Distinct Role of Adiposity and Insulin Resistance in Glucose Intolerance: Studies in Ventromedial Hypothalamic-Lesioned Obese Rats

Asako Kageyama, Tsutomu Hirano, Haruaki Kageyama, Toshimasa Osaka, Yoshio Namba, Masatomi Tsuji, Mitsuru Adachi, and Shuji Inoue

It remains unclear whether adiposity plays an important role in glucose intolerance independently of insulin resistance. We investigated whether adiposity and insulin resistance had distinct roles in glucose intolerance in rats. We examined glucose tolerance and insulin resistance using ventromedial hypothalamic (VMH)-lesioned rats in the dynamic and the static phases of obesity (2 and 14 weeks after lesioning, respectively). Rats were fed either normal chow or a fructose-enriched diet (60% of total calories). The intravenous glucose tolerance test (IVGTT) was performed by bolus injection of glucose solution (1 g/kg) and blood sampling after 0, 5 10, 30, and 60 minutes. Insulin resistance was evaluated from the steady-state plasma glucose (SSPG) value during continuous infusion of glucose, insulin, and somatostatin. SSPG was not increased in VMH-lesioned rats in the dynamic phase of obesity, but increased markedly in the static phase. The area under the glucose curve (glucose AUC) during IVGTT was increased in VMH-lesioned rats in the static phase, but not in the dynamic phase, when compared with their sham-operated counterparts. A fructose-enriched diet for 2 or 14 weeks increased SSPG values to a similar extent in both sham-operated and VMH-lesioned rats without inducing excess adiposity, but glucose intolerance was only developed in the obese rats. The plasma leptin level, an excellent indicator of adiposity, was significantly related to the glucose AUC independently of the insulin level. Insulin resistance or increased adiposity alone is not sufficient to impair glucose tolerance, but increased adiposity plays an important role in the development of glucose intolerance in an insulin-resistant state.

IT IS WELL KNOWN that glucose intolerance is frequently associated with obesity, and that obesity is an important risk factor for type 2 diabetes mellitus.[1] It is generally accepted that insulin resistance is a major factor in the development of glucose intolerance in obese subjects,[2-4] although mechanisms by which insulin resistance is associated with obesity still remain to be clarified.[4] Recently, circulating nonesterified fatty acids (NEFA) have attracted attention as an important factor in the onset of insulin resistance associated with obesity.[4,5] NEFA are secreted from adipose tissue via lipolysis by the action of hormone-sensitive lipase, and insulin suppresses the activity of this enzyme.[6,7] When insulin sensitivity is blunted, release of NEFA from adipose tissue is increased, resulting in an elevated plasma NEFA level. Elevation of the plasma NEFA level may impair glucose metabolism in muscle[8,9] and in liver.[10] In addition, apart from the action of insulin, release of NEFA from adipose tissue would also increase if the total adipose tissues mass is greatly increased.[4,11] Therefore, it is possible that increased adiposity may directly influence glucose tolerance independent of insulin resistance. However, little is known about how adiposity is directly related to glucose tolerance independent of insulin resistance, because obesity and insulin resistance usually develop simultaneously.

Bilateral lesions of the ventromedial nucleus of the hypothalamus (VMH) produce obesity in rats, and VMH-lesioned rats are frequently utilized as a representative animal model of obesity.[12,13] Unlike congenitally obese animals, VMH-lesioned obese rats have an initial rapid period of weight gain (the dynamic phase), after which plateau of body weight is reached (the static phase).[12,13] Frohman et al[14] observed many years ago that hyperinsulinemia without insulin resistance occurred in VMH-lesioned rats in the early weeks after the lesion. We also confirmed that insulin resistance does not exist in lesioned rats during the dynamic phase, but develops in the static phase.[15] This potentially allows us to investigate the distinct roles of insulin resistance and adiposity in glucose intolerance by assessing the pathophysiology of these rats during the different phases of obesity. Dietary fructose can induce insulin resistance in rats without causing obesity.[16-18] We recently found that dietary fructose also induces insulin resistance in VMH-lesioned rats during the dynamic phase of obesity.[19] Investigation of how glucose tolerance is altered in lean and VMH-lesioned rats during the different phases of obesity when insulin resistance is modified by dietary fructose may be useful in identifying the distinct role of adiposity in glucose intolerance independent of the role of insulin resistance.

Leptin, the peptide encoded by the obesity gene, is secreted by adipose cells and plays an important role in regulating food intake, energy expenditure, and adiposity.[20,21] Although the physiologic effects of circulating leptin on organs outside the central nervous system are still unclear, it is generally accepted that the plasma leptin concentration closely reflects the amount of adipose tissue in the body,[22,23] suggesting that circulating leptin is an excellent indicator of whole body adiposity. We recently reported that plasma leptin levels closely reflect adiposity in lean control and VMH-lesioned obese rats.[15] In addition, we found that plasma leptin levels in rats were not affected by insulin resistance when this was induced by dietary fructose.[19] In the present study, we evaluated the role of adiposity in the development of glucose intolerance in lean and obese rats with or without insulin resistance using plasma leptin as a new indicator of adiposity.

From the First Department of Internal Medicine, Showa University School of Medicine, Tokyo; and the Division of Geriatric Health and Nutrition, National Institute of Health and Nutrition, Tokyo, Japan.

Submitted July 6, 2001; accepted December 3, 2001.

Address reprint requests to Tsutomu Hirano, MD, PhD, First Department of Internal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan.

0026-0495/02/5106-0010$35.00/0

doi:10.1053/meta.2002.32728

Table 1. General and Metabolic Characteristics of Sham-Operated Lean Control Rats and VMH-Lesioned Rats Given Either Normal Chow or Fructose-Enriched Diets (2 weeks)

	Sham		VMH	
	Normal	Fructose	Normal	Fructose
No.	26	17	29	17
Initial body weight (g)	257 ± 3	252 ± 6	266 ± 2	271 ± 6†
Final body weight (g)	277 ± 4	263 ± 6	350 ± 4*†	329 ± 11*†‡
BW gain (g/d)	0.9 ± 0.3	2.5 ± 0.5	4.1 ± 0.8*†	5.9 ± 0.7*†‡
Parametrial fat pad (g)	3.7 ± 0.8	3.2 ± 0.4	7.5 ± 0.6*†	6.0 ± 0.5*†
Food intake (g/d)	13.7 ± 0.4	18.2 ± 1.9	29.3 ± 3.5*†	29.4 ± 1.8*†
Glucose (mmol/L)	6.8 ± 0.3	6.8 ± 0.3	7.3 ± 0.6	6.3 ± 0.3
NEFA (μmol/L)	1,169.3 ± 111.7	1,389.3 ± 208.3	1,755.8 ± 154.3*	2,129.9 ± 229.7*‡
Triglyceride (mmol/L)	0.8 ± 0.1	1.6 ± 0.2*	1.4 ± 0.1*	2.9 ± 0.5*†‡
Insulin (ng/mL)	1.5 ± 0.3	2.3 ± 0.3	2.6 ± 0.4	4.6 ± 1.5*†‡
Leptin (ng/mL)	3.4 ± 0.7	3.5 ± 0.4	19.7 ± 1.7*†	17.1 ± 2.7*†

NOTE. Data represent mean + SEM, statistical significance by 1-way ANOVA at $P < .05$.

*v sham-operated rats fed normal chow;

†v sham-operated rats fed fructose-enriched diet;

‡v VMH-lesioned rats fed normal chow.

MATERIALS AND METHODS

Rats

Female Sprague-Dawley rats weighing 230 to 290 g (Japan SCL Inc, Hamamatsu, Japan) were kept in individual cages on a rotating 12-hour light-dark cycle and had free access to rat chow and water. The animals were anesthetized by inhalation of Isoflulene (Forane; Dinabott, Osaka, Japan), and electrolytic bilateral VMH lesions were produced by the method previously described.[12,13,24] Control animals received sham VMH lesions (no current passed through the electrode). Rats fed a fructose diet ingested the experimental diet (Oriental Food Co, Tokyo, Japan) that contained (as percent of calories) 60% fructose, 11% corn oil, and 29% animal protein. The chow-fed group was fed standard rat chow (Oriental Food Co) that contained (as percent of calories) 60% vegetable starch, 11% corn oil, and 29% animal protein. Fructose feedings were started immediately after the VMH operation, and the diets were given for 2 or 14 weeks. Food was withdrawn at 9:00 AM on the day of experiments, and all experiments were performed after a 5-hour fast (2:00 PM).

Steady-State Plasma Glucose Method for Evaluation of Insulin Resistance

Insulin resistance in the whole body was assessed by the steady-state plasma glucose (SSPG) method originally developed by Reaven's laboratory[18,25] and modified by Harano et al.[26] Rats were anesthetized with pentobarbital and then given a constant infusion of glucose (8 mg · kg^{-1} · min^{-1}), insulin (2.5 mU · kg^{-1} · min^{-1}, Humalin R; Eli Lilly-Shionogi, Osaka, Japan), and somatostatin (0.5 μg/min, Sigma, St Louis, MO) for 170 minutes through a cannula inserted into the right jugular vein. Blood samples were collected before the infusion was started and 150, 160, and 170 minutes after the infusion from a cannula, which was inserted into the femoral vein. Under these conditions, endogenous insulin release was inhibited by somatostatin, and SSPG was maintained during the last 20 minutes of the infusion. The mean of 150-, 160-, and 170-minute samples was used to determine the SSPG values. Since the SSPG response is a direct reflection of the efficiency of insulin-mediated glucose disposal, higher SSPG values imply proportionally greater insulin resistance.

Intravenous Glucose Tolerance Test

The intravenous glucose tolerance test (IVGTT) involved an injection of a 0.5 g/mL glucose solution (1.0 g/kg body weight) via catheter. Blood samples were collected before the injection and 5, 10, 30, and 60 minutes afterwards. The blood was immediately centrifuged at 4°C, and plasma was stored at -20°C until assayed. Glucose, insulin, and NEFA were measured at each point, and the area under the curve (AUC) for these variables during IVGTT were calculated.

Measurements

Plasma glucose levels were determined by the glucose oxidase method (Glucose-B test; Wako Pure Pharmaceutical, Osaka, Japan). Plasma triglyceride concentration was determined by the enzyme method using a commercially available kit (Triglyceride-G test; Wako Pure Pharmaceutical). Plasma NEFA concentration was determined by the enzyme method using a commercially available kit (NEFA-C test; Wako Pure Pharmaceutical). Immunoreactive insulin (IRI) concentrations were determined by a radioimmunoassay kit (RI-13K; Linco Research, St Charles, MO) standardized against rat insulin. Plasma leptin concentrations were determined by a radioimmunoassay kit (RL-83K, Linco Research) for specifically determining rat leptin.

Statistics

Data are expressed as means ± SEM. Statistical significance was assessed by 1-way analysis of variance (ANOVA), and $P < .05$ was accepted as a significant difference. The correlation coefficients between 2 parameters were determined by Pearson's simple linear regression analysis. Stepwise mutivariate regression analysis was performed to assess a correlation of independent variables with glucose-AUC as the dependent variable, and an F value greater than 4.0 was accepted as significant.

RESULTS

Profile of Sham-Operated and VMH-Lesioned Rats at 2 Weeks Postoperatively

Table 1 shows the general and metabolic characteristics of sham-operated and VMH-lesioned rats 2 weeks after the operations given either normal chow or a fructose-enriched diet at 2 weeks after surgery. VMH-lesioned rats showed a 4-fold greater weight gain and a 2.5-fold greater parametrial fat pad weight compared with sham-operated rats. Fructose feeding for 2 weeks did not affect weight gain or the parametrial fat pad in either sham-operated rats or VMH-lesioned obese rats. Food

Table 2. General and Metabolic Characteristics of Sham-Operated Lean Control Rats and VMH-Lesioned Rats Given Either Normal Chow or Fructose-Enriched Diets (14weeks)

	Sham		VMH	
	Normal	Fructose	Normal	Fructose
No.	18	14	23	13
Initial body weight (g)	258 ± 5	270 ± 7	261 ± 2	276 ± 5
Final body weight (g)	342 ± 9	315 ± 14	458 ± 16*†	395 ± 24*‡
BW gain (g/d)	0.7 ± 0.3	0.8 ± 0.3	0.7 ± 0.4*†	0.0 ± 0.5†‡
Parametrial fat-pad (g)	4.5 ± 0.4	5.9 ± 1.4	12.6 ± 1.2*†	9.3 ± 2.0*
Food intake (g/d)	14.8 ± 0.9	18.9 ± 1.5	17.9 ± 2.0	14.9 ± 1.2
Glucose (mmol/L)	6.8 ± 0.3	6.4 ± 0.4	8.0 ± 0.5*†	6.0 ± 0.4‡
NEFA (μmol/L)	1,368.2 ± 151.3	2,022.0 ± 277.8*	1,949.2 ± 207.0*	2,468.6 ± 241.5*
Triglyceride (mmol/L)	1.0 ± 0.1	3.0 ± 0.5*	2.5 ± 0.3*	4.1 ± 0.8*‡
Insulin (ng/mL)	1.5 ± 0.1	2.4 ± 0.5	4.1 ± 0.6*†	3.3 ± 0.8*
Leptin (ng/mL)	7.4 ± 1.3	6.6 ± 2.2	49.6 ± 3.8*†	33.0 ± 9.2*†‡

NOTE. Data represent mean + SEM, statistical significance by 1-way ANOVA at $P < .05$.
*v sham-operated rats fed normal chow;
†v sham-operated rats fed fructose-enriched diet;
‡v VMH-lesioned rats fed normal chow.

intake was increased significantly in VMH-lesioned rats when compared with sham-operated rats, but was comparable between the fructose-enriched and normal diets in both lean and obese rats. Plasma glucose levels were comparable between the 4 groups. VMH-lesioned rats fed normal chow had a 2-fold increase of plasma triglyceride level. Fructose feeding doubled the plasma triglyceride level in both lean and obese animals. Plasma NEFA levels were significantly increased in VMH-lesioned rats whem compared with control rats. Plasma NEFA levels were also significantly increased by the fructose feeding in VMH-lesioned obese rats. Plasma insulin levels were 1.7-fold higher in VMH-lesioned rats fed a normal diet when compared with controls. Fructose feeding caused elevation of plasma insulin levels in control rats and VMH-lesioned rats. The plasma leptin concentration was 6-fold higher in VMH-lesioned rats than in controls, but fructose feeding did not affect plasma leptin in control or VMH-lesioned rats.

Profiles of Sham-Operated and VMH-Lesioned Rats at 14 Weeks Postoperatively

Table 2 shows the general and metabolic characteristics of sham-operated and VMH-lesioned rats 14 weeks after the operations given either normal chow or a fructose-enriched diet at 14 weeks after surgery. In sham-operated animals, the body weight and fat pad weight were increased by 1.2-fold at 14 weeks after surgery when compared with the values at 2 weeks. Weight gain reached a plateau in VMH-lesioned rats, with the final body weight and the parametrial fat pad weight being 1.3-fold and 2.8-fold greater than those in sham-operated rats, respectively. Hyperphagia was not observed in VMH-lesioned rats of the static phase, and food intake was comparable between rats on the normal and fructose-enriched diets. Long-term feeding of a high-fructose diet caused a slight decrease of weight in VMH-lesioned rats. Plasma glucose levels were slightly increased in VMH-lesioned rats compared with sham-operated controls. Plasma NEFA levels were higher in VMH-lesioned rats than in control rats. Long-term fructose feeding elevated the NEFA level in control rats, but this was not observed in VMH-lesioned rats. VMH-lesioned rats fed a normal diet had 2.5-fold higher plasma triglyceride levels than those in sham-operated control rats. Long-term feeding with the high-fructose diet continued to elevate the plasma triglyceride level in both sham-operated and VMH-lesioned rats. Plasma insulin levels were higher in VMH-lesioned rats than in sham-operated control rats, while fructose feeding did not cause further hyperinsulinemia in VMH-lesioned or control rats. Plasma leptin concentrations were markedly elevated in VMH-lesioned obese rats. Fructose feeding did not affect the plasma leptin level in control rats, but this diet tended to decrease leptin levels in the obese rats.

Insulin Resistance Evaluated by the SSPG Method

SSPG values were determined as an indicator of insulin resistance (Fig 1). The SSPG value was not increased in VMH-lesioned rats on a normal diet 2 weeks after lesioning, but was instead decreased to below the level in sham-operated controls (9.6 *v* 12.1 mmol/L). Fructose feeding for 2 weeks slightly elevated the SSPG value in sham-operated rats (15.4 *v* 12.0, $P < .001$) compared with the normal diet. Fructose feeding for 2 weeks also significantly elevated the SSPG value in VMH-lesioned rats (14.2 *v* 9.6, $P < .001$) compared with the normal diet. SSPG values were markedly increased in VMH-lesioned rats at 14 weeks after lesioning. Fructose feeding for 14 weeks caused a significant increase of SSPG values in sham-operated rats (17.0 *v* 12.8, $P < .05$), but did not further exacerbate insulin resistance in VMH-lesioned rats (17.0 *v* 19.1).

Insulin, Glucose, and NEFA Responses in the IVGTT

Plasma glucose and insulin levels before and after glucose administration during the IVGTT are illustrated in Fig 2. At 2 weeks (the dynamic phase of obesity), the plasma glucose level peaked 5 minutes after glucose injection and then declined in both sham-operated and VMH-lesioned rats fed a normal diet. The response to glucose injection was similar in control rats fed either diet or VMH-lesioned obese rats fed a normal diet in the

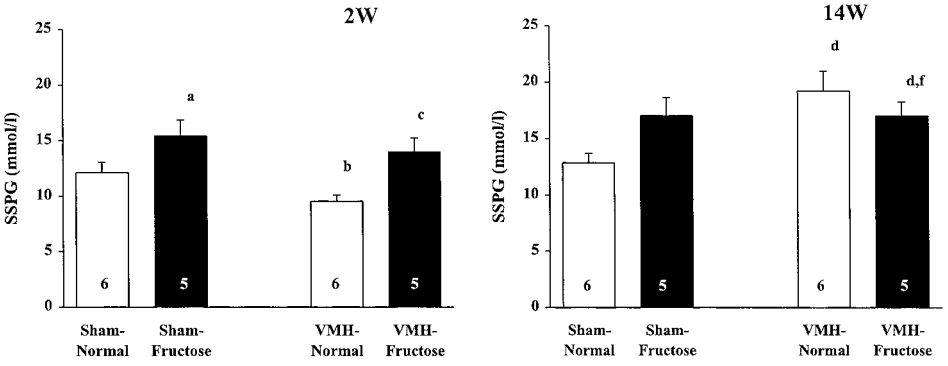

Fig 1. SSPG values, an indicator of insulin resistance, in sham-operated control and VMH-lesioned rats in the dynamic phase (2 weeks) and the static phase (14 weeks) of obesity fed either normal chow or fructose-rich diets for 2 or 14 weeks.

dynamic phase of obesity, while a substantial delayed decline after the peak was observed in VMH-lesioned rats fed fructose. Plasma insulin levels also peaked 5 minutes after glucose injection and then declined in lean rats on a normal diet. A marked increase of the insulin response was also observed in fructose-fed lean rats and in obese rats on both diets. In particular, fructose-fed VMH-lesioned rats showed marked hyperinsulinemia with a delayed peak. The glucose response was greater in VMH-lesioned rats than in sham-operated control rats at 14 weeks after the lesioning (the static phase of obesity). Fructose feeding for 14 weeks did not significantly affect the glucose response in control or VMH-lesioned obese rats, while a marked increase of insulin response was observed in fructose-fed control rats and obese rats on both diets.

Glucose AUC and insulin AUC in IVGTT are demonstrated in Figs 3 and 4, respectively. Fructose-fed VMH-lesioned rats in the dynamic phase of obesity and VMH-lesioned obese rats fed either diet in the static phase of obesity had higher glucose AUC values than the corresponding control rats fed either diet or VMH-lesioned rats in the dynamic phase on a normal diet (Fig 3). The insulin AUC values were greater in VMH-lesioned rats fed a normal diet in both phases than in corresponding controls. Insulin AUC values were increased by fructose feeding of control rats irrespective of the duration of feeding. Fructose feeding further increased the insulin AUC in VMH-lesioned rats in the dynamic phase of obesity, while this was not obvious in the VMH-lesioned rats in the static phase of obesity (Fig 4).

Plasma NEFA levels before and after intravenous glucose administration are shown in Fig 5. Before glucose injection, plasma NEFA levels were significantly higher in fructose-fed VMH-lesioned rats in both the dynamic and static phases of obesity when compared with the corresponding VMH-lesioned or sham-operated rats on a normal diet. Plasma NEFA levels declined 5 minutes after glucose injection and then gradually increased again from 10 minutes in VMH-lesioned rats fed a

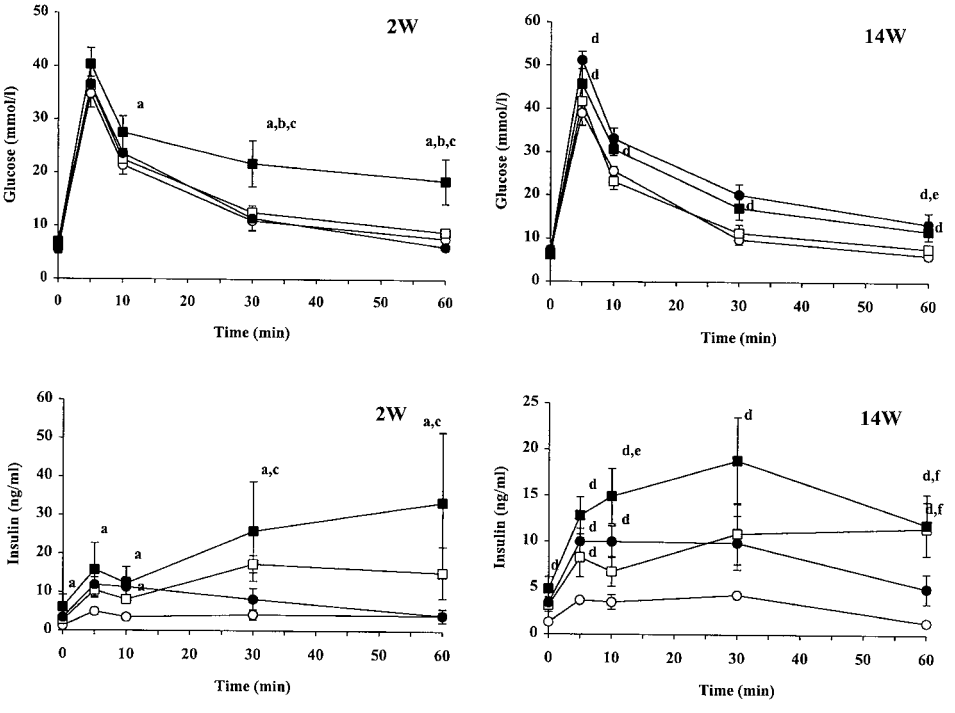

Fig 2. Plasma glucose and insulin levels before and after the IVGTT in sham-operated control and VMH-lesioned rats in the dynamic phase (2 weeks) and the static phase (14 weeks) of obesity fed either normal chow or fructose-rich diets for 2 or 14 weeks.

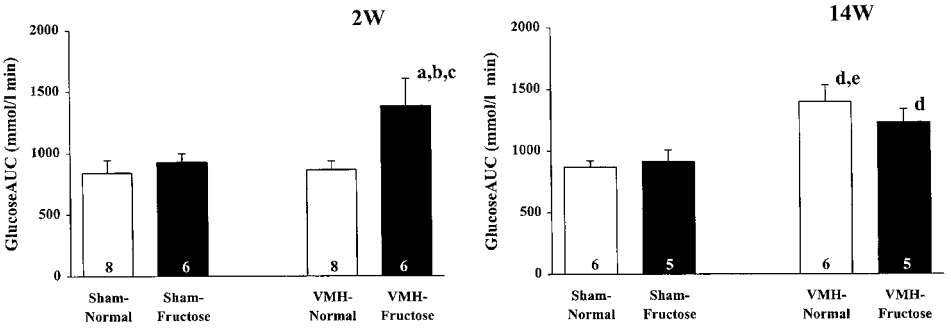

Fig 3. Glucose AUCs in IVGTT based on the data shown in Fig 2.

high-fructose diet, whereas NEFA levels decreased until 60 minutes in control rats on either diet. Before and 30 or 60 minutes after the glucose injection, plasma NEFA levels were higher in VMH-lesioned rats in the static phase of obesity than in their sham-operated counterparts; levels were almost normally suppressed in the early phase of the IVGTT (up to 10 minutes), but suppression became weaker in the late phase (after 30 minutes).

Figure 6 depicts NEFA AUC data obtained in the IVGTT. The values were greater in VMH-lesioned rats fed a high-fructose diet in both phases of obesity when compared with their normally fed counterparts. VMH-lesioned rats in the static phase of obesity and control rats fed fructose for 14 weeks had higher NEFA AUC values than control rats fed a normal diet.

Correlation of Adiposity With Glucose Intolerance

Table 3 shows the univariate correlation coefficients for glucose AUC and various parameters possibly related to glucose tolerance. (Note: parametrial fat pad weight was only measured in a few of the rats undergoing the IVGTT, so fat mass data are not available.) In all animals, the glucose AUC was significantly related to the levels of glucose, leptin, insulin, the insulin AUC, the NEFA AUC, the triglyceride level, and body weight. Basal NEFA levels were not correlated with the glucose AUC. Similarly, the glucose AUC was significantly related to glucose, leptin, NEFA AUC, and triglyceride, but not to body weight or basal NEFA in VMH-lesioned obese rats. In sham-operated control rats, the glucose AUC was only related to glucose and leptin levels. To confirm the independent relationship between adiposity and glucose tolerance, variables were assessed by stepwise regression analysis using glucose AUC as a dependent variable (Table 4). Stepwise mutivariate analysis revealed that leptin, glucose, and triglyceride levels were independently related to the glucose AUC. Of course, the basal glucose level has a strong influence on the glucose AUC because it is a component of AUC calculation. Therefore, we excluded glucose from the analysis. Leptin and triglyceride levels still showed an independent relationship with the glucose AUC. Finally, we examined the association of plasma leptin levels with adiposity. As shown in Table 5, plasma leptin was very closely associated with body weight and parametrical fat pad weight. Leptin was also significantly related to basal NEFA, NEFA AUC, and triglyceride, but not to glucose, insulin, or insulin AUC.

DISCUSSION

VMH-lesioned obese rats fed a normal diet arleady had a marked increased adiposity during the dynamic phase of obesity as indicated by substantial weight gain and enlarged parametrial fat pads, but insulin resistance was not observed in this phase.[19] Therefore, hyperinsulinemia at this time did not result from insulin resistance, but was largely due to derangement of the autonomic nervous system by VMH lesions.[12] The IVGTT revealed that VMH-lesioned obese rats still had a normal glucose response during this phase, although they were grossly obese, suggesting that increased adiposity alone does not impair glucose metabolism if insulin resistance is not present. Fructose feeding caused significant insulin resistance in lean (sham-operated control) rats, but these rats still showed a normal plasma glucose response. Zavaroni et al[18] have reported similar observations in lean rats fed a high-fructose diet and subjected to an oral glucose tolerance test. Fructose feeding

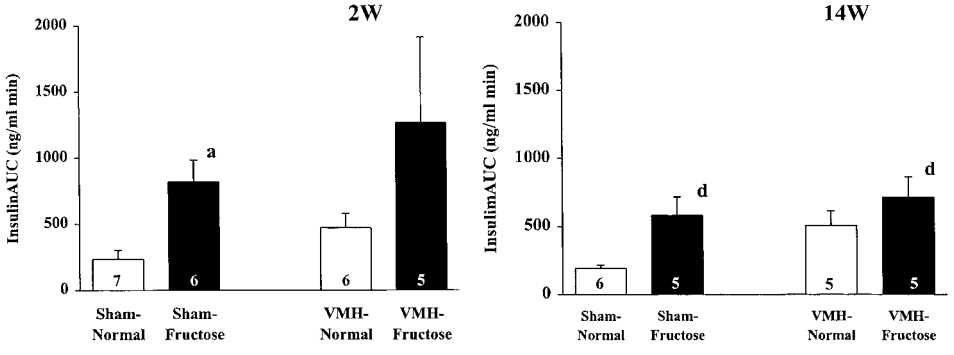

Fig 4. Insulin AUCs in IVGTT based on the data shown in Fig 2.

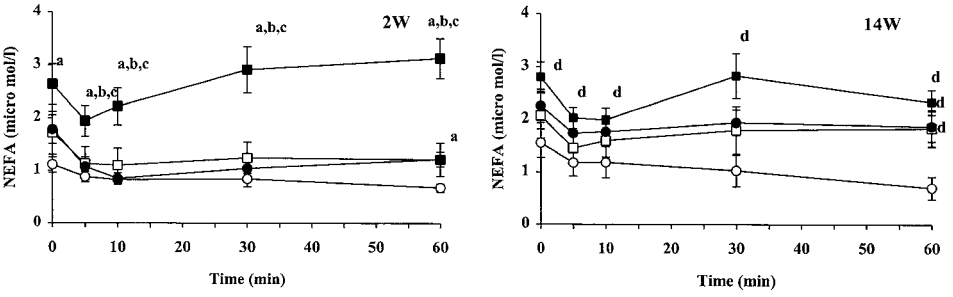

Fig 5. Plasma NEFA levels before and after the IVGTT in sham-operated control and VMH-lesioned rats in the dynamic phase (2 weeks) and the static phase (14 weeks) of obesity fed either normal chow or fructose-rich diets for 2 or 14 weeks.

for 2 weeks did not cause excessive weight gain or an increase of parametrial fat pad mass in either control or VMH-lesioned rats. Therefore, fructose-fed lean rats can be considered as an animal model of insulin resistance without obesity. On the other hand, VMH-lesioned rats fed a fructose-rich diet in the dynamic phase of obesity developed glucose intolerance. Interestingly, SSPG levels were comparable between fructose-fed lean rats and fructose-fed VMH-lesioned obese rats. Therefore, it is unlikely that glucose intolerance in the fructose-fed obese animals was attributable to the progression of insulin resistance compared with that in fructose-fed lean rats. We also found that insulin resistance developed in VMH-lesioned rats by 14 weeks after lesioning when body weight had reached a plateau and obesity was in a static state (similar to congenitally obese animals), and VMH-lesioned rats demonstrated impaired glucose tolerance at this time. VMH-lesioned obese rats in the static phase of obesity showed both increased adiposity and insulin resistance. We therefore hypothesized that both insulin resistance and increased adiposity are required to impair glucose tolerance, with either component alone being insufficient. In the present study, long-term fructose feeding (14 weeks) did not cause excess obesity in lean rats, although fructose-induced insulin resistance was noted. This is consistent with our hypothesis that glucose intolerance does not develop in lean rats even though long-term insulin resistance is present. Long-term fructose feeding did not have an additive effect on either adiposity or insulin resistance in VMH-lesioned obese rats. The similar degree of adiposity and insulin resistance seen in VMH-lesioned rats fed either diet in the static phase of obesity would explain why the degree of glucose intolerance was comparable between these animals.

Data from clinical and experimental studies strongly suggest that the plasma leptin level reflects whole body adiposity.[20,22,23] In fact, we observed that plasma leptin was closely related to the parametrial fat pad mass and body weight in this study. A novel finding of this study was the demonstration of a substantial correlation between plasma leptin levels and glucose AUC values in the IVGTT, and this correlation was independent of plasma insulin levels. In a previous study, we found that plasma leptin is not directly associated with insulin resistance in lean and VMH-lesioned obese rats.[19] Taken together, these data suggest that adiposity plays an important role in the development of glucose intolerance independent of insulin resistance. Whether this finding is specific for fructose-induced insulin resistance or can apply to insulin resistance in general needs to be confirmed by further studies.

It has been well recognized that an increase of NEFA impairs insulin-mediated glucose utilization in the peripheral tissues.[8,27] The importance of the glucose-fatty acid cycle, as initially proposed by Randle et al,[28] has been under intensive investigation in recent years.[8,29,30] The plasma NEFA level reflects the total mass of adipose tissue where fatty acids are stored as lipid droplets.[4] In the present study, fructose feeding for 2 weeks did not significantly increase basal NEFA and NEFA AUC in lean rats, suggesting that fructose-induced insulin resistance was unable to stimulate lipolysis in adipose tissues. On the other hand, fructose feeding substantially increased basal NEFA and NEFA AUC in VMH-lesioned rat in both the dynamic and static phases of obesity, suggesting that fructose-induced insulin resistance could stimulate lipolysis by adipocytes when the adipose tissue mass was remarkably enlarged. Because basal glucose levels were identical in all groups of rats, the glucose AUC was determined by the plasma clearance of exogenous glucose. Insulin-mediated glucose dis-

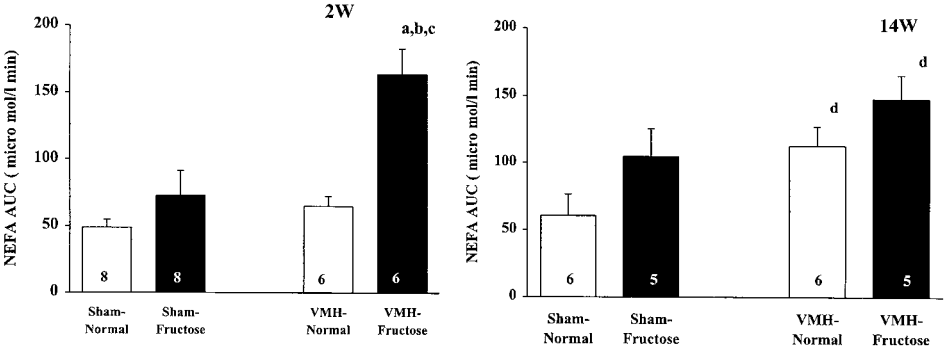

Fig 6. NEFA AUCs in IVGTT based on the data shown in Fig 5.

Table 3. Correlation Coefficients of Glucose AUC in IVGTT Various Parameters

	Total	VMH	Sham
No.	52	27	25
Glucose	0.424*	0.43*	0.673*
Insulin	0.380*	0.272	0.190
Insulin AUC	0.379*	0.348	0.229
Leptin	0.553*	0.413*	0.471*
Basal NEFA	0.137	0.210	0.283
NEFA AUC	0.343*	0.402*	0.247
Triglyceride	0.478*	0.540*	0.169
Body weight	0.372*	0.348	0.229

*$P < .05$.

Table 5. Correlation Coefficients of Plasma Leptin Levels With Various Parameters

	r Value	*P* Value
Body weight	.805	<.0001
Fat pads	.832	<.0001
Basal NEFA	.338	.014
NEFA AUC	.364	.008
Triglyceride	.287	.041
Glucose	.091	NS
Insulin	.253	NS
Insulin-AUC	.113	NS

NOTE. Total rats that underwent IVGTT (n = 52).
Abbreviation = NS, not significant ($P > .05$).

posal evaluated by the SSPG method or the glucose clamp test is not always related to the glucose response in a glucose tolerance test,[17,18,30,31] suggesting that plasma glucose clearance is also regulated by an insulin-independent pathway. The good correlation between glucose AUC and NEFA AUC suggests that the NEFA level during the IVGTT may be an important regulator of glucose disposal and that the weak suppression of NEFA during the test may impair glucose disposal. Further studies are required to elucidate whether NEFA can impair glucose clearance through an insulin-independent pathway.

Fructose feeding increases the plasma triglyceride level in rats,[12] so the increased plasma NEFA level in fructose-fed animals may be more attributable to increased hydrolysis of plasma triglyceride than to increased fatty acid mobilization from adipose tissue. Some clinical studies have demonstrated that severe hypertriglyceridemia impairs insulin sensitivity.[32,33] We found that the plasma triglyceride level was strongly related to glucose AUC independently of the insulin or leptin levels. This might suggest a possibility that triglyceride per se could play a role in glucose intolerance that is independent of adiposity or insulin resistance. However, because lean rats a fed high-fructose diet did not demonstrate glucose intolerance, although they became hypertriglyceridemic, we doubt that triglyceride can directly impair glucose tolerance. However, the plasma triglyceride level is probably a more stable and reliable marker of both insulin resistance and adiposity than is the NEFA level.

Table 4. Stepwise Multipule Regression Analysis for Glucose AUC

Independent Variable	*F* to Remove	Independent Variable (without glucose)	*F* to Remove
Leptin	14.2	Leptin	13.1
Glucose	12.3	Triglyceride	10.7
Triglyceride	11.7		
	$R^2 = .59$		$R^2 = .464$

NOTE. Total rats that underwent IVGTT (n = 52). Leptin, glucose, insulin, insulin AUC, NEFA AUC, triglyceride, and body weight were entered as independent variables. *F* values greater than 4.0 were accepted as significant.

In this study, we assessed insulin resistance by the SSPG method, which mainly reflects insulin-mediated glucose uptake by skeletal muscle. However, insulin resistance can also exist in adipose tissue and the liver, causing an increase of NEFA release and hepatic glucose output. Thus, it might be unreasonable to assume that insulin resistance in VMH-lesioned obese rats and fructose-fed rats is the same, unless all insulin resistance is shown to be comparable between these animals. Nevertheless, the present study suggests that increased adiposity plays an important role in glucose intolerance under insulin-resistant state, and that reduced suppression of NEFA during the IVGTT is involved in this mechanism.

REFERENCES

1. Haffner SM: Risk factors for non-insulin-dependent diabetes mellitus. J Hypertens Suppl 13:S73-76, 1995
2. Stewart MW, Humphriss DB, Berrish TS, et al: Features of syndrome X in first-degree relatives of NIDDM patients. Diabetes Care 18:1020-1022, 1995
3. Scheen AJ: From obesity to diabetes: Why, when and who? Acta Clin Belg 55:9-15, 2000
4. Kahn BB, Flier JS: Obesity and insulin resistance. J Clin Invest 106:473-481, 2000
5. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46:3-10, 1997
6. Holm C, Langin D, Manganiello VC, et al: Regulation of hormone-sensitive lipase activity in adipose tissue Methods Enzymol 286:45-67, 1997
7. Lafontan M, Sengenes C, Galitzky J, et al: Recent developments on lipolysis regulation in humans and discovery of a new lipolytic pathway. Int J Obes Relat Metab Disord 24:S47-52, 2000 (suppl 4)
8. Roden M, Price TB, Perseghin G, et al: Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest 97:2859-2865, 1996
9. Boden G, Jadali F, White J, et al: Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 88:960-966, 1991
10. Boden G, Chen X, Ruiz J, et al: Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest 93:2438-2446, 1994
11. Bruce R, Godsland I, Walton C, et al: Associations between insulin sensitivity, and free fatty acid and triglyceride metabolism independent of uncomplicated obesity. Metabolism 43:1275-1281, 1994
12. Bray GA, York DA: Hypothalamic and genetic obesity in experimental animals: An autonomic and endocrine hypothesis. Physiol Rev 59:719-809, 1979
13. Penicaud L, Larue-Achagiotis C, Le Magnen J: Endocrine basis for weight gain after fasting or VMH lesion in rats. Am J Physiol 245:E246-252, 1983

14. Frohman LA, Goldman JK, Bernardis LL: Studies of insulin sensitivity in vivo in weanling rats with hypothalamic obesity. Metabolism ;21:1133-1142, 1972

15. Suga A, Hirano T, Inoue S, et al: Plasma leptin levels and triglyceride secretion rates in VMH-lesioned obese rats: A role of adiposity. Am J Physiol 276:E650-657, 1999

16. Kazumi T, Hirano T, Odaka H, et al: VLDL triglyceride kinetics in Wistar fatty rats, an animal model of NIDDM: Effects of dietary fructose alone or in combination with pioglitazone. Diabetes 45:806-811, 1996

17. Wiggins D, Hems R, Gibbons GF: Decreased sensitivity to the inhibitory effect of insulin on the secretion of very-low-density lipoprotein in cultured hepatocytes from fructose-fed rats. Metabolism 44:841-847, 1995

18. Zavaroni I, Sander S, Scott S, et al: Effect of fructose feeding on insulin secretion and insulin action in the rat. Metabolism 29:970-973, 1980

19. Suga A, Hirano T, Kageyama H, et al: Effects of fructose and glucose on plasma leptin, insulin, and insulin resistance in lean and VMH-lesioned obese rats. Am J Physiol Endocrinol Metab 278:E677-683, 2000

20. Caro JF, Sinha MK, Kolaczynski JW, et al: Leptin: The tale of an obesity gene. Diabetes 45:1455-1462, 1996

21. Zhang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432, 1994

22. Dua A, Hennes MI, Hoffmann RG, et al:Leptin: A significant indicator of total body fat but not of visceral fat and insulin insensitivity in African-American women. Diabetes 45:1635-1637, 1996

23. Frederich RC, Lollmann B, Hamann A, et al: Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. J Clin Invest 96:1658-1663, 1995

24. Suga A, Hirano T, Kageyama H, et al: Rapid increase in circulating leptin in ventromedial hypothalamus-lesioned rats: Role of hyperinsulinemia and implication for upregulation mechanism. Diabetes 48:2034-2038, 1999

25. Wright DW, Hansen RI, Mondon CE, et al: Sucrose-induced insulin resistance in the rat: Modulation by exercise and diet. Am J Clin Nutr 38:879-883, 1983

26. Harano Y, Ohgaku S, Hidaka H, et al: Glucose, insulin and somatostatin infusion for the determination of insulin sensitivity. J Clin Endocrinol Metab 45:1124-1127, 1977

27. Paolisso G, Tataranni PA, Foley JE, et al: A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. Diabetologia 38:1213-1217, 1995

28. Randle PJ, Garland PB, Hales CN, et al: The glucose fatty-acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

29. Ferrannini E, Barrett EJ, Bevilacqua S, et al: Effect of fatty acids on glucose production and utilization in man. J Clin Invest 72:1737-1747, 1983

30. Kelley DE, Mokan M, Simoneau JA, et al: Interaction between glucose and free fatty acid metabolism in human skeletal muscle. J Clin Invest 92:91-98, 1993

31. Saad MF, Anderson RL, Laws A, et al: A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. Insulin Resistance Atherosclerosis Study. Diabetes 43:1114-1121, 1994

32. Steiner G: Altering triglyceride concentrations changes insulin-glucose relationships in hypertriglyceridemic patients. Double-blind study with gemfibrozil with implications for atherosclerosis. Diabetes Care 14:1077-1081, 1991

33. Steiner G, Morita S, Vranic M: Resistance to insulin but not to glucagon in lean human hypertriglyceridemics. Diabetes 29:899-905, 1980