Additive Hypoglycemic Effects of Drugs That Modify Free–Fatty Acid Metabolism by Different Mechanisms in Rats With Streptozocin-Induced Diabetes

GERALD M. REAVEN, HELEN CHANG, AND BRIAN B. HOFFMAN

In this study the effect of two drugs [etomoxir and nicotinic acid (NA)] on plasma glucose, free–fatty acid (FFA), and triglyceride (TG) concentrations was determined in rats with streptozocin (STZ)-induced diabetes. The two compounds modify FFA metabolism by different mechanisms, etomoxir (ethyl-2-[6-(4-chlorophenoxyl)-hexyl]oxirane-2-carboxylate) by inhibiting hepatic fatty acid oxidation, and NA by inhibiting lipolysis in adipose tissue. Diabetes was induced in male Sprague-Dawley rats, weighing ~400 g, by STZ injection (30 mg/kg i.v.), and the metabolic effects of the two drugs were studied 7–10 days later. The acute administration of either etomoxir or NA lowered plasma glucose concentrations in diabetic rats by ~150 mg/dl ($P < .001$) in 4 h. However, the two drugs differed dramatically in their effects on plasma FFA and TG concentrations. Specifically, etomoxir produced striking increases in plasma FFA and TG concentrations, whereas NA administration caused a marked decrease. However, when NA was given in conjunction with etomoxir, NA prevented the increase in plasma FFA and TG concentration seen with etomoxir; the combination of NA and etomoxir approximately doubled the decrease in plasma glucose concentration produced by NA or etomoxir when given alone. Because plasma insulin concentrations did not change in response to either drug, whether administered singly or in combination, these metabolic effects do not result from a change in insulin secretion. These results suggest that modulation of FFA metabolism at the level of the adipocyte or the liver can have dramatic effects on carbohydrate and lipid metabolism. Also, NA prevented the potentially adverse effects that etomoxir had on FFA and TG. The observation that the two drugs given in combination were capable of lowering plasma glucose from >400 mg/dl to essentially normal levels within 4 h raises the possibility that this pharmacologic approach may have relevance to the treatment of diabetes. *Diabetes* 37:28–32, 1988

The acute administration of either nicotinic acid (NA) or phenylisopropyladenosine (PIA), two potent antilipolytic agents, has been shown to lower elevated plasma free–fatty acid (FFA) concentrations in a rat model of non-insulin-dependent diabetes mellitus (NIDDM) (1). The fall in plasma FFA concentration was accompanied by a decrease in plasma triglyceride (TG) concentration, secondary to a reduction in very-low-density lipoprotein (VLDL)-TG secretion rate (1). More recently, we have presented evidence that plasma glucose concentrations also fall when rats with the same experimental form of NIDDM are given either NA or PIA (2). The latter observation emphasizes that changes in FFA metabolism can profoundly modulate glucose homeostasis, a notion introduced by Randle and colleagues >20 yr ago (3,4). Further evidence that changes in FFA metabolism can affect hepatic carbohydrate metabolism has come from the demonstration that compounds reducing the activity of the carnitine palmitoyltransferase (CPT) system, and thereby interfering with long-chain fatty acid oxidation, can lower plasma glucose levels in normal and diabetic rodents (5–7). It has been suggested that the hypoglycemic potency of agents that act in this manner is due to their ability to suppress hepatic gluconeogenesis (8), which also seems to contribute to the ability of NA to lower plasma glucose in diabetic rats (2). Thus, it appears that decreases in the rate of fatty acid oxidation, whether by specific inactivation of the CPT system or simply by decreasing FFA flow to the liver (NA and PIA), can reduce hyperglycemia in diabetic rodents. On the other hand, the impact of these two classes of compounds on FFA and TG metabolism in diabetic rodents is probably quite different. Our study was initiated to explore this issue by comparing

From the Department of Medicine, Stanford University School of Medicine, and the Geriatric Research, Education, and Clinical Center, Veterans Administration Medical Center, Palo Alto, California.
Address correspondence and reprint requests to Dr. Gerald M. Reaven, Veterans Administration (GRECC/182B), 3801 Miranda Ave., Palo Alto, CA 94304.
Received for publication 11 February 1987 and accepted in revised form 8 June 1987.

the acute effects of NA and a newly described inhibitor of the CPT system (9), etomoxir (ethyl-2-[6-(4-chlorophenoxyl)-hexyl]oxirane-2-carboxylate), on glucose, FFA, and TG metabolism in an experimental rat model of NIDDM.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 4 mo of age and weighing ~400 g, were used for all experiments. Diabetes was induced by injection of 30 mg/kg i.v. streptozocin (STZ) in citrate buffer, pH 4.5, and serum glucose concentration was measured 7 days later. The diabetic animals used for these studies had serum glucose concentrations >350 mg/dl, and experiments involving these animals were carried out 8–10 days after diabetes had been induced. Previous studies with this model have indicated that rats of this age do not become catabolic or ketotic when injected with comparable amounts of STZ and gain weight at the same rate as their nondiabetic littermates (1). All experiments were initiated at 1400 h, after the removal of food at 0800 h.

Etomoxir (50 mg/kg) or vehicle was administered by gastric tube, and blood was collected from the tail vein of unanesthetized rats for measurement of plasma glucose (10), insulin (11), FFA (12), and TG (13) concentration before and 90 and 180 min after the test substance was administered. When NA was given, either alone or in combination with etomoxir, 200 μmol/kg was injected subcutaneously at the start of the experiment and 60 and 120 min later. An equal volume of 0.9% NaCl was used as a control injection. The dose of nicotinic acid was based on results from our laboratory that have shown that plasma glucose levels fall in diabetic rats in response to this treatment schedule (2). The etomoxir was generously provided by H.P.O. Wolf (Byk Gulden Pharmazeutika) and administered at a dose demonstrated to lower plasma glucose concentration in rats with STZ-induced diabetes (9).

Data are expressed as means ± SE, and statistical significance was determined by Student's two-tailed *t* test and analysis of variance (14,15).

RESULTS

The effect of etomoxir on plasma glucose, FFA, and TG concentrations in diabetic and control rats is shown in Fig. 1. The data in Fig. 1, *left panel*, demonstrate that the plasma glucose concentration fell ~175 mg/dl in the 180 min after the gastric administration of etomoxir in rats with diabetes. In contrast, the fall in mean plasma glucose level 180 min after etomoxir in control rats was only 24 mg/dl. This difference was highly significant (*t* test, $P < .001$).

The results in Fig. 1, *middle panel*, indicate that plasma FFA concentrations increased substantially after the ingestion of etomoxir in both groups of rats, with increments above the baseline of 0.77 and 0.70 meq/L in diabetic and control rats, respectively. Although FFA levels rose to a similar degree in both groups, it is apparent that the absolute plasma FFA concentrations remained higher throughout in diabetic rats.

The effect of etomoxir on plasma TG levels is seen in Fig. 1, *right panel*, and it is clear that plasma TG concentrations more than doubled in the diabetic rats. In marked contrast, plasma TG levels did not increase above baseline values in etomoxir-treated control rats. The difference in the TG response of the two groups was also highly significant (*t* test, $P < .001$).

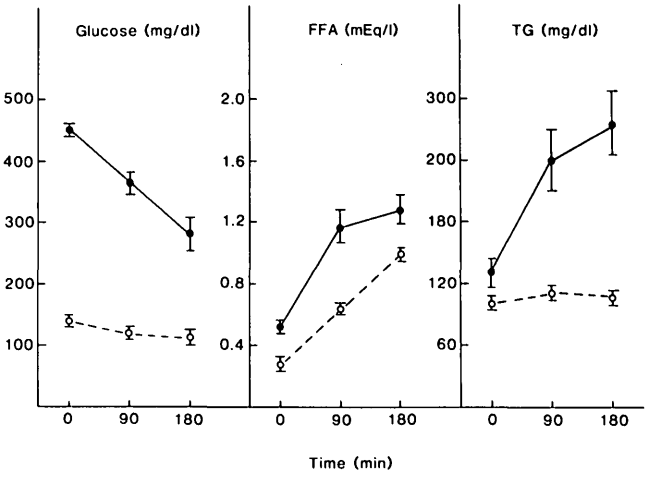

FIG. 1. Means ± SE of plasma glucose, free fatty acid (FFA), and triglyceride (TG) concentrations before (0 min) and 90 and 180 min after administration of 50 mg/kg etomoxir to normal (○) or diabetic (●) rats. *n* = 12 rats in each group.

The results in Fig. 2 compare the effects of administering vehicle, etomoxir, NA, or etomoxir plus NA to diabetic rats. The differences in the glucose, FFA, and TG responses to the four treatments shown were evaluated by two-way analysis of variance. The data in Fig. 2, *left panel*, indicate that plasma glucose concentrations were significantly ($P < .001$) lower after either etomoxir or NA than after the vehicle and that the magnitude of the fall was similar. However, when the two drugs were given together, plasma glucose concentrations were significantly ($P < .001$) lower than when either drug was given alone.

The pattern of the plasma FFA responses shown in Fig. 2, *middle panel*, were more complicated. Specifically, there was a significant ($P < .001$) increase in plasma FFA levels after etomoxir compared with administration of vehicle. In contrast, there was a significant ($P < .001$) decrease in plasma FFA concentrations when diabetic rats received either NA or NA plus etomoxir.

The data in Fig. 2, *right panel*, demonstrate that the changes in plasma TG concentrations paralleled the effects of the four treatments on plasma FFA levels. Thus, etomoxir administration led to a significant ($P < .001$) increase in plasma TG concentrations, whereas plasma TG concentrations fell significantly ($P < .001$) after either NA or etomoxir plus NA.

Table 1 lists the plasma insulin values in control and diabetic rats before and after administration of etomoxir, NA, or etomoxir plus NA. Apparently, plasma insulin concentrations are somewhat lower in the diabetic rats, but they did not change after any of the treatments. Thus, none of the drug effects on glucose, FFA, or TG metabolism seen in Figs. 1 and 2 were associated with changes in β-cell function.

DISCUSSION

The results demonstrate that etomoxir, a recently described inhibitor of the hepatic CPT system (9), is capable of acutely lowering plasma glucose concentrations in diabetic rodents. A similar fall in plasma glucose concentration after the

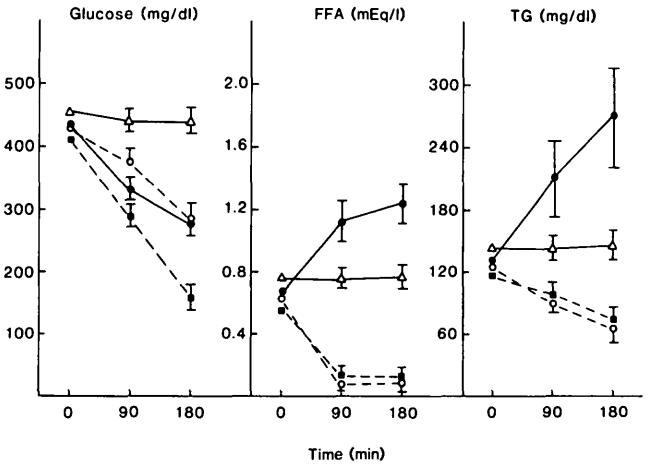

FIG. 2. Means ± SE of plasma glucose, free fatty acid (FFA), and triglyceride (TG) concentrations before (0 min) and 90 and 180 min after administration of 4 test substances to diabetic rats. *n* = 8 rats in each group. They were given either vehicle (△), nicotinic acid (○), etomoxir (●), or etomoxir plus nicotinic acid (■).

administration of two other compounds that have a comparable biochemical effect on the liver has been previously reported (5–7). The metabolic effect of all three drugs is probably related to the decrease in hepatic FFA oxidation and gluconeogenesis that is associated with inhibition of the hepatic CPT system (5–7). It is not surprising that plasma glucose concentration falls dramatically in diabetic rats when gluconeogenesis is reduced, but why this should occur when fatty acid oxidation is inhibited is less clear. It has been suggested that when hepatic oxidation of fatty acid is blocked, the liver must oxidize carbohydrate for this purpose (16,17). If this were the case, a diversion of carbohydrate from the gluconeogenic to the oxidative pathway could account for the observed fall in plasma glucose.

Less speculative is the explanation for the increase in TG concentrations observed after administration of etomoxir to the diabetic rats. Previous studies of rats with this form of experimentally induced diabetes have shown that hepatic VLDL secretion and plasma TG concentrations increase in response to an elevation of FFA concentration (18). This phenomenon was demonstrated in vitro with perfused rat liver as well as in vivo. Further evidence for the central role of elevated FFA levels as a cause of the increase in plasma TG concentration seen in etomoxir-treated diabetic rats is the ability of NA, which lowers plasma FFA, to prevent the rise in plasma TG concentration in response to etomoxir. Thus, inhibition of hepatic acid oxidation by etomoxir in rats with this form of diabetes would be expected to result in a rise in hepatic and plasma FFA concentration, which should lead to hypertriglyceridemia. If NA blocks the increase in FFA concentration in etomoxir-treated rats, TG concentrations will not rise. However, the observation that the increase in plasma FFA concentration after etomoxir administration to normal rats was not associated with hypertriglyceridemia is unexplained and worthy of further study.

In addition to providing insight into the in vivo consequences of inhibiting the hepatic CPT system, the results presented have important implications for both the pathogenesis and the treatment of NIDDM. In the former instance, these data provide further support for the view that defects in regulation of FFA metabolism may be responsible for the development of severe fasting hyperglycemia in patients with NIDDM. This notion evolved from the observation that both fasting and postprandial plasma FFA concentrations are directly related to levels of glycemia in patients with NIDDM (19,20), being normal in those with minimal fasting hyperglycemia (<140 mg/dl) and significantly greater than normal in those with severe fasting hyperglycemia (>240 mg/dl). Furthermore, results of several studies have documented the presence of a direct relationship between magnitude of hyperglycemia and elevation of plasma FFA concentration (20–22). Finally, there is considerable evidence that the degree of fasting hyperglycemia is directly related to hepatic glucose production rate in patients with NIDDM (21–24).

We believe that all these disparate observations can be integrated into one hypothesis based on the premise that elevated ambient FFA concentrations play an important role in stimulation of hepatic glucose output, via modulation of hepatic gluconeogenesis, leading to elevations of plasma glucose concentration. Within this framework, the effect on plasma glucose concentration of etomoxir, NA, and etomoxir plus NA can be explained. Inhibitors of the hepatic CPT system lead to a reduction in long-chain fatty acid oxidation and gluconeogenesis. If an increased rate of hepatic gluconeogenesis plays an important role in the development of fasting hyperglycemia in diabetes, the fall in plasma glucose concentration associated with etomoxir administration is easily understood. It can also be argued that NA exerts its hypoglycemic effect by decreasing hepatic gluconeogenesis, albeit less directly. We have recently shown that NA and PIA (an adenosine agonist), both of which act to decrease lipolysis and reduce plasma FFA concentration, also lower plasma glucose levels in rats, which is associated with a fall in hepatic glucose production (2). The apparent relationship between plasma FFA concentration and hepatic glucose production in NA-treated diabetic rats could be explained by the known effect of fatty acid oxidation on pyruvate dehydrogenase activity (25,26). Thus, a reduction in rate of hepatic and muscle fatty acid oxidation in diabetic rats, secondary to a fall in plasma FFA level, would be expected to lead to an increase in activity of pyruvate dehydrogenase, resulting in a decrease in both conversion of glucose to lactate in muscle and in hepatic gluconeogenesis.

TABLE 1
Plasma insulin concentrations

Group	Treatment	Insulin concentrations (μU/ml) Before	After 90 min	After 180 min
Control	Vehicle	27 ± 4	25 ± 5	25 ± 4
Diabetic	Etomoxir	16 ± 2	18 ± 1	19 ± 1
Diabetic	Nicotinic acid	17 ± 2	16 ± 1	14 ± 1
Diabetic	Etomoxir plus nicotinic acid	14 ± 1	13 ± 1	15 ± 1

Values are means ± SE. There were no significant differences in insulin concentration. *n* = 8 in each group.

Although this sequence of events is clearly speculative, it offers an explanation for the ability of NA to lower plasma glucose in diabetic rats. Because etomoxir and NA appear to modulate hepatic gluconeogenesis by different mechanisms, the observation that the ability of the two compounds to reduce plasma glucose was additive should not be surprising. It therefore seems apparent that abnormalities of FFA metabolism play a substantial role in the genesis of fasting hyperglycemia.

In addition to providing insight into the pathogenesis of NIDDM, our data are relevant to treatment of this syndrome. There is much experience with the use of NA in both animals and humans with diabetes (27–33). Unfortunately, the results of these studies are confusing, containing evidence indicating that NA has no effect, lowers blood glucose, or produces hyperglycemia. The reason(s) for this lack of consistency in the data is difficult to understand and probably results from, for example, differences in experimental protocol, dose of NA, or nutritional status of the diabetic subjects. We have found that NA consistently lowers the plasma glucose level in our experimental rat model of diabetes (2). Similarly, we have shown that the administration of the potent antilipolytic agent PIA, an α_1-adenosine receptor agonist, lowers plasma glucose concentration in diabetic rodents (2). Furthermore, the fall in plasma glucose concentration after PIA or NA is apparently associated with a fall in plasma FFA and TG concentrations. Because both plasma FFA and TG concentrations are often elevated in NIDDM, this combination of therapeutic events is desirable.

In contrast, the effect of etomoxir to lower plasma glucose was associated with a marked increase in plasma FFA and TG concentrations. Given that etomoxir inhibits long-chain FFA oxidation, it is not surprising that plasma FFA concentrations increase. Furthermore, if FFA oxidation is reduced, the possibility that this would lead to increased esterification of α-glycerophosphate and hypertriglyceridemia is clear. On the other hand, some of the findings with etomoxir were unexpected. For example, it has been reported that administration of the related compound clomoxir only leads to an increase in plasma FFA and TG concentrations in acute experiments in fasted animals (7). The results in Fig. 1 suggest that significant increases in plasma FFA concentrations were seen in control and diabetic rats when etomoxir was given to nonfasted rats. However, a significant rise in TG concentration was only seen in the diabetic rats. The reason for the difference in the effect of etomoxir on TG metabolism in control and diabetic rats is not clear, but the fact that hypertriglyceridemia occurred when diabetic rats were given etomoxir raises substantial questions about the use of this class of drug in the treatment of NIDDM. However, the data in Fig. 2 indicate that the untoward effects of etomoxir were prevented when given in combination with NA. Of even greater interest was the fact that the plasma glucose concentration fell from a mean of 420 ± 6 to 153 ± 14 mg/dl within 180 min of receiving etomoxir plus NA—a dramatic fall in plasma glucose concentration in the absence of any change in plasma insulin concentration.

Although the implications of our data in the treatment of NIDDM are suggestive, note the dangers of extrapolating data from animal to human studies. On the other hand, our animal model of experimental diabetes greatly resembles NIDDM. Old, overweight rats are insulin resistant and hyperinsulinemic (18,34,35), and we have shown that the administration of small doses of STZ leads to a dramatic increase in plasma glucose, FFA, and TG concentrations. At the same time, the fall in plasma insulin levels after low-dose STZ is modest (18), and animals thus treated are not catabolic and gain weight at the same rate as their age-matched controls. The combination of "normal" insulin levels, hyperglycemia, increased FFA levels, and hypertriglyceridemia replicates all the metabolic characteristics of patients with NIDDM. Therefore, we believe that the results of the animal data are greatly relevant to NIDDM.

In conclusion, we have shown that agents that modulate FFA metabolism by suppression of hepatic oxidation of long-chain fatty acids (etomoxir) or adipose tissue lipolysis (NA) can significantly lower plasma glucose concentration in an animal model of NIDDM. Furthermore, when etomoxir and NA were given together, they were capable of reducing plasma glucose concentration by ~300 mg/dl in diabetic rats. Finally, the acute deleterious effects of etomoxir on plasma FFA and TG concentrations were entirely prevented when it was administered in combination with NA. These results provide further support for the view that defects in FFA metabolism play a central role in the pathogenesis of NIDDM and focus on an approach to therapy in patients that may uniquely benefit.

ACKNOWLEDGMENTS

This study was supported in part by research grants from the Sandoz Research Institute and the National Institutes of Health (DK-30732).

B.B.H. is an Established Investigator of the American Heart Association.

REFERENCES

1. Hoffman BB, Dall'Aglio E, Hollenbeck C, Chang H, Reaven GM: Suppression of free fatty acids and triglycerides in normal and hypertriglyceridemic rats by the adenosine receptor agonist phenylisopropyladenosine. *J Pharmacol Exp Ther* 239:715–18, 1986
2. Reaven GM, Chang H, Ho H, Hoffman BB: Documentation of the hypoglycemic action of an adenosine agonist in insulin-deficient rats (Abstract). *Diabetes* 35 (Suppl. 1):89A, 1986
3. Randle PJ, Hales CN, Garland PB, Newsholme EA: The glucose fatty-acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1:785–89, 1963
4. Hales CN, Randle PJ: Effects of low-carbohydrate diet and diabetes mellitus on plasma concentrations of glucose, non-esterified fatty acids, and insulin during oral glucose tolerance tests. *Lancet* 1:790–94, 1963
5. Tutwiler GF, Kirsh T, Mohrbacher RJ, Ho W: Pharmacologic profile of methyl 2-tetradecylglycidate (McN-3716)—an orally effective hypoglycemic agent. *Metab Clin Exp* 27:1539–56, 1978
6. Tutwiler GF, Mohrbacher R, Ho W: Methyl 2-tetradecylglycidate, an orally effective hypoglycemic agent that inhibits long chain fatty acid oxidation selectively. *Diabetes* 28:242–48, 1979
7. Wolf HPO, Eistetter K, Ludwig G: Phenylalkyloxirane carboxylic acids, a new class of hypoglycaemic substances: hypoglycaemic and hypoketonaemic effects of sodium 2-[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate (B 807-27) in fasted animals. *Diabetologia* 22:456–63, 1982
8. Wolf HPO, Engel DW: Decrease of fatty acid oxidation, ketogenesis and gluconeogenesis in isolated perfused rat liver by phenylalkyl oxirane carboxylate (B 807-27) due to inhibition of CPT I (EC 2.3.1.21). *Eur J Biochem* 146:359–63, 1985
9. Wolf HPO: The effect of etomoxir on glucose tolerance in normal and diabetic rodents (Abstract). *Diabetes Res Clin Pract* (Suppl. 1):S607, 1987
10. Kadish AH, Litle RL, Sternberg JC: A new and rapid method for determination of glucose by measurement of rate of oxygen consumption. *Clin Chem* 14:116–31, 1968
11. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free

and antibody bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab* 33:732–38, 1971
12. Noma A, Okabe H, Kita M: A new colorimetric micro-determination of free fatty acids in serum. *Clin Chim Acta* 43:317–20, 1973
13. Wahlfeld AW: Triglyceride determination after enzymatic hydrolysis. In *Methods of Enzymatic Analysis*. Bergmeyer HV, Ed. New York, Academic, 1974, p. 1831–35
14. Winer BJ: *Statistical Principles in Experimental Design*. New York, McGraw-Hill, 1971, p. 514–99
15. Godfrey K: Statistics in practice: comparing the means of several groups. *N Engl J Med* 313:1450–56, 1985
16. Young JC, Bryan JE, Constable SH, Tutwiler GF, Holloszy JO: Effects of the oral hypoglycemic agent methyl palmoxirate on exercise capacity of rats. *Can J Physiol Pharmacol* 62:815–18, 1974
17. Young JC, Treadway JL, Fader EI, Caslin RF: Effects of oral hypoglycemic agent methylpalmoxirate on exercise capacity of streptozocin diabetic rats. *Diabetes* 35:744–48, 1986
18. Weiland O, Mondon C, Reaven GM: Evidence for multiple causality in the development of diabetic hypertriglyceridemia. *Diabetologia* 18:355–40, 1980
19. Fraze E, Donner CC, Swislocki ALM, Chiou Y-AM, Chen Y-DI, Reaven GM: Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance. *J Clin Endocrinol Metab* 61:807–11, 1985
20. Golay A, Chen Y-DI, Reaven GM: Effect of differences in glucose tolerance on insulin's ability to regulate carbohydrate and free fatty acid metabolism in obese individuals. *J Clin Endocrinol Metab* 62:1081–88, 1986
21. Bogardus C, Lillioja S, Howard BV, Reaven G, Mott D: Relationships between insulin secretion, insulin action and fasting plasma glucose concentration in nondiabetic and noninsulin-dependent diabetic subjects. *J Clin Invest* 74:1238–46, 1984
22. Golay A, Swislocki ALM, Chen Y-DI, Reaven GM: Relationships between plasma free fatty acid concentration, endogenous glucose production, and fasting hyperglycemia in normal and non-insulin dependent diabetic individuals. *Metabolism* 36:692–96, 1987
23. DeFronzo RA, Ferrannini E, Kovisto V: New concepts in the pathogenesis and treatment of noninsulin-dependent diabetes mellitus. *Am J Med* 74:52–81, 1983
24. Revers RR, Fink R, Griffin J, Olefsky JM, Kolterman OG: Influence of hyperglycemia on insulin's in vivo effects in type II diabetes. *J Clin Invest* 73:664–72, 1984
25. Olson MS, Scholz R, Buffington C, Dennis SC, Padma A, Patel TB, Waymack P, Debuysere MS: Regulation of α-ketoacid dehydrogenase multienzyme complexes in isolated perfused organs. In *The Regulation of Carbohydrate Formation and Utilization in Mammals*. Veneziale CM, Ed. Baltimore, MD, University Park, 1981, p. 153–89
26. Randle PJ: α-Ketoacid dehydrogenase complexes and respiratory fuel utilisation in diabetes. *Diabetologia* 28:479–84, 1985
27. Root MA, Ashmore J: The hypoglycemic activity of nicotinic acid in rats. *Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol* 248:117–23, 1964
28. Gross RC, Carlson LA: Metabolic effects of nicotinic acid in acute insulin deficiency in the rat. *Diabetes* 17:353–61, 1968
29. Felber JP, Büber V: Effect of nicotinic acid derivative on glucose levels and glucose tolerance in animal and in man. In *Metabolic Effects of Nicotinic Acid and Its Derivatives*. Gey KF, Carlson LA, Eds. Bern, Huber, 1970, p. 695–710
30. Stowers JM, Brewsher PD, Stein JM, Mowat J: Studies on the effects of nicotinic acid given orally or intravenously on oral and intravenous glucose tolerance in man. In *Metabolic Effects of Nicotinic Acid and Its Derivatives*. Gey KF, Carlson LA, Eds. Bern, Huber, 1970, p. 723–32
31. Dzedin T, Svedmyr N, Lundholm L: Influence of nicotinic acid on carbohydrate metabolism: relationship with adrenergic receptors. In *Metabolic Effects of Nicotinic Acid and Its Derivatives*. Gey KF, Carlson LA, Eds. Bern, Huber, 1970, p. 747–52
32. Froesch ER, Waldvogel M, Oelz O: Effects of nicotinic acid and structurally related drugs on adipose tissue in vitro and on carbohydrate and lipid metabolism of normal and diabetic rats in vivo. In *Metabolic Effects of Nicotinic Acid and Its Derivatives*. Gey KF, Carlson LA, Eds. Bern, Huber, 1970, p. 773–94
33. Ammon HPT, Estler C-J, Heim F: Alteration of carbohydrate metabolism in liver, skeletal muscle and brain by nicotinic acid in mice. In *Metabolic Effects of Nicotinic Acid and Its Derivatives*. Gey KF, Carlson LA, Eds. Bern, Huber, 1970, p. 799–810
34. Reaven E, Wright D, Mondon CE, Solomon R, Ho H, Reaven GM: Effect of age and diet on insulin secretion and insulin action in the rat. *Diabetes* 32:175–80, 1983
35. Narimiya M, Azhar S, Dolkas CB, Mondon CE, Sims C, Wright DW, Reaven GM: Insulin resistance in older rats. *Am J Physiol* 246:E397–404, 1984