Increased Hepatic Glucose Production and Decreased Hepatic Glucose Uptake at the Prediabetic Phase in the Otsuka Long-Evans Tokushima Fatty Rat Model

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To investigate the time course of the hepatic glucose metabolism in non–insulin-dependent diabetes (NIDDM), we measured hepatic glucose production (HGP) and first-pass uptake of portal glucose infusion by the liver (HGU) using dual-tracer methods in a NIDDM model, Otsuka Long-Evans Tokushima Fatty (OLETF) rats, and in normal controls, Long-Evans Tokushima Otsuka (LETO) rats, at 8, 14, and 28 weeks of age (n = 5, respectively). The fasting plasma glucose level in OLETF rats was significantly higher than in LETO rats at 28 weeks of age (8.9 ± 1.7 *v* 6.3 ± 0.4 mmol/L, $P < .01$), while there was no significant difference at 8 and 14 weeks. Hyperinsulinemia in OLETF rats appeared at ≥8 weeks of age. Basal HGP was significantly higher in OLETF than in LETO rats at 8 and 28 weeks (8 weeks, 12.7 ± 1.7 *v* 9.4 ± 1.8 mg · kg^{-1} · min^{-1}, $P < .05$; 28 weeks, 10.9 ± 1.6 *v* 7.1 ± 1.3 mg · kg^{-1} · min^{-1}, $P < .01$). At 14 weeks, basal HGP was not significantly different between OLETF and LETO rats. However, at all study points, HGU during a portal glucose infusion was significantly lower in OLETF than in LETO rats (8 weeks, 0.9 ± 0.2 *v* 2.3 ± 0.5, $P < .01$; 14 weeks, 0.8 ± 0.3 *v* 1.4 ± 0.3, $P < .05$; 28 weeks, 0.7 ± 0.2 *v* 1.4 ± 0.3 mg · kg^{-1} · min^{-1}, $P < .01$). Fasting plasma free fatty acid (FFA) levels were not significantly different between OLETF and LETO, except at 8 weeks. Suppression of plasma FFA levels by endogenous insulin during a portal glucose infusion was impaired in OLETF rats compared with LETO rats. In summary, this study demonstrates that derangement of hepatic glucose handling, such as increased basal HGP and decreased HGU, is observed in obese NIDDM model OLETF rats at the prediabetic phase when hyperglycemia is still not apparent. Furthermore, these derangements may be accompanied by impaired lipid metabolism.

NON–INSULIN-DEPENDENT DIABETES MELLITUS (NIDDM) is usually characterized by two abnormalities—anomalous pancreatic insulin secretion and insulin resistance in peripheral tissues, ie, reduced glucose uptake, particularly in the muscles, and acceleration of hepatic glucose production.[1,2] According to recent epidemiological studies, the order of onset of these derangements during the progress of NIDDM remains controversial,[3-5] but the overt diabetic condition is only apparent when pancreatic and peripheral defects coexist.

The liver plays a central role in the maintenance of normal glucose tolerance. After glucose ingestion, the liver switches from glucose production to glucose uptake.[6] This phenomenon is the result of two processes: suppression of hepatic glucose production (HGP) and enhancement of hepatic glucose uptake (HGU).[7] In NIDDM subjects with overt fasting hyperglycemia, the ability of insulin to suppress HGP is impaired and accelerated HGP shows good correlation with the degree of fasting hyperglycemia.[8] A recent study indicated that basal HGP in NIDDM is not higher than in normal subjects until the fasting plasma glucose concentration increases.[9] On the other hand, there is impaired suppression of HGP after an oral glucose load in impaired glucose tolerance, a precursor condition of NIDDM.[10] Studies using a dual-tracer technique[11-13] suggest that a defect in the suppression of HGP, not impaired HGU, is also responsible for postprandial glucose intolerance in NIDDM subjects. However, if the insulin resistance in peripheral tissue is severe, the impaired HGU may also worsen the metabolic derangement of NIDDM.

There are few reports on the time course of hepatic glucose handling such as glucose uptake and production before and after manifestation of diabetes. In the present study, we applied the dual-tracer technique using stable isotope (SI) to measure both HGP and HGU in NIDDM model rats and monitored the time course of these derangements.

MATERIALS AND METHODS

Animals

All procedures were approved by the Institutional Animal Experiments Committee of the Osaka University School of Medicine. Male Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Long-Evans Tokushima Otsuka (LETO) rats aged 4 weeks were kindly provided by Tokushima Research Institute, Otsuka Pharmaceutical (Tokushima, Japan). They were maintained on standard rat chow (Oriental Yeast, Tokyo, Japan) and housed in an environmentally controlled room with a 12-hour light/dark cycle.

Measurement of Metabolic and Hormonal Profiles Before and After Manifestation of Hyperglycemia

Twenty-nine OLETF and 31 LETO rats were randomly assigned to four groups for measurement of metabolic and hormonal profiles before and after manifestation of hyperglycemia (fasting blood sampling group). Three days before blood sampling, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg · kg^{-1}), and a polyethylene catheter (PE50; Nippon Becton Dickinson, Tokyo, Japan) was inserted into the left carotid artery. The catheter was filled with heparin solution, sealed, and tunneled subcutaneously around the side of the neck to the back of the head. The catheter was externalized through a skin incision through the back of the neck. To obtain the metabolic profile, blood samples for glucose, triglyceride, free fatty acids (FFA), insulin, and glucagon measurements were determined from conscious rats after a 24-hour fsat at 5, 8, 14, and 28 weeks of age.

Measurement of In Vivo HGP and First-Pass HGU

In vivo HGP and first-pass HGU by the liver were measured in 15 OLETF and 15 LETO rats aged 8, 14, and 28 weeks using a dual-tracer

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technique (hepatic glucose metabolism group). Ten days before the study, each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg · kg^{-1}), and a silicon catheter (Phicon tube; Fuji-Systems, Tokyo, Japan) was inserted into the portal vein from the inferior mesenteric vein. Three days before the study, another silicon catheter was inserted into the right jugular vein and a polyethylene catheter was inserted into the left carotid artery. Rats were used in the experiments only if they had a good appetite, normal stool, and no decrease in body weight after surgery.

Studies were performed with awake, unstressed, and chronically catheterized rats. All rats were fasted for 24 hours before the study. [6,6-$^2H_2$]glucose was infused into the jugular vein at primed (−120 to −110 minutes, 3.0 mg · kg^{-1} · min^{-1}) and constant rates (−110 to 0 minutes, 0.3 mg · kg^{-1} · min^{-1}) during basal condition. At time 0, infusion of glucose solution (90 mg/mL) containing 5% enriched [U-^{13}C]glucose was begun at a rate of 9.0 mg · kg^{-1} · min^{-1} into the portal vein. To clamp the plasma glucose concentration at 3 mmol/L above the basal level (−30 to 0 minutes), infusion of 20% glucose solution was started into the jugular vein. The infusion rate was periodically changed as described by Kraegen et al.[14] The infusion rate of [6,6-$^2H_2$]glucose increased to 1.0 mg · kg^{-1} · min^{-1} from 0 minutes to 120 minutes during portal glucose infusion to prevent decrease of plasma isotopic enrichment. SI-labeled glucose was purchased from Cambridge Isotope Laboratories (Woburn, MA).

Plasma samples for determination of enrichment of [6,6-$^2H_2$]glucose and [U-^{13}C]glucose were obtained at −120, −30, −15, 0, 60, 75, 90, 105, and 120 minutes during the study, and those for determination of plasma insulin were obtained at −120 and 120 minutes. Plasma samples for determination of FFA and triglyceride levels were obtained at the end of the study. The total volume of blood withdrawn, except for the last sampling, was less than 2.0 mL.

Analytical Procedure

Blood samples were immediately placed on ice and centrifuged at 4°C, and the separated plasma was frozen at −20°C until assay. Blood samples for measurement of glucose and immunoreactive insulin (IRI) were collected in dry heparinized tubes containing sodium fluoride. The plasma glucose level was determined by the glucose-oxidase method (Glucose Auto Analyzer II; Beckman, Fullerton, CA). Plasma IRI was determined by radioimmunoassay using an antirat insulin antiserum. Blood samples for the measurement of FFA and triglyceride were collected in tubes containing 275 μg/mL of diethyl *p*-nitrophenyl phosphate (Paroxon; Sigma, St Louis, MO) to inhibit the activity of lipoprotein lipase. Plasma FFA levels were determined by enzymatic calorimetric methods. Triglyceride was determined by an enzymatic microfluorometric method. Immunoreactive glucagon concentrations were determined with plasma samples containing 50 μL of aprotinin (Trasylol; Bayer, Leverkusen, Germany) by radioimmunoassay. After deproteinization with 0.8N perchloric acid, the whole-blood lactate and pyruvate concentrations were determined by the lactate oxidase method and the pyruvate oxidase method, respectively.

Measurement of SI-Labeled Glucose Enrichment

Both plasma SI-labeled glucose enrichments were simultaneously determined by the following method. Forty microliters of plasma was deproteinized with 120 μL of 99.5% ethanol. The supernatant was evaporated. The residue was derivatized for analysis by gas chromatography-mass spectrography (GCMS) by the following procedure. A 10-μL portion of MBTFA (*N*-methyl-bis[trifluoroacetamide]; Pierce, Rockford, IL) was added to the residue. Next, 10 μL pyridine was added and the mixture was heated for 1 hour at 60°C with occasional shaking. One microliter of the reaction product containing trifluoroacetylated (TFA) glucose was taken for the analysis by GCMS on TSQ-700 (Finnigan MAT, San Jose, CA) with a capillary column. The TFA glucose separated from the other compounds by gas chromatography was analyzed by 70-eV electron impact mass spectrometry.

In this study, the fragment ion peaks at mass per electrical charge (m/e) 319, 321, and 325 were selected to monitor the kinetics of unlabeled or labeled glucose ([6,6-$^2H_2$] and [U-^{13}C]), respectively. In general, ion peak m/e M has an isotopic ion peak m/e M+1, M+2 . . . , because of the natural abundance of stable isotopes. If N is the number of stable isotopes (N is 2 for [6,6-$^2H_2$]glucose and 6 for [U-^{13}C]glucose), we can define the isotopic peak ratio (IPR) of m/e M + N to m/e M as $IPR_{(M+N/M)}$ = (PI[M + N] + PI[M + N + 1] + PI[M + N + 2] + . . .) × 100/(PI[M] + PI[M + 1] + PI[M + 2] + . . . where PI is ion peak intensity. Linear standard curves were obtained when IPR was plotted against the [6,6-$^2H_2$]glucose or [U-^{13}C]glucose enrichment of the standard solution. Enrichments of the samples were determined by comparison with standard curves of SI-labeled glucose.

Calculations of HGP and HGU

We calculated HGP and HGU using the method of Radziuk et al[15] with modification of the steady-state conditions. The rate of appearance (Ra) in the systemic circulation was calculated using the following equation: Ra = ([Ei/E] - 1) × I, where I represents the infusion rate of the tracer, Ei represents the isotopic enrichment of the infused tracer, and E represents the isotopic enrichment of the plasma glucose. The rate of appearance of total glucose (Ra_{TG}) was obtained from the calculation of [6,6-$^2H_2$]glucose enrichment in the plasma glucose: $Ra_{TG} = [(Ei_{[6,6\text{-}^2H_2]}/E_{[6,6\text{-}^2H_2]}) - 1] \times I_{[6,6\text{-}^2H_2]}$, where $I_{[6,6\text{-}^2H_2]}$, $Ei_{[6,6\text{-}^2H_2]}$, and $E_{[6,6\text{-}^2H_2]}$ represent I, Ei, and E of [6,6-$^2H_2$]glucose, respectively. Ra_{TG} includes endogenously produced glucose (HGP) and exogenously infused glucose via the jugular vein (GIR) and the appearance rate of portally infused glucose to the systemic circulation (Ra_{PG}): Ra_{TG} = HGP + GIR + Ra_{PG}. Ra_{PG} was calculated from the plasma [U-^{13}C]glucose enrichment and Ra_{TG}: $Ra_{PG} = [(Ei_{[U\text{-}^{13}C]}/E_{[U\text{-}^{13}C]}) - 1] \times Ra_{TG}$, where $Ei_{[U\text{-}^{13}C]}$ and $E_{[U\text{-}^{13}C]}$ represent Ei and E of [U-^{13}C]glucose, respectively. During the basal period, Ra_{TG} identifies with HGP. HGU during a portal glucose infusion is obtained from the following equation: HGU = P_{inf} − Ra_{PG}, where P_{inf} is the rate of portally infused glucose (50 μmol · kg^{-1} · min^{-1}). The rate of disappearance of glucose (Rd) giving the peripheral glucose uptake is equal to Ra_{TG} in the steady-state. The results were represented as the averages of metabolic parameters from −30 to 0 minutes in the basal state and from 90 to 120 minutes during portal glucose infusion.

Statistical Analysis

Data are expressed as means ± SD. Statistical difference between mean values of both groups for the same age was assessed with the Mann-Whitney *U* test. Comparison of repeated measurement with each rats was assessed using ANOVA. Statistical significance was accepted at $P < .05$.

RESULTS

Body weight and plasma metabolic parameters in fasting blood sampling of OLETF and LETO rats are shown in Table 1. OLETF rats began to gain weight faster than LETO rats from 5 weeks, and the difference between two rats gradually increased with aging. Basal plasma glucose in OLETF rats was significantly higher than in LETO rats at 28 weeks of age. However, at 5 weeks of age, the basal plasma glucose of LETO rats was significantly higher. At 8 and 14 weeks of age, there was no significant difference between the two rat groups. Plasma insulin levels in OLETF were significantly higher than in LETO rats under fasting condition at 8, 14, and 28 weeks of age, and during basal state and portal glucose infusion at 14 and 28 weeks of age. There was no significant difference in basal

Table 1. Body Weight and Fasting Plasma Parameters in OLETF and LETO Rats

Parameter	5 Weeks		8 Weeks		14 Weeks		28 Weeks	
	OLETF	LETO	OLETF	LETO	OLETF	LETO	OLETF	LETO
n	9	9	7	7	6	8	7	7
Body weight (g)	121 ± 11†	97 ± 8	269 ± 28*	221 ± 13	428 ± 47†	343 ± 27	603 ± 39†	459 ± 25
Glucose (mmol/L)	5.5 ± 0.6*	6.2 ± 0.9	6.3 ± 0.5	6.1 ± 0.5	6.6 ± 0.4	6.7 ± 0.4	9.3 ± 1.5†	6.3 ± 0.4
Insulin (pmol/L)	50 ± 40	100 ± 75	344 ± 107*	155 ± 117	1,452 ± 459†	351 ± 141	2,029 ± 841†	317 ± 151
Glucagon (ng/L)	52 ± 24	62 ± 40	101 ± 70*	50 ± 19	129 ± 86	67 ± 16	166 ± 173	75 ± 53
Glucagon/insulin ratio	0.20 ± 0.06*	0.13 ± 0.07	0.05 ± 0.04	0.08 ± 0.06	0.01 ± 0.01†	0.04 ± 0.02	0.01 ± 0.01†	0.05 ± 0.03
Triglyceride (mmol/L)	0.61 ± 0.19†	0.35 ± 0.20	0.77 ± 0.35*	0.47 ± 0.20	1.26 ± 0.68*	0.52 ± 0.08	1.94 ± 0.65†	0.40 ± 0.11
FFA (mmol/L)	0.72 ± 0.30	0.63 ± 0.32	0.95 ± 0.23*	0.71 ± 0.15	0.68 ± 0.19	0.68 ± 0.25	0.58 ± 0.35	0.64 ± 0.10
Lactate (mmol/L)	516 ± 121	535 ± 177	563 ± 63	469 ± 159	620 ± 127	5.2 ± 0.9	1107 ± 715†	514 ± 89
Pyruvate (μmol/L)	60.7 ± 27.4	56.3 ± 16.6	83.4 ± 18.3*	57.9 ± 24.5	112.3 ± 31.8	93.6 ± 22.7	161.1 ± 82.1†	59.7 ± 13.5

NOTE. Results are expressed as means ± SD.
*$P < .05$, †$P < .01$ OLETF *v* LETO in the same age group.

plasma glucagon levels between OLETF and LETO rats except at 8 weeks of age. Fasting plasma lactate and pyruvate levels in OLETF rats were higher than in LETO rats only at 28 weeks.

Plasma metabolic parameters during the basal and portal glucose infusion state while measuring hepatic glucose metabolism are shown in Table 2. There was no significant difference in body weight between the fasting blood sampling group and the hepatic glucose metabolism group.

Plasma glucose levels and [6,6-$^2H_2$]glucose enrichment during basal and clamp state are shown in Fig 1. The steady-state of the tracer was achieved in all groups from −30 to 0 minutes in the basal state and from 90 to 120 minutes during portal glucose infusion.

HGP during the basal state is shown in Fig 2. At 8 weeks of age, OLETF rats had a significantly higher basal HGP than LETO rats (12.7 ± 1.7 *v* 9.4 ± 1.8 mg · kg^{-1} · min^{-1}, $P < .05$). However, at 14 weeks of age, there was no significant difference in basal HGP between OLETF and LETO rats (8.9 ± 0.4 *v* 7.5 ± 1.6 mg · kg^{-1} · min^{-1}). The difference of basal HGP between OLETF and LETO appeared again at 28 weeks of age (10.9 ± 1.6 *v* 7.1 ± 1.3 mg · kg^{-1} · min^{-1}, $P < .01$).

HGU is depicted in Fig 3. OLETF rats had a significantly lower HGU than LETO rats at all study points (8 weeks, 0.9 ± 0.2 *v* 2.3 ± 0.5, $P < .01$; 14 weeks, 0.8 ± 0.3 *v* 1.4 ± 0.3, $P < .05$; 28 weeks, 0.7 ± 0.2 *v* 1.4 ± 0.3 mg · kg^{-1} · min^{-1}, $P < .01$).

During portal glucose infusion, there was no significant difference in Rd between OLETF and LETO rats at all study points (8 weeks, 17.6 ± 1.3 *v* 16.0 ± 2.4; 14 weeks, 15.7 ± 1.0 *v* 14.2 ± 2.0; 28 weeks, 16.5 ± 1.1 *v* 14.3 ± 2.6 mg · kg^{-1} · min^{-1}). However, plasma insulin levels of OLETF rats were significantly higher than LETO rats at 14 and 28 weeks of age. In OLETF rats, therefore, the values of Rd divided by plasma insulin levels (Rd/IRI), which indicate insulin sensitivity of the peripheral tissue, were significantly lower than those of LETO rats (Fig 4).

OLETF rats had significantly higher fasting plasma triglyceride levels than LETO rats at all study points. Plasma FFA levels of OLETF rats during portal glucose infusion were significantly higher than LETO rats at all study points, while there was no significant difference in fasting plasma FFA levels between OLETF and LETO rats (Table 1 and Fig 5).

DISCUSSION

The OLETF rat was established by Kawano et al[16] as an obese NIDDM animal model. According to Shima et al,[17] the cumulative incidence of diabetes in male OLETF rats is 78% at 24 weeks of age. Oral glucose load showed manifestation of basal and postprandial hyperinsulinemia at 24 weeks of age, but not at 8 weeks of age,[16] which is thought to be caused by underlying insulin resistance.[18] In this study, high plasma glucose and IRI levels were observed at 28 weeks of age. At 8 and 14 weeks of age, hyperinsulinemia was observed at fasting state, while fasting plasma glucose levels were within the euglycemic range. However, at 5 weeks, OLETF rats did not show these two abnormalities. At 8 weeks of age, the fasting IRI

Table 2. Body Weight and Plasma Parameters During Basal and Portal Glucose Infusion State in OLETF and LETO Rats

	8 Weeks		14 Weeks		28 Weeks	
	OLETF	LETO	OLETF	LETO	OLETF	LETO
n	5	5	5	5	5	5
Body weight (g)	262 ± 30†	203 ± 14	402 ± 19†	321 ± 33	604 ± 45†	443 ± 25
Basal period						
Glucose (mmol/L)	6.2 ± 1.8	6.0 ± 0.8	5.7 ± 0.5	5.8 ± 0.5	10.1 ± 1.9†	6.5 ± 0.7
Insulin (pmol/L)	303 ± 81	214 ± 74	1212 ± 171†	279 ± 80	2197 ± 426†	420 ± 85
Portal glucose infusion period						
Glucose (mmol/L)	8.9 ± 0.6	8.2 ± 0.8	9.3 ± 2.1	8.4 ± 0.4	16.7 ± 1.4†	10.3 ± 1.3
Insulin (pmol/L)	603 ± 143	696 ± 262	2177 ± 1134†	441 ± 189	2359 ± 398†	1322 ± 183
FFA (mmol/L)	0.45 ± 0.12*	0.26 ± 0.02	0.57 ± 0.08†	0.37 ± 0.04	0.79 ± 0.04†	0.31 ± 0.05

NOTE. Results are expressed as means ± SD.
*$P < .05$, †$P < .01$ OLETF *v* LETO in the same age group.

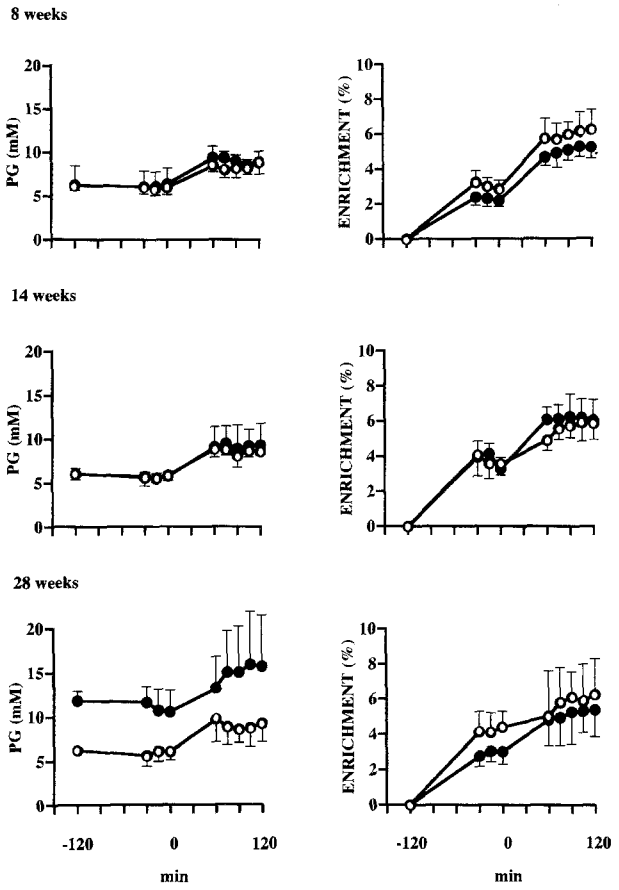

Fig 1. Plasma glucose levels and enrichment of [6,6-$^2H_2$]glucose during experiments with OLETF (●) and LETO (○) at 8, 14, and 28 weeks of age. Values are means ± SD. There were no significant variations in both plasma glucose levels and enrichment of [6,6-$^2H_2$]-glucose during the basal state and portal glucose infusion for all groups.

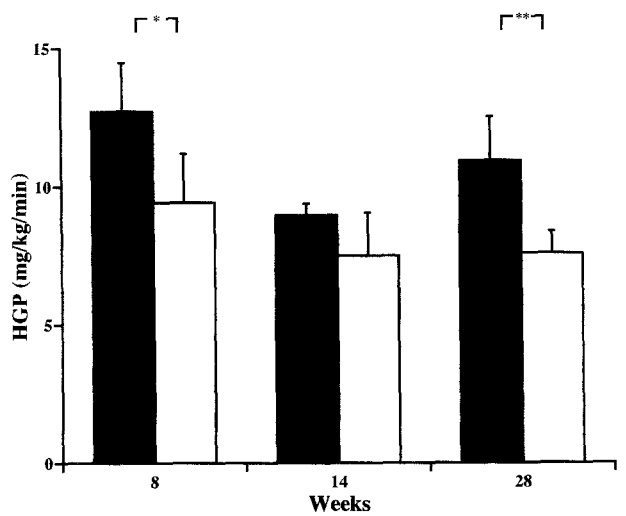

Fig 2. HGP in OLETF (■) and LETO (□) rats at 8, 14, and 28 weeks of age. Values are means ± SD. *$P < .05$, **$P < .01$ OLETF *v* LETO in the same age group.

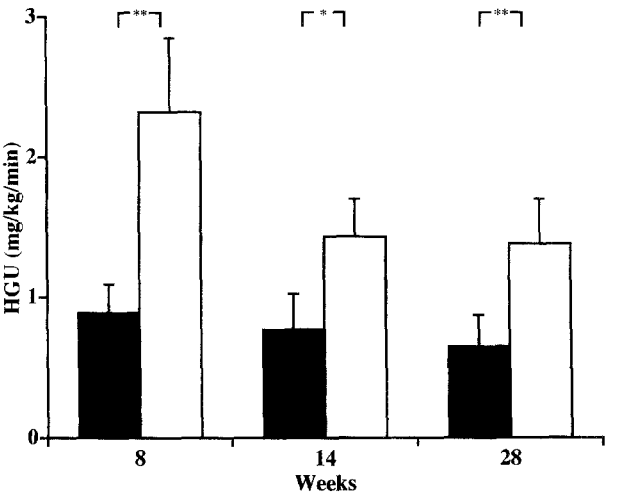

Fig 3. First-pass HGU in OLETF (■) and LETO (○) rats at 8, 14, and 28 weeks of age. Values are means ± SD. *$P < .05$, **$P < .01$ OLETF *v* LETO in the same age group.

in OLETF rats was 1.7 times higher than that in LETO rats. The present study agrees with the time courses of hyperglycemia and hyperinsulinemia, which were observed in a previous study,[17] except for the appearance of hyperinsulinemia at 8 weeks.

In this study, there was no significant difference in Rd between OLETF and LETO rats. However, at 14 and 28 weeks, the insulin levels of OLETF rats during the portal glucose infusion were 4.9 and 1.8 times higher than those of LETO rats, respectively. These findings implied that insulin resistance in the peripheral tissues already appears at 14 weeks. A recent report by Ishida et al[18] demonstrated that insulin-mediated whole-body glucose uptake measured by the hyperinsulinemic euglycemic clamp was significantly reduced in OLETF rats as compared with LETO rats at 16 weeks of age, but not at 10 weeks. In the present study, increased HGP develops at 8 weeks of age in OLETF rats. However, at 14 weeks, the difference of

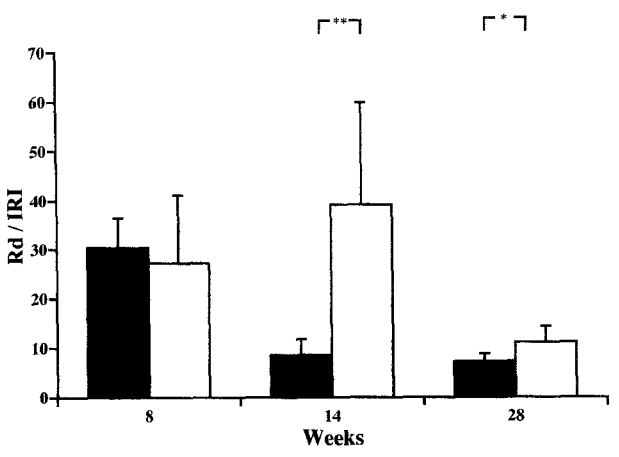

Fig 4. Insulin sensitivity of the peripheral tissue in OLETF (■) and LETO (○) rats at 8, 14, and 28 weeks of age. Values were calculated by dividing Rd values by plasma insulin levels (Rd/IRI). Values are means ± SD. *$P < .05$, **$P < .01$ OLETF *v* LETO in the same age group.

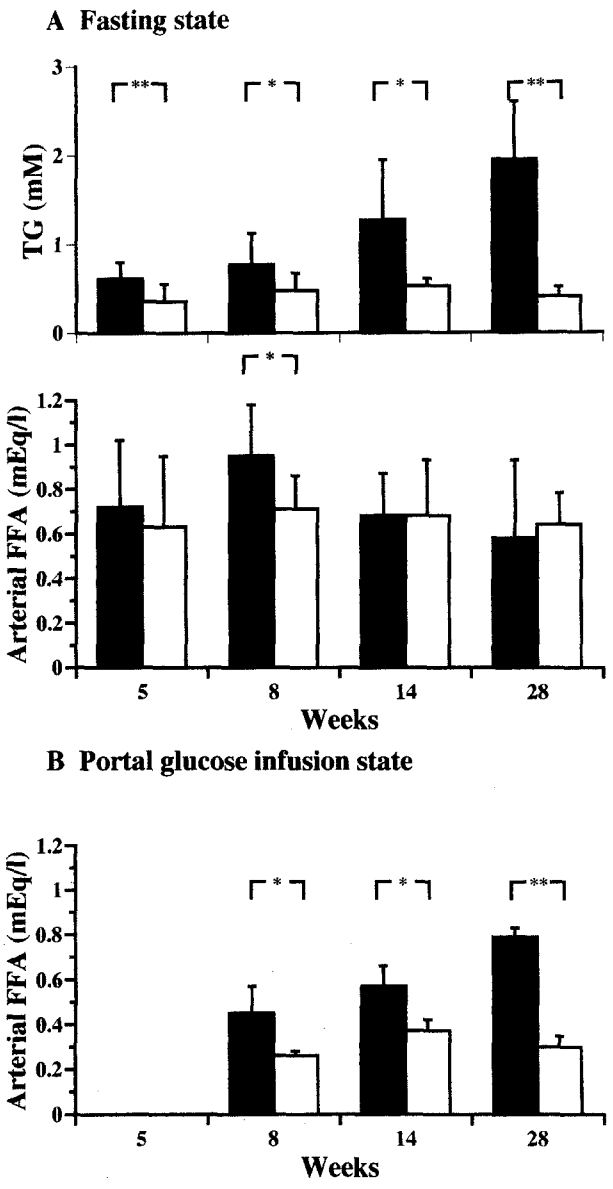

Fig 5. (A) Fasting plasma triglyceride and FFA in OLETF (■) and LETO (○) rats at 5, 8, 14, and 28 weeks of age. (B) Plasma free FFA in OLETF (■) and LETO (○) rats during portal glucose infusion state at 8, 14, and 28 weeks of age. Values are means ± SD. *$P < .05$, **$P < .01$ OLETF *v* LETO in the same age group.

HGP between OLETF and LETO rats disappears and then reappears at 28 weeks of age. This increased HGP is pronounced in overt NIDDM, which leads to their metabolic derangement.[19,20] Treatments with diet or sulfonylurea regimens reduced fasting hyperglycemia, accompanied by a reduction in hepatic glucose output.[21,22] Increased HGP is one of the major determinants of fasting hyperglycemia in NIDDM. These observations suggest that in NIDDM model rats, HPG is increased and might lead to later manifestation of hyperglycemia.

In OLETF rats, increased HGP appears before manifestation of the impaired metabolic clearance of glucose. This difference in the appearance of the two parameters disagreed with studies on other animal models of obese NIDDM such as Zucker fatty rats[23] and primates,[24] in which peripheral insulin resistance precedes the development of hepatic insulin resistance. Whether this discrepancy among animal models of NIDDM depends on the difference in animal models must be clarified further.

At 14 weeks of age, the increased HGP in OLETF rats disappeared temporarily. At this time, their fasting insulin level was 4.7 times higher than that of LETO rats. Olefsky et al[25] demonstrated that, in obese patients with insulin resistance, 100 μU/mL of insulin half-maximally inhibits HGP, but insufficiently stimulates glucose disposal in peripheral tissues. Thus, the liver might be more sensitive to insulin than peripheral tissue. Hyperinsulinemia is thought to suppress the increased HGP at 14 weeks of age in OLETF rats.

Although glucagon and/or the glucagon-to-insulin ratio play important roles in the regulation of HGP,[26] the increase of basal HGP in OLETF rats was not correlated to plasma glucagon levels and the glucagon-to-insulin ratio. Thus, glucagon plays a negligible role in augmented HGP in OLETF rats. Our results demonstrated in fasted OLETF rats that plasma FFA levels at 8 weeks of age and triglyceride level at ≥5 weeks of age were significantly higher than in LETO rats. Several reports[27-29] showed that FFA stimulates gluconeogenesis and thereby increases HGP. In addition, acute hypertriglyceridemia has been reported to diminish insulin-induced suppression of HGP.[30] Thus, the higher plasma FFA and triglyceride levels in OELTF rats might be a major cause of increased HGP.

At 8 weeks of age, when hyperglycemia is not apparent, OLETF rats showed significantly higher body weight than LETO rats. In obese women,[31] total-body fat mass correlates positively with basal HGP. The precise relationship between total-body fat mass and HGP has not been clearly shown yet. However, the increased HGP in OLETF rats might occur by a similar mechanism(s).

This study demonstrated that the first-pass HGU was impaired in OLETF rats compared with LETO rats even at 8 weeks of age. Portal glucose infusion raised plasma insulin 1.7 times higher in OLETF than in LETO rats. Regardless of hyperinsulinemia, OLETF rats showed decreased HGU much earlier than manifestation of hyperglycemia.

Hyperinsulinemia, hyperglycemia, or hypoglucagonemia alone does not stimulate glucose uptake by splanchnic tissues.[32,33] Furthermore, HGU remains minimal even when hyperinsulinemia and hyperglycemia are induced by peripheral glucose infusion.[32,34,35] However, when glucose is ingested or infused into the portal vein, the enhancement of either hepatic glucose removal or glycogen synthesis occurs in rats,[36] dogs[33,34] and humans.[37] Adkins et al[33] observed a good correlation between the arterial-portal glucose gradient and HGU. In this study, we did not measure the arterial-portal gradient due to the difficulty of portal blood sampling. Nonetheless, there is unlikely to be a significant difference in the arterial-portal gradient between OLETF and LETO rats, because exogenous glucose was infused directly into the portal vein at the identical rate. Also, hyperglycemia was not apparent at 8 weeks in OLETF rats. Therefore, neither the arterial-portal glucose

gradient nor hyperglycemia could explain the impaired first-pass HGU of OLETF rats. Hepatic insulin resistance might affect impaired HGU in OLETF rats. However, to confirm this requires further evaluation of the insulin dose dependency on HGU.

The present study indicated that at 8 weeks, the arterial plasma FFA levels in OLETF rats during a portal glucose infusion were significantly higher than that in LETO rats, while there was no significant difference in fasting values of FFA. It has been demonstrated in patients with NIDDM that insulin fails to reduce plasma FFA concentrations.[38] As OLETF rats showed impaired peripheral glucose uptake at 28 weeks of age, they might exhibit impaired insulin-induced reduction of FFA before the appearance of insulin resistance on glucose metabolism in the peripheral tissues.

Elevated plasma FFA levels can inhibit insulin-stimulated glucose uptake and HGP suppression.[28] At 8 weeks, OLETF rats showed elevated plasma FFA levels, increased HGP, and decreased HGU. Whether elevated plasma FFA levels can lead to these impairments of hepatic glucose handling remains to be clarified.

In summary, the present findings demonstrate that HGP is increased and HGU is decreased in the obese NIDDM OLETF rat model much earlier than the appearance of insulin resistance in peripheral tissue. Possible defect(s) in glucose and lipid metabolisms that increase the plasma FFA levels might lead to hepatic insulin resistance in the NIDDM rat model.

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