

Lipotoxicity in Human Pancreatic Islets and the Protective Effect of Metformin

Roberto Lupi,¹ Silvia Del Guerra,¹ Vanna Fierabracci,² Lorella Marselli,¹ Michela Novelli,² Giovanni Patanè,³ Ugo Boggi,⁴ Franco Mosca,⁴ Salvatore Piro,³ Stefano Del Prato,¹ and Piero Marchetti¹

Human pancreatic islets from eight donors were incubated for 48 h in the presence of 2.0 mmol/l free fatty acid (FFA) (oleate to palmitate, 2 to 1). Insulin secretion was then assessed in response to glucose (16.7 mmol/l), arginine (20 mmol/l), and glyburide (200 µmol/l) during static incubation or by perfusion. Glucose oxidation and utilization and intra-islet triglyceride content were measured. The effect of metformin (2.4 µg/ml) was studied because it protects rat islets from lipotoxicity. Glucose-stimulated but not arginine- or glyburide-stimulated insulin release was significantly lower from FFA-exposed islets. Impairment of insulin secretion after exposure to FFAs was mainly accounted for by defective early-phase release. In control islets, increasing glucose concentration was associated with an increase in glucose utilization and oxidation. FFA incubation reduced both glucose utilization and oxidation at maximal glucose concentration. Islet triglyceride content increased significantly after FFA exposure. Addition of metformin to high-FFA media prevented impairment in glucose-mediated insulin release, decline of first-phase insulin secretion, and reduction of glucose utilization and oxidation without significantly affecting islet triglyceride accumulation. These results show that lipotoxicity in human islets is characterized by selective loss of glucose responsiveness and impaired glucose metabolism, with a clear defect in early-phase insulin release. Metformin prevents these deleterious effects, supporting a direct protective action on human β-cells. *Diabetes* 51 (Suppl. 1):S134–S137, 2002

Free fatty acids (FFAs) act as potent signaling molecules in several cellular processes, including insulin secretion (1,2). Experimental evidence indicates that prolonged exposure to high FFA concentrations has detrimental effects on β-cells (lipotoxicity), including reduced glucose-stimulated insulin release, suppressed proinsulin biosynthesis, and β-cell loss by apoptosis (2,3). Thus, prolonged exposure to high FFAs may generate alterations of β-cells, as in type 2 diabetes (4). How this applies to humans, however, is still

controversial. The 24-h infusion of triglyceride inhibited insulin release in control subjects (5), and reduction of plasma FFAs in first-degree relatives of type 2 diabetic subjects improved acute insulin secretion (6). On the other hand, Boden et al. (7) found that prolonged fat infusion stimulated insulin release. Zhou and Grill (8) reported decreased glucose-induced insulin release in human islets incubated for 48 h with 0.125 mmol/l palmitate or oleate. Finally, elevated FFA concentrations were associated with an increased proinsulin/insulin ratio (9), a marker for β-cell dysfunction.

In the present report, we restudied the effect of elevated FFAs on isolated human pancreatic islets. Moreover, because metformin was recently shown to protect rat islets from lipotoxicity (10), its effect on human islets was determined.

RESEARCH DESIGN AND METHODS

Pancreatic islets were isolated from eight donors and cultured in M199 medium as reported (11). Islet aliquots were maintained for 48 h at 37°C in M199 with or without 2.0 mmol/l FFA (oleate to palmitate, 2 to 1; Sigma, St. Louis, MO), with 2% human albumin. Then insulin secretion was studied in batch incubations (6–8 independent pancreases) or by perfusion (four pancreases), as described (11,12). Insulin was measured by immunoradiometric assay (Pantec Forniture Biomediche, Turin, Italy). Glucose utilization and oxidation were assessed (islets from five pancreases) by measuring the formation of ³H₂O from [5-³H]glucose and ¹⁴CO₂ from [U-¹⁴C]glucose, respectively, as described (13). Intra-islet triglyceride concentration was measured by a commercial kit (GPO-Trinder; Sigma) after extraction (13). The effect of a therapeutic concentration (2.4 µg/ml) of metformin (Laboratori Guidotti, Pisa, Italy) was assessed by adding the drug (11) to the 48-h incubation media.

Results are given as means ± SD. Statistical analysis was performed by the

TABLE 1
Insulin release from control, FFA-treated, and FFA-plus-metformin-treated islets

	Insulin release (pmol · islet ⁻¹ · 45 min ⁻¹)			
	3.3 G	16.7 G	3.3 G + arginine	3.3 G + glyburide
Control	22.1 ± 3.1	56.7 ± 12.6*	47.2 ± 9.4*	44.1 ± 9.4*
FFA	18.9 ± 6.3	28.3 ± 6.9†	50.4 ± 12.6*	53.5 ± 11.6*
Metformin	20.7 ± 2.9	46.5 ± 6.4*	—	—
FFA + metformin	22.0 ± 6.3	47.2 ± 6.3*	—	—

Data are means ± SD. Experiments were performed in triplicate for each islet isolation; the number of independent pancreases used was 6–8. Indications of 3.3 and 16.7 G equal 3.3 and 16.7 mmol/l glucose, respectively. Values at 16.7 G were significantly different between groups ($P < 0.01$ by ANOVA); * $P < 0.01$ vs. 3.3 G by Student's *t* test; † $P < 0.05$ vs. control, metformin and FFA + metformin at 16.7 G by the Bonferroni correction.

From the ¹Department of Endocrinology and Metabolism, Metabolic Unit, University of Pisa, Pisa, Italy; ²Department of General Pathology, University of Pisa, Pisa, Italy; ³Department of Endocrinology, University of Catania, Catania, Italy; and ⁴Department of Oncology, University of Pisa, Pisa, Italy.

Address correspondence and reprint requests to marchant@immr.med.unipi.it.

Accepted for publication 30 June 2001.

ANOVA, analysis of variance; FFA, free fatty acid.

The symposium and the publication of this article have been made possible by an unrestricted educational grant from Servier, Paris.

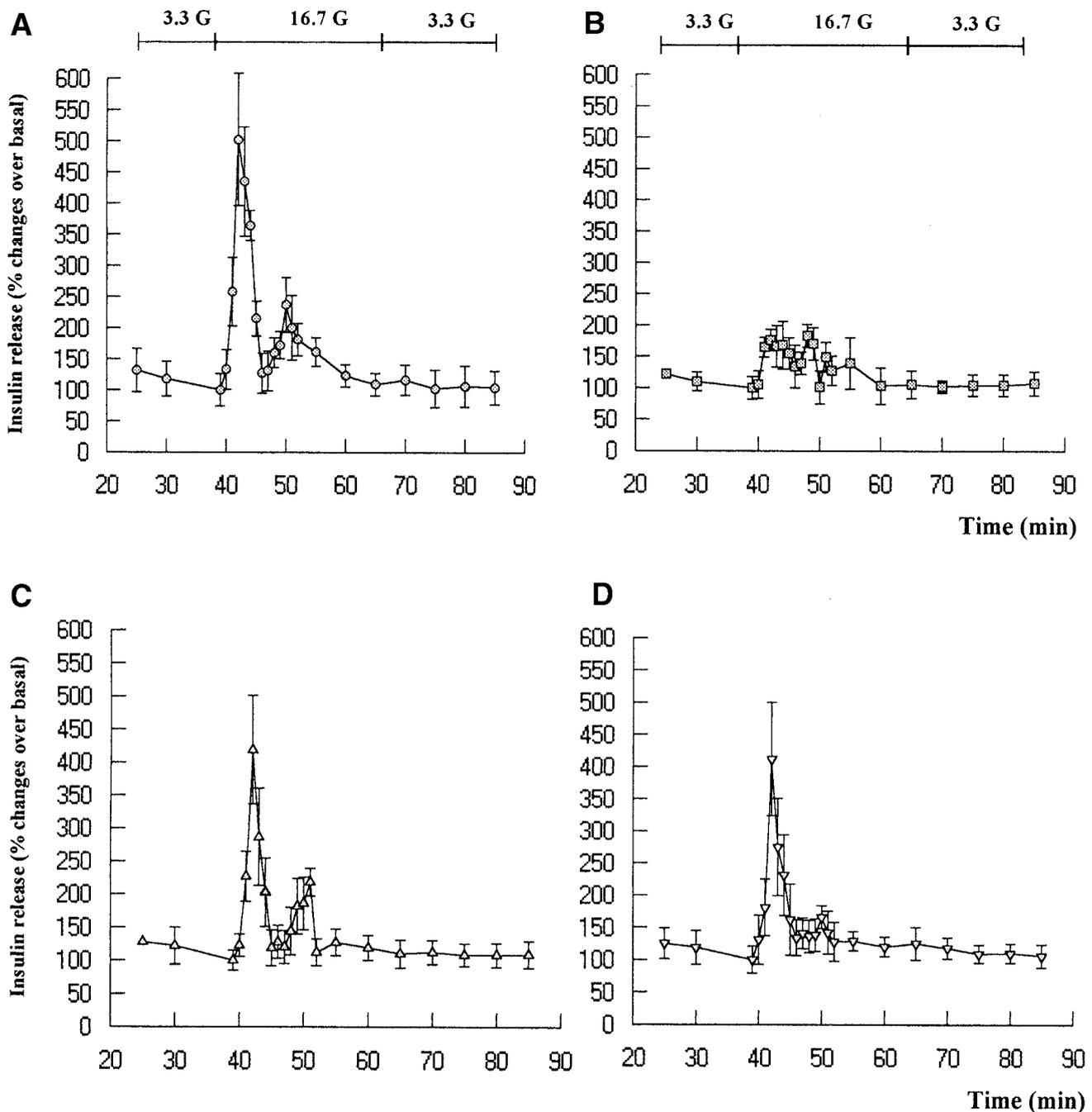


FIG. 1. Islet perfusion experiments (from four different pancreases) showing the dynamics of insulin secretion from control islets (A), islets pre-exposed for 48 h to 2.0 mmol/l FFA (B), metformin (2.4 μ g/ml) alone (C), and FFA (2.0 mmol/l) plus metformin (2.4 μ g/ml) (D). The flow rate was 1 ml/min.

two-tailed Student's *t* test or analysis of variance (ANOVA) (plus the Bonferroni correction).

RESULTS

Incubation of human islets in high-FFA medium was associated with a significant impairment of glucose-stimulated insulin release (56.7 ± 12.6 vs. 28.3 ± 6.9 pmol \cdot islet $^{-1} \cdot 45$ min $^{-1}$; $P < 0.05$), whereas the response to arginine or glyburide was unchanged (Table 1). Metformin did not affect insulin secretion in response to glucose alone. When the drug was added with high FFA, the inhibition of glucose-induced insulin release was pre-

vented (Table 1). The results of perfusion experiments are shown in Fig. 1. These confirmed that pre-exposure to FFAs decreased insulin secretion, mainly accounted for by a reduction of early-phase insulin release (Fig. 1A and B). As in batch incubations, metformin alone did not affect insulin release (Fig. 1C), whereas it abolished the suppressive effect of high FFAs (Fig. 1D).

In control islets, glucose utilization and oxidation increased significantly in response to 16.7 mmol/l glucose (Table 2). The 48-h incubation with 2.0 mmol/l FFA blunted both glucose utilization (71.5 ± 22.6 vs. 135.8 ± 47.7 pmol \cdot islet $^{-1} \cdot 120$ min $^{-1}$) and oxidation (24.7 ± 7.0 vs. $44.9 \pm$

TABLE 2

Glucose utilization and oxidation in control, FFA-treated, and FFA-plus-metformin-treated islets

	Glucose utilization (mmol/l glucose)		Glucose oxidation (mmol/l glucose)	
	3.3	16.7	3.3	16.7
Control	42.5 ± 13.9	135.8 ± 47.7†	19.5 ± 8.7	44.9 ± 13.3*
FFA	26.6 ± 11.4	71.5 ± 22.6‡	17.4 ± 11.2	24.7 ± 7.0‡
Metformin	43.3 ± 10.1	127.4 ± 25.4†	18.7 ± 8.8	37.2 ± 5.0*
FFA + metformin	47.1 ± 14.9	130.0 ± 37.7†	18.3 ± 8.6	32.8 ± 8.7*

Data are means ± SD of five separate experiments and are given in picomoles per islet per 120 min. Values of glucose utilization and oxidation at 16.7 mmol/l glucose differed significantly between groups ($P = 0.01$ and $P = 0.02$ by ANOVA, respectively); * $P < 0.05$ vs. control, 3.3 mmol/l glucose, and † $P < 0.02$ vs. control, 3.3 mmol/l glucose by the Student's t test; ‡ $P < 0.05$ vs. control, 16.7 mmol/l glucose, by the Bonferroni correction.

13.3 pmol · islet⁻¹ · 120 min⁻¹; $P < 0.05$ for both). Metformin had no effect in control incubations, but its addition to high-FFA medium was associated with normalization of glucose fluxes.

In control islets, triglyceride content was 15.0 ± 2.1 ng/islet and increased to 25.4 ± 2.5 ng/islet upon culture in a high-FFA medium ($P < 0.05$). In the islets cultured in the presence of both FFA and metformin, triglyceride content (21.3 ± 3.0 ng/islet) remained higher than that in control islets ($P < 0.05$).

DISCUSSION

We show that in human islets, high FFA levels cause selective loss of glucose responsiveness, which is particularly manifest during the first minutes after glucose challenge (early-phase insulin release). In contrast, insulin secretion in response to arginine and glyburide was not affected. This suggests that in human islets, FFAs selectively impair the release of insulin mediated by glucose, possibly by interfering with its metabolism. In our study, we measured both glucose utilization and oxidation to confirm that high FFA levels hampered both processes. The hypothesis is therefore supported that FFA-induced inhibition of glucose-stimulated insulin release may be related, at least in part, to the glucose-FFA (Randle) cycle (14), i.e., increased availability of FFAs favors their oxidation, leading to impaired glucose metabolism by substrate competition. Indeed, reduced pyruvate-dehydrogenase activity has been observed in islets from rodents exposed to high FFA concentrations (15). In our experiments, incubation with high FFAs was associated with triglyceride accumulation in islets. The role of triglyceride content on islet function remains controversial (16,17) because several studies have shown marked impairment of insulin secretion by FFAs independent from islet triglyceride content. Also in this study, improvement of islet function by metformin was not associated with a significant reduction of triglyceride content, but with normalization of glucose utilization and oxidation. These effects of metformin on glucose metabolism in islets are similar to effects at the level of peripheral tissues (18). Our results with the therapeutic concentration of metformin are similar to findings in rat islets, in which metformin caused reduction of FFA oxidation (10). Moreover, metformin has

been reported to prevent the development of diabetes in the Zucker diabetic fatty rat (19). Finally, treatment with drugs able to reduce plasma FFA levels or their oxidation, such as acipimox (6) or glitazones (19–21), can improve β -cell function, supporting the concept of interplay between FFAs and glucose metabolism.

Overall, the results of the present study are consistent with the concept that raised concentrations of circulating FFAs may contribute to the development of abnormalities in human islet function. Drugs improving glucose utilization and oxidation at the islet level, such as metformin, can prevent the deleterious effects of FFAs. From a clinical point of view, these results lend support to intervention aiming at normalizing lipid metabolism and reducing plasma FFA levels.

ACKNOWLEDGMENTS

This work was supported in part by the Italian Ministero Università e Ricerca Scientifica e Tecnologica (COFIN 2000).

REFERENCES

- Crespin SR, Greeough WB, Steinberg D: Stimulation of insulin secretion by long-chain free fatty acids: a direct pancreatic effect. *J Clin Invest* 52:1979–1984, 1973
- McGarry JD, Dobbins RL: Fatty acid, lipotoxicity and insulin secretion. *Diabetologia* 42:128–138, 1999
- Shimabukuro M, Higa M, Zhou YT, Wang MY, Newgard CB, Unger RH: Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats: role of serine palmitoyltransferase overexpression. *J Biol Chem* 273:32487–32490, 1998
- Unger RH: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications. *Diabetes* 44:863–870, 1995
- Paolisso G, Gambardella A, Amato L, Tortorello R, D'Amore A, Varricchio M, D'Onofrio F: Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* 38:1295–1299, 1995
- Paolisso G, Tagliamonte MR, Rizzo MR, Gualdiero P, Saccomanno G, Gambardella A, Giugliano D, D'Onofrio F, Howard BV: Lowering fatty acids potentiates acute insulin response in first degree relatives of people with type 2 diabetes. *Diabetologia* 41:1127–1132, 1998
- Boden G, Chen X, Rosner J, Barton M: Effects of a 48h fat infusion on insulin secretion and glucose utilization. *Diabetes* 44:1239–1242, 1995
- Zhou YP, Grill V: Long-term exposure to fatty acids and ketones inhibits B-cell functions in human pancreatic islets of Langerhans. *J Clin Endocrinol Metab* 80:1584–1590, 1995
- Bjorklund A, Grill V: Enhancing effects of long-term elevated glucose and palmitate on stored and secreted proinsulin-to-insulin ratios in human pancreatic islets. *Diabetes* 48:1409–1414, 1999
- Patanè G, Piro S, Rabuazzo AM, Anello M, Vigneri R, Purrello F: Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. *Diabetes* 49:735–740, 2000
- Lupi R, Del Guerra S, Tellini C, Giannarelli R, Coppelli A, Lorenzetti M, Carmellini M, Mosca F, Navalesi R, Marchetti P: The biguanide compound metformin prevents desensitization of human pancreatic islets induced by high glucose. *Eur J Pharmacol* 8:205–209, 1999
- Marchetti P, Dotta F, Ling Z, Lupi R, Del Guerra S, Santangelo C, Realacci M, Marselli L, Di Mario U, Navalesi R: The function of pancreatic islets isolated from a type 1 diabetic patient. *Diabetes Care* 23:701–703, 2000
- Purrello F, Buscema N, Rabuazzo AM, Caltabiano V, Forte F, Vinci C, Vetri M, Vigneri R: Glucose modulated glucose transporter affinity, glucokinase activity and secretory response in rat pancreatic beta-cells. *Diabetes* 42:199–205, 1993
- Zhou YP, Grill VE: Long-term exposure of rat pancreatic islets to fatty acid inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. *J Clin Invest* 93:870–876, 1994
- Zhou YP, Berggren PO, Grill VE: A fatty acid-induced decrease in pyruvate dehydrogenase activity is an important determinant of beta-cell dysfunction in the obese diabetic db/db mouse. *Diabetes* 45:580–586, 1996
- Segall L, Lameloise N, Assimacopoulos-Jeannet F, Roche E, Corkey P, Thumelin S, Corkey BE, Prentki M: Lipid rather than glucose metabolism

- is implicated in altered insulin secretion caused by oleate in INS-1 cells. *Am J Physiol* 277:E521-E258, 1999
17. De Souza CJ, Capotorto JV, Cornell-Kennon S, Wu YJ, Steil GM, Trivedi N, Weir GC: Beta-cell dysfunction in 48h glucose-infused rats is not a consequence of elevated plasma lipid or islet triglyceride levels. *Metabolism* 49:755-759, 2000
18. Riccio A, Del Prato S, Vigili de Kreutzenberg S, Tiengo A: Glucose and lipid metabolism in non-insulin-dependent diabetes: effect of metformin. *Diabete Metab* 17:180-184, 1991
19. Sreenan S, Sturis J, Pugh W, Burant CF, Polonski KS: Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin or troglitazone. *Am J Physiol* 271:E742-E747, 1996
20. Oakes ND, Camilleri S, Furler SM, Chisholm DJ, Kraegen EW: The insulin sensitizer BRL 49653 reduces systemic fatty acid supply and utilization and tissue lipid availability in the rat. *Metabolism* 46:935-942, 1997
21. Buckingham RE, Al-Barazanji KA, Toseland CD, Slaughter M, Connor SC, West A, Bond B, Turner NC, Clapham JC: Peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, protects against nephropathy and pancreatic islet abnormalities in Zucker fatty rats. *Diabetes* 47:1326-1334, 1998