Adenosine monophosphate-activated protein kinase (AMPK) plays a central role in the regulation of glucose and lipid metabolism as an intracellular energy sensor. Upon activation by allosteric binding of AMP or phosphorylation at Thr<sup>172</sup> of its catalytic subunit, AMPK accelerates ATP-generating catabolic pathways, including glucose uptake, and glucose and fatty acid oxidation. In many reports, AMP-activators such as aminoimidazole carboxamide ribonucleotide (AICAR), metoformin and berberine stimulated muscle glucose uptake by the increased GLUT4 translocation.<sup>12–14)</sup>

In the present study, we have examined the effect of MC on blood glucose, and we also investigated the AMPK activation of the muscle in order to elucidate the mechanism of the antidiabetic effect.

## MATERIALS AND METHODS

The fruit of the MC used in this experiment was obtained in a market in Kagoshima Japan. One hundred grams of the fruit was extracted with 2 l of water (40°C, 2 hr, 2 times). The water extracts were lyophilized (MC) and stored at room temperature until use. The yield was 18.7%.

**Animals**—KK-Ay mice (Clea, Tokyo, Japan), 9 weeks old, were used. KK-Ay mice with blood glucose level above 300 mg/100 ml were considered to be diabetic in this study. The mice were housed in an air-conditioned room at 22 ± 2°C with a 12 hr light-12 hr dark cycle (light: 9:00 am to 9:00 pm). The animals were kept in an experimental animal room for 7 days with free access to food (CE-2, Clea) and water (tap water). Blood samples were drawn from the cavernous sinus with a capillary to determine blood glucose levels under non-anesthesia and non-fasting. MC was dissolved in distilled water. The oral administration of MC was given on a compulsory basis. Animals were treated

\*To whom correspondence should be addressed: Department of Clinical Nutrition, Suzuka University of Medical Science, 1001-1 Kishioka, Suzuka, Mie 510-0293, Japan. Tel.: +81-59-383-8991; Fax: +81-59-383-9666; E-mail: miura@suzuka-u.ac.jp

in accordance with the Guideline for the Care and Use of Laboratory Animals (the Prime Minister's Office No. 6, 1980).

**Determination of Blood Glucose** — Blood glucose levels in mice were determined by the glucose oxidase method.<sup>15)</sup>

**Isolation of Hindlimb Muscle** — The mice were given MC (100 mg/kg) or distilled water (as controls) orally and, 4 and 7 hr later, the hindlimb muscle was resected for the experiment.

**Analysis of AMPK<sup>16)</sup>** — Small segments of muscle (20–50 mg) were homogenized in 100  $\mu$ l of ice-cold 50 mM Tris-HCl (pH 7.5), 50 mM NaF, 5 mM Na pyrophosphate, 1 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 1 mM benzamide, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1% (vol/vol) Triton X, and 10% (vol/vol) glycerol with a glass homogenization tube. The homogenate was kept on ice and then centrifuged at 4000 *g* for 30 min, 4°C. The supernatants were removed and their protein concentrations were determined by a commercial kit (Protein Assay kit, Bio-Rad Laboratory Inc., CA, U.S.A.).

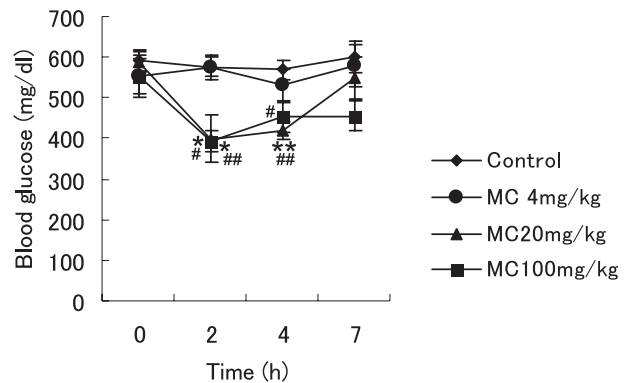
**Western Blot Analysis** — Anti-AMPK, anti-Phosphorylated AMPK and anti-GAPDH were purchased from Cell Signaling Co. Ltd (Danvers, MA, U.S.A.). The proteins (30  $\mu$ g) prepared were suspended in 1% sodium dodecyl sulfate (SDS) and 50 mM dithiothreitol and subjected to SDS-polyacrylamide (9%) gel electrophoresis. Electrophoretic transfer to nitrocellulose paper and detection of the immunocomplex with enhanced chemiluminescence (Amersham, Buckinghamshire, U.K.) were carried out as has been previously described.<sup>17)</sup> The sheet was exposed on RX X-ray film and an intensifying screen (Fuji, Tokyo, Japan). The prestained molecular weight standard (Bio-Rad, Richmond, VA, U.S.A.) was used for estimation of the molecular weight. The experiments were performed at least twice for each tissue with similar results.

**Statistical Analysis** — All the data were expressed as mean  $\pm$  S.E., and Student's *t*-test and analysis of variance (ANOVA) were used for the statistical analysis. The values were considered to be significant when the *p* value was less than 0.05.

## RESULTS

### The Effect of MC on Blood Glucose in KK-Ay

The effect of MC injected p.o to KK-Ay mice



**Fig. 1.** Effects of MC on Blood Glucose in KK-Ay Mice

MC 4, 20 and 100 mg/kg were administered orally to KK-Ay diabetic mice. After 2, 4 and 7 hr, blood samples were taken to determine blood glucose level. Each value represents the mean  $\pm$  S.E. of 4–5 mice. Significantly different from prevalence, \**p* < 0.05, \*\**p* < 0.01 (by ANOVA). Significantly different from corresponding control, #*p* < 0.05, ###*p* < 0.01 (by ANOVA).

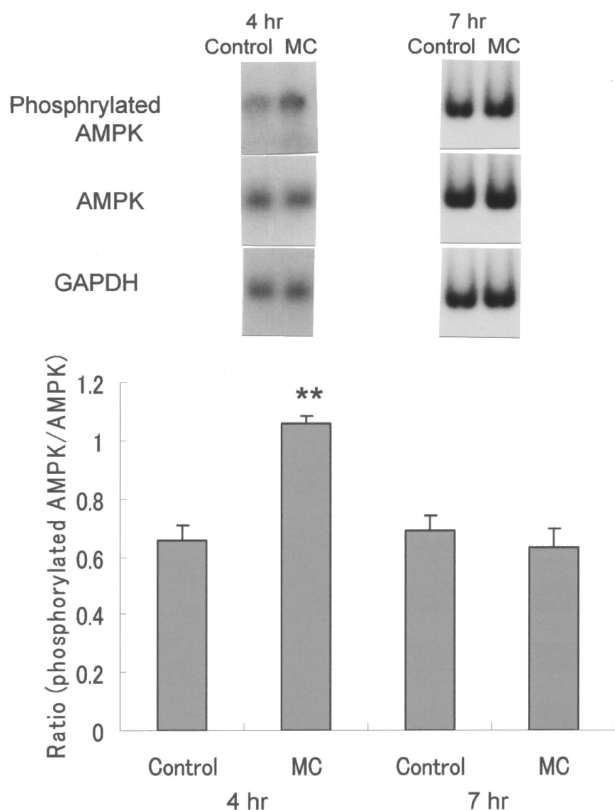
is shown in Fig. 1. MC-treated animals (100 mg/kg body weight) showed lower blood glucose levels from 2 to 4 hr after the administration (2 hr; *p* < 0.01, 4 hr; *p* < 0.05) when compared with controls. MC (20 mg/kg) also decreased blood glucose 2 and 4 hr after administration (2 hr; *p* < 0.05, 4 hr; *p* < 0.01). These data is similar to the results of previous study.<sup>11)</sup>

### Muscle AMPK Phosphorylation

Effects of MC on muscle AMPK phosphorylation in both control and MC-treated KK-Ay mice are shown in Fig. 2. The quantitation of AMPK protein in muscle was assessed by Western blotting in the mice. Quantitation of the AMPK band isolated from nitrocellulose paper demonstrated that the ratio (phosphorylated AMPK/AMPK) in the muscle from MC treated mice was 161% of that observed in the control mice (*p* < 0.01) 4 hr after administration. No differences in the ratio were observed at 7 hr after administration (Fig. 2).

## DISCUSSION

This study clearly showed that the water extract of the fruits of MC produced a consistent hypoglycemic effect. We examined the therapeutic effects of MC on hyperglycemia in KK-Ay mice, an animal model with type 2 diabetes mellitus. KK-Ay mice, which are known for genetically induced diabetes, including ob/ob mice<sup>18)</sup> and KK mice,<sup>19)</sup> are hyperinsulinemic as a result of insulin resistance.<sup>20)</sup>



**Fig. 2.** Effect of MC on PMPK activity in Mouse Skeletal Muscle

MC (100 mg/kg) was administered to mice orally, and after 4 and 7 hr muscle samples were obtained. Western blotting analysis of AMPK protein content in 30  $\mu$ g protein was done from muscle of mice, followed by quantitation of AMPK protein content abundance in the muscle. Autoradiographic bands from hybridizations shown were quantitated by densitometry. Each value represents the mean  $\pm$  S.E. of 5 mice. Significantly different from control, \*\* $p < 0.01$ .

The insulin resistance in peripheral tissues is known to be one of the major pathogenic factors of type 2 diabetes. The finding that MC decreases blood glucose level in KK-Ay mice, is important. In addition, we confirmed that MC did not affect the blood glucose of ddY normal mice (data not shown).<sup>11)</sup> These findings indicate that MC is useful for type 2 diabetes.

As for the hypoglycemic compounds of MC, Ali *et al.* reported that MC contained nonsapogenin, which may contribute to the reduction of blood glucose in streptozotocin-induced diabetic rats.<sup>9)</sup> Lolkar *et al.* reported that the hypoglycemic principle was charantin.<sup>5)</sup> In the present study, these compounds could be related to the antidiabetic activity.<sup>5)</sup>

This study was further extended to determine the modulation of upstream regulators involved in the GLUT4 translocation signaling pathway such as AMPK, which was an important marker involved in

the stage of nutrient-sensing. Phosphorylation of AMPK at Thr<sup>172</sup>, the active site of the AMPK  $\alpha$ -subunit and which was essential for enzyme activity, was demonstrated by Western blotting. The levels of AMPK were normalized with glyceraldehydes 3-phosphate dehydrogenase (GAPDH). AMPK was found to have been significantly phosphorylated after the cells were treated with MC (100 mg/kg). After 7 hr, AMPK phosphorylation was restored, while no difference in blood glucose were observed between control and MC.

In a previous study, we examined the effect of MC on GLUT4 glucose transporter in KK-Ay mouse muscle, since it has been reported that GLUT4 plays a crucial role in the muscle process of glucose uptake. MC increased GLUT4 protein content of muscle in KK-Ay mice. From these findings, it is likely that the hypoglycemic effect of MC is derived, at least in part, from the increase in GLUT4 translocation, presumably due to the increase of AMPK activity in muscle.

Further study would show how MC could become a useful drug in the treatment of diabetes through this unique therapeutic mechanism. The above experimental results suggest that the antidiabetic activity of MC supports its traditional medical use for type 2 diabetes.

**Acknowledgements** This work was supported by MEXT. HAITEKU (2005).

## REFERENCES

- 1) Aslam, M. (1979) Interaction between curry ingredient and drug. *Lancet*, **17**, 607.
- 2) Cupts, S. S. and Seth, C. B. (1982) Effect of *Momordica charantia* Linn. (karela) on glucose tolerance in albino rats. *J. Indian Med. Assoc.*, **39**, 581–584.
- 3) Wang, W., Miura, T., Shi, H., Ma, D. M., Zhao, Q. D., Zhang, W. P., Ishihara, E., Kanehara, M., Zhang, B. L., Gao, X. M., Zhang, D. Q. and Ishida, T. (2008) Effect of Tangzhiqing on glucose and lipid metabolism in genetically type 2 diabetes KK-Ay mice. *J. Health Sci.*, **54**, 203–206.
- 4) Upadhyaya, G. L., Ajai, K. and Pant, M. C. (1985) Effect of Karela as hypoglycemic and hypocholesterolemic agent. *Journal of diabetes association India*, **25**, 12–15.
- 5) Lolkar, M. M. and Rajaramn Rao, M. R. (1966) Pharmacology of a hypoglycaemic principle iso-

- lated from the fruits of *Momordica charantia* Linn. *Indian J. Pharm.*, **28**, 129–133.
- 6) Day, C., Cartwright, T., Provost, J. and Bailey, C. J. (1990) Hypoglycemic effect of *Momordica charantia* extracts. *Planta Med.*, **56**, 426–429.
  - 7) Higashino, H., Suzuki, A., Tanaka, Y. and Pootakham, K. (1992) Hypoglycemic effect of Siamese *Momordica charantia* and *Phyllanthus urinaria* extracts in streptozotocin-induced diabetic rats. *Folia pharmacol. Japon.*, **100**, 415–421.
  - 8) Srivastava, Y., Venkatakrishna-Bhatt, H., Verma, Y. and Venkaiah, K. (1993) Antidiabetic and adaptogenic properties of *Momordica charantia* extract: an experimental and clinical evaluation. *Phytother. Res.*, **7**, 285–289.
  - 9) Ali, L., Azad Khan, A. K., Rouf Mamun, M. I., Mosihuzzaman, M., Nahar, N., Nur-e-Alam, M. and Rokeya, B. (1993) Studies on hypoglycemic effects of fruit pulp, seed, and whole plant of *Momordica charantia* on normal and diabetic model rats. *Planta Med.*, **59**, 408–412.
  - 10) Srivastava, Y., Venkatakrishna-Bhatt, H. and Verma, Y. (1988) Effect of *Momordica charantia* Linn. Pomous aqueous extract on cataractogenesis in murrin alloxan diabetics. *Pharmacol. Res. Commun.*, **20**, 201–209.
  - 11) Miura, T., Itoh, C., Iwamoto, N., Kato, M., Kawai, M., Park, S. R. and Suzuki, I. (2001) Hypoglycemic Activity of the fruit of the *Momordica charantia* in type 2 diabetic mice. *J. Nutr. Sci. Vitaminol. (Tokyo)*, **47**, 340–344.
  - 12) Cuthbertson, D. J., Babraj, J. A., Mustard, K. J., Towler, M. C., Green, K. A., Wachterhage, H., Leese, G. P., Baar, K., Thomason-Hughes, M., Southerland, C., Hardie, D. G. and Rennie, M. J. (2007) 5-aminoimidazole-4-carboxamide 1-beta-D-ribofuranoside acutely stimulates skeletal muscle 2-deoxyglucose uptake in healthy men. *Diabetes*, **56**, 2078–2084.
  - 13) Sell, H., Dietze-Schroeder, D., Eckardt, K. and Eckel, J. (2006) Cytokine secretion by human adipocytes is differentially regulated by adiponectin, AICAR and troglitazone. *Biochem. Biophys. Res. Commun.*, **343**, 700–706.
  - 14) Cheng, Z., Pang, T., Gu, M., Gao, A. H., Xie, C. M., Li, J. Y., Nan, F. J. and Li, J. (2006) Berberine-stimulated glucose uptake in L6 myotubes involves both AMPK and P38 MAPK. *Biochim. Biophys. Acta*, **1760**, 1682–1689.
  - 15) Stevens, J. F. (1951) Determination of blood glucose by glucose oxidase method. *Clin. Chim. Acta*, **32**, 199–201.
  - 16) Durante, P. E., Mustard, K. J., Park, S. H., Winder, W. W. and Hardie, D. G. (2002) Effects of endurance training on activity and expression of AMP-activated protein kinase isoform in rat muscles. *Am. J. Physiol.*, **283**, E178–E186.
  - 17) Perez, C., Albert, I. and DeFay, K. (1990) A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. *Cell*, **63**, 251–258.
  - 18) Coleman, D. L. (1982) Diabetes-obesity syndromes in mice. *Diabetes*, **31**(Suppl. 1), 1–6.
  - 19) Nakamura, M. (1962) A diabetic strain of the mouse. *Proc. Jpn. Acad.*, **38**, 348–352.
  - 20) Nishimura, M. (1969) Breeding of mice strains for diabetes mellitus. *Exp. Anim.*, **18**, 147–157.