Influences of the *Grifola Gargal* extracts on insulin resistance induced by high-fructose diet in rats

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It has been well known that the high fructose diet causes the insulin resistance. And it has been reported that certain components of mushrooms effects on insulin resistance. The purpose of this study was to evaluate the effects of *Grifola Gargal*（mushroom）extracts on insulin resistance induced by high-fructose diet. Male rats of strain aged 7 weeks were divided into 3 groups, such as high-fructose diet（Fru）, high-fructose diet ＋ *Grifola Gargal* extracts（1.5% in diet; Fru ＋ G）, and standard chow-diet（Control）groups. For these rats, after one overnight fasts, a sequential euglycemic clamp experiment with two different insulin infusion rates of 3.0（L-clamp）and 30.0 mU/kg BW/min（H-clamp）was performed. Glucose infusion rates（GIRs）, calculated respectively from 60 to 90 min in L-clamp, and 150 to 180 min in H-clamp, were recognized an sign of whole body insulin action. High-fructose feeding markedly reduced GIRs in both L- and H-clamp experiments compared with Control. In L-clamp experiment, the effects of Grifola Gargal extracts were not shown. However, in H-clamp, GIR were increased by *Grifola Gargal* extracts compared with Fru, and reached the same levels as in Control. Therefore, we demonstrated that the administration of *Grifola Gargal* extracts did not affect insulin sensitivity, but produced a significant rise in insulin responsiveness.

Keywords : *Grifola Gargal*, euglycemic clamp, high-fructose diet, insulin resistance

Introduction

Insulin resistance plays a fundamental role in the incidence of the metabolic syndrome, defined as a series of abnormalities including impaired glucose tolerance, hypertension and dyslipidemia[1)−4)].

A diet high in fructose（>60/100g）induces insulin resistance in animals, and rats that are fed a high dose of fructose are considered in forming a nutritional model for insulin resistance[5)]. Fructose uptake has been shown to induce dyslipidemia, low grade hepatic inflammation and the activation of stress-sensitive pathways in the liver. Therefore, these instances indicate the evidence of lipotoxicity in the livers of fructose-fed animals[6)].

It has been well known that mushrooms are rich in fiber, minerals, vitamins and low in lipids[7)−8)]. And the effects of the mushrooms have been examined in the many researches. *Grifola Gargal* is one of mushroom that grows on the upper part of dead trees（*Nothofagus*

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oblique, *Nothofagus alpina*, *Nothofagus domberi*, *Laurelia philipiana*) at the southern Chilean coast. Moreover, it has been possible that the aqueous extracts of *Grifola Gargal* decrease the value of plasma glucose and triglyceride. However, unfortunately, the mechanism of these findings is not elucidated.

The purpose of this study was to clarify the effects of *Grifola Gargal* extracts on insulin resistance induced by high-fructose feeding, using the sequential euglycemic clamp experiment in rats.

Materials and Methods

Materials

Total of 12 male rats of Wistar strain aged 6 weeks were obtained from Chubu Kagakushizai, Nagoya Japan. The *Grifola Gargal* extracts were kindly supplied to us by Iwade Research Institute of Mycology Co.,Ltd. Insulin suspension was purchased from Novo Nordisk (100U/ml). And we used 20% (wt/vol.) glucose solution.

Animal Care

Male rats of Wistar strain were individually caged, in a room with constant temperature (20-22℃). They had free access to food and water. All procedures were in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals.*

At 7 week of age, they were allocated to one of the following 3 groups, and each group was fed the corresponding diet for 3 weeks; 1) high-fructose diet (contained 60% glucose; Nippon Bio-Supp.Center, Nagoya Japan; Fru, n=4), 2) high-fructose diet + *Grifola Gargal* extracts (Fru + G, n=4) and 3) standard chow-diet (powdered rodent diet MF; Control, n=4). The caloric composition of the standard chew-diet was 59% vegetable starch, 29% protein, and 12% fat. The corresponding composition for the high-fructose diet was 60% fructose, 28% protein, and 12% fat. The *Grifola Gargal* extracts was given mixed to the food (1.5%) in small amounts every 2-3 days. At 10 weeks of age, all rats were subjected to the euglycemic clamp test.

The procedures of euglycemic clamp experiment

The euglycemic clamp technique was originally described by Defronzo et al.[9] as a mean to evaluate tissue sensitivity to exogenous insulin. Food was withdrawn 16 hours before the experiment to assess whole body insulin action. Before the euglycemic clamp experiment, rats were anesthetized intraperitoneally and prepared surgically for continuous infusion of glucose and insulin. A midline ventral incision was made on the neck, two catheters (Silascon SH tubing: No.00, Kaneka Medix, Osaka Japan) were implanted in the right jugular vein and left carotid artery respectively. The right catheter was applied to the collection of blood sample. The left catheter was connected with infusion pumps (model TE-331S, TERUMO, Tokyo, Japan) for the infusion of glucose and insulin. The value of basal blood glucose concentration (before the clamp) was immediately checked by means of Accu-Chek compact (Roche Diagnostics Japan, Tokyo, Japan). A two-step sequential euglycemic clamp experiment with two different insulin rate of 3.0 (L-clamp; physiological insulin concentration) and 30.0mU/kg BW/min (H-clamp; ten times of the physiological insulin concentration) was performed. The glucose solution was also injected with the insulin. For measurement of the blood glucose concentration, blood was sampled every 10 min for the duration of the clamp experiment. Blood glucose concentration was kept constantly at the basal level with a variable infusion of glucose solution (Fig 1). Each clamp experiment was performed for 90 min. Glucose infusion rate (GIRs: mg/kg/min) of the L- and H-clamp were measured during the last 30 min, when the values of blood glucose concentration was stabilized in this period (Fig 2). The means of GIR values from 60 to 90 min and 150 to 180 min in the two-step sequential euglycemic clamp experiments were regarded as an sign of whole body insulin action since a plateau in the glucose infusion rate was achieved during 60-90 and 150-180 min[10)-11)].

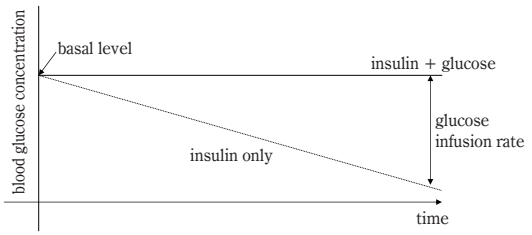

Fig 1. Glucose infusion rate

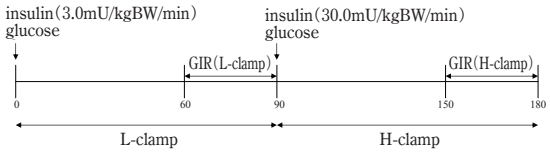

Fig 2. Time course of the experiments

Statistical analysis

All Data obtained were indicated as means ± S.E. Statistical analysis was performed by means of the one-way analysis of variance followed by Fisher's PLSD test. Values of $P<0.05$ were considered significant.

Results

Body weight and blood glucose

The final body weights in the high-fructose-fed rats (Fru group) tended to be lower than those of the standard chow-fed (Control group) and high-fructose + *Grifola Gargal* extracts fed rats (Fru + G group) (Table 1). There was no significance between Control and Fru + G group in final body weights.

The levels of basal blood glucose concentrations were almost same in 3 groups, and blood glucose concentrations during the clamp experiments were well maintained at basal levels. In all groups, significance was observed slightly.

Table 1. Body weight and blood glucose concentrations during the clamp experiment

Group	BW (g)	Blood glucose (mg/dl)		
		Basal	Clamp	
			3.0	30.0
Control	297±8	81±2	78±3	71±2
Fru	270±9	76±2	78±2	64±1
Fru+G	299±13	74±3	72±2	64±2

Means±S.E.

GIR

The average of GIRs for the last 30 min in the L-clamp and the H-clamp experiments was shown in Table 2. In the experiments of the insulin infusion rate of 3.0mU/kg BW/min (L-clamp), it was resulted that GIR in Fru group decreased markedly, when compared with that of Control group. However, there were no differences between the GIRs of Fru and Fru + G group. In the experiment of the insulin infusion of 30.0mU/kg BW/min, the GIR in Fru group were lower than that of Control group. On the other hands, the GIR of Fru + G was higher than that of Fru group. The values of GIR in Fru + G indicated almost same level of GIR in Control group.

Table 2. Glucose infusion rate (GIR) during the euglycemic clamp experiment (insulin infusion rate 3.0 and 30.0 mU/kg body weight/min)

Group	GIR (mg/kg/min)	
	L-clamp (3.0 mU/kg/min)	H-clamp (30.0mU/kg/min)
Control	4.4 ± 0.2^{a}	14.0 ± 1.8^{a}
Fru	1.8 ± 0.1^{b}	9.1 ± 1.5^{b}
Fru+G	1.0 ± 0.4^{b}	13.9 ± 0.7^{a}

Values with different letters are significantly different ($P<0.05$)
Means±S.E.

Discussions

This study designed to evaluate the effects of *Grifola Gargal* extracts on insulin resistance induced by high-fructose feeding. The GIR at the insulin infusion rate of 3.0 mU/kg BW/min (physiological insulin concentration) reflect primarily insulin sensitivity in peripheral tissues such as skeletal muscle and adipose tissue. Changes in insulin sensitivity are thought to be caused mainly by changes in insulin receptor binding activities and certain kinds of signaling factor. The infusion rate of 30.0 mU/kg BW/min leads to maximal insulin action, insulin responsiveness, indicating predominantly the capacity of post-receptor binding mechanism[12)-13)]. The amounts of GLUT4 in the cell seem to be arrived at the surface of the cell membrane, and show the maximal glucose intake. In H-clamp, *Grifola Gargal* extracts were made to increase the GIR and reach to the similar levels of the standard chow-fed rats. We demonstrated that the administration of *Grifola Gargal* extracts did not affect insulin sensitivity, but produced a significant increase in insulin responsiveness, similar to the levels of the Control group.

Huang et al. reported the activity of certain components of the immune system is altered by dietary composition and/or excess nutrients[14]. Shapiro et al. indicated that chronic fructose consumption induces leptin resistance[15]. And there was the report that insulin resistance induced by a high-fructose diet potentiates thioacetamide hepatotoxicity[16]. However, the mechanism underlying the fructose-induced insulin resistance is not known clearly.

On the other hands, mushrooms have been considered as an edible and medicinal resources for thousands of years. Many studies have demonstrated the effectiveness of mushrooms. For example, whole maitake (*Grifola frondosa*) mushroom and crude fractions of the mushroom were shown to favorably affect hypertension and glucose-insulin metabolism in rodent[17)-21)]. It is speculated that these effects are derived from polysaccharides and polyphenols in mushrooms. However, the detail of the mechanisms does not clarify. Moreover, few reports on the effects of *Grifola Garga*l have ever presented.

We proved that the *Grifola Gargal* extracts cure insulin resistance by means of the improvement of insulin responsiveness. There may be the possibility that certain components of *Grifola Gargal*, such as the polysaccharides or polyphenols have causes. Additional investigations are necessary to obtain the detailed data such as the identification of main components in the mushroom and determination of effective site in the insulin pathway.

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