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Surgical Removal of Visceral Fat Decreases Plasma Free Fatty Acid and Increases Insulin Sensitivity on Liver and Peripheral Tissue in Monosodium glutamate (MSG)-obese Rats

In order to evaluate the role of visceral and subcutaneous fat tissue in insulin sensitivity and lipid metabolism, we measured the fasting levels of plasma free fatty acid (FFA) and insulin, glucose disappearance rate (Rd), and hepatic glucose production rate (HGP) after surgical removal of visceral (VF) or subcutaneous (SF) fat tissue in monosodium glutamate-obese (MSG-Ob) rats. Monosodium glutamate obesity was induced in rats by neonatal injection of MSG. Surgery to remove fat was done at 15 weeks of age. The experiments were done four weeks after the surgery. MSG-Ob rats showed increased levels of FFA, insulin, and HGP and decreased Rd compared to normal rats. In the VF group, the FFA level and HGP were decreased to normal values, Rd was partially normalized, but the level of insulin did not change significantly compared to MSG-Ob. In the SF group, FFA and Rd were partially normalized, but HGP was not suppressed significantly compared to MSG-Ob. These results suggest that visceral fat affects the insulin sensitivity of liver and FFA concentration more than subcutaneous fat; however, no significant difference was shown on whole body insulin sensitivity and fasting insulin concentration.

Key Words: *Obesity; Rats; Viscera; Adipose tissue; Insulin resistance; Liver; Fatty acids, nonesterified; Glucose clamp technique*

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INTRODUCTION

Obesity, as an important risk factor for non-insulin-dependent diabetes mellitus (NIDDM), has been noted in a number of prospective studies (1, 2). Moreover, the distribution of fat to particular regions of the body seems to be a more important risk factor than obesity itself (3-5). Studies have found that patients with a type of fat distribution that reflects a greater adiposity in the abdominal region, particularly in visceral tissues, showed more pronounced abnormalities in the metabolic profile such as insulin resistance, abnormal insulin removal, and disorders in lipoprotein regulation which predisposes the patients to NIDDM compared to patients with fat distributed in the lower part of the body, the gluteofemoral region. The mechanisms of the relationship between the abnormalities in the metabolic profile and fat distribution were studied from several angles; however, they are still not fully understood. First, differences in the metabolic characteristics of visceral and subcutaneous adipose tissue have been observed. Compared to subcutaneous fat, visceral fat showed a high lipolytic activity (6) and low response to the antilipolytic effect of insulin (7). These characteristics would result in a high release of free fatty acid (FFA) from visceral adipose tissues and induce lipotoxicity in liver, pancreas, and skeletal muscles. Second, leptin and TNF-α, which are secreted in obese adipocyte and act on hepatocyte, pancreatic β-cell, and skeletal muscle, and would modify insulin action, were reported as factors linking obesity and insulin resistance (8, 9). Although some controversies still exist, there was a higher secretion rate of leptin in the subcutaneous than in the visceral fat tissue. However, no site-specific difference has been reported in the secretion rate of TNF-α between visceral and subcutaneous fat tissue (10). Finally, we thought that the anatomical site-specific secretion of these factors act mainly on different organs and produce different effects. However, not many reports evaluated those effects from this aspect. Furthermore, these factors affect secretion and/or action of other factors. In order to evaluate the overall effect of these factors, in vivo data is needed.

To provide direct evidence to support the cause-effect relationship between site specific fat tissue and insulin

action, we examined the impact of surgical removal of visceral or subcutaneous fat pads on hepatic and whole body insulin sensitivity.

MATERIALS AND METHODS

Animal care

Pregnant female Sprague-Dawley rats were purchased from Daehan Experimental Animal Center for this study. Only the male offsprings were used. At two days after birth, these neonatal male rats were divided into normal (n=5) and MSG-injected group. For the MSG-injected group, obesity was induced by injection (2, 4, 6, 8, 10 days) of monosodium glutamate (4 g/kg, subcutaneously) for 15 weeks. At 15 weeks of age, the MSG-obese rats of similar body weights were divided into three groups: control (MSG-Ob, n=6), surgical visceral fat removal (VF, n=6), and surgical subcutaneous fat removal (SF, n=6) group. The rats in the experimental groups went through surgery at this time, and the experiments were performed at four weeks after the surgery.

At the time of surgery to remove fat deposits, MSG-obese rats showed severe fat deposits in the subcutaneous tissue and intraabdominal cavity. Surgery was done aseptically under pentothal sodium (40 mg/kg BW) anesthesia. For the VF group, visceral fat, especially the epididymal and retroperitoneal fat, was removed. For the SF group, abdominal subcutaneous fat was removed. The sham operation was performed in normal and MSG-Ob rats. A similar percentage of body weight was removed from VF and SF removal operations in the rats. The rats were treated daily with antibiotics for two weeks intraperitoneally.

Euglycemic hyperinsulinemic clamp

At four weeks after the surgery, and 7 hr of fasting (from 0800 to 1500), the rats were anesthetized with pentothal sodium, then, the insulin and glucose clamp technique was performed according to Kim et al. (11). As briefly described, one catheter was inserted into the right external jugular vein to infuse insulin, 25% glucose, and [3-^{3}H] glucose (NEN, Boston, U.S.A.), and another catheter was inserted into the left femoral artery to take a blood sample. Thirty min after catheterization, blood sampling was taken to determine the basal state for plasma insulin, FFA, glucose, TG and cholesterol. Insulin and glucose were clamped at two levels, basal and hyperinsulinemic euglycemic levels.

At first, a primed (13.3 μCi/kg) and continuous (0.67 μCi/kg · min) infusion of [3-^{3}H] glucose was initiated and continued throughout the study (about 150 min). During the initial 60 min, isotope was infused, and this period was denoted as the isoinsulinemic isoglycemic clamp. For the next 90 min, a prime (204 pmol/kg) and maintenance dose (10.2 pmol/kg · min) of insulin (Velosulin, Bagsvaed, Denmark) was infused, and glucose was infused at variable rates with a peristaltic pump (Gilson, France) to adjust the glucose level at ~ 4.4 mM.

Blood sampling to determine the glucose level was taken at 10 min interval, and the blood glucose concentration was measured immediately with a glucose analyzer (YSI 1410, Ohio, U.S.A.). The blood sample for insulin and tritiated glucose was done at 50, 60, 140, 150 min. Tritiated glucose was measured with a liquid scintillation counter (Pharmacia, Sweden). The glucose disappearance rate (Rd, mg/kg · min) at steady state was calculated by the method below (12):

$$Rd = \frac{[3\text{-}^3H]\ \text{glucose infusion rate (DPM/kg} \cdot \text{min)}}{\text{Steady state value of glucose specific activity (DPM/mg)}}$$

The steady state value of glucose specific activity was expressed as the ratio of radioactivity in plasma to plasma glucose concentration. The hepatic glucose output rate was calculated by subtracting the Rd by the glucose infusion rate (GIR).

Measurement of the glycogen synthase (GS) activity

GS activities were measured in the liver and soleus by modifying the method of Thomas et al. (13). The tissue samples (50 mg) were homogenized with Polytron homogenizer (Kinematica GmbH, Switzerland) in 1.0 mL of 50 mM Tris-HCl buffer, 4℃, pH 7.8, containing 20 mM EDTA, and 25 mM NaF. The homogenate was centrifuged at 10,000 g for 30 min at 4℃, and a supernatant was used for the GS assay. GS assay was measured by the incorporation of UDP-[U-^{14}C]-glucose into glycogen in the absence of glucose-6-phosphate (G-6-P) to measure the active GS and in the 10 mM G-6-P for total GS.

Measurement of plasma insulin, FFA, triglyceride (TG), and cholesterol

Insulin concentration was determined by radioimmunoassay. The concentrations of FFA, TG and total cholesterol were analyzed with diagnostic kits.

Measurement of body fat and length

Visceral fat was determined by weighing excised fat in the intraabdominal cavity, specifically divided to epi-

dydimal, retroperitoneal and omental fat. Subcutaneous fat means fat on the subcutaneous area of abdominal wall. Body length was measured by length from upper canine to anus. Tail length was measured by length from anus to tip of tail.

Statistical analysis

All values are represented as the mean ±S.E.. Statistical analyses were performed with unpaired or paired t-test.

RESULTS

The growth curve of MSG-Ob rats shifted downward and body weights of MSG-Ob rats lowered significantly from seven to 15 weeks of age compared to normal rats (Fig. 1). At the time of study, the MSG-Ob rats were lighter in weight and shorter in body and tail length, but had approximately four times the abdominal fat than normal rats. Approximately equal amounts of total VF and SF were removed (Table 1). The fasting levels of FFA, insulin and TG increased significantly in the MSG-obese rats compared to normal rats ($p<0.01$ for FFA and insulin; $p<0.05$ for TG). But the fasting levels of glucose and total cholesterol did not change significantly. These elevated FFA levels were completely normalized in VF, but partially normalized in SF. The elevated levels of insulin and TG in MSG-obese rats did not recover significantly in VF and SF (Table 2).

At the time of surgery, the levels of FFA and insulin were increased in MSG-Ob compared to normal rats. During the post-operation period, the levels of FFA and insulin were significantly increased ($p<0.05$) in MSG-Ob and unchanged in normal and SF rats. But in the VF rats, the FFA level decreased although the insulin level did not change (Fig. 2, 3).

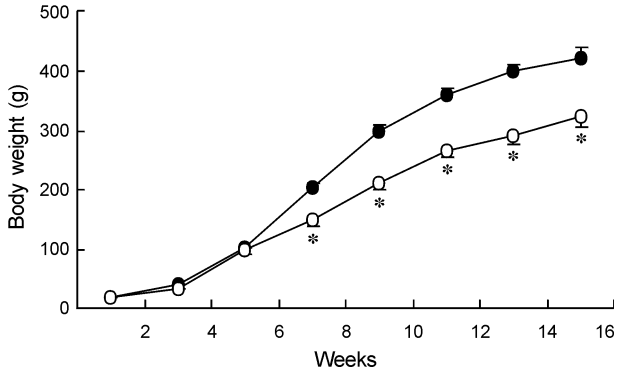

Fig. 1. Changes of body weight in normal (closed circle) and MSG-Ob rats (open circle) during 15 weeks of age. *$p<0.05$ vs normal rats

The results of the two-step, insulin-glucose clamp study are shown in Table 3. At the basal state, the Rd of MSG-Ob rats decreased compared to normal rats and did not increase with VF and SF. During the hyperinsulinemic euglycemic clamp study, the Rd of MSG-Ob rats decreased compared to normal rats, but increased with VF and SF. The HGP of MSG-Ob rats during the hyperinsulinemic euglycemic clamp study increased compared to normal rats, but decreased to the normal level in VF. The HGP of SF showed a decreasing tendency compared to MSG-Ob, but did not change significantly.

The GS activity of soleus muscle decreased in MSG-Ob compared to normal rats in the total activity but was normalized by VF and SF. The GS activity of liver showed no significant changes among the experimental groups (Fig. 4).

Table 1. Physical characteristics of experimental groups

	Normal	MSG-Ob	VF	SF
Body weight (g)	461±20.5	372±27.1*	301±23.5†	320±31.73†
Body length (cm)	24.4±0.510	21.5±0.500†	21.5±0.540†	20.5±0.289†
Tail length (cm)	21.6±0.245	13.8±0.601†	14.7±0.520†	14.0±0.707†
Total abdominal fat (%)	3.41±0.293	13.11±0.947†	7.40±0.825*,§	7.94±0.638*,§
Subcutaneous fat	1.30±0.155	6.28±0.341†	4.53±0.164*,§	1.85±0.463§,¶
Visceral fat	2.11±0.146	6.83±0.341†	2.96±0.710§	6.08±0.322*,†,¶
Omental fat	0.42±0.025	1.33±0.063†	0.73±0.114§	0.98±0.051†
Retroperitoneal fat	0.60±0.039	2.67±0.130†	1.14±0.290§	2.23±0.197
Epididymal fat	1.09±0.088	2.83±0.278†	1.08±0.351§	2.88±0.140
Amounts of fat removal	0	0	2.83±0.107	2.59±0.219

Values are mean±S.E.
VF, group for surgical removal of visceral fat tissue; SF, group for surgical removal of subcutaneous fat tissue of abdominal area
*$p<0.05$, †$p<0.01$, vs normal; †$p<0.05$, §$p<0.01$, vs MSG-Ob; ‖$p<0.05$, ¶$p<0.01$, vs VF

Table 2. Metabolic characteristics of experimental groups

	Normal	MSG-Ob	VF	SF
FFA (μEq/L)	828±135.7	2,340±410.0†	790±431.2§	1,138±99.9†,‖
Insulin (pM)	120±8.3	219±21.5†	192±20.6*	196±17.6*
Glucose (mM)	4.8±0.38	4.7±0.25	4.7±0.14	4.9±0.16
TG (mg/dl)	17.6±3.47	46.2±7.81*	37.7±4.83†	34.0±2.01†
Cholesterol (mM)	2.4±0.26	2.2±0.27	2.9±0.23	2.1±0.21

Values are mean±S.E.
VF, group for surgical removal of visceral fat tissue; SF, group for surgical removal of subcutaneous fat tissue of abdominal area; TG, triglyceride
*$p<0.05$, †$p<0.01$, vs normal; †$p<0.05$, §$p<0.01$, vs MSG-Ob; ‖$p<0.05$, vs VF

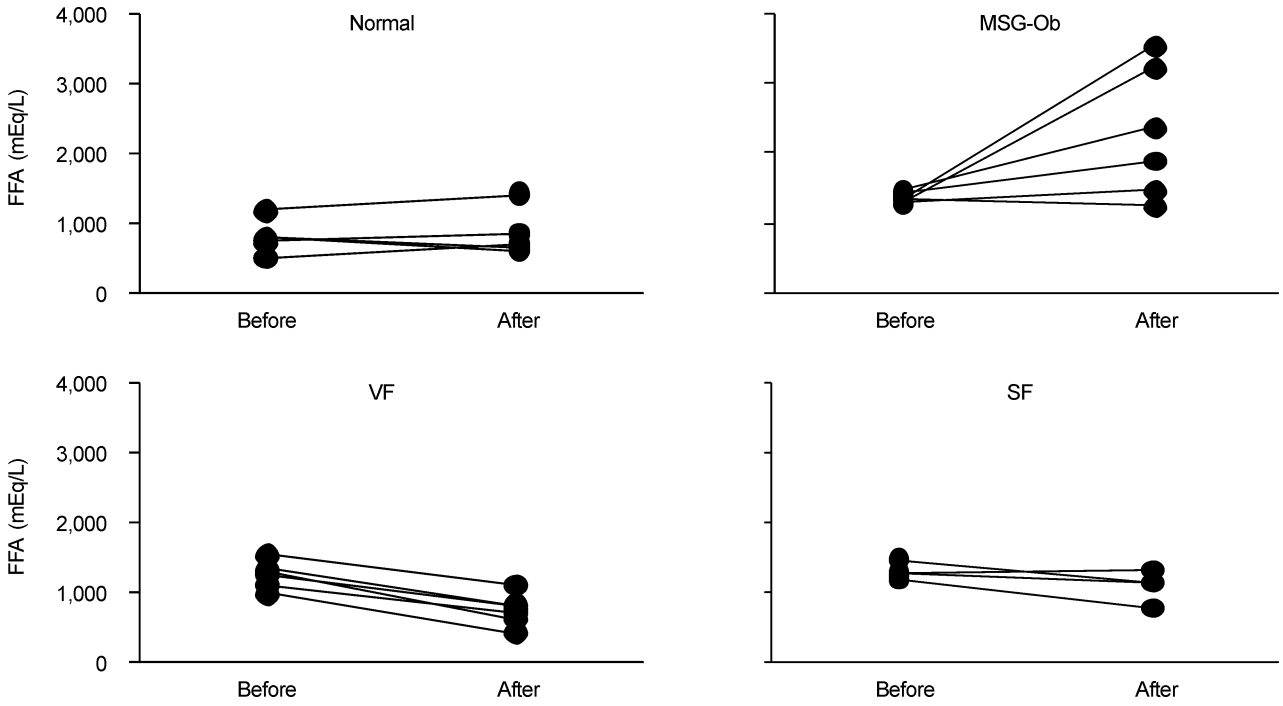

Fig. 2. Changes of FFA before and after surgery in experimental groups.

DISCUSSION

It is well known that neonatal treatment of MSG results in a specific lesion on the ventromedial hypothalamus and induces obesity (14). In this study, the neonatal MSG-treated rats showed severe obesity with short stature, increased levels of FFA, insulin and TG, decreased Rd, increased HGP, and decreased total glycogen synthase activity in skeletal muscle.

To evaluate the role of site specific fat tissue in these changes, we measured these parameters after surgical removal of visceral or subcutaneous fat tissue in MSG-obese rats.

Compared to normal rats, the 15-week-old, MSG-obese rats showed an elevated level of FFA, which is an important causative factor of insulin resistance in liver and skeletal muscle (15). The elevated FFA level was increased further in the sham-operated control rats, but the level was completely normalized in VF and partially recovered in SF rats. These results demonstrated that visceral fat plays a more prominent role in the plasma FFA level and resulted from a greater turnover rate of FFA in visceral fat pad compared to subcutaneous fat pad (6, 7).

In addition to the high turnover rate of FFA, visceral fat depots are drained by portal circulation. Therefore, FFAs released from these depots reach the liver in high concentrations. High levels of portal FFAs may eventually result in an enhancement of hepatic triglyceride synthesis, causing hyperlipidemia, and would also induce hepatic insulin resistance, resulting in an increase of HGP (16). HGP, whose elevation is a pathophysiological hallmark of type II diabetes, is regulated by insulin and FFA level, directly or indirectly (17-19). In this study, we

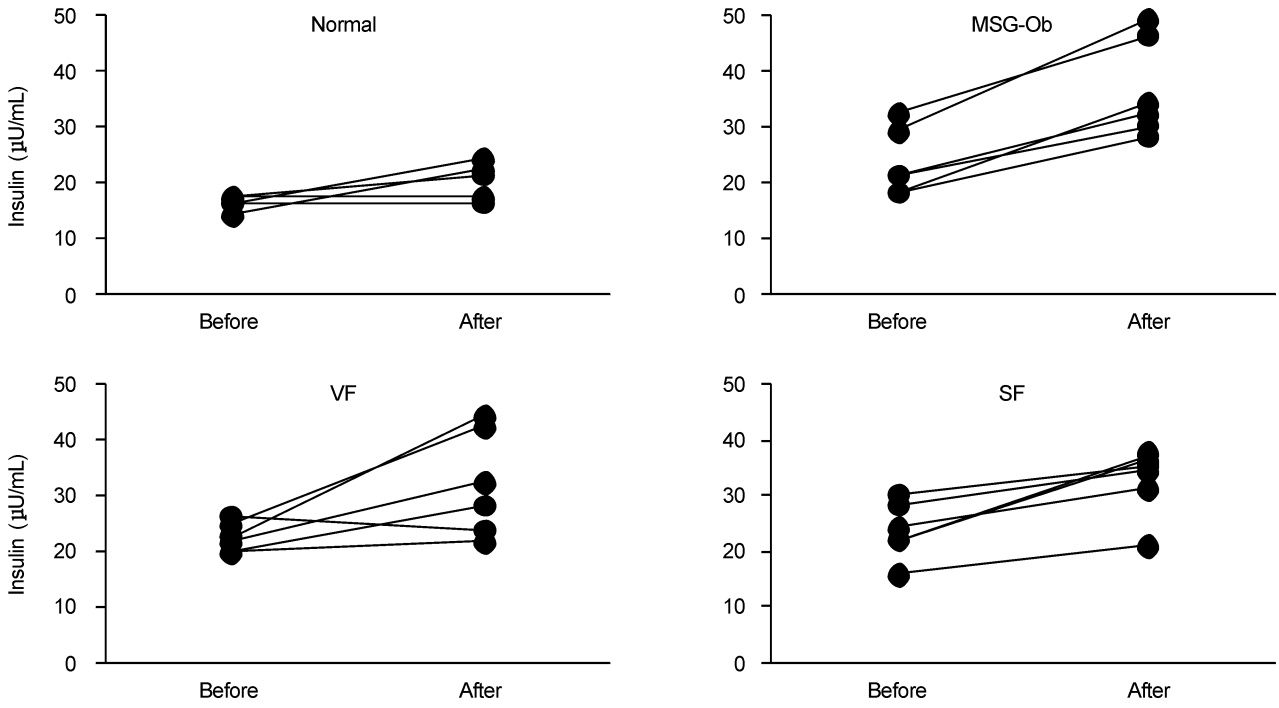

Fig. 3. Changes of insulin before and after surgery in experimental groups.

Table 3. Steady state values of glucose disappearance rate (Rd), hepatic glucose production rate (HGP), glucose infusion rate (GIR), glucose, and insulin at hyperinsulinemic euglycemic clamp studies in experimental groups

	Normal	MSG-Ob	VF	SF
Basal state				
Glucose (mM)	4.4±0.19	4.4±0.19	4.3±0.22	4.3±0.13
Insulin (pM)	103±7.7	195±20.1†	181±17.0*	178±16.8*
Rd (mg/kg · min)	5.1±0.37	3.6±0.22†	3.4±0.10*	3.9±0.16*
HGP (mg/kg · min)	5.1±0.37	3.6±0.22†	3.4±0.10*	3.9±0.16*
Hyperinsulinemic				
Euglycemic clamp				
Glucose (mM)	4.5±0.10	4.4±0.12	4.3±0.10	4.3±0.09
Insulin (pM)	636±59.8	672±61.8	630±53.0	600±49.6
Rd (mg/kg · min)	10.8±1.26	5.6±0.20*	7.5±0.57†	6.7±0.15*,§
HGP (mg/kg · min)	-0.0±1.39	3.3±0.75†	0.0±0.86†	1.7±0.11‖
GIR (mg/kg · min)	10.8±1.48	2.3±0.80†	7.5±1.02§	5.1±0.16†,†,¶

Values are mean±S.E.
VF, group for surgical removal of visceral fat tissue; SF, group for surgical removal of subcutaneous fat tissue of abdominal area
*$p<0.05$, †$p<0.01$, vs normal; ‡$p<0.05$, §$p<0.01$, vs MSG-Ob; ‖$p<0.05$, ¶$p<0.01$, vs VF

measured the insulin sensitivity in hepatic and peripheral tissue at two levels of insulin through glucose-insulin clamp technique. The HGP of MSG-Ob rats was not suppressed by insulin, which meant the hepatic insulin resistance existed in MSG-Ob rats. Hepatic insulin resistance in MSG-Ob rats was completely normalized by visceral fat removal, and partially recovered by subcutaneous fat removal.

The increased FFA level also increased the availability and oxidation of FFAs in the skeletal muscle by the FFA/glucose cycle, which impairs glucose utilization (20). The Rd during the hyperinsulinemic euglycemic clamp was mainly reflected by the glucose uptake of the skeletal muscle. The Rd of MSG-Ob was decreased to ~70% during the basal state in spite of an elevated basal insulin level and to ~51% during the hyperinsulinemic clamp compared to the normal rats. During basal state, no significant improvement was observed in VF and SF, but during the hyperinsulinemic clamp, the Rds of VF and SF were increased to 69% and 62% of normal rats,

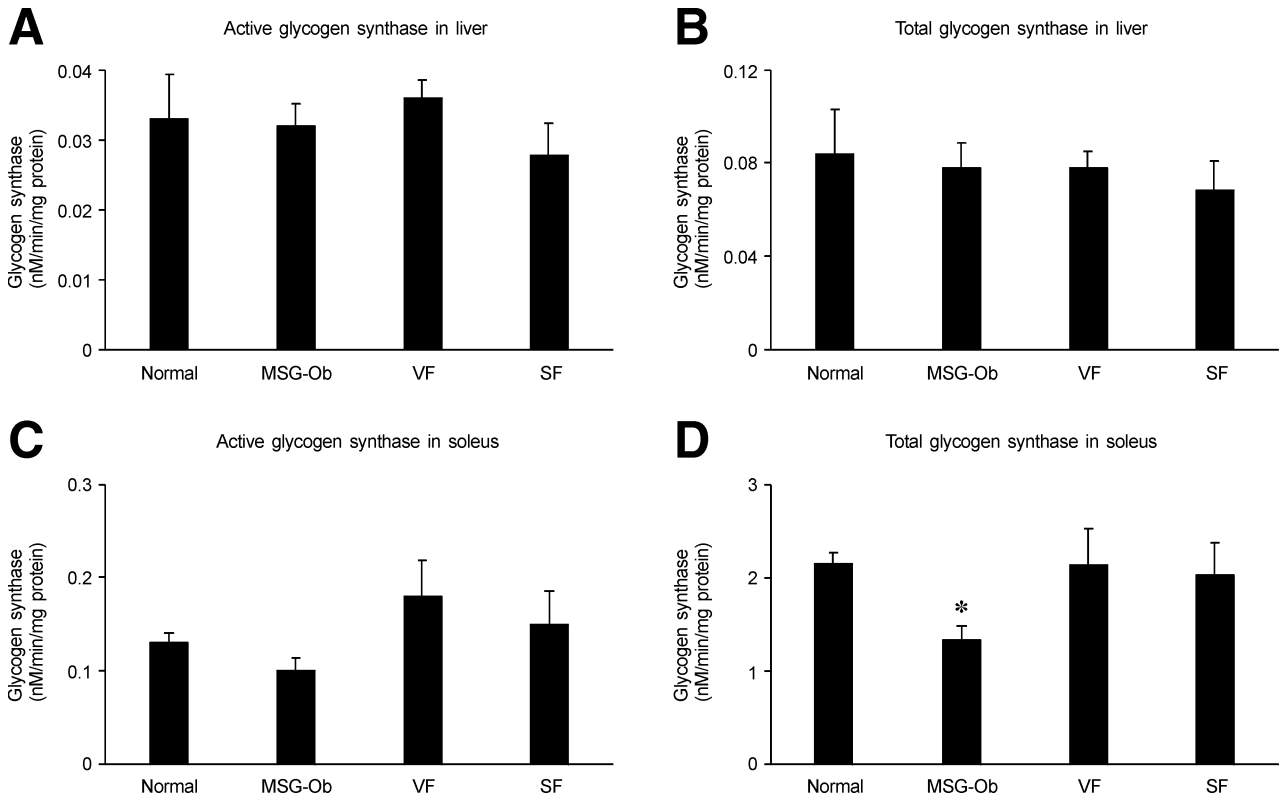

Fig. 4. Active and total glycogen synthase activity in liver and soleus muscle in experimental groups. *$p<0.05$ vs normal group.

respectively. As these results indicated, we thought that the insulin resistance occurred in the hepatic and peripheral tissue in MSG-Ob rats, and this hepatic insulin resistance was recovered more in VF than in SF. However, the peripheral insulin resistance was recovered similarly in VF and SF. These results demonstrated that the insulin action on hepatic and skeletal muscle was regulated differently and that insulin resistance associated with increased fat pad was affected by releasing site as well as FFA level. Thus, this study provides direct evidence of the causal role of increased visceral adiposity in hepatic insulin resistance.

Compared to normal rats, the plasma TG concentration was elevated in MSG-Ob rats. Although the FFA levels were decreased, no significant lowering effect was observed in both VF and SF rats. In the obese animal, the plasma insulin level was elevated in spite of normoglycemia due to insulin resistance, low hepatic clearance rate of insulin, and insulinotrophic effect of FFA (21). In this study, the fasting insulin concentration was significantly elevated by compensatory mechanisms in MSG-Ob rats, but the elevated insulin level did not decrease significantly by either VF and SF in spite of significantly decreased FFA levels and improved peripheral insulin sensitivity in both VF and SF.

Glycogen synthase (GS) is a rate-limiting step in insulin action in skeletal muscle, and its activity was inhibited in high FFA or insulin resistant state (22). In the present study, the total GS activity in skeletal muscle was decreased in MSG-Ob rats compared to normal rats. This decrease in GS activity was observed in other reports (23) as well, but it was completely normalized in VF and SF rats in spite of an incomplete recovery of glucose uptake by insulin in skeletal muscle. Compared to muscle, relatively little is known of glycogen metabolism in liver in obesity and NIDDM. Liver glycogen may be of crucial importance in regulating the basal glucose concentration of autoregulation between gluconeogenesis and glycogen breakdown and in controlling the rate of hepatic glucose production. Increased liver glycogen levels have been found in NIDDM subjects (24) and in some animal models of diabetes, i.e, obese fa/fa Zucker rats (25), and db/db mice (26). In the obesity models, GS activity of the liver had some controversies. Chen et al. (27) reported that the GS activity was higher in GTG-obese mice, however, Thorburn et al. (28) reported that active GS was lower in adult New Zealand obese mice. In this study, despite elevated HGP, no change in the GS activity of the liver was seen in MSG-obese rats compared to normal rats. The glycogen concentration of MSG-Ob rats was slightly increased in the liver but decreased in skeletal muscle (data not shown). These results meant that the GS activity was regulated by different processes in the skeletal muscle and liver.

In conclusion, these results suggest that visceral fat affects the insulin sensitivity of liver and FFA concentration more than subcutaneous fat. However, no significant difference was shown in whole body insulin sensitivity and fasting insulin concentration.

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