

# Wasabi 摂取によるフルクトース負荷インスリン抵抗性糖尿病モデルラットにおけるインスリン抵抗性の改善と糸球体過剰濾過及び蛋白尿軽減効果に関する研究

Wasabi increases insulin sensitivity and attenuates glomerular hyperfiltration and proteinuria in insulin-resistant diabetes of fructose-fed Wistar rats

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## 邦文要約

過剰なフルクトース負荷は2型糖尿病を発症させることが報告されている。本研究では、わさびの水抽出エキ스가フルクトース負荷ラットでみられるインスリン抵抗性を軽減するか否かについて検討した。Wistar ラットを、1) 15% フルクトース溶液群、2) 15% のフルクトース溶液及び 10% わさび水抽出物群、または 3) 水投与群の3群に分け、6週間経過観察した。実験最終に24時間尿を採取し、麻酔下に血液及び臓器を摘出し以後の実験に用いた。3群間で体重及び収縮期血圧に変化はみられなかったが、フルクトース負荷群では大動脈壁の重量が増加し、これはわさび投与群で有意に軽減した。フルクトース負荷では、血糖及び血漿インスリン濃度が有意に増加し、インスリン抵抗性の指標である HOMA-R 値は水投与群に比して有意に高値であった。血糖及びインスリン濃度の上昇はわさび群では有意に軽減し、水投与群との間に差がみられなかった。フルクトース負荷によるインスリン感受性の低下は、糸球体濾過量の増加を伴っており、尿量、尿中ナトリウム排泄量及び尿中タンパク排泄量が有意に高値を示した。わさび抽出物の投与により、尿量、尿中ナ

トリウム排泄量及びタンパク排泄量は低下し、水投与群との間で差はみられなかった。高速液体クロマトグラフィー分析により、わさび抽出物には強力な酸素ラジカル・スカベンジャーである6-メチルスルフィニルヘキシルイソチオシアン酸(6-MSITC)が含まれることが示されたが、わさび抽出物中の6-MSITC量はインスリン感受性の改善に必要とされる量より低いことが明らかとなったことから、わさび抽出物中のインスリン抵抗性の改善には、6-MSITC以外の物質の関与も示唆された。

## ABSTRACT

Fructose loading results in the development of type-2 diabetes. In the present study we examined whether Wasabi extract attenuates insulin resistance in fructose-loaded Wistar rats. The rats were randomly assigned to one of the following groups: 1) rats given 15% fructose solution, 2) rats given 15% fructose and 10% Wasabi extracts, and 3) rats given water. They were maintained for 6 weeks. There were no differences in body weight or systolic blood pressure among the three experimental groups. Weights of aortic walls increased with fructose

loading and this was abolished with Wasabi extracts treatments. Fructose loading significantly increased plasma insulin concentrations in association with increased plasma glucose levels, thereby increasing levels of HOMA-R, an indicator of insulin resistance. These alterations were reversed with Wasabi extract to normal levels as in control rats. Decreased insulin sensitivity was associated with glomerular hyperfiltration and increases in urine volume, and urinary sodium and protein excretion. These altered urinary parameters in fructose loading were significantly attenuated with treatment with Wasabi extracts. Wasabi extracts contain a potent oxygen radical scavenger, 6-methylsulfinyl hexyl isothiocyanate (6-MSITC) as assessed by  $C_{18}$  reversed phase HPLC; however, the amount of 6-MSITC present was much lower than the amount required to restore insulin sensitivity. These results suggest that Wasabi extracts attenuate insulin resistance induced by fructose loading, and this is associated with decreased glomerular hyperfiltration and urinary protein excretion. These benefits may be attributed to substances other than 6-MSITC in Wasabi extracts.

## INTRODUCTION

According to National Health and Nutrition Surveillance in Japan, the prevalence of diabetes has increased over the past 2 decades<sup>1)</sup>. More than 90% of the patients have type-2 diabetes, whereas Caucasian people in the US and Europe commonly suffer from type-1 diabetes. The reason for this difference is not clear; however, it may be due to differences in genetic background, life style, or food culture. Recent studies suggested that excessive fructose-intake causes type-2 diabetes in rodents<sup>2,5)</sup>.

However, it is not clear whether this also occurs in humans. In general, elderly people in Japan consume more than 150g of sweet fruits per day<sup>6)</sup>. That corresponds to an intake of more than 22g sucrose per day if the fruits contain brix 15% fructose. Such a high intake for a long time potentially becomes a risk for type-2 diabetes. Diabetes is a main cause of renal impairment and subsequent hemodialysis or kidney transplant. Glycosylation or hypersecretion of insulin, and proteinuria all play a critical role in renal impairment that occurs with diabetes.

On the other hand, Wasabi is a member of the Brassicaceae group along with *Armoracia rusticana*, known as horseradish in western countries. Wasabi is essentially Japanese horseradish, and bears a resemblance to horseradish in western countries (Fig. 1). Wasabi was first described as food in the Asuka era of Japan, 592-710 AD<sup>7)</sup>. Since then, it has been an integral cuisine in Japanese food culture. It is often used as a spice in Sushi or Sashimi with raw fish. However, it is not clear whether Wasabi is useful to promote health.

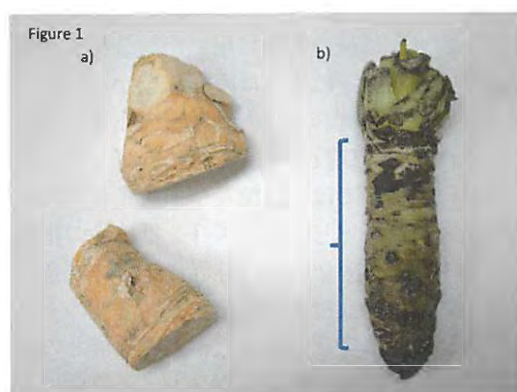


Figure 1. Appearance of horse radish and Wasabi. Left panel represents horse radish (*Armoracia rusticana*) and the right panel depicts Japanese horse radish, Wasabi (*Wasabi* or *Eutrema*). The root indicated was used for this experiment.

Recently, however, it was reported that Wasabi contains potent oxygen radical scavenger, 6-methylsulfinyl hexyl isothiocyanate (6-MSITC), and works as a bactericidal substance<sup>8)</sup>. Moreover, hot water extract from Wasabi attenuates body weight gain by suppressing PPAR- $\gamma$  and decreasing body fat of mice<sup>9)</sup>. To this context, Fukuchi et al. reported that oral administration of 6-MSITC ameliorates diabetic nephropathy in type 2 diabetic mice<sup>10)</sup>.

Considering these results, present study investigated the influence of long-term Wasabi intake on the early stage of type-2 diabetes induced with fructose loading in Wistar rats, especially with regard to insulin resistance and glomerular hyperfiltration.

## MATERIALS and METHODS

### 1) Design

Wistar male rats aged 5 weeks were purchased from Sankyo Laboratory, Tokyo, Japan. The rats were fed regular chow (0.75%NaCl, w/v) (Oriental Kobo Co., Ltd., Tokyo, Japan). At the age of 7 weeks, the rats were randomly assigned to 1) control group given water, 2) fructose group given 15% (w/v) fructose solution (Wako Pure Chemicals, Tokyo, Japan) or 3) Wasabi group given 15% (w/v) fructose and 10% (w/v) Wasabi solutions (Marutoh Co., Shizuoka, Japan).

Wasabi root was homogenized in water by Polytron blender (KINEMATIKA, Luzern, Switzerland) and the homogenates were filtered through a cotton filter. The water-soluble extract was given to the rats as Wasabi solution. The regular chow used in the present study was made according to the recommendation for animal research reported by American Institute of Nutrition (AIN-76 and AIN-93). Each solution and the chow were available ad

libitum.

At the end of study, the rats were placed in a metabolic cage to obtain urine for 24-h<sup>11)12)</sup>. After 12-h of fasting, the rats were anesthetized with pentobarbital (75mg/kg body weight) and then blood samples and organs of interest were obtained. The samples were stored at -80°C until use.

### 2) Determinations of parameters

Systolic blood pressure was determined by the tail-cuff method (Natsume Manometer-Tachometer model KN-210-1, Tokyo, Japan)<sup>13)</sup>. Protein excretion in urine was determined using Protein assay kit, BioRad, Tokyo, Japan. Electrolytes in blood and urine were determined using an autoanalyzer.

### 3) C<sub>18</sub> reversed phase high performance chromatography (C<sub>18</sub>-HPLC)

Ten percent Wasabi extract was analyzed using C<sub>18</sub>-HPLC system equipped with Crest Pak C18S column, PU-4180 pump and UV detector UV-4075 controlled by LC-NetII/ADC (JASCO Co., Ltd., Tokyo, Japan). Ten microliter of the extract was applied onto C<sub>18</sub>-HPLC prewashed with 0.1% formic acid. The moving solvent system was 0.1% formic acid with a 0-70% methanol gradient for 20 min. Flow rate was 1mL/min. For comparison, we analyzed C<sub>18</sub>-HPLC pattern of 6-MSITC (Cayman Chemical, Ann Arbor, USA).

### 4) Statistical analysis

All statistical analyses were performed using STATISTICA software (StatSoft, Tulsa, OK). Values were expressed as means $\pm$ SD. Differences were assessed by one-way or two way factorial analysis of variance followed by post-hoc least significant difference (LSD) test

or Mann-Whitney U test. P-values less than 0.05 were considered statistically significant.

## 5) Guidelines for handling rats for experiments

We followed the guidelines for experimental animal handling, and our study was approved by the Animal Care Committee of the Kyoritsu Women's University (#15001). The experiment was conducted in accordance with the National Institutes of Health (NIH) guidelines.

## RESULTS

### 1) Effects of Wasabi on insulin sensitivity

The rats gained body weight in a time-dependent manner during the experimental period. However, there were no differences in body weight among the three experimental groups (Fig. 2).

The rats given 15% fructose solution had 59% higher fasting plasma glucose concentrations

than control rats (Fig. 3-a). These higher plasma glucose concentrations were associated with significantly increased fasting plasma insulin concentrations compared with those in control rats (Fig. 3-b). Accordingly, the homeostasis model assessment insulin resistance (HOMA-R), a parameter of insulin resistance, was higher in the fructose group than in the control group, suggesting that insulin sensitivity was significantly decreased upon fructose loading (Fig. 3-c) <sup>14</sup>.

In contrast, in the Wasabi group, the increased fasting plasma glucose concentrations caused by fructose-loading declined in association with an attenuation of increased plasma insulin

Figure 2

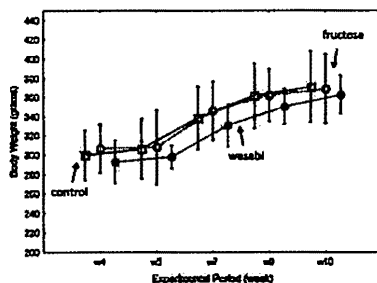


Figure 2. Effects of Wasabi on rat body weight upon fructose loading

Open circle, fructose group (fructose); closed circle, Wasabi group (wasabi); open square, control (saline) group (control). The values are expressed as means  $\pm$  SD. The differences were assessed by two-way ANOVA. Rat body weight was significantly increased in a time-dependent manner in the three experimental groups; however, difference among groups was not significant.

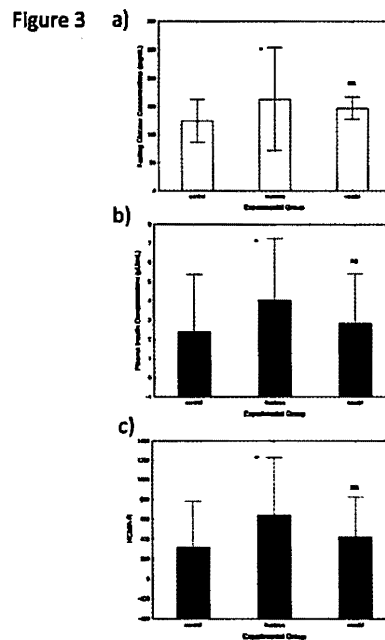


Figure 3. Effects of Wasabi on glucose metabolism upon fructose loading in rats

Fasting glucose concentration (a), plasma insulin concentrations (b), and HOMA-R (c) are shown. control, control (saline) group; fructose, fructose group and wasabi, Wasabi group. The values are expressed as means  $\pm$  SD. Differences were assessed by one-way ANOVA. \* $p < 0.05$  vs control (saline) group. Ns represents statistically not significant.

concentrations toward the control levels (Fig. 3-a,b). Thus, HOMA-R in the Wasabi group was almost equal to that in the control group, suggesting improvement of insulin sensitivity upon Wasabi consumption (Fig. 3-c).

## 2) Hemodynamic changes

At the end of the experiment, systolic blood pressures were  $139 \pm 7$  mmHg for control group,  $143 \pm 9$  mmHg for fructose group, and  $144 \pm 4$  mmHg for Wasabi group; there were no significant differences among the three groups. Moreover, the changes in systolic blood pressure during the study did not differ among the groups (Fig. 4).

The organ weights are shown in Table 1. Weights of aortic walls were significantly increased with in the fructose-loading group compared with weights in the control group, and this increase was almost completely abolished in Wasabi group. However, weights of the heart and kidney did not differ among the three experimental groups. The weights of the aortic walls were positively correlated with plasma insulin concentrations ( $r=0.465$ ,  $p<0.05$ ).

## 3) Renal function

Plasma creatinine concentrations were significantly increased in the fructose group; how-

Figure 4

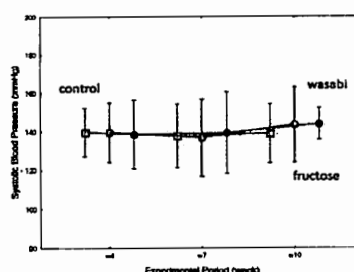


Figure 4. Effects of Wasabi on systolic blood pressure upon fructose loading in rats

Open circle, fructose group (fructose); closed circle, Wasabi group (wasabi); open square, control (saline) group. The values are expressed as means  $\pm$  SD. The differences were assessed by two-way ANOVA.

ever, creatinine clearance rate (Ccr) was significantly increased with in fructose-loading rats compared with Ccr in the control group, suggesting that hyperfiltration was occurring in the fructose group (Fig 5-a). Wasabi treatment attenuated this hyperfiltration, and there was no difference in Ccr between Wasabi and control groups.

Similarly, urine volume, urinary sodium excretions and sodium clearance rate were significantly increased with fructose-loading whereas fractional excretions of sodium (FENa) did not differ between fructose and control groups

Table 1. Organ weight of the experimental group

Group	Aortic weight (wet mg/mm <sup>2</sup> )	Heart weight (g/100g BW)	Kidney weight (g/100g BW)
Control	$0.148 \pm 0.026$	$0.943 \pm 0.143$	$1.176 \pm 0.052$
Fructose	$0.188 \pm 0.029^{**}$	$0.953 \pm 0.038$	$1.146 \pm 0.086$
Wasabi	$0.162 \pm 0.014^{\#}$	$0.940 \pm 0.072$	$1.200 \pm 0.072$

The values are expressed as means  $\pm$  SD. The differences were assessed by one-way ANOVA.  $^{**}p<0.01$  vs control group;  $^{\#}p<0.05$  vs fructose group

Figure 5

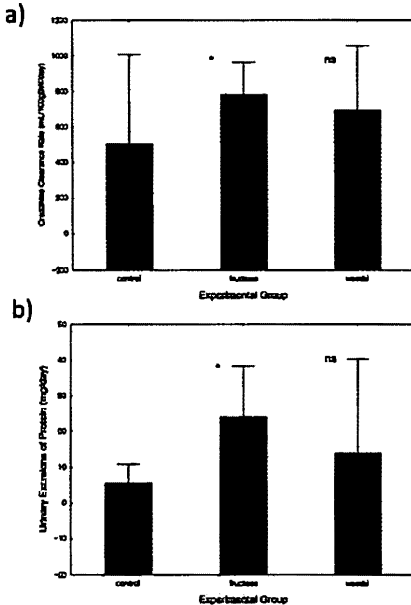


Figure 5. Effects of Wasabi on creatinine clearance rate and urinary protein excretions

Creatinine clearance rate (a) and urinary excretions of protein (b) are shown. control, control (saline) group; fructose, fructose group and wasabi, Wasabi group. The values are expressed as means  $\pm$  SD. The difference was assessed by one-way ANOVA. \* $p < 0.05$  vs control (saline) group. Ns represents statistically not significant.

(Table 2). In the Wasabi group, urine volume, urinary sodium excretions and sodium clearance rate were all decreased to the control levels. FENa did not differ between the fructose and Wasabi groups.

We examined urinary protein excretions (Fig. 5-b). Fructose loading significantly increased urinary protein excretions compared with excretion levels in control rats, and this increase was significantly attenuated in the Wasabi group.

We demonstrate the  $C_{18}$ -HPLC pattern of the Wasabi extract (10  $\mu$ L of 10% extract) in Figure 6. More than 31 peaks were detected, and one of them corresponded to the retention time (18.89 min) of the standard 6-MSITC as indicated by an arrow in the figure.

## DISCUSSION

The most important finding in this study was that fructose-loading increases insulin resistance in Wistar rats, and this was attenuated by the intake of Wasabi extracts. The decrease

Table 2. Plasma and urinary electrolytes concentrations

variables	control	fructose	Wasabi
<b>Plasma</b>			
pNa (mEq/L)	144.6 $\pm$ 2.3	142.7 $\pm$ 0.8*	143.4 $\pm$ 0.7
pK (mEq/L)	4.32 $\pm$ 0.19	4.06 $\pm$ 0.19*	3.94 $\pm$ 0.23***
pCr (mg/dL)	0.37 $\pm$ 0.01	0.42 $\pm$ 0.03**	0.39 $\pm$ 0.03
<b>Urine</b>			
UV (mL/100gBW/day)	1.04 $\pm$ 0.50	16.26 $\pm$ 8.03**	10.04 $\pm$ 11.3
UNaV (mEq/100gBW/day)	0.12 $\pm$ 0.07	0.29 $\pm$ 0.13**	0.14 $\pm$ 0.09 <sup>#</sup>
CNa (mL/100gBW/day)	0.45 $\pm$ 0.27	1.11 $\pm$ 0.53**	0.52 $\pm$ 0.33 <sup>#</sup>
FENa (%)	0.19 $\pm$ 0.13	0.26 $\pm$ 0.11	0.15 $\pm$ 0.11
UKV (mEq/100gBW/day)	0.40 $\pm$ 0.25	0.34 $\pm$ 0.12	0.38 $\pm$ 0.14
CK (mL/100gBW/day)	1.52 $\pm$ 0.97	1.29 $\pm$ 0.48	1.39 $\pm$ 0.49
UNa/K	0.73 $\pm$ 0.47	0.87 $\pm$ 0.37	0.51 $\pm$ 0.33

pNa, plasma sodium concentrations; pK, plasma potassium concentrations; pCr, plasma creatinine concentrations; UV, urine volume; UNaV, urinary sodium excretions; CNa, sodium clearance rate; FENa, fractional excretion of sodium; UKV, urinary potassium excretions; CK, potassium clearance rate; UNa/K, ratio of urinary sodium to potassium. The values are expressed as means  $\pm$  SD. The differences were assessed by one-way ANOVA. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$  vs control group, # $p < 0.05$  vs fructose group.

Figure 6

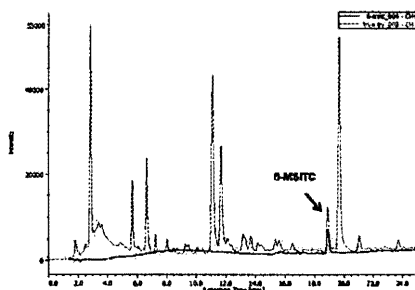


Figure 6. C<sub>18</sub> reversed-phase HPLC analysis of Wasabi extract

Wasabi extract was analyzed as detailed in the text by high-performance liquid chromatography (HPLC). Ten microliter of 1mg/mL standard 6-MSITC is indicated by an arrow.

in insulin sensitivity in fructose-loaded rats was associated with glomerular hyperfiltration and proteinuria. The intake of Wasabi extracts reversed the glomerular hyperfiltration toward normal levels and attenuated proteinuria. Thus, the data clearly suggest that the intake of Wasabi extracts attenuates insulin resistance and glomerular hyperfiltration induced by fructose loading.

Hyperfiltration in insulin resistance is mediated by increased permeability of the glomerular basement membranes or elevation of glomerular blood pressure due to inappropriate insulin secretion<sup>15-17</sup>. Increased glomerular blood pressure causes albuminuria and massive proteinuria, and the leak of blood protein has a critical role in tubular and glomerular impairment in diabetes. In this sense, it is important to normalize hyperfiltration along with restoring insulin sensitivity, as we demonstrated with Wasabi extract. This suggests that Wasabi extract is useful to prevent progression from insulin resistance to overt type-2 diabetes.

Urinary sodium excretion was increased with fructose loading. Since FENa was not changed upon fructose loading, the increased glomerular filtration may be related to the increase in urinary sodium excretion. Wasabi consumption decreased the sodium excretion toward control levels, which supports that Wasabi reverses hyperfiltration to normal levels.

Interestingly, we demonstrated that weights of aortic walls were significantly increased with fructose loading compared with weights of control groups. This was significantly attenuated upon feeding with Wasabi extract. It is reported that high insulin secretion causes vascular hypertrophy through enhancing vascular smooth muscle proliferation. In fact, we demonstrated that the weights of aortic walls positively correlated to plasma insulin concentrations.

The attenuation of aortic hypertrophy by Wasabi may be explained by the presence of 6-MSITC, a potent antioxidant substance<sup>18,20</sup>. According to HPLC analysis, Wasabi extracts contained 0.25mg/mL 6-MSITC in this experiment, resulting in approximately 12.5mg/day per rat (Fig. 6). This is far below the dose required to decrease blood glucose levels in genetic NIDDM mice<sup>10</sup>. Antioxidants enhance nitric oxide generation through eNOS activation, and thereby decrease systemic blood pressure. If 6-MSITC is involved in the increased insulin sensitivity observed in the Wasabi group, systolic blood pressure would have declined. Considering these results, the restoration of insulin sensitivity by Wasabi extracts may not be mediated by 6-MSITC and reduction of oxidative stress. The exact mechanism remains to be elucidated.

In conclusion, we demonstrated in the present study that consuming of Wasabi extracts at-

tenuate insulin resistance in fructose-fed rats with decreases in urinary protein excretions and aortic wall weights. These may be related to decreases in plasma insulin levels. Substances responsible for such benefits remain to be elucidated; however, such benefits may be reflected by the fact that Wasabi has been used as assortment for a long time in Japanese food culture.

*Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.*

## ACKNOWLEDGEMENTS

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