Defective Fatty Acid-mediated β-Cell Compensation in Zucker Diabetic Fatty Rats

PATHOGENIC IMPLICATIONS FOR OBESITY-DEPENDENT DIABETES*

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Although obesity is associated with insulin resistance, most obese humans and rodents remain normoglycemic because of compensatory hyperinsulinemia. This has been attributed to β-cell hyperplasia and increased low Km glucose metabolism of islets. Since free fatty acids (FFA) can induce these same β-cell changes in normal islets of Wistar rats and since plasma FFA are increased in obesity, FFA could be the signal from adipocytes that elicits β-cell compensation sufficient to prevent diabetes. To determine if compensation is impaired in islets of rats with a diabetic mutation, the Zucker diabetic fatty (ZDF) rat, we cultured islets from 6-week-old obese (fa/fa) rats that had compensated for obesity and apparently normal islets from lean ZDF rats (fa/+) in 0, 1, or 2 mM FFA. Low Km glucose usage rose 2.5-fold in FFA-cultured control islets from age-matched Wistar rats, but failed to rise in either the precompensated islets of ZDF rats or in islets of lean ZDF rats. Bromodeoxyuridine incorporation increased 3.2-fold in Wistar islets but not in islets from obese or lean ZDF rats. Insulin secretion doubled in normal islets cultured in 2 mM FFA (p < 0.01) but increased only slightly in islets from lean ZDF rats (not significant) and declined in islets from obese ZDF rats (p < 0.05). We conclude that, unlike the islets of age-matched Wistar rats, islets of 6-week-old heterozygous and homozygous ZDF rats lack the capacity for FFA-induced enhancement of β-cell function.

Pancreatic islets from obese rodents are enlarged and exhibit a marked increase in low Km glucose metabolism (1), which may account for their high output of insulin even at low concentrations of glucose (1-3). The hyperinsulinemia is regarded as a compensatory response that prevents hyperglycemia despite the insulin resistance that invariably accompanies obesity.

There is evidence that free fatty acids (FFA) may be the signal from adipocytes that mediates this compensatory insulin secretion (1). Plasma FFA are elevated in obesity (4, 5) and have long been known to stimulate insulin secretion (6-8). Moreover, the compensatory triad observed in islets from obese rats can be induced in normal islets by culturing them for 7 days in the presence of 1 or 2 mM FFA; low Km glucose metabolism rises dramatically (1), there is evidence of increased β-cell replication (1), and insulin secretion increases (1), confirming the earlier description of FFA-induced hyperinsulinemia (9).

If the FFA-induced compensation in obesity is, at least in part, responsible for preventing diabetes, it follows that FFA-induced compensation may be impaired at or before the onset of diabetes. In this study we assess the ability of FFA to induce the compensatory triad of enhanced low Km glucose metabolism, increased β-cell replication, and insulin hypersecretion in islets from obese rats with a diabetic mutation, the Zucker diabetic fatty rat (ZDF-drt). We observe that FFA induction of these compensatory changes is impaired, not only in islets from obese homozygous rats, but also in islets from lean heterozygous ZDF rats.

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MATERIALS AND METHODS

Animals—Four groups of rats were studied at 6 weeks of age. Male Wistar rats were purchased from Charles River Laboratories (Wilmington, MA). Homozygous obese ZDF-drt rats (fa/fa) and heterozygous lean ZDF littermates (fa +/-) were bred in our laboratory from ZDF/drt-fa(F10) rats purchased from Dr. Richard Peterson (University of Indianapolis School of Medicine, IN). Rats from our colony exhibit the previously described phenotype (10). Obesity is discernible at 6 weeks of age. Since all obese (fa/fa) male ZDF rats develop hyperglycemia (>200 mg/dl) and glycosuria by 8–10 weeks of age, all 6-week-old obese males were considered to be prediabetic; not only was their 9 a.m. plasma glucose normal (135 ± 3 mg/dl) but other β-cell hallmarks of diabetes, such as impaired glucose-stimulated insulin secretion and reduced GLUT-2, had not yet appeared (11, 12). By contrast, obese homozygous females and lean heterozygous males almost never become hyperglycemic (9 a.m. plasma glucose 123 ± 7 mg/dl).

All rats received standard rat chow (Teklad F6664, Teklad, Madison, WI) ad libitum and had free access to water. All institutional guidelines for animal care and use were followed.

Islet Culture—Pancreatic islets of 6-week-old rats were isolated by the method of Naber et al. (13) as modified by Lee et al. (14) and maintained for 7 days in suspension culture in 60-mm glass Petri dishes at 37 °C in a humidified atmosphere of 5% CO2 and 95% air as described previously (1, 14); however, the glucose concentration of the medium was reduced to 2 mM, the minimum glucose level in which more than 80% of the islets will be present at the end of 7 days of culture. The low chain fatty acid mixture added to the culture medium was oleic palmitate = 2:1 (sodium salts, Sigma).

Perfusion Experiments—For perfusion experiments 50–100 islets oxyuridine; ZDF, Zucker diabetic fatty; PPAR, peroxisome proliferator-activated receptor.

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The abbreviations used are: FFA, free fatty acid; BrdUrd, bromodeoxyuridine; ZDF, Zucker diabetic fatty; PPAR, peroxisome proliferator-activated receptor.
were hand-picked under a stereoscopic microscope, washed twice with Krebs-Ringer bicarbonate-Hepes buffer (pH 7.4, 3 mM glucose) and loaded into 13 mm chambers containing an 8-μm nylon membrane filter (Millipore, Bedford, MA). Perifusion was carried out as described previously (14). Immunoreactive insulin was determined by radioimmunoassay (15) using charcoal separation (16).

**Insulin Content of Islets—** Twenty islets each were washed twice with PBS and put into 500 μl of acid ethanol (0.18 M HCl in 95% ethanol). Insulin was extracted overnight at 4°C after sonication for 1 min (Laboratory Supplies Co. Inc., Hicksville, NY).

Measurements of Glucose Usage—Glucose usage in cultured islets was measured by the method of Zawalich and Matchinsky (17) and Zawalich et al. (18), as described previously in full detail (1), except that 50–100 islets were counted rather than pipetted and transferred to small vials.

Measurements of Cell Viability—After the 7-day culture period, ~20 islets were randomly selected from culture dishes of 0, 1, and 2 mM FFA of Wistar and ZDF (fa+/+) rats. Islets were washed twice with PBS and stained with fluorescein diacetate and ethidium bromide for 1 min. Five to six hundred cells from each of the six groups were counted randomly under a fluorescence microscope (Nikon Optiphot UFX II-A, Garden City, NY). Green cells were counted as viable and red cells as dead, and the data were expressed as percent viability.

**Bromodeoxyuridine (BrdUrd) Incorporation—** After a 3-day culture period, islets were fixed in Bouin's solution, immobilized in 6% gelatin and embedded in paraffin. Five-μm thick serial sections were processed for insulin staining (Dako, Carpinteria, CA) and BrdUrd quantitation using an anti-BrdUrd antibody (19) (Boehringer Mannheim). BrdUrd-positive nuclei of insulin-positive cells were counted using a fluorescence microscope. Results were expressed as BrdUrd+ nuclei/total nuclei × 100.

Statistical Analyses—All results are expressed as mean ± S.E. Statistical significance was evaluated using two-way analysis of variance followed by Scheffe’s multiple comparison test.

**RESULTS**

Effects of FFA on Basal Hyperinsulinemia of Prediabetic Islets—As originally reported by Zhou and Grill (9) and confirmed by our laboratory (1), exposure of islets from normal
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Wistar rats to 1 and 2 mM FFA resulted in a concentration-de-
scretion associated with exposure to FFA.

increase in basal insulin secretion after culture in 2 mM FFA
prior to the isolation of islets.

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its magnitude was less. By contrast, in the obese ZDF groups
cultured islets and therise in insulin occurred sooner, although
was small and not statistically significant; glucose-stimulated
secretion was significantly reduced, suggesting an in-
trinsic resistance to the effects of FFA on islet function in rats
with a single ZDF allele.

To avoid the influence of precompensation, we examined the
effects of FFA on the islets of lean heterozygous ZDF animals in
which obesity, insulin resistance and compensatory hyperinsu-
linemia were absent and no prior compensatory changes in
islets had occurred. As shown in Fig. 1B, the FFA-induced
increase in basal insulin secretion after culture in 2 mM FFA
was small and not statistically significant; glucose-stimulated
insulin secretion was significantly reduced, suggesting an in-
trinsic resistance to the effects of FFA on islet function in rats
with a single ZDF allele.

To exclude insulin depletions as the cause of the impaired
FFA-mediated effects on insulin secretion, insulin content was
measured in all islet groups (Table I). There were no signifi-
cant differences in change of insulin content between the lean
Wistar and ZDF groups. Moreover, since the secreted insulin in
all groups (Table I) represented at most only 3.8% of the total
insulin content, it is unlikely that differences in pancreatic
insulin content account for the effect of FFA on insulin
secretion.

To exclude the possibility that elevated concentrations of
FFA in vitro might have killed a greater number of cells in
the islets of obese and lean ZDF rats than in the lean Wistar
controls, we tested cell viability using fluorescein incorporation.
As indicated in Table II, exposure to 2 mM FFA reduced
viability by 7% in Wistar islets and 12% in ZDF islets, not
nearly enough to account for the large differences in insulin
secretion associated with exposure to FFA.

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Effect of FFA on BrdUrd Incorporation in Islet Cells of
ZDF—We have previously observed a 3-fold increase in BrdUrd
incorporation in islets of normal Wistar rats cultured for 7 days
in 2 mM FFA (1). Islets from ZDF rats were isolated at the age
of 6 weeks, approximately 3 weeks before the expected onset of
diabetes, and cultured in 0 or 2 mM FFA. BrdUrd incorporation,
which in the absence of FFA was 1.6 times higher than in the
smaller islets of lean controls, did not increase further, perhaps
because -cells had already expanded maximally in vivo. How-
ever, in islets of lean heterozygous males, in which no prior
increase in -cell volume had taken place, BrdUrd incorpor-
lation was not enhanced by culture in 2 mM FFA (Fig. 2, Table I).

Effect of FFA on Low Km Glucose Usage by Prediabetic Is-
lettes—Previously we reported a 3-fold increase in low Km
Km glucose usage in precompensated islets of
based prediabetic rats could be further enhanced by exposure to
FFA, we cultured such islets for 1 week in the presence of 1 or 2
mM FFA. As shown in Table III, no further enhancement of
low Km glucose usage was induced in these precompensated
islets. In islets of lean heterozygous ZDF rats which are devoid
of compensatory changes, low Km glucose usage in the ab-
ence of FFA was no greater than in islets of Wistar rats.
However, in contrast to the Wistar islets, no increase in low Km
Km glucose usage had occurred after 7 days of culture at 1 and 2
mM FFA (Table III).
changes" that are induced in islets from normal Wistar rats by rozygous ZDF rats do not develop any of the "compensatory
increased low insulin secretion, increased BrdUrd incorporation, and in-
These changes include increased basal and glucose-stimulated
increased only slightly (NS) in the lean ZDF group, and glucose-stimulated insulin secretion was reduced by the 2 mM FFA. Thus, high FFA levels comparable to those observed in plasma of prediabetic ZDF rats (14) did not elicit a normal compensatory insulin response. The ZDF colony may thus have an intrinsic β-cell defect that interferes with FFA-mediated compensation. In as much as islets from 6-week-old obese homozygous ZDF rats appear to have reached a fully compensated state in vivo prior to their isolation, either more than 7 days are required in vitro for FFA-mediated induction of compensatory events in ZDF rats or, more likely in ZDF rats a time window during which compensation can occur close before the age of 6 weeks.

The mechanism by which FFA induce the changes that in islets of normal rats result in compensatory hyperinsulinemia has not as yet been identified. In prediabetes, a fatty acid-activated receptor with homology to the peroxisome proliferator-activated receptor (PPAR) was recently cloned (20). An isoform of PPAR is expressed in islets. It may be involved in up-regulation of glycolytic enzymes responsible for low Km glucose usage. However, changes in alternative fuel use could be the cause of the increase in low Km glycolysis (21).

The mechanism of the normal FFA-induced increase in BrdUrd incorporation is also unknown. To our knowledge, a mitogenic effect of FFA in vitro has not been described previously in mammalian cells. However, intracellular levels of palmitoyl-CoA within the known physiologic range are able to potentiate protein kinase C activity in vitro (22, 23) and stimulate protein kinase C-catalyzed phosphorylation of epidermal-growth factor receptor (24).

If the compensatory hyperinsulinemia in normal islets is the result of FFA-mediated induction of enzymes, what is the mechanism of the failed compensation in islets of ZDF rats? Given the fact that islets from obese prediabetic rats have an abnormally high lipid content in vivo (14), lipid overload seems plausible. It has long been known that increased long-chain fatty acyl-CoA impedes glucose metabolism at multiple levels (25–29), as recently reviewed by McGarry (30). A novel additional mechanism, excessive acylation, also warrants consideration; just as unregulated overglycation resulting from hyperglycemia can modify the function of certain proteins, excessively high FFA levels causing overacylation might similarly alter protein functions (31).

In summary, these results confirm our earlier report (1) that normal islets cultured in the presence of elevated FFA levels develop the same changes in DNA synthesis, glucose usage and insulin secretion that are present in vivo in nonobese rats. We further demonstrated that islets from obese prediabetic and nonprediabetic rats do not develop the foregoing compensatory changes when cultured under these same conditions, perhaps because they had already occurred earlier in

| Glucose | pmol/h/islet | pmol/h/ng DNA |
|---------|--------------|---------------|
| Wistar male | | |
| 0 mM FFA | 18.6 ± 0.7 | 5.75 ± 0.23 |
| 1 mM FFA | 37.4 ± 6.0 | 11.19 ± 1.79 |
| 2 mM FFA | 44.0 ± 5.9 | 14.51 ± 1.95 |
| ZDF (fa/+) male | | |
| 0 mM FFA | 18.7 ± 3.6 | 4.95 ± 0.78 |
| 1 mM FFA | 13.6 ± 1.8 | 3.59 ± 0.43 |
| 2 mM FFA | 45.6 ± 8.2 | 2.50 ± 0.45 |
| ZDF (fa/−) male | | |
| 0 mM FFA | 44.8 ± 11.8 | 2.42 ± 0.64 |
| 1 mM FFA | 55.9 ± 8.4 | 2.54 ± 0.38 |
| 2 mM FFA | 45.6 ± 8.2 | 2.50 ± 0.45 |
| ZDF (fa/−) male | | |
| 0 mM FFA | 80.4 ± 14.1 | 4.73 ± 0.83 |
| 1 mM FFA | 49.8 ± 7.1 | 2.86 ± 0.41 |
| 2 mM FFA | 70.5 ± 7.8 | 3.27 ± 0.36 |

a Values are mean ± standard error of the mean (n = 3).
b p < 0.05 versus 0 mM FFA.
c p < 0.05 and d p < 0.01 versus Wistar male.
° p < 0.05 and f p < 0.01 versus ZDF (fa/+) male.

DISCUSSION

The results indicate that islets from homozygous and heterozygous ZDF rats do not develop any of the "compensatory changes" that are induced in islets from normal Wistar rats after 7 days of culture in 1 or 2 mM long chain fatty acids (FFA). These changes include increased basal and glucose-stimulated insulin secretion, increased BrdUrd incorporation, and increased low Km glucose metabolism. Since compensatory hyperinsulinemia is required to prevent hyperglycemia in the face of worsening insulin resistance, it follows that the propensity for obesity-dependent diabetes in ZDF rats could be the consequence of the failure of FFA to induce the changes in β-cells that result in the necessary degree of hyperinsulinemia.

In the case of islets from 6-week-old obese homozygous ZDF rats a measure of compensation had already occurred in vivo prior to their isolation for the culture experiments, making it impossible to differentiate between precompensation that had reached a maximum and intrinsic resistance of ZDF β-cells to actions of FFA. The islets of 6-week-old obese rats are 3.3 times larger than those of lean controls (12) and their insulin secretion is 3.2 times greater (12). Lean heterozygous ZDF rats, by contrast, do not differ from Wistar islets with respect to β-cell volume density, low Km glucose usage or insulin secretion. Nevertheless, despite the absence of any antecedent compensatory changes in vivo, they too were completely unresponsive to culture in FFA. BrdUrd incorporation, which increased 3.2-fold in islets of Wistar males in the presence of 2 mM FFA, did not increase at all in the islets of lean ZDF rats. Similarly, low Km glucose usage, which increased 2.5-fold in the Wistar animals, did not increase in the lean ZDF group. Finally, insulin secretion at 3 mM glucose, which more than doubled in the Wistar islets, increased only slightly (NS) in the lean ZDF group, and glucose-stimulated insulin secretion was reduced by the 2 mM FFA. Thus, high FFA levels comparable to those observed in plasma of prediabetic ZDF rats (14) did not elicit a normal compensatory insulin response. The ZDF colony may thus have an intrinsic β-cell defect that interferes with FFA-mediated compensation. In as much as islets from 6-week-old obese homozygous ZDF rats appear to have reached a fully compensated state in vivo prior to their isolation, either more than 7 days are required in vitro for FFA-mediated induction of compensatory events in ZDF rats or, more likely in ZDF rats a time window during which compensation can occur close before the age of 6 weeks.

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In summary, these results confirm our earlier report (1) that normal islets cultured in the presence of elevated FFA levels develop the same changes in DNA synthesis, glucose usage and insulin secretion that are present in vivo in nonobese rats. We further demonstrated that islets from obese prediabetic and nonprediabetic rats do not develop the foregoing compensatory changes when cultured under these same conditions, perhaps because they had already occurred earlier in

H. Hirose, J. Milburn, R. H. Unger, and Y.-T Zhou, unpublished observations.
vivo. Consequently, if insulin resistance worsens, such islets would be incapable of further insulin secretion and hyperglycemia would supervene. However, islets from lean heterozygous ZDF rats without β-cell precompensation also failed to respond normally to FFA enriched culture. It is possible that the β-cell unresponsiveness to FFA present at 6 weeks of age and thereafter accounts for the inability to compensate fully for the insulin resistance and thereby prevent diabetes.

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REFERENCES
1. Milburn, J. L., Hirose, H., Lee, Y. H., Nagasawa, Y., Ogawa, A., Ohneda, M., Beltrandel Rio, H., Ohneda, M., Johnson, J. H., and Unger, R. H. (1995) J. Biol. Chem. 270, 1295–1299
2. Unger, R. H. (1995) Diabetes 44, 863–870
3. Johnson, J. H., Ogawa, A., Chen, L., Ord, L., Newgard, C. B., Alam, T., and Unger, R. H. (1990) Science 250, 546–549
4. Gorden, E. S. (1960) Am. J. Clin. Nutr. 8, 740–747
5. Björntorp, P., Bergman, H., and Varnauskas, E. (1969) Acta Med. Scand. 185, 351–356
6. Greenough, W. B., Crespin, S. R., and Steinberg, D. (1967) Lancet 2, 1334–1336
7. Madison, L. L., Seyffert, W. A., Unger, R. H., and Barker, B. (1968) Metabolism 17, 301–304
8. Crespin, S. R., Greenough, W. B., III, and Steinberg, D. (1969) J. Clin. Invest. 48, 1934–1943
9. Zhou, Y. P., and Grill, V. E. (1994) J. Clin. Invest. 93, 870–876
10. Peterson, R. G., Shaw, W. N., Neel, M., Little, L. A., and Eichberg, J. (1990) ILAR News 32, 16–19
11. Ohneda, M., Johnson, J. H., Lee, Y. H., Nagasawa, Y., and Unger, R. H. (1994) Am. J. Physiol. 267, E988–E974
12. Ohneda, M., Inman, L. R., Unger, R. H. (1995) Diabetologia 38, 173–179
13. Naber, S. P., McDonald, J. M., Jaret, L., McDaniel, M. L., Ludvigsen, C. W., and Lacy, P. E. (1980) Diabetologia 19, 439–444
14. Lee, Y., Hirose, H., Ohneda, M., Johnson, J. H., McGarry, J. D., and Unger, R. H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10878–10882
15. Yalow, R. S., and Berson, S. A. (1960) J. Clin. Invest. 39, 1157–1175
16. Herbert, V., Lau, K. S., Gottlieb, C. W., and Bleicher, S. J. (1965) J. Clin. Endocrinol. Metab. 25, 1375–1384
17. Zawalich, W. S., and Matchinsky, F. M. (1977) Endocrinology 100, 1–8
18. Zawalich, W. S., Pagliara, A. S., and Matchinsky, F. M. (1977) Endocrinology 100, 1276–1283
19. Schutte, B., Reynolds, M. J., Bosman, F. T., and Blijham, G. H. (1987) J. Histochem. Cytochem. 35, 1343–1345
20. Amri, E. Z., Bonino, F., Allahu, G., Abumrad, N. A., and Grimaldi, P. A. (1995) J. Biol. Chem. 270, 2367–2371
21. Randle, P. J. (1986) Biochem. Soc. Trans. 14, 799–806
22. Orellana, A., Hidalgo, P., Morales, M. N., Mezzano, D., and Bronfman, M. (1990) Eur. J. Biochem. 190, 57–63
23. Bronfman, M., Morales, M. N., and Orellana, A. (1988) Biochem. Biophys. Res. Commun. 159, 987–992
24. Orellana, A., Hidalgo, P., Morales, M. N., Mezzano, D., and Bronfman, M. (1993) Eur. J. Biochem. 215, 903–906
25. Schmidt, M. F. G. (1989) Biochim. Biophys. Acta 988, 411–426
26. Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A. (1963) Lancet 1, 785–789
27. Weber, G., Convey, H., Lee, M. A., and Stamm, N. B. (1966) Science 154, 1357–1360
28. Ferrannini, E., Barrett, E. J., Bevilacqua, S., and DeFronzo, R. A. (1983) J. Clin. Invest. 72, 1737–1747
29. Capito, K., Hansen, S. E., Hedekov, C. J., Ilsen, H., and Thams, P. (1992) Acta Diabetol. 28, 193–198
30. McGarry, J. D. (1992) Science 258, 766–770
31. McIlhinney, R. A. J. (1990) Trends Biochem. Sci. 15, 387–391