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Combined Trial of Fish Oil and Exercise Training Prevents Impairment in Insulin Action on Glucose Transport of Skeletal Muscle Induced by High-Fat Diet in Rats

Ji Hyun Lee[1], Jong Yeon Kim, Yong Woon Kim, So Young Park, Woon Ki Youn, Eung Chan Jang, Deok-Il Park, Suck Jun Kim, Eun Jung Kim, and Suck Kang Lee

Department of Physiology, Yeungnam University College of Medicine; [1]Department of Internal Medicine, School of Medicine, Catholic University of Taegu-Hyosung, Taegu 705-053, Korea

The purpose of the present study was to determine the preventive effects of combined interventional trial of fish oil treatment and exercise training on insulin resistance of skeletal muscle in high-fat fed rats. Male Wistar rats were randomly divided into chow diet (CD), high-fat diet (HF), high-fat diet with fish oil (FO), high-fat diet with exercise training (EX), and FO+EX groups. The rats in control group were fed chow diet containing, as percents of calories, 58.9% carbohydrate, 12.4% fat, and 28.7% protein. High-fat diet provided 32% energy as lard, 18% as corn oil, 27% as carbohydrate and 23% as casein. The fish oil diet had the same composition as the high fat diet except that 100 g menhaden oil was substituted for corn oil. Insulin sensitivity was assessed by *in vitro* glucose transport in the soleus muscle after diet treatment and treadmill running for 4 weeks. While the FO or EX only partially prevented insulin resistance on glucose transport and visceral obesity induced by high-fat diet, these interventions completely corrected hyperinsulinemia and hyperglycemia from the high-fat diet. The rats in the FO+EX showed normalized insulin action on glucose transport, plasma chemicals and visceral fat mass. Insulin-mediated glucose transport was negatively associated with total visceral fat mass ($r=-0.734$; $p<0.000$), plasma triglyceride ($r=-0.403$; $p<0.05$) and lepin ($r=-0.583$; $p<0.001$) concentrations with significance. Multiple stepwise regression analysis showed that only total visceral fat mass was independently associated with insulin-mediated glucose transport ($r=-0.668$; $p<0.000$). In conclusion, combined interventional trial of FO+EX recovered insulin resistance on glucose transport of skeletal muscle induced by high-fat diet. Visceral fat mass might be more important factor than plasma TG and leptin to induce insulin resistance on glucose transport of skeletal muscle in high-fat fed rats.

Key Words: Plasma triglyceride, Leptin, Visceral fat

INTRODUCTION

Impairments of insulin action on glucose metabolism in skeletal muscle is a characteristic feature of type II diabetes and several other related metabolic disorders like visceral obesity. Despite of many studies on insulin resistance of the skeletal muscle, its precise mechanism is not clear. However, it is likely that both genetic and environmental factors such as diet and physical activity, are involved (DeFronzo, 1988). Dietary factors such as high-fat or high-sucrose diets have been implicated in the development of hepatic and peripheral tissue insulin resistance in rats (Sucini & Lavau, 1978; Pagliassotti et al, 1994a). High-fat feeding causes widespread peripheral insulin resistance, hyperglycemia, hyperlipidemia and visceral obesity (Kraegen et al, 1986; Storlin et al, 1986; Pagliassotti et al, 1994b; Han et al, 1997). The effects

Corresponding to: Suck Kang Lee, Department of Physiology, Yeungnam University College of Medicine, Taegu 705-053, Korea. (Tel) 82-53-620-4330, (Fax) 82-53-651-3651

of several intervention such as diet composition change on the development of diet induced insulin resistance were studied (Storlin et al, 1991; Macho et al, 1993; Luo et al, 1996). In additon, numerous interventional trials (Kraegen et al, 1989; Mourier et al, 1997; Oakes et al, 1997; Owens et al, 1999) have explored the impact of exercise training on the recovery of insulin resistance in high-fat diet induced insulin resistance. However, there was no report of combined interventional trial of diet composition change and exercise training on insulin resistance of skeletal muscle in high-fat fed rats.

Therefore, we investigated the preventive effects of the combined interventional trial of fish oil ingestion and exercise training on glucose transport of skeletal muscle and its related factors in the skeletal muscle of high-fat fed rats.

METHODS

2-[1,2-^{3}H]-deoxy-D-glucose (2-DG), and D-[1-^{14}C] mannitol were obtained from New England Nuclear (Boston, MA, USA). Porcine insulin was purchased from Novo Nordisk (Denmark). All other reagents were obtained from Sigma Chemical (St. Louis, MO, USA).

Animals and treatments

Male (~50 g) Wistar rats were obtained from Animal Care Unit at Yeungnam University College of Medicine and placed on either a chow diet (CD) as a control group or high-fat diet for 4 weeks. The rats on high-fat diet were divided into 5 groups: high-fat diet (HF), high-fat diet with fish oil (FO), high-fat diet with exercise (EX), and high-fat diet with fish oil and exercise (FO+EX) groups. The high-fat diet contained, as percents of total calories, 32% lard, 18% corn oil, 5% sucrose, 22% starch, and 23% casein, supplemented with vitamins, 22 g/kg vitamin mix (Teklad #40077), and minerals, 51 g/kg mineral mix (Teklad #170915). The fish oil diet had the same composition as the high-fat diet except that 100 g/kg menhaden oil (18% of total calories) was substituted for corn oil. The chow diet contained as percents of calories, 58.9% carbohydrate, 12.4% fat, and 28.7% protein. The energy content of the high-fat diet was 5.1 kcal/g, while the chow diet contained 3.3 kcal/g. The rats were provided the diets and water ad libitum.

The treadmill running was loaded as an exercise training five days a week for 4 weeks. A running speed of 20 m/min with slope zero for 50 minutes in a day at the first week, 22 m/min with slope 2° for 60 minutes at the second and third weeks, and 22 m/min with slope 5° for 60 minutes at the last week.

Insulin-stimulated glucose transport in soleus muscle

Food was removed after 6 : 00 PM the day before the experiment. Rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (65 mg/kg body weight) and blood samples for measurements of glucose, insulin, triglycerides and leptin concentrations were drawn from the tail vein, and then, the soleus muscles were removed. Before incubation, the soleus muscles were split longitudinally into strips with an average weight of ~25 mg (Kim et al, 1999).

To allow recovery from the dissection and splitting procedures, the muscles were incubated for 30 min at 35°C in shaking incubator in 1.5 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% bovine serum albumin (BSA). After the 30 min recovery period, the soleus strips were incubated for 60 min at 35°C in the same buffer in the presence or absence of a maximally effective concentration of porcine insulin (2 mU/ml) before measurement of 2-DG transport activity. Glucose transport was measured using 2-DG, as described previously (Kim et al, 1999). After incubation with insulin, muscles were incubated at 30°C for 20 min in 1.5 ml KHB containing 4 mM 2-[1,2-^{3}H]-deoxyglucose (1.5 μCi/ml), 36 mM [^{14}C] mannitol (0.2 μCi/ml), 0.1% BSA, and insulin if it was present in the previous incubation. Extracellular space and intracellular 2-DG concentration (μmol/ml intracellular water/20 min) were determined as previously described (Kim et al, 1999).

Visceral fat mass and blood chemicals

After the muscle dissection was completed, the abdominal cavity was opened, and the epididymal, mesenteric, and retroperitoneal fat mass were removed and weighed. Plasma (from the tail vein blood) glucose concentration was determined, using the glucose oxidase method (YSI Sidekick 1500, Yellow Springs Instruments, Ohio, USA). Plasma insulin and leptin were measured by radioimmunoassay. Plasma triglyceride concentration was measured using a kit (Sigma

Chemical, St. Louis, MO, USA).

Statistical analysis

Values were expressed as means±SE. Difference between the groups was analyzed with a one-way analysis of variance (ANOVA) followed by LSD t-test. Simple linear regression analysis and stepwise multiple regression analysis were used for correlation between and among the factors. All statistical analyses were performed using SPSS system.

RESULTS

Insulin-stimulated glucose transport in soleus muscles

The insulin responsiveness of the glucose transport process was measured in the soleus muscles in vitro using 2-deoxyglucose. The soleus is a slow-twitch muscle that consists of approximately 87% type I fibers and 13% type II fibers (Armstong & Phelps, 1984).

Fig. 1 showed glucose transport (expressed by μmol/ml/20 min) in the basal and maximally insulin stimulated state. There were no differences in glucose transport of basal state among all the groups. In the HF (1.93 ± 0.04) insulin-mediated 2-DG transport was about 63% of that in the CD (3.06 ± 0.24). This data demonstrates that the HF resulted in a significant decrease ($p<0.05$) in the insulin responsiveness of glucose transport in the soleus muscles compared with the CD. The insulin-stimulated glucose transport in the FO (2.37 ± 0.19) was increased by 22% than in those of the HF ($p<0.05$). However it was only 77% of the level of CD ($p<0.05$). The insulin-stimulated glucose transport averaged 2.39 ± 0.09 in EX which was 78% and 124% of the CD ($p<0.05$) and the HF ($p<0.05$), respectively. The results showed that replacement of the corn oil in the diet with menhaden oil and exercise training for 4 weeks provided significant, but only partial, protection against the muscle insulin resistance on glucose transport induced by high-fat diet. However insulin-stimulated glucose transport of the FO+EX (2.77 ± 0.08) was recovered to the level of the CD and significantly higher than those of the HF, FO, and EX ($p<0.05$).

Body weights and visceral fat mass

There was no significant difference in body weight among all groups (Table 1). Total visceral fat mass (% ratio to body weight) was increased in the HF by

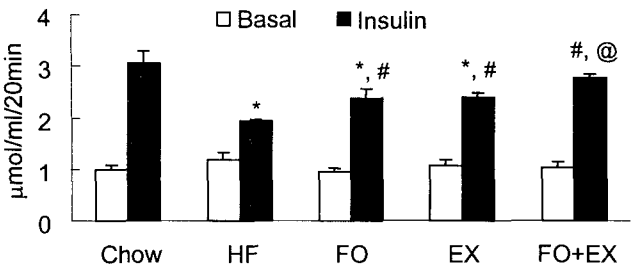

Fig. 1. Basal and insulin stimulated glucose transport activity in chow and high fat diet rats. HF: high-fat, FO: high-fat with fish oil, EX: high-fat with exercise, FO+EX: high-fat with fish oil and exercise. *$p<0.05$ vs chow, $^{\#}p<0.05$ vs HF, $^{@}p<0.05$ vs FO and EX.

Table 1. Body weight and percent visceral fat mass expressed by percent ratio to body weight in chow and high-fat diet rats

	Chow	HF	FO	EX	FO+EX
Body weight (g)	248.3±3.3	252.4±3.1	249.3±4.8	250.4±3.6	245.0±3.5
%Epididymal	0.97±0.08	1.29±0.06*	1.09±0.06$^{\#}$	1.09±0.07$^{\#}$	0.90±0.06$^{\#,@}$
%Mesenteric	1.19±0.05	1.67±0.07*	1.44±0.09$^{*,\#}$	1.40±0.09$^{\#}$	1.11±0.09$^{\#,@}$
%Retroperitoneal	0.86±0.06	1.97±0.08*	1.33±0.08$^{*,\#}$	1.30±0.07$^{*,\#}$	1.04±0.07$^{\#,@}$
%Total visceral fat	3.02±0.15	4.94±0.15*	3.86±0.19$^{*,\#}$	3.79±0.21$^{*,\#}$	3.05±0.21$^{\#,@}$

Values are expressed mean±SE. The number of rats in each group is 8. HF: high-fat, FO: high-fat with fish oil, EX: high-fat with exercise, FO+EX: high-fat with fish oil and exercise. *$p<0.05$ vs Chow, $^{\#}p<0.05$ vs HF and $^{@}p<0.05$ vs FO and EX.

Table 2. Plasma concentrations of glucose, insulin, leptin and triglyceride (TG)

	Chow	HF	FO	EX	FO+EX
Glucose (mg/dl)	79.8±3.9	85.4±4.4	78.5±8.3	82.5±2.9	81.5±2.8
Insulin (μU/ml)	11.7±0.7	19.6±1.8*	13.3±1.9$^{\#}$	13.1±2.5$^{\#}$	10.5±1.2$^{\#}$
Leptin (ng/ml)	0.9±0.1	1.8±0.2*	1.4±0.1*	1.5±0.3*	1.1±0.1$^{\#}$
TG (mg/dl)	58.4±2.7	67.6±4.4*	47.3±2.2*$^{,\#}$	55.1±2.9$^{\#}$	48.1±2.7*$^{,\#}$

Values are expressed mean±SE. The number of rats in each group is 8. HF: high-fat, FO: high-fat with fish oil, EX: high-fat with exercise, FO+EX: high-fat with fish oil and exercise. *$p<0.05$ vs Chow, $^{\#}p<0.05$ vs HF.

Table 3. Simple correlation coefficients for glucose transport activity and the indicative variables in pooled experimental cases from all groups (n=40)

	Total visceral fat	Glucose	Insulin	Leptin	Triglyceride
r	−.734	−.236	−.372	−.583	−.403
P<	.000	.142	.056	.001	.020

Table 4. Multiple stepwise regression analysis; standardized regression coefficient for glucose transport activity and indicated covariates in pooled experimental cases from all groups (n=40)

Covariate	Standardized Regression Coefficient	t	p<
Total visceral fat	−.668	−4.216	.000
Glucose	−.007	−.046	.964
Insulin	−.108	−.585	.565
Leptin	−.087	−.360	.723
Triglyceride	−.105	−.596	.558
r^2	.447		

63% compared with the CD. Each of the visceral fat depots, epididymal, mesenteric and retroperitoneal, was heavier in the HF than in the CD ($p<0.05$). Combination trial of FO and EX lowered total visceral fat mass to 78% ($p<0.05$) and 77% ($p<0.05$), respectively, compared with the HF, although they had still higher total visceral fat mass than the CD ($p<0.05$). The total visceral fat mass of the FO+EX was as similar as CD and it was significantly lower than that of FO and EX ($p<0.05$) (Table 1).

Plasma chemicals

There was no significant difference in plasma glucose among all groups. Plasma insulin levels were increased in the HF by 68% ($p<0.05$) compared with the CD and recovered to the level of the CD by the FO, the EX and FO+EX. While intervention trial with the EX reduced high plasma triglyceride levels in high-fat diet to the levels of the CD, the FO and FO+EX produced even lower plasma triglyceride than the CD. Leptin levels increased by high-fat diet tended to be reduced by the FO or EX intervention and was significantly decreased in FO+EX ($p<0.05$) (Table 2).

Regression analysis

Simple linear regression analysis showed that significant association of muscle 2-DG transport activity with total visceral fat mass ($r=-0.734$; $p<0.000$), and plasma triglyceride ($r=-0.403$; $p<0.05$) and lepin ($r=-0.583$; $p<0.001$) (Table 3). Multiple linear regression analysis demonstrated only total visceral fat mass among the variables was independently associated with glucose transport activity ($r=-0.668$; $p<0.000$) (Table 4).

DISCUSSION

High-fat diet produced profound insulin resistance as was assessed by glucose transport in isolated skeletal muscle in vitro, which is consistent with previous studies (Storlien et al, 1986). The mechanism of this reaction remain unclear, but increased visceral fat mass and aberrant plasma chemicals have been implicated (Han et al, 1997; Hansen et al, 1997; Goodfriend et al, 1998). Compatibly with this implication, simple linear regression analysis showed significant association of glucose transport with plasma leptin and triglyceride concentrations, and visceral fat mass.

Fish oil intake and exercise training have been used to prevent diet-induced insulin resistance but the complete prevention has been disputable (Kern et al, 1990; Knowler, 1995; Fasching, 1996; Luo et al, 1996). Furthermore combined trials of fish oil treatment and exercise training had not been tried before this experiment. In our study, both fish oil treatment and exercise training achieved partial prevention in insulin resistance by high-fat diet, which is consistent with studies of Mori et al (1999) and Kern et al (1990). However combined trials of these interventions prevented insulin impairments in insulin-stimulated glucose transport with normal plasma chemical levels and visceral fat mass.

Interestingly, while any one of the two trials prevented aberrant changes in insulin and triglyceride concentrations, visceral fat mass was normalized only by combined trials. Although causality of visceral fat mass and plasma chemicals are not clear, visceral obesity with decreased serum triglyceride can cause insulin resistance and dyslipidaemia (Kim-Motoyama et al, 1997). Furthermore, our study showed fish oil treatment resulted in significantly lowered triglyceride concentrations than those of chow diet although visceral fat mass was still increased. Multiple stepwise regression analysis demonstrating visceral fat mass as a powerful determinant for glucose transport also supports this notion. Visceral fat is metabolically unique when compared with subcutaneous adipose tissue. It is more sensitive to lipolytic stimuli, such as norepinehrine, and in proximity to hepatic portal circulation (Ostman et al, 1979). By supplying free fatty acids to the liver, visceral fat leads to increase in circulating triglyceride, increased hepatic glucose production and decreased hepatic insulin extraction (Gower et al, 1999). Therefore, visceral fat is hypothesized to be responsible for the strong positive association with dyslipidemia, hyperinsulinemia and glucose intolerance (Kissebah et al, 1976; Bjorntorp, 1990). Reducing visceral fat accompany decreased triglyceride and insulin, and improving insulin resistance (Johannsson et al, 1997; Kim et al, 1999).

Chronic exercise training results in preferential loss of visceral fat and attenuating fat accumulation as well through expending more energy, therefore it may contribute to prevent or alleviate insulin resistance (Mourier et al, 1997; Owens et al, 1999). Fish oil has also been reported to prevent or reduce visceral fat accumulation (Ikemoto et al, 1996; Hun et al, 1999) and the implicated possible mechanisms underlying these responses by fish oil ingestion are reduction in the intestinal absorption of glucose and other lipid (Thomson et al, 1993) and antiadipogenic effects via decreased expression of enzyme involving adipocyte metabolism such as fatty acid synthase, hormone sensitive lipase and lipoprotein lipase (Parrish et al, 1990; Clarke & jump, 1997; Raclot et al, 1997). However, more investigations are needed to determine the mechanisms in this model.

Finally, we speculate that combined interventional trials of fish oil and exercise training might prevent insulin resistance by high-fat diet, otherwise single intervention of either fish oil or exercise training might not enough to recover muscle insulin resistance on glucose transport. Visceral fat mass might be more important factor than plasma triglyceride and leptin concentration to induce muscle insulin resistance on glucose transport in high-fat fed rats.

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