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A High Carbohydrate Diet Induces Insulin Resistance Through Decreased Glucose Utilization in Ovariectomized Rats

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Background : Recent research has reported that high sugar diets increase insulin resistance, without abdominal obesity, in male, but not female Wister rats. Whether a high sucrose (SU) diet increased insulin resistance in ovariectomized (OVX) rats was determined.

Methods : Female Sprague Dawley rats, weighing 273±20 g, had either an ovariectomy or a sham operation (sham). OVX and sham rats were divided into two groups: one group had a 68 En% SU diet and the other a 68 En% starch (ST) diet for 8 weeks.

Results : The body weight was higher in the OVX than the sham rats, regardless of dietary carbohydrate subtype. The fasting serum glucose levels did not differ according to diet and ovariectomy. However, the fasting serum insulin levels were higher in the OVX than the sham rats, and in the OVX rats, a high SU diet increased the serum insulin levels more than a high ST diet. The whole body glucose disposal rates, which referred to the state of insulin sensitivity, were lower in the OVX rats fed both the high SU and ST diets, compared to sham rats. Glycogen deposits in the soleus and quadriceps muscles were lower in the OVX rats fed high SU and ST diets than in sham rats. The glucose transporter 4 content and fraction velocity of glycogen synthase in muscles showed similar glucose disposal rates. However, the triacylglycerol content in the muscles were higher in the OVX rats with a high SU diet than those with a high ST diet.

Conclusion : These results suggested that an OVX increased the weight gain due to higher food intakes, regardless of dietary carbohydrate subtypes. OVX-induced obesity may be involved in the induction of insulin resistance from an increased triacylglycerol content, decreased glucose uptake and glycogen synthesis in skeletal muscles, regardless of dietary carbohydrate subtypes.

Key Words : Ovariectomy, Sucrose, Euglycemic hyperinsulinemic clamp, Glycogen synthase activity, Glucose transporter 4

INTRODUCTION

Insulin resistance is a characteristic feature of type 2 diabetes. The development of insulin resistance can be linked to both genetic and environmental factors[1]. A key environmental factor in the development of insulin resistance is diet composition. Diet-induced insulin resistance is characterized by fasting normoglycemia, accompanied by hyperinsulinemia, impaired suppression of hepatic glucose production by insulin, and impaired insulin-stimulated glucose uptake in muscle and adipose tissues[2-4]. Multiple previous studies have reported that male rats developed diet induced insulin resistance after 1~8 weeks of high-sucrose diet feeding[3-5]. However, Stark et al[6]. demonstrated that the glucose tolerance curves were no different among healthy male Sprague Dawley rats fed high fat and high fructose diets for 3 months, and the fasting glucose and insulin levels were not

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significantly different at any time point during the experiment. Fasting serum triacylglycerols levels and glycogen synthase activities in soleus muscle were not significantly elevated by high fat and fructose diets. They suggested that rats fed either high fat or fructose diets adapted to the nutritional intervention, without developing classical signs of insulin resistance and impaired glucose tolerance.

It has been reported, unlike with high fat diets, that high-sucrose diets (SU) cause insulin resistance in the absence of an increase in visceral fat, but increase the triacylglycerol contents of the liver and muscles, leading to increased insulin resistance in male rats[7]. Another point with regard to a high fat diet is that a high SU diet develops insulin resistance only in male rats. However, it is controversial whether high SU feeding results in elevated insulin resistance and hypertriglyceridemia in female rats, compared to those with a high starch (ST) diet[8, 9], even though results with regard to a high SU diet have not been reported. Thus, the effect of estrogen withdrawal and dietary carbohydrate subtypes on insulin resistance in female Sprague Dawley rats were determined over an 8 week period.

MATERIALS AND METHODS

1. Experimental animals

All surgical and experimental procedures were performed according to the guidelines of the Animal Care and Use Review Committee at Konkuk University, Korea. Female Sprague Dawley rats, 39 weighing 273±20 g, were randomly divided into two groups by flipping a coin, with one of the groups randomly assigned for OVX, and the other for sham operations. After the ovariectomies[10], the rats were allowed 7 days to recover from the stress of surgery. Ovariectomized (OVX) and sham rats were randomly divided into two groups. Each group of OVX and sham rats was assigned either a high ST or SU diet. Rats were housed individually in stainless steel cages in a controlled (23℃: 12 hour light and dark cycle) environment. The sample numbers of each group are as follows: OVX-SU, 10; OVX-ST, 9; sham-SU, 10 and sham-ST, 10.

Semi-purified diets for each group were made by a modified method for experimental diets[11]. The high SU diet consisted of 68 energy percentage (En%) sucrose, 12 En% corn oil and 20 En% casein; in the high ST diet, the sucrose in the SU diet was replaced with starch. The animals were allowed free access to the diets for 8 weeks. The blood glucose levels, food intake and body weight were measured weekly at an assigned time.

2. Euglycemic hyperinsulinemic (EH) clamp

Indwelling catheters were inserted into the jugular vein and carotid artery at the seventh week of the experimental period[12]. After 7 days of insertion, euglycemic hyperinsulinemic (EH) clamp studies were performed on the rats in an awake, unstressed and fasting state, as described in a previous paper[13, 14]. The glucose infusion rate was calculated, and expressed in terms of mg of glucose per kg of body weight per minute. The glucose disposal rate indicates an index of the whole-body response to the response to exogenous insulin. After EH clamping, the rats were killed by decapitation. The soleus and quadriceps muscles were rapidly dissected, weighed, and then frozen in liquid nitrogen. Tissues were stored at -70℃ until further analysis.

3. Biochemical measurements

The serum glucose levels were analyzed by a glucose analyzer II (Beckman, Fullerton, CA) and the serum insulin and leptin levels measured using commercial radioimmunoassay kits (Linco Research, St Charles, MO). The serum 17-β-estradiol concentrations were measured by an ELISA Kit (Research Diagnostics Inc, NJ). In order to determine the glycogen levels in the liver and soleus and quadriceps muscle tissues, these tissues were homogenized, and deproteinized with 1.5 M perchloric acid. The glycogen was digested into glucose with α-amyloglucosidase in acid buffer. The glycogen levels were expressed as the glucose levels derived from the glycogen in the soleus and quadriceps muscles and liver tissues[15]. Interstitial fat from the soleus and quadriceps muscles was removed, and the muscle triacylglycerol extracted with a chloroform-methanol solution (2:1, vol/vol). Subsequently, the extracted triacylglycerol was resuspended in pure chroloform[16], and the concentration determined using a Trinder kit (Sigma, St. Louis, MO). The glycogen synthase activity was measured by a modification to the methods of Thomas et al.[17] and Rossetti et al[13]. After centrifugation at 15,000 rpm for 25 min of the muscle homogenates, the supernatant was incubated at 37℃ for 10 min. with a physiological concentration of the substrate (0.3 mM UDPG-[^{3}H] glucose) in the absence or presence of 10.0 mM glucose 6-phosphate (G-6-P). The glycogen synthase activity was determined by the radioactivity in glycogen synthesized during the incubation. Maximal glycogen synthase activity was observed in the presence of 10 mM G-6-P; whereas, the independent form activity was assayed in the absence of G-6-P. The total glycogen synthase activity is the sum of the G-6-P dependent and independent activities. The total glycogen synthase activity was expressed as nanomoles per mg protein per minute and as the fractional velocity (FV), a percentage of the ratio of the independent form and total activities. Total membranes from the soleus and quadriceps muscles were prepared for determining the glucose transporter 4 (GLUT4) content by the methods of Walker et al[18]. The

GLUT4 contents in the total membranes was measured by Western blotting, with the rabbit GLUT4 antibody (Chemicon, Temecula, CA). A muscle standard (an unrelated crude membrane fraction) was run on every gel for comparison of the samples from different immunoblots. Quantification was performed with a scanning laser densitometer (BioRad, Richmond, CA).

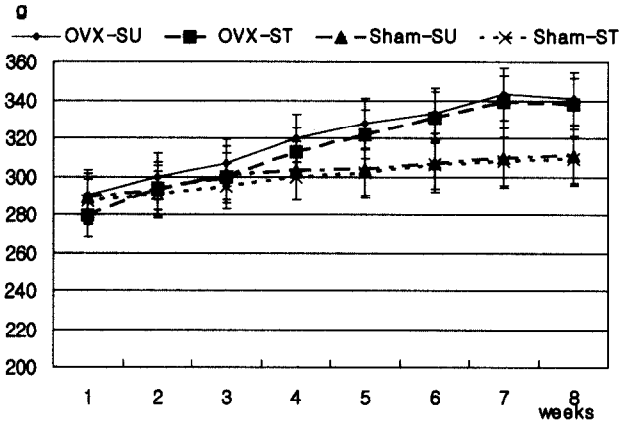
4. Statistical analysis

All results are expressed as the mean±standard deviation. Statistical analysis was performed using the SAS statistical analysis program[19]. Two-way analyses of variance (ANOVA) were carried out to determine whether the main effects were of the diet, ovariectomy or the interaction between the two. The main effects of diet and ovariectomy were tested only if there was no interaction between diet and ovariectomy. Since all factors showed no interaction between diet and ovariectomy, the main effects of the diet and the ovariectomy were determined. Differences with a $p<0.05$ were considered statistically significant.

RESULTS

1. Body weight, food intake and serum estradiol and leptin levels

The body weights were no different during the first three weeks according to ovariectomy and dietary carbohydrate subtypes (Figure 1). From the 4th week of experimental diets feeding, the body weight increased dramatically in the OVX rats, independent of the dietary carbohydrate subtype (Table 1). However, the serum leptin levels did not differ in the OVX and sham, as well as by dietary carbohydrate subtype after 8 weeks of the experimental diets. The average food intake during the experimental diet period was highest in the OVX rats fed the high ST diet, whereas the OVX rats fed the high SU diet consumed less energy than those fed the high ST diet. The OVX rats consumed more than the sham rats fed either diet. The serum estradiol levels of the OVX rats decreased to one third of those of the sham rats, regardless of diet.



*There was a significant difference by ovariectomy at $p<0.05$ (by two way ANOVA).

Figure 1. Changes in the body weight during the experimental period

Table 1. Body weight, energy intake, and serum leptin, estradiol, glucose, and insulin after 8 weeks of the experimental diets

	OVX-SU	OVX-ST	Sham-SU	Sham-ST
Body weight (g)	330±59	328±47	278±39	281±45**
Energy intake (kcal/day)	22.4±2.3	25.9±1.8	18.5±1.7	19.4±2.1*†
Serum leptin (ng/mL)	3.4±1.1	3.3±0.9	2.9±0.9	3.0±1.0
Serum estradiol (pg/mL)	2.4±0.9	2.5±1.2	6.5±2.2	6.1±1.8**
Serum glucose (mmoL/L)	5.7±0.5	5.5±0.6	5.4±0.7	5.4±0.6
Serum insulin (pmoL/L)	243±34	209±29	161±25	173±31*†

*There was a significant difference by ovariectomy at $p<0.05$ (by two way ANOVA), **$p<0.01$
†There was a significant difference by carbohydrate subtypes at $p<0.05$ (by two way ANOVA)

Table 2. Glucose disposal rate, serum glucose and insulin concentrations at the baseline of euglycemic hyperinsulinemic clamp

	OVX-SU	OVX-ST	Sham-SU	Sham-ST
Glucose disposal rate (mg/kg/min)	50.6±6.4	55.4±7.1	64.7±8.9	68.4±7.3*
Serum glucose (mmoL/L)	6.4±0.9	6.1±0.8	5.7±0.9	5.9±1.0
Serum insulin (pmoL/L)	269±31	232±32	183±28	195±36*†

*There was a significant difference by ovariectomy at $p<0.05$ (by two way ANOVA)
†There was a significant difference by carbohydrate subtypes at $p<0.05$ (by two way ANOVA)

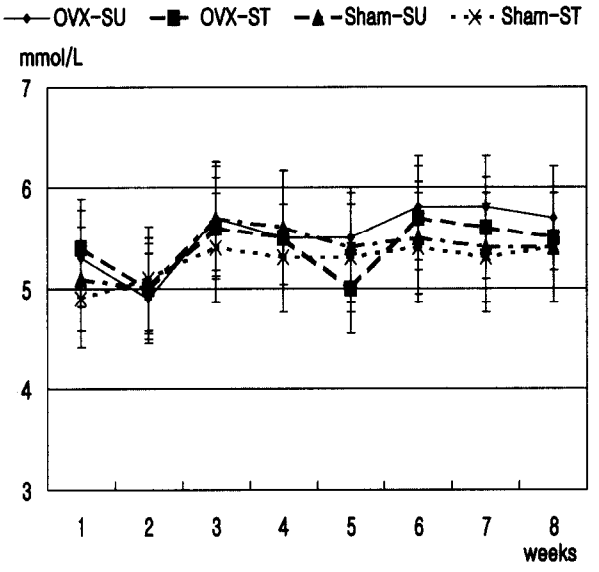


Figure 2. Changes in the serum glucose levels during the experimental period

2. Serum glucose and insulin levels and glucose disposal rates

There were no differences in the serum glucose levels between the groups during the entire experimental period (Figure 2). The serum insulin levels were higher in the OVX rats than the sham rats, and the levels of the OVX rats fed the high SU diet were higher than those fed the high ST diet (Table 1). The whole body glucose disposal rates were lower in the OVX rats, regardless of dietary carbohydrate subtypes, compared to the sham rats (Table 2). In the OVX rats, the glucose disposal rates were lower in the high SU than in the high ST diet, but these differences were not statistically significantly (p=0.08).

3. Glycogen, triglyceride and GLUT4 contents, and glycogen synthase activities in muscles

To determine the main factors in the decreased whole body glucose disposal rates in the OVX rats, compared to those in the sham rats, the glucose utilization was measured in the muscles, one of the important insulin stimulated glucose utilizing tissues. The glucose utilization in the muscles was assessed by the capacity of glucose uptake and the storage of glucose as

Table 3. Glycogen and triglyceride contents in soleus and quadriceps muscles

	OVX-SU	OVX-ST	Sham-SU	Sham-ST
Glycogen (mg/g tissue)				
Soleus M	2.2±0.6	2.5±0.8	2.8±0.6	3.0±0.7*
Quadriceps M	2.0±0.7	2.1±0.6	2.1±0.8	2.3±0.7
Triglyceride (mg/g tissue)				
Soleus M	302.4±48.9	282.2±32.6	259.3±41.2	257.7±33.8
Quadriceps M	412.3±45.1	369±49.2	322.8±52.9	313.4±47.6*†

*There was a significant difference by ovariectomy at p<0.05 (by two way ANOVA)
†There was a significant difference by carbohydrate subtypes at p<0.05 (by two way ANOVA)

Table 4. Glycogen synthase activities and GLUT4 contents in soleus and quadriceps muscles

	OVX-SU	OVX-ST	Sham-SU	Sham-ST
Total glycogen synthase (nmoL/mg protein/min)				
Soleus M	33.5±+2.5	32.7±3.6	34.4±3.7	37.2±4.2
Quadriceps M	32.7±3.8	33.4±2.7	34.1±3.5	35.4±4.3
Fraction velocity of glycogen synthase (% of total glycogen synthase)				
Soleus M	7.9±1.2	8.3±0.9	8.6±1.1	9.5±0.9*†
Quadriceps M	7.7±0.9	8.1±1.2	8.4±0.8	8.5±1.0
GLUT4 (%)‡				
Soleus M	129.8±29.5	153.8±35.9	160.3±39.6	221.6±42.8*†
Quadriceps M	91.4±24.8	115.2±29.7	118.1±33.9	142.8±38.0*

*There was a significant difference by ovariectomy at p<0.05 (by two way ANOVA)
†There was a significant difference by carbohydrate subtypes at p<0.05 (by two way ANOVA)
‡The percentage band density in the sample membrane fraction to an unrelated crude membrane fraction

glycogen. The capacity of glucose uptake was determined by the GLUT4 content in the cell membrane, which was directly correlated with the glucose uptake into muscle cells. The glycogen content represent the net glucose storage in the muscles, while the glycogen synthase activity showed the capacity for glycogen synthesis. The FV represents the capacity for glycogen synthesis independent of the cellular G-6-P levels. Thus, the higher the FV, the higher the glycogen synthesis, regardless of the G-6-P level. Since an increased triglyceride content in the muscle cells inhibited insulin stimulated glucose utilization, the triglyceride content in the muscles was measured.

The glycogen content in the soleus muscle was lower in the OVX than the sham rats, and the dietary carbohydrate subtypes did not significantly change the glycogen content (Table 3). The level was not significantly different in the quadriceps muscles, regardless of an ovariectomy and the dietary carbohydrate subtypes. In contrast to the glycogen content, the triacylglycerol content of the quadriceps muscles was higher in the OVX rats, regardless of the dietary carbohydrate subtypes, compared to the sham rats. The triacylglycerol content of the soleus muscle showed the same tendency, but there was no statistically significantly difference (p=0.07).

There were no changes in the total glycogen synthase activities by ovariectomy and dietary carbohydrate subtype in both the soleus and quadriceps muscles (Table 4). The lowest fractional velocity of glycogen synthase in the soleus muscle among the groups was in the OVX rats fed the high SU diet. However, there was no significant difference in the FV in the quadriceps muscles among the groups.

The GLUT4 contents in the soleus and quadriceps muscle membranes were affected by an ovariectomy and the dietary carbohydrate subtypes (Table 4). In the soleus muscle, the GLUT4 content was the lowest in OVX rats fed the high SU diet, with the highest in the sham rats fed the high ST diet. The lowest GLUT4 content in quadriceps muscle among the groups was in the OVX rats fed the high SU diet.

DISCUSSION

Our results suggest that an ovariectomy increases the insulin resistance in parallel with a greater weight gain than in sham-operated rats. OVX-induced obesity reduced the glycogen and elevated the triacylglycerol syntheses in the muscles with both the high SU and ST diets. The fractional velocity of glycogen synthase and the triglyceride and glycogen contents showed slight differences between the soleus and quadriceps muscles. These differences were due to the distinct characteristics of each muscle: the soleus muscle is a slow twitch muscle, which has more mitochondria, uses more fat as an energy source, stores more glycogen and less fat; whereas, the quadriceps muscle is a fast twitch muscle, has less mitochondria, uses more glucose as an energy source, and stores more fat and less glycogen.

In our study, neither the dietary carbohydrate subtypes nor an ovariectomy changed the serum leptin levels, which was consistent with other studies[20, 21]. An ovariectomy produced hyperplasia and increased the weight gain, without altering the serum leptin levels in either the high SU or ST diets. Pelleymounter et al.[20] suggested that estradiol does not directly regulate leptin secretion or effect on the fat mass. An ovariectomy did not alter the leptin levels in lean mice, but estradiol administration for 14 days reduced the serum leptin levels in a dose response manner (0.05~17 μg/day). However, this did not significantly reduce the leptin levels, although they were corrected with the estradiol-induced reduction in body fat. Furthermore, neither estradiol reduction, via an ovariectomy, nor addition, via exogenous administration, significantly altered the ability of leptin to reduce the fat mass. Leptin was equally effective in reducing the body weight in lean and obese OVX mice and in intact controls. Chen and Heiman[21] showed that the OVX rats increased their food intake, but did not change their energy expenditure or total level of fuel utilization, leading to gains in both lean and fat masses. Daily leptin injections initially significantly decreased the food intake, but feeding gradually increased to a stable level by the 16th day, and remained at that level for the duration of study. A body composition analysis indicated that a chronic injection of leptin to OVX rats dramatically decreased the fat mass due to an increased fat utilization, and also prevented a reduction in the calorie expenditure. The loss of ovarian function in rats is not associated with a change in leptin sensitivity. Thus, the weight gain of OVX rats was mainly due to hyperplasia, independent of the leptin secretion and sensitivity.

Kim et al.[8] showed that insulin resistance occurred in male, but not female, Wister rats fed a high SU diet. In the OVX rats, the high SU diet also induced insulin resistance by reducing the insulin-stimulated glucose utilization, but the suppression of the hepatic glucose output was normal. Sucrose-fed OVX rats developed fasting and glucose-stimulated hyperinsulinemia. However, in our study, not only the high SU, but also a high ST diet induced insulin resistance through decreased glucose uptake and glycogen synthesis in the skeletal muscle of OVX rats. Meanwhile, the whole body glucose disposal rates in the OVX rats on the high SU diet tended to be higher than those on the high ST diet, but this was not significant. This may have been due to the significant higher energy intake with the high ST than the high SU diet. Thus, an isocaloric intake in both groups may lead to higher body weights and lower glucose

disposal rates with the high SU compared to the high ST diet. Kumagai et al.[22] demonstrated that in OVX rats, insulin resistance was induced in parallel with a decreased insulin-stimulated content of 2-deoxyglucose (2-DG) in muscles, which is an index of glucose transport. The glycogen synthesis in the muscle was also decreased. Estradiol alone, and in combination with progesterone, but not progesterone alone, restored the insulin sensitivity to the values of intact controls. Thus, estradiol plays an important role in the maintenance of normal insulin sensitivity.

Horton et al.[9] reported that no difference was observed in the plasma and tissue triacylglycerol or tissue glycogen between high SU- and ST-fed, 7-week old, female Wister rats. They concluded that female rats, in contrast to males, do not develop sucrose-induced insulin resistance and hypertriglyceridemia. However, Kim et al.[8] demonstrated that insulin responsiveness of 2-DG transport in epitrochlearis and soleus muscles *in vitro* was decreased in both male and female rats fed a high SU diet compared to feeding on a chow diet. There was a highly significant negative correlation between the stimulated muscle 2-DG transport and visceral fat mass. Compared with rat chow, semipurified high SU and high ST diets, similarly to high-fat diets, causes an increased visceral fat accumulation and severe resistance of skeletal muscle glucose transport due to stimulation by insulin and contractions. In summary, OVX rats had increased energy intakes with both high SU and ST diets, which led to weight gains. OVX-induced obesity with both the high SU and high ST diets, increased intracellular fat storage in muscles and deceased glycogen synthesis, which contributed to the development of insulin resistance in OVX rats. However, OVX rats fed the high SU diet had a higher chance of weight gain and greater insulin resistance when they consumed isocaloric diets containing either high SU or ST.

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