Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes

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Submitted 17 November 2006; accepted in final form 6 February 2007

Cusi K, Kashyap S, Gastaldelli A, Bajaj M, Cersosimo E. Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes. Am J Physiol Endocrinol Metab 292: E1775–E1781, 2007. First published February 13, 2007; doi:10.1152/ajpendo.00624.2006.—Elevated plasma FFA cause β-cell lipotoxicity and impair insulin secretion in nondiabetic subjects predisposed to type 2 diabetes mellitus (T2DM; i.e., with a strong family history of T2DM (FH+)) but not in nondiabetic subjects without a family history of T2DM. To determine whether lowering plasma FFA with acipimox, an antilipolytic nicotinic acid derivative, may enhance insulin secretion, nine FH+ volunteers were admitted twice and received in random order either acipimox or placebo (double-blind) for 48 h. Plasma glucose/insulin/C-peptide concentrations were measured from 0800 to 2400. On day 3, insulin secretion rates (ISRs) were assessed during a +125 mg/dl hyperglycemic clamp. Acipimox reduced 48-h plasma FFA by 36% (P < 0.001) and increased the plasma C-peptide relative to the plasma glucose concentration or ΔC-peptide/Δglucose AUC (+177%, P = 0.02), an index of improved β-cell function. Acipimox improved insulin sensitivity (M/I) 26.1 ± 5% (P < 0.04). First- (+19 ± 6%, P = 0.1) and second-phase (+31 ± 6%, P = 0.05) ISRs during the hyperglycemic clamp also improved. This was particularly evident when examined relative to the prevailing insulin resistance [1/(M/I)], as both first- and second-phase ISR markedly increased by 29 ± 7 (P < 0.05) and 41 ± 8% (P = 0.02). There was an inverse correlation between fasting FFA and first-phase ISR (r² = 0.31, P < 0.02) and acute (2–4 min) glucose-induced insulin release after acipimox (r² = 0.52, P < 0.04). In this proof-of-concept study in FH+ individuals predisposed to T2DM, a 48-h reduction of plasma FFA improves day-long meal insulin resistance (10.220.33.4 on August 28, 2017 http://ajpendo.physiology.org/ Downloaded from

Address for reprint requests and other correspondence: K. Cusi, The Univ. of Texas H. S. C. at San Antonio, Diabetes Division, Rm. 3.3805,7703 Floyd Curl Drive, San Antonio, TX 78284-3900 (e-mail: cusi@uthscsa.edu).

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FH+ subjects may improve insulin secretion in a population with a very high genetic susceptibility to the deleterious effect of FFA (26), such as in FH+ subjects of Hispanic ancestry, an ethnic group that has never been carefully studied before in this regard.

The aim of the present study was to perform a placebo (PBO)-controlled study that could serve as proof of principle on whether a reduction of plasma FFA levels may enhance insulin secretion in Hispanic subjects genetically predisposed to develop diabetes (i.e., with a strong family history of T2DM), a finding that may have clinical implications for future prevention strategies in Hispanic subjects prone to T2DM.

RESEARCH DESIGN AND METHODS

Subjects. Nine healthy Hispanic FH+ subjects participated in the study (Table 1). A strong family history of T2DM was defined as having both parents with T2DM. All participants had an initial screening visit, including a normal 75-g oral glucose tolerance test (OGTT). Body weight and physical activity were stable in all subjects for ≥3 mo prior to enrollment. Each subject gave written, informed consent before participation. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center, San Antonio, TX.

Study design. Subjects were admitted to the General Clinical Research Center (GCRC) at 1700 on two separate occasions to receive either APX (250 mg orally every 4 h) or PBO in a double-blind, randomized fashion. On days 1 and 2 of each admission they underwent a metabolic profile in which plasma glucose, FFA, and hormone concentrations were measured during the next 48 h every 2 h from 0800 to 2400 (i.e., “metabolic profile”; Fig. 1). Blood was drawn via a catheter inserted into the antecubital vein. Subjects were fed a eucaloric standardized diet and allowed to ambulate freely within the Center, San Antonio, TX.

Institutional Review Board of the University of Texas Health Science

Table 1. Clinical and laboratory characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>FH+ Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (M/F)</td>
<td>9 (5/4)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>38±4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.3±1.1</td>
</tr>
<tr>
<td>LBM, %</td>
<td>69±4</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>4.9±0.2</td>
</tr>
<tr>
<td>FPG, mg/dl</td>
<td>95±10</td>
</tr>
<tr>
<td>2-h Plasma glucose, mg/dl</td>
<td>122±4</td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/ml</td>
<td>9±1</td>
</tr>
<tr>
<td>2-h insulin, μU/ml</td>
<td>74±15</td>
</tr>
<tr>
<td>Fasting FFA, μmol/l</td>
<td>498±24</td>
</tr>
</tbody>
</table>

Data are means ± SE. FH+, subjects with a strong family history of type 2 diabetes mellitus; M, males; F, females; BMI, body mass index; LBM, lean body mass; FPG, fasting plasma glucose; FFA, free fatty acid.

Fig. 1. Plasma glucose, free fatty acid (FFA), and C-peptide during the 48-h metabolic profile in response to acipimox (○) or placebo (●) during the in-patient stay.

Hyperglycemic clamp. After an overnight fast, subjects underwent a hyperglycemic clamp on day 3 (16). Briefly, after collection of baseline samples, plasma glucose was acutely raised by 125 ± 5 mg/dl above baseline and maintained for 120 min by periodic adjustment of a 20% dextrose infusion based upon the negative feedback principle. Plasma samples were obtained every 2 min from 0 to 10 min (first-phase insulin) and every 5 min from 10 to 120 min (second phase). Subjects voided immediately before and at the end of the study for measurement of urinary glucose loss.

Analytical determinations. The plasma glucose concentration was determined by the glucose oxidase method with a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin and C-peptide concentrations were determined by radioimmunoassays. Plasma FFA concentration was measured by standard colorimetric methods.

Calculations. The C-peptide increase above baseline (above fasting) was expressed as the area under the curve (AUC) after breakfast (0800–1200), lunch (1200–1800), and dinner (1800–2400) was calculated using the trapezoidal method. To better represent β-cell function, we calculated the increase above baseline in C-peptide AUC following breakfast, lunch, and dinner in relation to the increase above baseline in plasma glucose AUC. During the hyperglycemic and insulin clamp studies, basal (–30 to 0 min) and steady-state (80 to 120 min) plasma glucose, FFA, insulin, and C-peptide represent the mean of values drawn at 10-min intervals. The steady-state glucose infusion...
rate during the hyperglycemic clamp represents the mean glucose infusion rate from 80 to 120 min, corrected for urinary glucose losses. Estimation of insulin secretion rates. ISRs were estimated from peripheral plasma C-peptide levels by deconvolution analysis and linear regularization using a two-compartment model with standard parameters for C-peptide kinetics (45). As validated by Van Cauter et al. (45), use of standard parameters for C-peptide clearance and distribution results in ISR that differ only slightly from those obtained with individual parameters. Subjects genetically predisposed to T2DM are insulin resistant, and insulin secretion is best represented in relation to the degree of insulin resistance (11, 12, 15, 20, 23, 26, 28, 29, 30, 46, 50). We (26) have shown that β-cell function responds to the presence of insulin resistance in FH+ subjects with a compensatory increase in insulin secretion. Insulin sensitivity differed significantly between PBO and APX administration as estimated by the amount of glucose metabolized (M) per unit of plasma insulin concentration (M/I) (Fig. 2) (16). This measure of tissue sensitivity to endogenously secreted insulin during the hyperglycemic clamp (M/I) keeps in our hands a close correlation with the euglycemic insulin (rate of glucose disposal) clamp [r = 0.94, P < 0.0001 (26)].

To more properly assess ISR between PBO and APX treatments while having different insulin sensitivities, we related the ISR to the severity of insulin resistance (quantified during the hyperglycemic clamp as M/I). During the hyperglycemic clamp, insulin resistance is the inverse of M/I [the rate of glucose disposal (M) per unit of plasma insulin concentration (I/I)], i.e., the lower the M/I the greater the insulin resistance. Thus, ISR related to peripheral insulin resistance (ISRMI) was expressed as

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\text{ISR}_{\text{MI}} = \frac{\text{ISR}}{\text{insulin resistance [1/(M/I)]}}
\]

To assess β-cell function in response to the prevailing plasma glucose observed during the 48-h administration of APX or PBO, we evaluated, for each individual, the relationship between plasma C-peptide and glucose concentrations measured at the same time points (every 2 h from 0800 to 2400, e.g., fasting and after meals). The slope of the curve can be considered as an index of insulin secretion “dose response” to the prevailing plasma glucose concentration [i.e., β-cell glucose sensitivity (22)].

Statistical analysis. All values represent means ± SE. Within-group differences were determined by the paired two-tailed Student’s t-test. Differences between basal and insulin clamp periods and between groups (FH+ vs. control) were tested by two-way ANOVA for repeated measures. Normal distribution was checked prior to all analyses, and nonparametric estimates were used when appropriate. Comparisons were considered statistically significant if the P value was <0.05.

RESULTS

Patient characteristics. FH+ subjects were very insulin resistant and kept the plasma glucose concentration within the normal range at the expense of a two- to threefold higher plasma insulin concentration during the screening OGTT (74 ± 15 vs. 24 ± 6 μmol/ml, P < 0.001) and 48-h metabolic profile (26 ± 8 vs. 13 ± 3 μmol/ml, P < 0.001) compared with matched controls without a family history of T2DM studied in our laboratory (Table 1) (26).

Metabolic profile (48-h): plasma glucose, hormone, and FFA concentrations. Treatment with APX compared with PBO significantly lowered the fasting (APX: 281 ± 42 vs. PBO: 498 ± 24 μmol/ml, P < 0.001) and mean 48-h plasma FFA concentrations (APX: 194 ± 35 vs. PBO: 283 ± 45 μmol/ml, P < 0.001). The 48-h plasma insulin concentration was slightly but significantly reduced from 18 ± 6 to 14 ± 5 μU/ml (P < 0.05). Plasma adiponectin, leptin, and TNF-α were unchanged by APX (data not shown). The mean C-peptide concentration increase above the fasting C-peptide level during the 48-h metabolic profile was not significantly higher after APX compared with PBO, likely due to the improvement in insulin sensitivity (M/I during the hyperglycemic clamp; see below) and the secondary reduction in the 48-h plasma glucose (~7%, P < 0.001). However, despite all participants having a normal OGTT at study entry and the plasma glucose excursions ranging within a narrow range during the 0800–2400 glucose profile (between 88 and 120 mg/dl), APX significantly reduced the postprandial plasma glucose concentration (increase above baseline or Δ) AUC after breakfast, lunch, and dinner (1,731 ± 527 vs. 1,024 ± 282 (−41%) mg/dl × 240 min, 1,286 ± 339 vs. 600 ± 296 mg/dl × (−53%), and 4,479 ± 989 vs. 2,539 ± 650 (−43%) mg/dl × 360 min, respectively, P = 0.05–0.02).

The fasting plasma glucose was unchanged (day 3: APX: 87 ± 2 vs. PBO: 89 ± 1 μmol/l, P = NS). When the relation between the increase in plasma C-peptide AUC and the glucose concentration AUC was taken into account (ΔC-peptide AUC/Δglucose AUC) there was a significant increase following APX administration (+177%, P = 0.02), indicating improved β-cell function in response to mixed meals.

Insulin secretion and insulin action during the hyperglycemic clamp (day 3). The fasting plasma FFA concentration was reduced by 43% as a result of APX administration (APX: 281 ± 42 vs. PBO: 498 ± 24 μmol/l, P < 0.001) and remained lower compared with placebo during the hyperglycemic clamp (APX: 109 ± 13 vs. PBO: 147 ± 14 μmol/l, P = 0.047). The increment in plasma glucose concentration by intravenous glucose during the hyperglycemic clamps was nearly identical between both groups (APX: +127 ± 5 vs. PBO: +123 ± 4 mg/dl, from 87 ± 2 to 214 ± 2 and 89 ± 2 to 212 ± 3, respectively, P = NS). M/I, a measure of tissue sensitivity to endogenously secreted insulin, was reduced to ~50% of that previously reported in subjects without a family history of T2DM (26). Insulin sensitivity improved following acipimox treatment compared with placebo (APX: 10.2 ± 0.6 vs. 8.1 ± 0.6 mg·kg·LBM−1·min−1 per μU/ml, P < 0.04; Fig. 2). Lowering of plasma FFA concentration for 48 h with APX enhanced first-phase (+19 ± 6%, P = 0.1), but in particular
improved second-phase insulin secretion by 31 ± 5% (second phase: from 275 ± 36 to 361 ± 55 pmol·min⁻¹·m², P = 0.05) during the hyperglycemic clamp (Fig. 3A). When the ISR was examined relative to the prevailing insulin resistance (as the inverse of insulin sensitivity measured as M/I and calculated as $\text{ISR}_{\text{M/I}} = \text{ISR} \times [1/\text{M/I}]$), both first- and second-phase $\text{ISR}_{\text{M/I}}$ markedly increased by 29 ± 7 (P = 0.048) and 41 ± 8% (P = 0.021), respectively (Fig. 3B).

Correlations. There was an inverse correlation between fasting plasma FFA levels and first- ($r^2 = 0.31, P < 0.02$) and second-phase insulin secretion ($r^2 = 0.20, P < 0.06$). After treatment, subjects with the highest fasting plasma FFA had the lowest acute (2–4 min) insulin release ($r^2 = 0.52, P < 0.04$).

DISCUSSION

Numerous studies have demonstrated that elevated plasma FFA levels cause hepatic and muscle insulin resistance (8, 15, 27, 33). More recently, we have reported that elevating the FFA levels cause hepatic and muscle insulin resistance (8, 15, 27, 33). These results highlighted the importance in FH+ subjects of β-cell lipotoxicity for the development of T2DM later in life. The present study has examined, for the first time, the effect of a reduction in plasma FFA concentration for 48 h on insulin secretion and insulin action in Hispanic FH+ subjects. Acipimox, a nicotinic acid derivative, is a potent inhibitor of lipolysis that lowers plasma FFA concentration by specifically targeting adipose tissue by activating the nicotinic acid/acipimox G protein-coupled receptor (1, 14, 25). The receptor is found almost exclusively in adipocytes (31, 41, 51). Therefore, acipimox has no direct effects on muscle, liver, or pancreatic β-cells. Rather than significantly enhancing adipose tissue insulin sensitivity, administration of acipimox is associated with an acute inhibition of hormone-sensitive lipase and prevention of FFA release by the adipocyte (1, 14, 25).

In FH+ subjects a sustained reduction in plasma FFA concentration for just 2 days with acipimox caused a significant improvement in insulin sensitivity (M/I) in the 0800–2400 ΔC-peptide/Δglucose AUC ratio (a measure of insulin secretion relative to the glucose concentration) and in glucose-stimulated ISRs during the hyperglycemic clamps. Moreover, given that ISR is greatly conditioned by the presence of insulin resistance (11, 12, 15, 20, 23, 26, 28–30), when ISR was examined in relation to the prevailing insulin resistance, both first- and second-phase ISR improved by 29 and 41% (P < 0.05 and P = 0.02, respectively). In Caucasian first-degree relatives of subjects with T2DM, 4 wk of acipimox treatment have been reported to enhance the acute insulin response to intravenous glucose (36). In another elegant set of studies involving subjects with established T2DM, acipimox enhanced glucose-stimulated insulin secretion only in patients with less advanced T2DM in whom the HbA1c was lower (38), suggesting that lowering plasma FFA levels may be of potential benefit in the early stages of the disease, but not later on when there is little residual pancreatic β-cell function. Taken together, they suggest that early intervention to lower plasma FFA concentration may be essential for optimal pancreatic β-cell function in subjects at risk of T2DM.

Although insulin action and insulin secretion improved, the changes were rather modest. It is possible that a greater effect may have been achieved if treatment would have been extended for a longer period of time, rather than just 48 h. However, it is well established that chronic treatment with even low-dose nicotinic acid for just 2 wk (2, 13, 37, 47, 49) or short-term use of acipimox (40, 52) may lead to rebound elevations in plasma FFA concentration and potentially worsen insulin resistance (2, 13, 37, 47, 49). To avoid any potential plasma FFA rebound in our studies, we used acipimox for only 2 days. Although our study design allowed us to perform a “proof-of-concept” study on the role of reducing plasma FFA in FH+ subjects, the possible rebound of plasma FFA between doses and the fact that its predominant effect appears to be a prevention of FFA release by the adipocyte (25), rather than an insulin-sensitizing effect on fat cells, limits its long-term clinical potential in this population. This is in sharp contrast to thiazolidinediones that lower plasma FFA by stimulating glyceroneogenesis (i.e., phosphoenolpyruvate carboxykinase [PEPCK] and glyceraldehyde-3-phosphate dehydrogenase [GPDH]) as well as FFA uptake and reesterification (6), increasing plasma adiponectin levels [which were low and unchanged by acipimox treatment in this cohort of insulin-resistant FH+ subjects (data not shown)] (5), and improving

Fig. 3. Insulin secretion during the hyperglycemic clamp in FH+ subjects treated with acipimox or placebo. A: insulin secretion rate (ISR) measured by deconvolution analysis of the plasma C-peptide concentration. B: ISR in relation to the prevailing degree of insulin resistance (as the inverse of insulin sensitivity, measured as M/I). Data are means ± SE.
insulin sensitivity in adipocytes (48). The clinical implication would be that thiazolidinediones may be better suited to ameliorate β-cell lipotoxicity in FH+ subjects and, as suggested by recent clinical trials, to potentially prevent T2DM (18, 53).

There are additional reasons that may have limited a further improvement in insulin secretion in FH+ subjects. Participants were lean and plasma FFA levels lower than the levels seen in obesity and T2DM, which typically range from ~700 to 800 μmol/l (8, 15, 27, 33). This could have minimized the impact of lowering plasma FFA with acipimox compared with previous studies, in which obese nondiabetic offspring of T2DM parents (36) and obese new onset T2DM patients (38) were studied. Adipose tissue insulin resistance was evident in our cohort of lean FH+ subjects, as the plasma FFA level was “normal” (rather than suppressed) in the presence of marked hyperinsulinemia; however, insulin secretion was increased sufficiently to maintain normal glucose tolerance and prevent a further deleterious rise in the plasma FFA concentration. McGarry (33) clearly demonstrated the important role of plasma FFA in the regulation of pancreatic β-cell function and in maintaining chronic hyperinsulinemia in insulin-resistant obese subjects. In the setting of obesity, but not in lean subjects, removal of this FFA stimulus by overnight reduction of plasma FFA with nicotinic acid impairs glucose-induced insulin secretion (17, 33). However, in our experience (26), and that of others (7, 17, 32), β-cell adaptation to changing plasma FFA levels in lean healthy subjects without a genetic predisposition to T2DM is normal. In ongoing studies we are examining the role of plasma FFA in overweight FH+ subjects but would predict on the basis of our previous work (26) that elevated plasma FFA may also be deleterious in obese FH+ subjects, in contrast to the reported role (17) of enhancing insulin secretion in obese individuals without any family history of T2DM.

Of mention is that improvement in insulin sensitivity by acipimox may have in part accounted for the modest enhancement of glucose-stimulated insulin secretion with treatment, as β-cells couple very tightly insulin release with insulin action (11, 12, 15, 20, 23, 26, 28–30). In the setting of improved insulin sensitivity, the need for an increase in insulin output is reduced, and any improvement in insulin secretion may be blunted by the physiological adaptation to improved insulin sensitivity. However, whereas insulin sensitivity improved, FH+ patients remained significantly insulin resistant after acipimox treatment, as insulin sensitivity was ~20–50% lower than in healthy, lean, insulin-sensitive individuals assessed by the same the M/I index used in the present study (2, 12, 16, 26). Improvement in insulin secretion in the face of enhanced insulin action suggested a positive change in β-cell adaptation. Therefore, we also examined β-cell function as the relationship between plasma C-peptide and glucose concentration during the 48-h administration of acipimox or placebo as an index of insulin secretion dose response to the prevailing plasma glucose concentration, also known as β-cell glucose sensitivity (22). As observed in Fig. 4, there was a significant improvement in β-cell adaptation with acipimox that resulted in higher C-peptide for any given plasma glucose concentration. Reduction in plasma FFA concentration appears as the most likely mechanism for improved β-cell adaptation. However, exposure of macrophages to elevated fatty acids activates NF-κB inflammatory pathways (43) and may alter the immune response (42).

Because macrophages have nicotinic acid receptors (25), one cannot exclude the possibility that acipimox may have ameliorated within the pancreatic islet FFA-induced macrophage activation and cytokine production that could be contributing to β-cell lipotoxicity. Nevertheless, plasma inflammatory cytokine levels were normal in FH+ subjects prior to treatment and unchanged by acipimox.

In summary, in this proof-of-concept study, we have demonstrated that short-term treatment with the antilipolytic agent acipimox may improve insulin action and insulin secretion in normal-glucose-tolerant FH+ subjects who are genetically predisposed to develop T2DM. A larger improvement of pancreatic β-cell function in FH+ subjects may require long-term treatment with agents that both reduce plasma FFA concentration (thus ameliorating β-cell lipotoxicity in subjects particularly susceptible to elevated FFA and genetically predisposed to T2DM) and improve adipose tissue insulin resistance (thus decreasing the chronic insulin secretory demand; i.e., thiazolidinediones). Taken together, our results emphasize the role of adipose tissue as a therapeutic target in populations at high risk of T2DM.

ACKNOWLEDGMENTS

We thank all volunteers and the invaluable efforts of the GCRC nursing staff, Celia Darland (research dietician) and associated personnel, and the skilled nursing assistance of James King, John Kincade, Norma Diaz, and Tricia Wolff, who performed the metabolic studies.

GRANTS

This work was supported by a Veterans Administration Merit Award (K. Cusi), a GCRC Audie L. Murphy Veterans Hospital Grant (M01-RR-01346), and the Veterans Affairs Medical Research Fund.

REFERENCES

E1780

ACIPIMOX AND INSULIN SECRETION IN SUBJECTS PRONE TO T2DM


