Acute lowering of circulating fatty acids improves insulin secretion in a subset of type 2 diabetes subjects

ELISABETH QVIGSTAD, INGRID L. MOSTAD, KRISTIAN S. BJERVE, AND VALDEMAR E. GRILL
Departments of Endocrinology and Clinical Chemistry, Faculty of Medicine, Norwegian University of Science and Technology, N-7489 Trondheim, Norway

Submitted 13 March 2002; accepted in final form 9 September 2002

Qvigstad, Elisabeth, Ingrid L. Mostad, Kristian S. Bjerve, and Valdemar E. Grill. Acute lowering of circulating fatty acids improves insulin secretion in a subset of type 2 diabetes subjects. Am J Physiol Endocrinol Metab 284: E129–E137, 2003; 10.1152/ajpendo.00114.2002.—We tested the effects of acute perturbations of elevated fatty acids (FA) on insulin secretion in type 2 diabetes. Twenty-one type 2 diabetes subjects with hypertriglyceridemia (tripalicylglycerol >2.2 mmol/l) and 10 age-matched nonobese subjects participated. Glucose-stimulated insulin secretion was monitored during hyperglycemic clamps for 120 min. An infusion of Intralipid and heparin was added during minutes 60–120. In one of two tests, the subjects ingested 250 mg of Acipimox 60 min before the hyperglycemic clamp. A third test (also with Acipimox) was performed in 17 of the diabetic subjects after 3 days of a low-fat diet. Acipimox lowered FA levels and enhanced insulin sensitivity in nondiabetic and diabetic subjects alike. Acipimox administration failed to affect insulin secretion rates in nondiabetic subjects and in the group of diabetic subjects as a whole. However, in the diabetic subjects, Acipimox increased integrated insulin secretion rates during minutes 60–120 in the 50% having the lowest levels of hemoglobin A1c (379 ± 34 vs. 326 ± 30 pmol·kg⁻¹·min⁻¹ without Acipimox, P < 0.05). A 3-day dietary intervention diminished energy from fat from 39 to 23% without affecting FA levels and without improving the insulin response during clamps. Elevated FA levels may tonically inhibit stimulated insulin secretion in a subset of type 2 diabetic subjects. Acipimox; low-fat diet

AN ELEVATED PLASMA LEVEL of fatty acids (FA) is a risk factor for type 2 diabetes (8, 24). The risk has been ascribed to an insulin resistance-inducing effect of FA in skeletal muscle and in liver (3). Randle and colleagues proposed many years ago that resistance may be due to a glucose-fatty acid cycle, i.e., a reciprocal relationship between the metabolism of FA and glucose in which abundance of FA decreases the uptake and metabolism of glucose (reviewed in Refs. 27, 28). The pyruvate dehydrogenase (PDH) complex played a key role in this concept. It was demonstrated in liver, heart, and skeletal muscle that FA decreased PDH activity through activation of PDH kinase (27, 28). Evidence was presented that an ambient effect of FA on PDH kinase activity was supplemented by a time-dependent one. Many data support the operation of the glucose-fatty acid cycle (27), although alternative concepts have also been put forward (17, 37).

More recent evidence indicates that elevated FA can exert negative effects on pancreatic β-cells and that such effects add to the diabetogenicity of elevated FA. In the obese and diabetic Zucker rat, triacylglycerols accumulate in β-cells in conjunction with elevated plasma FA (for review see Ref. 19). Such accumulation has been associated with β-cell damage, with proposed implications in humans (19). Our animal studies show that long-term elevated FA inhibit glucose-induced insulin secretion and insulin biosynthesis in vitro and ex vivo (30, 40). Similar effects were also found in human pancreatic islets in vitro (41). Blocking FA oxidation swiftly improved glucose-induced insulin secretion, implying that on-going accelerated FA oxidation was a negative factor (30, 39, 40). By analogy with results from other tissues, long-term elevated FA downregulated PDH and upregulated PDH kinase activities in pancreatic islets (42).

The negative effects of FA on β-cells were recognized much later than corresponding effects on liver and muscle. This was because FA acutely stimulate insulin secretion (19). The mechanisms behind FA-induced stimulation of insulin secretion are complex and not fully elucidated (19, 35) but are dependent on the formation of fatty acyl-CoAs, FA esterification, and FA oxidation. The stimulatory effects of FA on insulin secretion confound the demonstration of inhibitory effects of FA and complicate the elucidation of mechanisms that underlie inhibition.

Against this background, we envisage two negative components of long-term elevated FA on insulin secretion. One is a decrease in glucose-induced insulin secretion and the other a decrease, absence, or even reversal of FA-induced stimulation of insulin secretion. With the assumption of the operation of a glucose fatty cycle in β-cells similar to other tissues (for which evidence, but no proof, is available), both effects could...
involve the PDH enzyme complex. If PDH-kinase activity is time dependently upregulated by long-term elevated FA, then glucose oxidation and its metabolic signal for secretion would be reduced. Activation of PDH kinase by an acute increase in ambient FA concentrations (27, 28) could then further enhance total PDH kinase activity through an increase in acetyl-CoA-to-CoA ratios. The total (ambient and long term) effects on PDH kinase activity would then inhibit the metabolic signal for glucose-induced insulin secretion to a greater extent than in the absence of elevated FA. Such a putative negative effect might possibly be stronger than any concomitant stimulatory effect of FA (whatever the underlying mechanism of stimulation). Given this scenario, chronically elevated FA could exert a tonic inhibitory effect on glucose-induced insulin secretion.

Although it is hypothetical, we thought the aforementioned concept worthwhile to serve as a basis for investigations in type 2 diabetic subjects. Also, no other study in humans has, to our knowledge, attempted to test the impact of acute perturbations of FA levels on insulin secretion in type 2 diabetic subjects. The results of such a study would complement previous studies in humans that have evaluated long-term effects of FA by recording insulin responses after 24- to 48-h infusions of triacylglycerols such as Intralipid (4, 6, 7, 22).

We therefore designed a protocol wherein elevated levels of FA were acutely lowered and then reintroduced on a background of stable hyperglycemia. Specifically, the nicotinic acid derivative Acipimox was used to lower FA before a 2-h hyperglycemic clamp to which an Intralipid plus heparin infusion was added during the 2nd h. The effects were compared with the patients on their usual diet and again on a fat-restricted one. Because subjects with hypertriglyceridemia usually have elevated FA, we chose such diabetic subjects for the study. Recognizing the heterogeneity of type 2 diabetes even among hypertriglyceridemic patients, we included a large number of subjects in the study. The results were compared with those of age- and sex-matched nondiabetic subjects.

SUBJECTS AND METHODS

Subjects. Twenty-one subjects with type 2 diabetes (11 male, 10 female) participated. These subjects were recruited from the outpatient clinic of our Department of Endocrinology. Inclusion criteria were type 2 diabetes as defined by clinical criteria, age 40–75 yr, and hypertriglyceridemia with fasting triacylglycerol concentrations >2.2 mmol/l. Exclusion criteria were insulin treatment, proliferative retinopathy, pregnancy or lactation, heart failure grade III or IV, allergy to fish or other aliment prohibiting diet intervention, alcoholism, and other serious diseases affecting the possibility to participate.

Eighteen of the diabetic subjects were being treated with metformin in doses varying from 500 to 3,000 mg/day. Fifteen subjects were being treated with glipizamide in doses varying from 2.5 to 20 mg/day. One person was being treated with glibenclamide (10.5 mg/day). The last dose of glipizide was taken at bedtime and the last dose of glibenclamide at dinner. The majority of subjects were being treated with more than one antidiabetic medication. Thirteen subjects treated with metformin were on combination therapy with glibenclamide or glipizide. Four of the subjects were receiving antihyperlipemic treatment in the form of one or more drugs from the classes of ACE inhibitors, Ca2+ antagonists, α-agonists, β-blockers, loop diuretics, and thiazides. Three of the female diabetic subjects were receiving per oral estrogen. Sixteen subjects were receiving one or more other medications such as salicylates, nitroglycerine, proton pump inhibitor, allopurinol, antihistamine, potassium substitution, and antiasthmatic medication (excluding per oral glucocorticoids).

Nondiabetic subjects (5 male, 5 female) matched for age and sex with the diabetic subjects were recruited among blood donors from the local blood donor unit. None of the nondiabetic subjects received any continuous medication.

One of the control subjects and four of the diabetic subjects were habitual smokers.

All participants gave written, informed consent. The local ethics committee and the Norwegian Drug Control Authority approved the protocols that were used in the study.

Experimental design. On the day of inclusion, a physical examination was performed in all subjects. Diet and physical activity at inclusion were recorded with a frequency questionnaire. All subjects underwent hyperglycemic clamp testing (11) on two occasions: one without and one with pretreatment with a single capsule (250 mg) of Acipimox. Subsequently, 17 of the 21 diabetic subjects ingested a low-fat diet for 3 days, after which they again underwent hyperglycemic clamping with Acipimox. The interval between the tests varied between 2 and 6 wk. The order of the two first test occasions was randomized. A blinding design was not possible due to the flushing that usually follows ingestion of Acipimox.

Test procedures. The subjects reported to the clinic between 8 and 9 AM. Body weight and blood pressure were measured. A cannula (Venflon; Viggo, Helsingborg, Sweden) was inserted into an antecubital vein for the sampling of blood. An electric blanket was used to partially arterialize venous blood. A second cannula was inserted into the antecubital vein of the contralateral arm for infusions. Fasting blood samples were collected. Then, according to randomization, the subjects received either 250 mg Acipimox or no medication. Sixty minutes later, a hyperglycemic clamp was started, aiming at a blood glucose concentration 6 mmol/l above the fasting glucose concentration in each individual subject. The clamp was initiated by a bolus injection of 0.25 g/kg body wt of glucose, followed by an infusion of a 10% solution of glucose.

Blood glucose was measured every 5 min during the 120-min clamp period. The infusion rate of glucose was adjusted according to these measurements. At minute 60 of the ongoing hyperglycemic clamp, an infusion of Intralipid (20%, 1 ml/min; Pharmacia, Uppsala, Sweden) and heparin (0.4 U·kg−1·min−1; Leo, Ballerup, Denmark) was started and maintained for another 60 min until the end of the clamp. Blood samples were collected at standardized intervals. The samples destined for FA measurements were collected in tubes containing EDTA.1 For glucagon measurements, 0.55

1We did not routinely add other preservatives to samples for FA measurements. Samples were stored at −80°C, a procedure that decreases artifactual lipolysis compared with storage at −20°C (38). During the clamp, we checked in vitro lipolysis by adding the lipase inhibitor Paraoxon (Sigma Chemical, St. Louis, MO) to tubes before blood sampling. We found no effect of Paraoxon in samples in the
ml of aprotinin (Trasylol; Bayer, Leverkusen, Germany) was added to chilled, preheparinized tubes. All plasma samples were frozen and kept at −80°C for later analysis.

**Assays.** Glucose in blood and urine during the clamps was determined by a glucose oxidase method using a YSI Glucose Analyzer (Yellow Springs Instrument, Yellow Springs, OH). Insulin, C-peptide, and glucagon were measured by RIA. The insulin assay was specific for human insulin (Linco Research, St. Charles, MO). According to the manufacturer, the interassay coefficient of variation (CV) of the insulin RIA is 9.7% and the intra-assay CV 5.0%. Cross-reactivity with proinsulin is ~0.2%. The human C-peptide assay was from Linco Research. Cross-reactivity was <4% against proinsulin and nondetectable against insulin. The intra-assay CV varied between 3.4 and 6.4% according to C-peptide level; interassay CV was between 2.4 and 9.3%. Proinsulin was determined by ELISA (Dako, Oslo, Norway). Cortisol was determined by competitive immunoassay using a commercial kit (DPC, Los Angeles, CA). Concentrations of FA were determined by an enzymatic colorimetric method (NEFA-C kit, Wako Pure Chemical Industries, Osaka, Japan). Plasma phospholipid FA (PL-FA) were determined by gas chromatography (5) and triacylglycerols, cholesterol, HDL cholesterol, and glycosylated hemoglobin (Hb Alc) by standard laboratory techniques.

**Physical activity, diet registration, and intervention.** At inclusion, physical activity and food intake were registered in all subjects with a validated questionnaire (20). For the dietary intervention (performed by the diabetic subjects only), food-weighing records were obtained during 3 days on the usual diet as well as during 3 days of dietary intervention.

The intervention diet was a low-fat, fiber-rich diet that was isoenergetic with each patient’s ordinary diet. At the start of the diet intervention, subjects were told to reduce all fats but to increase the intake of fish, cereals, potatoes, rice, pasta, vegetables, and fruits. The intake of energy and nutrients was computed by means of a food database (AKF96) and software systems (BEREGN) developed at the Institute of Nutrition Research, University of Oslo. The food database was based mainly on the official Norwegian Food Table (29).

**Presentation of results.** Values are given as means ± SE. BMI, body mass index; Hb A1c, glycosylated hemoglobin. *P < 0.01; †P < 0.001, diabetic compared with nondiabetic subjects. ‡Range of Hb A1c was 5.2–11.2%.

**RESULTS**

**Clinical characteristics of diabetic and nondiabetic subjects**. The diabetic subjects were obese, albeit to a varying extent (Table 1). The metabolic control as assessed by Hb A1c was fair, albeit with large variations among subjects. The average duration of known diabetes was between 6 and 7 yr. The levels of triacylglycerols were, by design, markedly elevated. The levels of cholesterol were above normal according to current guidelines (2), as were the cholesterol-to-HDL ratios.

Nondiabetic subjects were age and sex matched to the diabetic group (Table 1). Nondiabetic subjects were fairly lean. They had normal triacylglycerol levels. Differences between diabetic and nondiabetic subjects included body mass index (BMI), triacylglycerols, and blood pressure (Table 1).

Energy intake, as assessed by questionnaire, did not differ between the groups. The diabetic subjects consumed on average 8.7 ± 0.7 MJ/day, with a mean of 48 energy percentage (E%) from carbohydrates, 33 E% from fat, 18 E% from protein, and 1 E% from alcohol. The nondiabetic subjects consumed 8.9 ± 0.9 MJ/day, with a mean of 51 E% from carbohydrates, 30 E% from fat, 15 E% from protein, and 4 E% from alcohol. Intake of carbohydrates was significantly lower in the diabetic subjects than in the nondiabetic group (P < 0.05), whereas intake of protein and fat was significantly higher (P < 0.05 and P < 0.001, respectively).

The frequency of physical activity did not differ between diabetic and nondiabetic subjects. Thirty-three percent of the diabetic subjects exercised more than

Table 1. Clinical characteristics of diabetic and nondiabetic subjects at inclusion

<table>
<thead>
<tr>
<th></th>
<th>Diabetic</th>
<th>Nondiabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>55.9 ± 2.4</td>
<td>54.5 ± 3.5</td>
</tr>
<tr>
<td>Male/female</td>
<td>11/10</td>
<td>5/5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.2 ± 1.0</td>
<td>25.3 ± 0.6†</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>144 ± 4</td>
<td>118 ± 4†</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>84 ± 2</td>
<td>75 ± 2</td>
</tr>
<tr>
<td>Duration of diabetes, yr</td>
<td>6.5 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/l</td>
<td>10.6 ± 0.5</td>
<td>4.9 ± 0.1†</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>7.9 ± 0.3‡</td>
<td>5.3 ± 0.1†</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>6.5 ± 0.3</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.12 ± 0.05</td>
<td>1.35 ± 0.12</td>
</tr>
<tr>
<td>Triacylglycerols, mmol/l</td>
<td>4.6 ± 0.7</td>
<td>1.2 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; Hb A1c, glycosylated hemoglobin. *P < 0.01; †P < 0.001, diabetic compared with nondiabetic subjects. ‡Range of Hb A1c was 5.2–11.2%.

To the glucose-only hour of the clamp. Statistical analysis was done using Statistical Package for the Social Sciences, version 10.0 (SPSS, Chicago, IL). Significance testing was done by Student’s paired t-test, by independent samples t-test, and, for nonnormally distributed variables, by the Wilcoxon matched pairs signed rank sum test or Mann-Whitney test. For repeated analyses, ANOVA was done. Spearman’s correlation coefficients were used to evaluate bivariate correlations. Linear regression was performed using the Enter, Stepwise, and Backward models.

**Physical activity, diet registration, and intervention.** At inclusion, physical activity and food intake were registered in all subjects with a validated questionnaire (20). For the dietary intervention (performed by the diabetic subjects only), food-weighing records were obtained during 3 days on the usual diet as well as during 3 days of dietary intervention.

The intervention diet was a low-fat, fiber-rich diet that was isoenergetic with each patient’s ordinary diet. At the start of the diet intervention, subjects were told to reduce all fats but to increase the intake of fish, cereals, potatoes, rice, pasta, vegetables, and fruits. The intake of energy and nutrients was computed by means of a food database (AKF96) and software systems (BEREGN) developed at the Institute of Nutrition Research, University of Oslo. The food database was based mainly on the official Norwegian Food Table (29).

**Presentation of results.** Values are given as means ± SE if not stated otherwise. Insulin levels are presented as absolute levels or increments relative to 60-min values, as evident from table and text. Insulin secretion rates (ISR) were computed from the C-peptide data by a regression model (ISEC, version 3.4a). The model derives parameters of C-peptide kinetics from the subject’s sex, age, type (normal, obese, type 2 diabetes), body weight, and height (15). Insulin sensitivity was assessed by the M/I ratio, where M equals the amount of glucose infused (mg·kg⁻¹·min⁻¹) minus urinary loss, and I equals the mean level of insulin during a specified interval, expressed as microunits per milliliter. Because in our protocol the within-subject changes in insulin levels were minor, we additionally used M values alone to assess insulin sensitivity. The time periods 20–59, 60–79, and 80–120 min were chosen for the M and MI calculations. These intervals were chosen arbitrarily to obtain a degree of steady state of hyperglycemia after initiation of the hyperglycemic clamp and to compare effects during Intralipid infusion at time intervals corresponding to the partition of the glucose-only hour of the clamp. Statistical analysis was done using Statistical Package for the Social Sciences, version 10.0 (SPSS, Chicago, IL). Significance testing was done by Student’s paired t-test, by independent samples t-test, and, for nonnormally distributed variables, by the Wilcoxon matched pairs signed rank sum test or Mann-Whitney test. For repeated analyses, ANOVA was done. Spearman’s correlation coefficients were used to evaluate bivariate correlations. Linear regression was performed using the Enter, Stepwise, and Backward models.

**RESULTS**

**Clinical characteristics of diabetic and nondiabetic subjects**. The diabetic subjects were obese, albeit to a varying extent (Table 1). The metabolic control as assessed by Hb A1c was fair, albeit with large variations among subjects. The average duration of known diabetes was between 6 and 7 yr. The levels of triacylglycerols were, by design, markedly elevated. The levels of cholesterol were above normal according to current guidelines (2), as were the cholesterol-to-HDL ratios.

Nondiabetic subjects were age and sex matched to the diabetic group (Table 1). Nondiabetic subjects were fairly lean. They had normal triacylglycerol levels. Differences between diabetic and nondiabetic subjects included body mass index (BMI), triacylglycerols, and blood pressure (Table 1).

Energy intake, as assessed by questionnaire, did not differ between the groups. The diabetic subjects consumed on average 8.7 ± 0.7 MJ/day, with a mean of 48 energy percentage (E%) from carbohydrates, 33 E% from fat, 18 E% from protein, and 1 E% from alcohol. The nondiabetic subjects consumed 8.9 ± 0.9 MJ/day, with a mean of 51 E% from carbohydrates, 30 E% from fat, 15 E% from protein, and 4 E% from alcohol. Intake of carbohydrates was significantly lower in the diabetic subjects than in the nondiabetic group (P < 0.05), whereas intake of protein and fat was significantly higher (P < 0.05 and P < 0.001, respectively).

The frequency of physical activity did not differ between diabetic and nondiabetic subjects. Thirty-three percent of the diabetic subjects exercised more than
three times a week, with a minimum duration of 20 min, compared with 40% of the control subjects [not significant (NS)]. However, the intensity of exercise differed between the groups, as only 24% of the diabetic subjects exercised enough to feel warm or short of breath, compared with 70% of the control subjects ($P < 0.04$).

Fasting levels of FA, insulin, C-peptide, glucagon, and proinsulin. These measurements showed the expected differences between nondiabetic and obese type 2 diabetic subjects. Fasting levels of FA were thus significantly higher in the diabetic than in the nondiabetic subjects (0.68 ± 0.04 vs. 0.51 ± 0.07 mmol/l, $P < 0.05$). Fasting levels of insulin were similar in diabetic and nondiabetic subjects (14.4 ± 1.5 vs. 19.5 ± 2.6 μU/ml), as were C-peptide levels (1.11 ± 0.11 vs. 0.86 ± 0.13 nmol/l). Glucagon levels were higher in the diabetic subjects (40.9 ± 4.1 vs. 19.6 ± 1.7 pmol/l, $P < 0.05$), as were proinsulin levels (17.4 ± 2.6 vs. 8.8 ± 2.2 pmol/l, $P < 0.05$) and proinsulin-to-insulin ratios (0.24 ± 0.05 vs. 0.08 ± 0.02, $P < 0.05$).

Hyperglycemic clamps: blood glucose and FA. Blood glucose was increased ~6 mmol/l above the fasting blood glucose concentration (Fig. 1, top). The glucose levels during the clamps were similar in experiments with and without Acipimox. The mean ± SE of concentrations achieved in diabetic subjects was 15.4 ± 0.6 mmol/l without Acipimox and 15.5 ± 0.6 mmol/l with Acipimox. The corresponding values in nondiabetic subjects were 11.1 ± 0.2 and 10.7 ± 0.2 mmol/l (NS).

During the 1st h of the clamp, FA levels were reduced by 29% in the diabetic subjects and by 67% in the nondiabetic subjects (Fig. 1, bottom). During Intralipid infusion, i.e., during the 2nd h of the clamp, the FA levels were increased threefold in the diabetic and sevenfold in the nondiabetic subjects. Mean FA levels at the end of the Intralipid infusion clamp were 48% higher in the diabetic than in the nondiabetic group. This difference was, however, not significant ($P < 0.07$).

In the Acipimox experiments, 250 mg of Acipimox were taken by mouth 60 min before the start of the hyperglycemic clamp. This resulted in a 52% reduction of FA in the diabetic subjects at the start of the clamp and a 77% reduction in nondiabetic subjects (Fig. 1, bottom). During the 1st h of the clamp, FA levels were further reduced by 59% in the diabetic subjects and by 62% in the nondiabetic subjects.

Hyperglycemic clamps: insulin levels and insulin secretion without Acipimox. An early insulin response to glucose elevation was expectedly missing in the diabetic subjects (Fig. 2, top). Insulin concentrations during Intralipid infusion increased modestly in diabetic subjects and more markedly so in nondiabetic subjects. In the diabetic subjects, there was a positive correlation with fasting levels of triacylglycerols and insulin levels ($r = 0.39, P < 0.05$) and a tendency toward a negative correlation with fasting levels of FA ($r = -0.36, P < 0.1$). We found no such correlations in the control group.

The difference between diabetic and nondiabetic subjects during Intralipid infusion was even more marked when β-cell function was assessed from C-peptide data (Fig. 2, middle) or from ISR (Fig. 2, bottom). The latter parameter decreased from 4.6 ± 1.0 pmol·kg⁻¹·min⁻¹ at minute 60 to 3.7 ± 0.7 pmol·kg⁻¹·min⁻¹ at minute 120, a 13% decrease in the diabetic subjects. In contrast, in the nondiabetic subjects, ISR increased from 6.0 ± 0.4 pmol·kg⁻¹·min⁻¹ at minute 60 to 8.1 ± 0.7 pmol·kg⁻¹·min⁻¹, i.e., a 25% increase ($P < 0.05$ for difference between the groups).

The insulin responses during the total clamp period varied substantially among the diabetic subjects. The ISR parameter correlated positively with fasting C-peptide data (Fig. 2, middle) or from ISR (Fig. 2, bottom). The latter parameter decreased from 4.6 ± 1.0 pmol·kg⁻¹·min⁻¹ at minute 60 to 3.7 ± 0.7 pmol·kg⁻¹·min⁻¹ at minute 120, a 13% decrease in the diabetic subjects. In contrast, in the nondiabetic subjects, ISR increased from 6.0 ± 0.4 pmol·kg⁻¹·min⁻¹ at minute 60 to 8.1 ± 0.7 pmol·kg⁻¹·min⁻¹, i.e., a 25% increase ($P < 0.05$ for difference between the groups).
peptide \((r = 0.51, P < 0.02)\) for the entire clamp period. ISRs correlated negatively with fasting glucose levels \((r = -0.44, P < 0.05)\), with Hb A1c \((r = -0.53, P < 0.01)\), and with serum cortisol \((r = -0.50, P < 0.03)\). A negative correlation was also found with fasting FA for minutes 60–120 \((r = -0.47, P < 0.03)\).

Insulin secretion after Acipimox. In the diabetic subjects, Acipimox enhanced insulin levels marginally \((17\%\,P < 0.03)\) during the ensuing hyperglycemic clamp (Fig. 2, top left). However, Acipimox failed to significantly affect ISR \((513\,P < 0.03)\) for the 0- to 120-min period without and 543 with Acipimox; Fig. 2, bottom). Also, there was no significant effect of Acipimox when the periods minutes 0–5 (corresponding to first-phase secretion), 0–59, or 60–120 were analyzed separately.

In the nondiabetic subjects, previous Acipimox failed to significantly affect insulin, C-peptide, or ISR during any time of the clamp (Fig. 2, right).

It seemed possible that diabetic subjects with comparably good metabolic control would retain a greater potential for nutrient-induced regulation of insulin secretion than subjects with poor metabolic control. The size of our study group allowed us to perform post hoc analysis on the basis of metabolic control. There was no effect of Hb A1c during the glucose-only part of the clamp \((P > 0.5)\). However, an effect of Acipimox on insulin secretion during Intralipid infusion correlated negatively with Hb A1c \((P = -0.64, P < 0.04, \text{Fig. 3})\). When the diabetic subjects were dichotomized on the basis of their Hb A1c, those with the lower Hb A1c concentrations exhibited a positive effect of Acipimox on the integrated insulin secretion rates \((P = 0.05; \text{Fig. 4})\). For those with higher Hb A1c, values were 242 and 278 pmol·kg\(^{-1}\)·min\(^{-1}\) with and without Acipimox, respectively \((P < 0.1)\). Levels of FA and triacylglycerols in the dichotomized groups at inclusion did not differ significantly \((\text{FA, 0.66 ± 0.07 mmol/l for those with lower Hb A1c vs. 0.63 ± 0.06 mmol/l for those with higher Hb A1c and for triacylglycerols, 4.24 ± 1.14 vs. 4.92 ± 0.74 mmol/l})

nor did age of apparent onset of diabetes \((6.3 ± 1.1 \text{ vs. 6.8 ± 1.3 yr})\) or BMI \((31.5 ± 1.4 \text{ vs. 31.0 ± 1.7})\) differ significantly.

Fig. 2. Insulin, C-peptide, and insulin secretion rates during hyperglycemic clamps. For insulin and C-peptide, ○, tests without Acipimox; ●, tests with Acipimox. Data points for insulin secretion rate (ISR) during hyperglycemic clamps are model derived, dashed lines denoting tests without Acipimox and bold lines with Acipimox. Brackets denote the duration of Intralipid + heparin infusions. No. of experiments are as for Fig. 1.

\[E133\]
M and M/I before and after Acipimox. As expected, the diabetic subjects were insulin resistant with low M/I values compared with the nondiabetic subjects (Fig. 5). Lowering of FA with Acipimox resulted in moderate increases in M and M/I in both the diabetic and nondiabetic subjects (Fig. 5). In the diabetic subjects, Acipimox improved insulin sensitivity (P < 0.05 or less) for parts of the clamp indicated in Fig. 5. We found a positive correlation between the insulin-sensitizing effect of Acipimox and Hb A1c concentrations (for change of M 20–59 min, r = 0.65, P < 0.002 and for change of M/I 20–59 min, r = 0.75, P < 0.001; i.e., those subjects with higher Hb A1c had greater effect of Acipimox on insulin sensitivity than those with lower Hb A1c levels.

Effects of a low-fat diet on energy and nutrient intake. We tested the effects of a low-fat diet in 17 of the diabetic subjects. The dietary intervention led to a mean reduction of fat from 39 to 23 E% (range 9–40). Carbohydrates were increased from 42 to 52 E% (range 37–68) and proteins from 18 to 23 E% (range 18–30). The mean change in total energy intake was −0.7 ± 0.3 MJ/day (NS).

Effects of a low-fat diet on glucose, FA, lipids, and hormones. The 17 diabetic subjects who completed the low-fat diet protocol did not differ significantly from the whole group of patients in the clinical characteristics of Table 1 (results not shown). The dietary intervention did not significantly affect fasting concentrations of blood glucose measured on test days (9.4 ± 0.7 mmol/l after the usual diet compared with 8.9 ± 0.6 mmol/l after low-fat diet).

The low-fat diet did not significantly affect fasting concentrations of C-peptide, insulin, proinsulin, glucagon, and cortisol or concentrations of FA and triacylglycerols. Total cholesterol was significantly reduced from 6.3 ± 0.3 to 5.8 ± 0.3 mmol/l (P < 0.001). HDL tended to decrease from 1.11 ± 0.04 to 1.07 ± 0.04 mmol/l (P < 0.1). Total plasma PL-FA were unchanged, as were also saturated and monounsaturated FA fractions. The unsaturated n-6 FA fraction of PL-FA was reduced by the dietary intervention from 34.2 ± 0.9 to 31.6 ± 1.0 g/100 g PL-FA (P < 0.005), and the n-3 FA fraction was increased from 12.9 ± 0.9 to 15.1 ± 1.0 g/100 g PL-FA (P < 0.01). The ratio of n-6 to n-3 FA decreased from 3.0 ± 0.3 to 2.3 ± 0.2 (P < 0.005).

Effects of low-fat diet on clamp parameters. Glucose concentrations and FA concentrations achieved during the clamps were the same during the tests after the low-fat diet compared with usual diet (results not shown). The low-fat diet failed to affect the glucose-stimulated rise in insulin levels above the effect exerted by Acipimox when the subjects were on their usual diet. Thus the integrated ISRs during minutes 0–120 were 569 ± 58 pmol·kg\(^{-1}\)·min\(^{-1}\) before and 570 ± 52 pmol·kg\(^{-1}\)·min\(^{-1}\) after the low-fat diet. There was no difference between subjects with high and low Hb A1c in this respect. The low-fat diet reduced insulin sensitivity as assessed by M/I during the 20- to 59-min period, i.e., during the infusion of glucose alone (from 0.11 ± 0.03 to 0.07 ± 0.02, P < 0.03). There was no such effect during the additional infusion of Intralipid. The reduction in HDL correlated with the change in M/I during the 20- to 59-min period of the...
clamp \((r = 0.62, P < 0.01)\). The following variables were not reciprocally correlated and entered in multiple regression analysis: change in vegetable intake, change in PL-FA, physical activity, BMI, cholesterol, HDL, FA, and fasting insulin levels. Reduction in HDL remained the single explaining factor of the reduction of insulin sensitivity, explaining 29% of the variance, \(P < 0.03\).

**DISCUSSION**

The notion of “lipotoxicity” as a factor behind attenuated insulin secretion in type 2 diabetes is controversial. Experimental studies in vivo in humans have been inconclusive as to the influence of FA. Infusion of Intralipid in nondiabetic subjects led to reduction of glucose-induced insulin secretion in one study (22) but not in another (4). A third study (7) found no effect; the lack of a positive effect was interpreted as dysfunctional, because the attendant FA-induced insulin resistance should have enhanced insulin secretion. In another study from the same group (6), there was a definite negative effect in obese nondiabetic subjects but a positive one in type 2 diabetic subjects. A previous study indicated that 1 wk of Acipimox treatment improved insulin responses in insulin-resistant subjects with elevated FA (23). Because treatment lowered FA levels, the results suggest a tonic inhibitory effect of elevated FA. However, effects of Acipimox on hormonal parameters, such as levels of glucagon, growth hormone, and cortisol, make the interpretation of results difficult. All together, these studies do not give clear-cut evidence for a generalized negative effect of elevated FA on insulin secretion.

In the present study, we used a different approach by measuring, for the first time, glucose-induced insulin secretion in relation to acute decreases, followed by increases, in ambient FA. Our protocol was designed to test the hypothesis that chronic elevation of FA in type 2 diabetes would influence the effect of ambient FA on glucose-induced insulin secretion. At first glance, our results failed to support this hypothesis. There was thus no effect on ISR in the whole group of diabetic subjects during the hyperglycemic clamp despite the preclamp lowering of FA by Acipimox.

It could be argued that the time frame of FA lowering was too short to expect any change in \(\beta\)-cell metabolism and/or secretion. However, previous Acipimox did increase insulin sensitivity, implying effects on intermediary metabolism within the time frame of measurements. The effects on insulin sensitivity confirm findings with Acipimox found in most studies (25, 31) albeit with exceptions (32).

We did note a small effect of Acipimox on peripheral insulin levels in the diabetic subjects that were compatible with an effect on insulin clearance by the liver. The effect is opposite to that expected from a study in normal dogs that concluded that FA impair hepatic clearance of insulin (36). Differences in experimental design and species differences, as well as the presence of diabetes in our study, could be important for the discrepancy.

In a post hoc analysis, we found a negative correlation between Hb A1c levels on the one hand and a stimulatory effect of Acipimox on the other during the Intralipid infusion. Furthermore, a significant positive ISR response to Acipimox treatment was found in the 50% of the subjects having the lowest Hb A1c levels. The positive association with metabolic control may be indirect, since we did not see any enhancement by Acipimox of insulin secretion in the group of normoglycemic nondiabetic subjects. However, the nondiabetic subjects were normotriglyceridemic, whereas patients were hypertriglyceridemic, so there could still be an impact of elevated blood glucose in the context of hypertriglyceridemia. Further studies will have to test whether correction of hyperglycemia in type 2 diabetes will influence insulin responses in the present or in a similar protocol. Intriguingly, the apparent effects of
Acipimox on insulin sensitivity showed a relation to metabolic control that was opposite to that on insulin secretion. An increase in insulin sensitivity would, in principle, lead to a decrease in insulin secretion. To the best of our knowledge, such coupling would, however, appear to require a longer period of induction than that of the present protocol. Therefore, it seems unlikely (although not impossible) that the effects on insulin sensitivity explain the effects on insulin secretion.

It is of interest to compare our results in diabetic subjects with those of Carpentier et al. (6), who tested the effects of a 48-h infusion of Intralipid on subsequent glucose-induced insulin secretion. Those authors found that Intralipid infusion enhanced, rather than decreased, insulin secretion in type 2 diabetic subjects. As in our study, a wide variability of response was noted in the seven diabetic subjects in the study of Carpentier et al. (6). It is obvious from the present and previous studies that type 2 diabetes is heterogeneous in terms of lipid influences on insulin secretion. A large group (21 in our study) was necessary to allow the distinctions between the two subgroups that we found. A recent study indicates that genetic variability also could influence FA interactions with 6-cells (33).

In the present context, it is noteworthy that our diabetic subjects appear representative in many respects of type 2 diabetes in Norway (9), as well as of Northern European eating habits (10, 18). Also, the variability in several diabetes-related characteristics between individuals that we find can be considered typical for type 2 diabetes (21).

Interestingly, ISR increased in the nondiabetic subjects during the Intralipid part of the protocol (without previous Acipimox), whereas no increase was seen in the diabetic subjects. In another study, also in normotriglyceridemic nondiabetic subjects, we compared insulin levels in protocols with and without Intralipid (26). It could then be shown that Intralipid infusion increased insulin levels above any (minor) effect during a glucose plus saline infusion. Extrapolating this information to the present study, one may infer that FA induce an insulin response in nondiabetic normotriglyceridemic subjects but not in hypertriglyceridemic type 2 diabetic subjects. This conclusion is at least compatible with an inhibitory influence of FA linked to chronically elevated FA.

The relative proportion of fat in the diet affects FA oxidation in different tissues (14), possibly including 6-cells. One source of variation of FA interactions with 6-cells in diabetic subjects could thus be the FA content of each individual’s diet. We evaluated the influence of FA in the diet for insulin release by comparing insulin secretion in the Acipimox protocol during the usual diet with that during a fat-restricted diet. The 3-day dietary intervention failed to affect insulin secretion despite the fact that it was successful by all measured criteria (1) and did significantly alter lipid parameters. It cannot be ruled out that a longer period of dietary intervention would give different results. Also, the possible influence of the relative increase in n-3 FA, which may have a particularly negative influence on insulin secretion (12), has to be considered.

The low-fat diet led to a decrease in insulin sensitivity during the glucose-only part of the clamp. Other dietary studies in humans are divided as to effects on sensitivity. A major finding was the variability of insulin response in the diabetic subjects to the acute perturbations of FA. From other studies, we know that type 2 diabetes progresses with time, the metabolic control worsening and insulin secretion decreasing with the duration of the disease (34). The observation in our study that good metabolic control was associated with a positive insulin response (albeit a moderate one) to pretreatment with Acipimox could indicate that lowering of FA may be beneficial when the disease has not progressed too far. We speculate that a “window” for therapeutic intervention may exist early in type 2 diabetes. Such a window could include the prediabetic stage, as obese prediabetic individuals have been shown to be particularly susceptible to negative effects of long-term elevated FA (6).

Can clinical implications be drawn from this study? A major finding was the variability of the insulin response in the diabetic subjects to the acute perturbations of FA. From other studies, we know that type 2 diabetes progresses with time, the metabolic control worsening and insulin secretion decreasing with the duration of the disease (34). The observation in our study that good metabolic control was associated with a positive insulin response (albeit a moderate one) to pretreatment with Acipimox could indicate that lowering of FA may be beneficial when the disease has not progressed too far. We speculate that a “window” for therapeutic intervention may exist early in type 2 diabetes. Such a window could include the prediabetic stage, as obese prediabetic individuals have been shown to be particularly susceptible to negative effects of long-term elevated FA (6).

We are grateful to Ellen G. Lystad and Harriet Solle for assistance during the clamp procedures and to Prof. Erol Cerasi for valuable comments about the manuscript.

This study was supported by the Norwegian Research Council (Grant no. 11290/320), the Norwegian Diabetes Association, the Norwegian Endocrine Society, and Torstein Erbo’s Foundation.

REFERENCES


AJP-Endocrinol Metab • VOL 284 • JANUARY 2003 • www.ajpendo.org


