Mechanism of Abnormal Oral Glucose Tolerance of Genetically Obese *fa/fa* Rats

F. ROHNER-JEANRENAUD, J. PROIETTO, E. IONESCU, AND B. JEANRENAUD

SUMMARY

The genetically obese *fa/fa* rat is glucose intolerant when tested in a conscious state after the spontaneous ingestion of a glucose solution. The aim of this study was to investigate the mechanism(s) underlying the abnormal oral glucose tolerance test of obese animals with the non-steady-state measurement of glucose turnover proposed by Steele et al. in 1968. Our results show that the total rate of glucose appearance is enhanced in obese compared with lean animals. This abnormality is not due to an increased gut glucose absorption but to a lack of suppression and even a transient stimulation of hepatic glucose production after the ingestion of glucose. The rate of glucose utilization by the obese animals is somewhat increased compared with controls or unchanged when expressed as glucose metabolic clearance rate, thus excluding this parameter from the factors contributing to the observed glucose intolerance. The results obtained with genetically obese rats agree with those reported for type II diabetes in humans. The observed defect of the obese group could be related to an abnormal regulation of insulin counterregulatory hormone(s) or of hepatic innervation as well as to other defects of hepatic glycogen handling. DIABETES 1986; 35:1350–55.

The genetically obese fatty rat (*fa/fa*) first described by Zucker and Zucker[1] is mostly claimed to be an animal model of obesity, hyperinsulinemia, and hyperlipoproteinemia. Contradictory results exist regarding their basal glycemia and their glucose tolerance.[2,3] The reported normoglycemia[2,3] and the normal glucose tolerance, as tested by intravenous glucose administration,[4,5] in adult obese *fa/fa* rats are unusual given the marked insulin resistance found in various tissues of these animals.[6–9] However, it has recently been shown that 13- to 14-wk-old conscious obese rats have a significantly elevated basal glycemia, whereas this is not the case for younger obese animals 6–7 wk old.[5] More important, when oral glucose tolerance tests were performed in freely moving genetically obese rats 6–7 or 13–14 wk old, a marked glucose intolerance was observed; this pathological trait worsened with the duration of the syndrome, i.e., it became more marked at 13–14 wk old than at the earlier age.[5] The assessment of glucose tolerance in a conscious state is by far the most physiological one when compared with intravenous glucose infusion, intraperitoneal injection, or glucose intubing in anesthetized animals. Because adult obese *fa/fa* rats have a high basal glycemia and an abnormal glucose tolerance, they are a good model of impaired glucose tolerance associated with insulin resistance.

The aim of this study was to investigate the mechanism(s) underlying the abnormal oral glucose tolerance test in conscious adult obese *fa/fa* rats by a method allowing for the measurement of non-steady-state glucose metabolism in vivo. The question is whether the abnormal oral glucose tolerance test of obese animals is related to an impaired glucose utilization, to an increased gut absorption, to a lack of suppression of hepatic glucose production, or to a combination of these defects.

MATERIALS AND METHODS

Animals. Male monozygotic lean (*FA/FA*) and genetically obese (*fa/fa*) rats 13–15 wk old weighing 308 ± 6 and 396 ± 12 g, respectively, were used throughout the experiments. The animals, originally purchased from the Centre de Sélection et d'Elevage d'Animaux de Laboratoire (Centre National de la Recherche Scientifique, Orléans, France), were bred in our laboratories. They were maintained at a constant temperature (22°C) in an animal quarter with a fixed (12-h) artificial light cycle.

All animals had free access to water and a standard laboratory chow (12% H_2O, 17% protein, 3% lipid, 59% carbohydrate, 4% cellulose, and 5% mineral, UAR laboratory

From the Laboratoires de Recherches Métaboliques, Faculty and the Department of Medicine, University of Geneva, Geneva, Switzerland.
Address reprint requests to Dr. F. Rohner-Jeanrenaud, Laboratoires de Recherches Métaboliques, 64 avenue de la Roseraie, 1211 Geneva 4, Switzerland.
Received for publication 20 March 1986 and in revised form 13 June 1986.

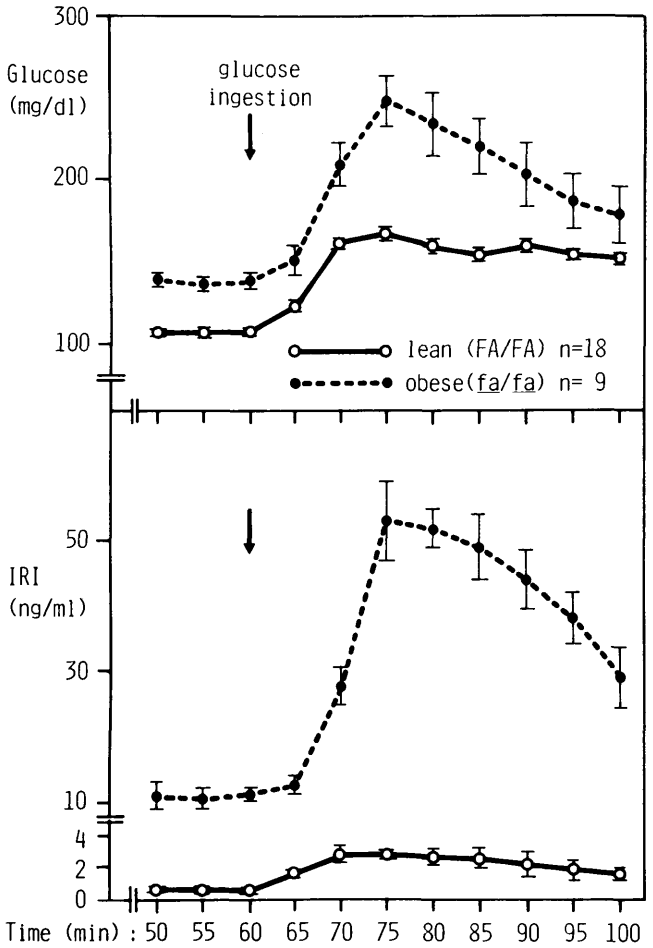

FIG. 1. Plasma glucose and immunoreactive insulin (IRI) levels after oral glucose load in lean and obese Zucker (*fa/fa*) rats. Glucose load, 2 g/kg lean body wt presented at 60 min (*arrow*). Values are means ± SE of 18 lean and 9 obese rats. All intergroup differences were statistically significant at at least $P < .05$, except for plasma glucose levels at 100 min.

chow, Epinay, Villemoisson, France). At 12 wk of age, the rats were implanted, under nembutal anesthesia (70 mg/kg body wt), with a heart catheter placed via the right jugular vein for blood sampling and with another catheter placed in the left jugular vein for tracer infusion. Both catheters were fixed on the skull with acrylic cement as previously described.[10,11] Before the experiments, the rats were allowed to recover from these surgical procedures for ~1 wk, at which time they had returned to their preoperative weights. The catheters were rinsed daily with 1 ml saline solution containing 25 U heparin and 20,000 U penicillin. After recovery, the rats were deprived of food and water for 6–7 h (0800 to 1400 or 1500 h), and on 2 successive days they were trained to drink 1 ml of a 60% glucose solution within 1 min. On the day of the experiment, 6–7 h after food and water removal, the catheters were rinsed and connected to the infusion and sampling pumps.

Experimental design. After a resting period of ~15–30 min, a baseline blood sample was taken (210 μl, 1-min sampling, dead vol = half of vol withdrawn; Ismatec peristaltic pump ip 4, Zurich, Switzerland). A primed constant infusion of [^{3}H]3D-glucose (New England Nuclear, Boston, MA) then commenced at 15 μCi/h by a peristaltic pump (Gilson Minipulse 2, Villiers, Lebel, France). The priming dose was given over the first 5 min; the infusion rate was five times the constant infusion rate (15 μCi/h, 1 ml/h) for the first 2 min (min 0–2), four times the rate for the second 2 min (min 2–4), and three times the rate for the 5th min (min 5). This allowed tracer equilibrium to be reached within 50 min. At min 50, blood samples (210 μl, 1-min sampling, as before) were withdrawn every 5 min (min 50, 55, and 60) for measurement of glucose specific activity and plasma glucose and insulin levels. At min 60, the rats were presented with a dish containing 1 ml of 60% glucose labeled with [^{14}C]1D-glucose (Amersham, Amersham, UK) (~2 g/kg lean body wt containing 30 μCi of [^{14}C]1D-glucose), which they drank within 1 min. Blood samples continued to be withdrawn every 5 min for 40 min, up to min 100. At the end of the experiments, three 5-min samples were taken to accurately measure the delivery rate of [^{3}H]3D-glucose, and aliquots of the 60% [^{14}C]1D-glucose solution were taken for precise measurement of specific activity. The total amount of blood taken was 2.5 ml over 1 h, which has been shown to alter neither the basal glycemia nor the hematocrit.

Analytical procedures. Glucose was measured with a Beckman glucose analyzer 2 (Beckman, Palo Alto, CA). Plasma insulin concentrations were measured by radioimmunoassay with dextran-coated charcoal separation of the bound and free fractions.[12] For determination of [^{3}H]3D-glucose and [^{14}C]1D-glucose specific activities, 30 μl of plasma were deproteinized in duplicate by 60 μl of $ZnSO_4$ (0.3 M) and 60 μl of $Ba(OH)_2$ (0.3 M); 100 μl of the supernatant were passed through an ion-exchange resin (Bio-Rad Ag-2X8, Bio-Rad, Richmond, CA) to remove ^{14}C-labeled charged metabolites of glucose. The columns were washed with distilled water, and the eluant was collected in scintillation vials that were dried in an oven to remove tritiated water. Distilled water was then added to redissolve the glucose, and the samples were separated into two aliquots. The first aliquot was used after luma gel (Lumac/3M, Schaesberg, The Netherlands)

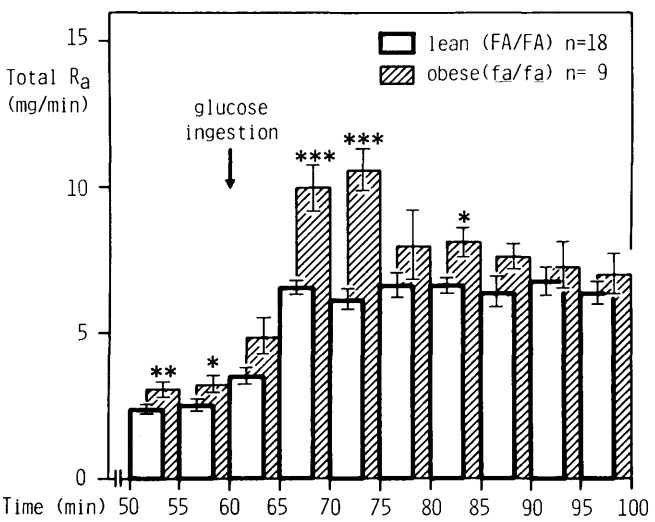

FIG. 2. Total rate of glucose appearance (total Ra) after oral glucose load in lean and obese Zucker (*fa/fa*) rats. Glucose load, 2 g/kg lean body wt. Values are means ± SE of 18 lean and 9 obese rats. *$P < .05$; **$P < .025$; ***$P < .001$. Other differences are not statistically significant.

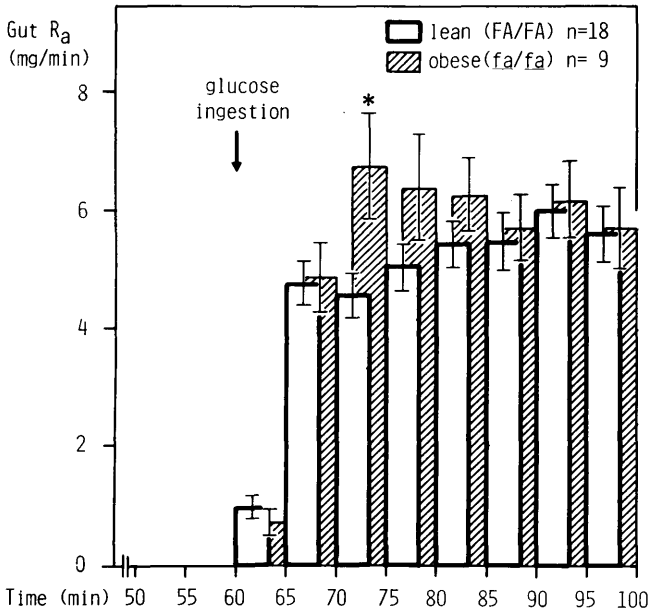

FIG. 3. Gut-derived appearance rate of glucose (gut Ra) after oral glucose load in lean and obese Zucker (*fa/fa*) rats. Glucose load, 2 g/kg lean body wt. Values are means ± SE of 18 lean and 9 obese rats. Note scale is not same as in Fig. 2.
***P* < .05. Other differences are not statistically significant.**

addition to count ^{3}H and ^{14}C with a two-channel liquid scintillation counter (1217 Rackbeta, LKB Wallac) with a dual-label–counting program that corrected for spillover. The second aliquot was used to determine the rate of randomization of ^{14}C from position 1 to other carbon positions of glucose (measurement of Cori cycle) by isolating the ^{14}C carbon at position 6 and multiplying the counts obtained by a factor of four as previously described.[13–15]

Calculations. The Steele equation[16] was used to calculate the total rate of glucose appearance (total Ra) as well as the rate of glucose disappearance (Rd) from the [^{3}H]3D-glucose specific activity. We have recently validated this equation in the rat and found that the optimal pool fraction (fraction of total glucose pool that is in rapidly mixing compartment) is 0.5. The specific activity of [^{14}C]1D-glucose labeling the 1 ml of glucose ingested allowed, by transposition of the Steele equation, for the measurement of the rate of gut glucose absorption (gut Ra). The rate of hepatic glucose production (HGP) was calculated as the difference between the total rate of glucose appearance and the gut glucose absorption.[17] The metabolic clearance rate of glucose (MCRg) was calculated as the rate of glucose disappearance divided by glycemia.

Statistical analysis. The two-tailed Student's *t* test for unpaired data was used throughout the study.

RESULTS

In a preliminary set of experiments, the basal rate of total glucose appearance measured over 50 min with [^{3}H]3D-glucose infusion only was found to be constant as a function of time in both lean and obese rats, as was the glycemia [lean, Ra = 2.59 ± 0.12 mg/min and glycemia = 113 ± 3 mg/dl (*N* = 4); obese, Ra = 3.22 ± 0.19 mg/min and glycemia = 136 ± 2 mg/dl (*N* = 4)]. Both parameters were statistically different in lean versus obese rats. (Ra, $P < .05$; glycemia, $P < .001$).

Figure 1 shows the evolution of plasma glucose and insulin levels before and after the 1 ml of 60% glucose ingestion in lean and obese rats. In the basal state and compared with lean animals, obese rats had a higher glycemia [obese, 139 ± 4 mg/dl (*N* = 9); lean, 108 ± 2 mg/dl (*N* = 18); $P < .001$] and were hyperinsulinemic [obese, 10.4 ± 1.5 ng/ml (*N* = 9); lean, 0.6 ± 0.1 ng/ml (*N* = 18); $P < .001$]. Furthermore, after glucose ingestion, obese rats reached much higher peaks of plasma glucose and insulin levels than did controls. However, although the lower glycemia of lean animals remained constant from its peak value up to the end of the test, the hyperglycemia of obese animals decreased, and glycemia was no longer statistically different from controls at the last experimental point. The total rate of glucose appearance (total Ra) in the two groups of animals is shown in Fig. 2. The total Ra was statistically higher in obese than in lean animals in the basal state as well as after the glucose ingestion, especially over the first 15 min after the glucose load. Figure 3 depicts the gut glucose absorption (gut Ra) as measured by [^{14}C]1D-glucose appearance. In the basal state, gut Ra was shown to be 0 on the basis of additional experiments in which simultaneous portal and peripheral blood samples were withdrawn from lean and obese rats deprived of food and water for 6–7 h. The portal-peripheral glucose gradient was −5 ± 3 mg/dl (*N* = 4) in lean rats and 4 ± 4 mg/dl (*N* = 4) in obese animals, indicating a lack of gut absorption in both groups. As can also be seen in Fig. 3, except for one time interval (70–75 min), gut Ra was the same in lean and obese rats over the whole experimental period. By subtracting the rate of gut glucose absorption from the total rate of glucose appearance, HGP could be calculated (Fig. 4). As shown for total Ra, basal HGP was

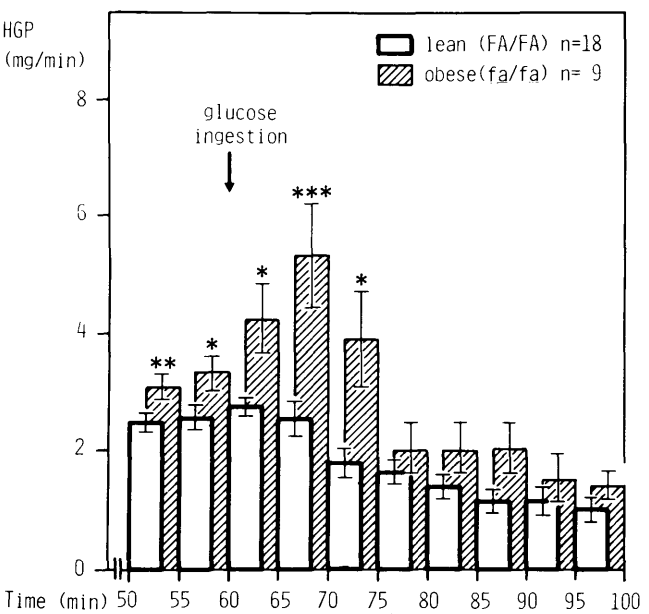

FIG. 4. Hepatic glucose production (HGP) after oral glucose load in lean and obese Zucker (*fa/fa*) rats. Glucose load, 2 g/kg lean body wt. Values are means ± SE of 18 lean and 9 obese rats. Note scale is same as in Fig. 3.
***P* < .05; ****P* < .02; *****P* < .005. Other differences are not statistically significant.**

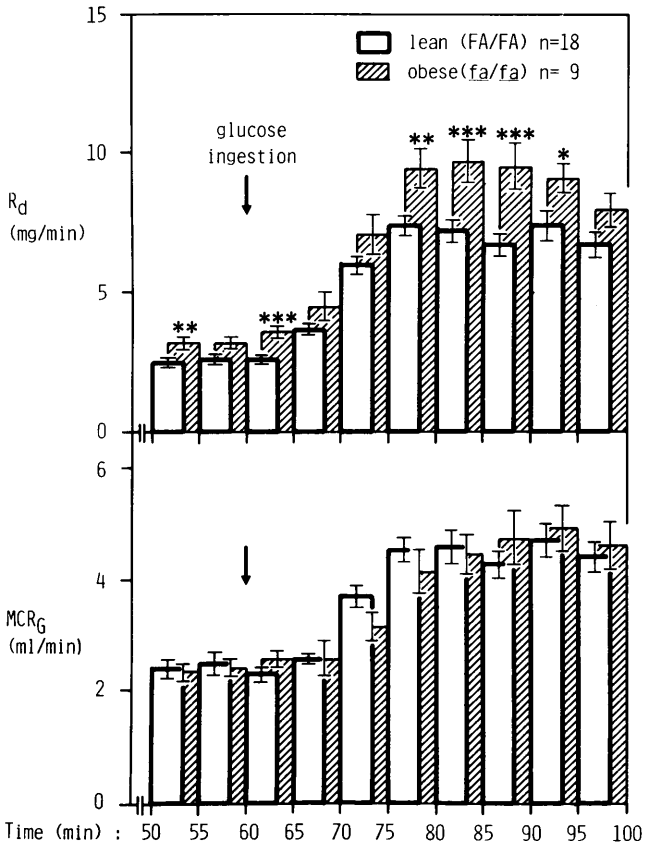

FIG. 5. Rate of glucose disappearance (Rd) and metabolic clearance rate of glucose (MCRg) after oral glucose load in lean and obese (*fa/fa*) rats. Glucose load, 2 g/kg lean body wt. Values are means ± SE of 18 lean and 9 obese rats.
***P* < .05; ****P* < .02; *****P* < .005. Other differences are not statistically significant.**

significantly higher in obese than in lean rats. Moreover, although HGP of lean rats started to decrease during the first 15 min after the oral glucose load, that of obese animals showed a 70% increase during the same period (65–70 min); thereafter it was almost identical to that of the lean group through the end of the test. Figure 5 shows the rate of glucose utilization (Rd) as well as the metabolic clearance rate of glucose (MCRg), which partly corrects for the impact of glycemia (different in the 2 groups) on the actual glucose disposal. In the basal state, Rd of obese animals was higher than that of lean ones, although the difference was not marked. After the glucose meal, Rd of obese rats increased more than did that of lean rats, especially during the second half of the test. When expressed in terms of MCRg, however, the two groups of animals displayed identical curves, which increased as a function of time, due to the increase in insulin levels.

The percent glucose recycling via the Cori cycle, as measured by the presence of ^{14}C on position 6 (see MATERIALS AND METHODS) is shown in Table 1. The amount of glucose recycled was very small, always <10% of the total ^{14}C counts. However, note that from the 25th min after the glucose ingestion (min 85) onward, the amount of glucose recycled was slightly higher in obese than in lean rats, although the difference reached statistical significance at only $1P < .05$.

TABLE 1
Percent recycling after oral glucose load in lean and obese Zucker *fa/fa* rats

Time (min)	Recycling (%)	
	Lean	Obese
65	2.8 ± 1.6	2.7 ± 2.7
70	3.8 ± 0.5	3.7 ± 1.9
75	3.5 ± 0.7	3.7 ± 0.7
80	2.8 ± 1.3	5.3 ± 0.7
85	3.8 ± 0.9	7.7 ± 1.5*
90	5.0 ± 0.7	7.7 ± 0.9*
95	6.5 ± 0.9	9.0 ± 1.0
100	7.0 ± 0.7	9.7 ± 0.9*

Results are calculated as percent ^{14}C counts recycled of total ^{14}C counts measured in each sample and expressed as means ± SE of 4 lean and 4 obese rats.
*$1P < .05$.

Table 2 summarizes the data presented in Figs. 2–5 by expressing the areas under the curves for the first 15 min after glucose ingestion for the different parameters, i.e., total Ra, gut Ra, HGP, and Rd. As expected from the dynamic curves, total Ra of obese animals was higher than that of lean animals. This can be attributed to the increased HGP of obese rats because gut Ra was identical in both groups. The area under the curve of Rd, but not that of MCRg, was significantly higher in obese than in lean rats.

DISCUSSION

Ionescu et al.[5] found that genetically obese *fa/fa* rats are glucose intolerant when tested under physiological conditions in which conscious animals spontaneously drink a glucose solution. Our study was aimed at investigating the mechanism of this glucose intolerance. Two tracers were used to measure not only the total rate of glucose appearance and disappearance but also the rate of gut glucose absorption and HGP.[16,17] Our results show that the total rate of glucose appearance is enhanced in obese compared with lean animals. This abnormality is not due to an increased gut glucose absorption but to a lack of suppression and even a stimulation of HGP after the glucose load (see Table 2). Note that the basal HGP was higher in obese than in lean animals, which accounts for the basal hyperglycemia mea-

TABLE 2
Areas under curves of total rate of glucose appearance (total Ra), gut-derived rate of glucose appearance (gut Ra), hepatic glucose production (HGP), and rate of glucose disappearance (Rd) after oral glucose load in lean and obese Zucker *fa/fa* rats (mg/15 min)

	Total Ra*	Gut Ra†	HGP‡	Rd§
Lean (*FA/FA*)	83.9 ± 3.4	52.0 ± 4.2	33.0 ± 2.5	61.4 ± 2.5
Obese (*fa/fa*)	128.7 ± 8.6	62.1 ± 7.2	67.2 ± 10.4	76.1 ± 5.3

Areas have been calculated over 15 min because peak glycemia occurs at 15 min. Values are means ± SE of 18 lean and 9 obese rats.
*$P < .001$ in lean vs. obese rats.
†Difference between lean and obese rats not statistically significant.
‡$P < .005$ in lean vs. obese rats.
§$P < .02$ in lean vs. obese rats.

sured in the obese group. The rate of glucose utilization by obese animals was increased when compared with controls, excluding this parameter from being even partly responsible for the glucose intolerance. This may seem surprising in view of the marked insulin resistance of adult obese animals that has been found to prevail in vitro in adipose tissue[6] and in various muscle preparations.[7,8] However, measurement of in vivo glucose metabolism (M) with the clamp technique by Terrettaz et al.[9] showed that M was the same in lean and obese rats, but at the cost of plasma insulin levels that were about four times higher in obese rats than in controls. This is precisely what happened in our study: the increased rate of glucose utilization in obese rats was achieved at the expense of much higher insulin as well as glucose levels. Thus, although in vitro or in vivo studies with the clamp technique are able to demonstrate intrinsic defect(s) of glucose metabolism, in vivo studies as close to physiological situations as in our study clearly suggest that the defect of glucose utilization may have little impact on actual glucose intolerance because it is compensated for by high plasma insulin and glucose levels. It should be stressed that the increased rate of glucose disappearance in obese rats, compared with that in lean ones, became significant at a time when both glycemia and insulinemia had reached their highest values.

The Cori cycle, involving glucose metabolism and gluconeogenesis, was low in both lean and obese rats over the 40-min experimental period. This agrees with other studies showing that glucose recycling is almost negligible in humans.[18] However, the amount of glucose recycled is slightly higher in obese compared with lean animals, especially over the second half of the test. This may be correlated with their elevated rate of glucose disappearance and with an increased rate of gluconeogenesis, as previously hypothetized.[19]

Thus, the glucose intolerance of obese *fa/fa* rats is due to a high basal HGP, which is even further stimulated after a glucose meal.

Note that HGP in lean rats was not suppressed by >38% over the 40-min experimental period. However, in other studies in which HGP was measured after glucose ingestion, it was observed that this process was completely suppressed by the 60th min after the glucose load.[18,20] Therefore a more complete suppression of HGP may have occurred in lean rats, if we had prolonged the experiment. Moreover, the animals were studied after 6–7 h of food deprivation, at which time they still had glycogen in their livers.[21] In addition, under these conditions, it has been found that the peripheral glucagon levels were slightly stimulated in lean rats at 5 min and then remained constant at their basal levels through the end of the test (F. Rohner-Jeanrenaud, unpublished observations). Therefore, minimal increases of portal glucagon levels after the glucose load acting on the liver may partly explain why HGP was not suppressed by more than ~40%.

The mechanism underlying the actual stimulation of HGP in obese rats after an oral glucose load is unknown at present. Interestingly, this defect of HGP is similar to that found in type II diabetes in humans.[22] A lack of suppression or even a stimulation of glucagon secretion after the sugar ingestion could be one of the causes. This possibility should be explored by measuring portal glucagon levels, a technically difficult task in freely moving rats. Other counterregulatory hormones such as glucocorticoids or catecholamines could also play a role in the stimulation of HGP in obese *fa/fa* rats because their control is reportedly abnormal in several animal models of obesity.[19] Finally, this defect could be directly or indirectly mediated via the autonomic nervous system, whose modulation is known to be altered in obese compared with lean rats.[23]

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of Catherine Camenzind and thank Christiane McVeigh for typing this manuscript.

This work was supported by Grants 3.851.083 and 3.822.086 of the Swiss National Science Foundation and by a grant-in-aid of Nestlé S.A.

J.P. holds a National Health and Medical Research Council (Australia) Neil Hamilton Fairley Fellowship.

REFERENCES

[1] Zucker, L. M,. and Zucker, T. F.: Fatty, a new mutation in the rat. J. Hered. 1961; 52:275–78.

[2] Bray, G. A.: The Zucker-fatty rat: a review. Fed. Proc. 1977; 36:148–53.

[3] Herberg, L., and Coleman, D. L.: Laboratory animals exhibiting obesity and diabetes syndrome. Metabolism 1977; 26:59–99.

[4] Crettaz, M., Horton, E. S., Wardzala, L. J., Horton, E. D., and Jeanrenaud, B.: Physical training of Zucker rats: lack of alleviation of muscle insulin resistance. Am. J. Physiol. 1983; 244:E414–20.

[5] Ionescu, E., Sauter, J. F., and Jeanrenaud, B.: Abnormal oral glucose tolerance in genetically obese (*fa/fa*) rats. Am. J. Physiol. 1985; 248:E500–506.

[6] Cushman, S. W., Zarnovski, M. J., Franzusoff, A. J., and Salans, L. B.: Alterations in glucose metabolism and its stimulation by insulin in isolated adipose cells during the development of genetic obesity in the Zucker fatty rat. Metabolism 1978; 27:1930–40.

[7] Kemmer, F. W., Berger, M., Herberg, L., Gries, F. A., Wirdeyer, A., and Becker, K.: Glucose metabolism in perfused skeletal muscle. Demonstration of insulin resistance in the obese Zucker rats. Biochem. J. 1979; 178:733–41.

[8] Crettaz, M., Prentki, M., Zaninetti, D., and Jeanrenaud, B.: Insulin resistance in soleus muscle from obese Zucker rats. Biochem. J. 1980; 186:525–34.

[9] Terrettaz, J., and Jeanrenaud, B.: In vivo hepatic and peripheral insulin resistance in genetically obese (*fa/fa*) rats. Endocrinology 1983; 112:1346–51.

[10] Steffens, A. B.: A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. Physiol. Behav. 1969; 4:833–36.

[11] Berthoud, H. R., Trimble, E. R., Siegel, E. G., Bereiter, D. A., and Jeanrenaud, B.: Cephalic-phase insulin secretion in normal and pancreatic islet-transplanted rats. Am. J. Physiol. 1980; 238:E336–40.

[12] Herbert, V., Lau, K. S., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. 1965; 25:1375–84.

[13] Dunn, D. F., Friedmann, B., Maass, A. R., Reichard, G. A., and Weinhouse, S.: Effects of insulin on blood glucose entry and removal rates in normal dogs. J. Biol. Chem. 1957; 225:225–37.

[14] Reichard, G. A., Jr., Moury, N. F., Jr., Hochella, N. J., Patterson, A. L., and Weinhouse, S.: Quantitative estimation of the Cori cycle in the human. J. Biol. Chem. 1963; 238:495–501.

[15] Forbath, N., and Hetenyi, B., Jr.: Metabolic interrelations of glucose and lactate in unanaesthetized normal and diabetic dogs. Can. J. Physiol. Pharmacol. 1970; 48:115–22.

[16] Steele, R.: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann. NY Acad. Sci. 1959; 82:420–30.

[17] Steele, R., Bjerknes, C., Isbel Rathgeb, B. A., and Altszuler, N.: Glucose uptake and production during the oral glucose tolerance test. Diabetes 1968; 17:415–21.

[18] Ferrannini, E., Bjorkman, O., Reichard, G. A., Jr., Pilo, A., Olsson, M., Wahren, J., and DeFronzo, R. A.: The disposal of an oral glucose load in healthy subjects. Diabetes 1985; 34:580–88.

[19] Jeanrenaud, B., Halimi, S., and van de Werve, G.: Neuro-endocrine disorders seen as triggers of the triad: obesity—insulin resistance—abnormal glucose tolerance. Diabetes Metab. Rev. 1985; 1:261–91.

[20] Pilo, A., Ferrannini, E., Biörkman, O., Wahren, J., Reichard, G. A.,

Jr., Felig, P., and DeFronzo, R. A.: Analysis of glucose production and disappearance rates following an oral glucose load in normal subjects: a double tracer approach. *In* Carbohydrate Metabolism. Cobelli, C., and Bergman, R. N., Eds. New York, Wiley, 1981:221.

[21] Postle, A. D., and Bloxham, D. P.: The use of tritiated water to measure absolute rates of hepatic glycogen synthesis. Biochem. J. 1980; 192: 65–73.

[22] Firth, R. G., Bell, P. M., Marsh, H. M., Hansen, I., and Rizza, R. A.: Postprandial hyperglycemia in patients with noninsulin-dependent diabetes mellitus. Role of hepatic and extrahepatic tissues. J. Clin. Invest. 1986; 77:1525–32.

[23] Rohner-Jeanrenaud, F., Bobbioni, E., Ionescu, E., Sauter, J. F., and Jeanrenaud, B.: CNS regulation of insulin secretion. *In* Advances in Metabolic Disorders. Vol. 10. Szabo, A. J., Ed. New York, Academic, 1983:193.