Elevated plasma nonesterified fatty acids are associated with deterioration of acute insulin response in IGT but not NGT

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Stefan, Norbert, Michael Stumvoll, Clifton Bogardus, and P. Antonio Tataranni. Elevated plasma nonesterified fatty acids are associated with deterioration of acute insulin response in IGT but not NGT. Am J Physiol Endocrinol Metab 284: E1156–E1161, 2003. First published February 11, 2003; 10.1152/ajpendo.00427.2002.—High concentrations of nonesterified fatty acids (NEFA) are a risk factor for developing type 2 diabetes in Pima Indians. In vitro and in vivo, chronic elevation of NEFA decreases glucose-stimulated insulin secretion. We hypothesized that high fasting plasma NEFA would increase the risk of type 2 diabetes by inducing a worsening of glucose-stimulated insulin secretion in Pima Indians. To test this hypothesis, fasting plasma NEFA concentrations, body composition, insulin action (M), acute insulin response (AIR, 25-g IVGTT), and glucose tolerance (75-g OGTT) were measured in 151 Pima Indians [107 normal glucose tolerant (NGT), 44 impaired glucose tolerant (IGT)] at the initial visit. These subjects, participants in ongoing studies of the pathogenesis of obesity and type 2 diabetes, had follow-up measurements of body composition, glucose tolerance, M, and AIR. In NGT individuals, cross-sectionally, high fasting plasma NEFA concentrations at the initial visit were negatively associated with AIR after adjustment for age, sex, percent body fat, and M (P = 0.03). Longitudinally, high fasting plasma NEFA concentrations at the initial visit were not associated with change in AIR. In individuals with IGT, cross-sectionally, high fasting plasma NEFA concentrations at the initial visit were not associated with AIR. Longitudinally, high fasting plasma NEFA concentrations at the initial visit were associated with a decrease in AIR before (P < 0.0001) and after adjustment for sex, age at follow-up, time of follow-up, change in percent body fat and insulin sensitivity, and AIR at the initial visit (P = 0.0006). In conclusion, findings in people with NGT indicate that fasting plasma NEFA concentrations are not a primary etiologic factor for β-cell failure. However, in subjects who have progressed to a state of IGT, chronically elevated NEFA seem to have a deleterious effect on pancreas-beta-cell capacity.

β-cell; insulin secretion; insulin sensitivity; diabetes; oral and intravenous glucose tolerance tests; normal glucose tolerance; impaired glucose tolerance

Nonesterified Fatty Acids (NEFA) have long been recognized for their contribution to decreasing insulin-mediated glucose disposal (1, 22, 27). The relationship between plasma NEFA and insulin secretion, however, is still debated. A 48-h lipid infusion has been shown to increase insulin secretion in humans (3). Moreover, short-term experimental reduction in plasma NEFA after 12–24 h of fasting has been shown to decrease glucose-stimulated insulin secretion (2, 7) in humans, indicating that NEFA play a role in sustaining β-cell function under this condition. Conversely, elevation of NEFA was shown to decrease glucose-stimulated (6, 18), but not arginine-stimulated, insulin secretion in humans (5). This concept is supported by findings in animals (24) and studies in vitro (4, 8, 26) and has been referred to as lipotoxicity (17, 29).

In Pima Indians, insulin resistance and insulin-secretory dysfunction are independent risk factors of type 2 diabetes (14). Moreover, high fasting plasma NEFA concentrations have been shown to be a risk factor for development of type 2 diabetes independent of adiposity and whole body insulin sensitivity. Interestingly, this predictive effect was not independent of glucose-stimulated insulin secretion (19). Thus it can be reasoned that high plasma NEFA confer increased risk of type 2 diabetes by decreasing glucose-stimulated insulin secretion. Our longitudinal studies indicate that the transition from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) is characterized by substantial worsening in insulin-secretory function (14). Therefore, the etiologic role of plasma NEFA concentrations on decrease in insulin secretion was examined specifically in subjects with NGT. To assess the relationship between elevated plasma NEFA concentrations and acute insulin response (AIR) in individuals with a preexisting β-cell defect, we performed the same analyses in subjects with IGT.

Materials and Methods

Subjects

A total of 151 Pima Indians who were participants in ongoing studies of the pathogenesis of obesity and type 2 diabetes were included in this analysis. Some data from these individuals were included in earlier reports (19). All subjects were between 18 and 50 yr of age and were nonsmokers at the time of the study. They were healthy according to a physical examination and routine laboratory tests. Subjects were then invited back at approximately annual intervals for repeated oral glucose tolerance tests (OGTTs).
and assessment of insulin sensitivity and insulin secretion. For this analysis, the initial visit and the last visit of each subject were included. Subjects who had not been diagnosed with type 2 diabetes before the initial visit to the research clinic were selected. Because offspring of diabetic mothers were shown to have impaired insulin-secretory function (9), they were excluded from the analyses. Eight of the 107 NGT and 16 of the 44 IGT subjects were diagnosed with diabetes at their last visit. The average time of follow-up was 5.8 ± 3.4 yr (means ± SD), with a minimum of 0.6 and a maximum of 15 yr. Fasting plasma NEFA concentrations were available only at the initial visit. The protocol was approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases, and all subjects provided written informed consent before participation.

Cross-sectional analyses were carried out in all subjects at the initial visit who were characterized for plasma NEFA concentrations and had initial and follow-up measurements of glucose tolerance, percent body fat, insulin sensitivity [rate of total insulin-stimulated glucose disposal (M)], and acute insulin response (AIR).

Longitudinal analyses were performed in all subjects who had NGT or IGT at the initial visit. Subjects had either NGT or IGT or were diabetic.

Subjects were admitted for 8–10 days to the National Institutes of Health Clinical Research Unit in Phoenix, AZ, where they were fed a weight-maintaining diet (50% of calories as carbohydrate, 30% as fat and 20% as protein) and abstained from strenuous exercise. After ≥3 days on the diet, subjects underwent a series of tests for the assessment of body composition, glucose tolerance, insulin sensitivity, and AIR.

Methods

Body composition. Body composition was estimated by underwater weighing with determination of residual lung volume by helium dilution (10) or by total body dual-energy X-ray absorptiometry (DPX-L; Lunar, Madison, WI) (15, 28). Percent body fat, fat mass, and fat-free mass were calculated as previously described (25), and a conversion equation (28) was used to make measurements comparable between the two methods.

OGTT and analytical procedures. After a 12-h overnight fast, subjects underwent a 75-g OGTT. Baseline blood samples were drawn for the determination of fasting plasma glucose, insulin, and NEFA concentrations. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA) in the fasting state and 2 h after glucose ingestion for assessment of glucose tolerance according to the 1997 World Health Organization diagnostic criteria (8a). Plasma insulin concentrations were determined by the modification of Herbert et al. (11) of the radioimmunoassay of Yalow and Berson (31). Blood samples for the measurement of fasting plasma NEFA concentrations were drawn with prechilled syringes and stored at −20°C until they were analyzed according to Miles et al. (16).

Intravenous glucose tolerance test. Early-phase insulin secretion was measured in response to a 25-g intravenous glucose bolus with calculation of the AIR as the average incremental plasma insulin concentration from the 3rd to the 5th min after the glucose bolus over baseline (14).

Hyperinsulinemic euglycemic glucose clamp. Insulin action was assessed at physiological insulin concentrations during a hyperinsulinemic euglycemic glucose clamp, as previously described (13, 30). In brief, after an overnight fast, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 40 mU·m body surface area·min−1 leading to steady-state plasma insulin concentrations. Plasma glucose concentrations were maintained at ~5.5 mmol/l with a variable infusion of a 20% glucose solution. M values were calculated for the last 40 min of insulin infusion. M values were additionally adjusted for endogenous glucose production (measured by a primed (30 μCi) continuous (0.3 μCi/min) [3-3H]glucose infusion) and steady-state plasma glucose and insulin concentrations, as previously described (13) and normalized to estimated metabolic body size (EMBS = fat-free mass + 17.7 kg).

Statistical analyses. Statistical analyses were performed using the software of the SAS Institute (Cary, NC). Results are given as means ± SD. Fasting plasma insulin concentrations, M, and AIR were logarithmically transformed to approximate a normal distribution. Because some of the subjects were related, all analyses were performed after adjustment for family membership in generalized estimating equation regression models (PROC GENMOD) of the SAS procedure that account for nuclear family membership and thus allow analyses with all individuals in a sibship (32). A P value of <0.05 was considered to be statistically significant. Differences between anthropometric and metabolic characteristics at the initial visit and follow-up were assessed by Student’s t-test. In cross-sectional analysis, the relationship between fasting plasma NEFA concentrations and AIR, adjusted for age, sex, percent body fat, and M, was examined in linear models. In prospective analyses, the predictive effect of fasting plasma NEFA concentrations at the initial visit on change (follow-up adjusted for baseline) in AIR was evaluated separately in NGT and IGT subjects who had baseline as well as follow-up measurements of percent body fat, glucose tolerance, M, and AIR by use of linear models. In these models, change in AIR was adjusted for sex, follow-up age, changes in percent body fat and M, and time of follow-up. Changes in M and in 2-h plasma glucose concentrations were adjusted for age, sex, follow-up age, and change in percent body fat.

RESULTS

Anthropometrics and metabolic characteristics of the study population are presented in Table 1. During the initial visit, individuals with IGT were older and had higher fasting and 2-h plasma glucose and insulin concentrations and lower M than individuals with NGT. Individuals with IGT also tended to have lower AIR than individuals with NGT (P = 0.09). There was no difference in fasting plasma NEFA concentrations between the two groups. Except for fasting plasma glucose and AIR in individuals with NGT and fasting glucose, fasting insulin, 2-h insulin, and M in individuals with IGT, the changes in anthropometrics and metabolic characteristics between the initial visit and the follow-up visit were statistically significant.

Individuals with NGT

In individuals with NGT, cross-sectionally, high fasting plasma NEFA concentrations at the initial visit were not associated with AIR before but were negatively associated after adjustment for age, sex, percent body fat, and M (P = 0.03; Table 2). Longitudinally, high fasting plasma NEFA concentrations at the initial

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Table 1. Anthropometrics and metabolic characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>NTG n = 107 (72 M/35 F)</th>
<th></th>
<th>IGT n = 44 (7 M/37 F)</th>
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<tbody>
<tr>
<td></td>
<td>Initial Follow-up    P value</td>
<td>Initial Follow-up    P value</td>
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<tr>
<td>Age, yr</td>
<td>25 ± 6</td>
<td>32 ± 6</td>
<td>&lt;0.0001</td>
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<tr>
<td>Weight, kg</td>
<td>94 ± 23</td>
<td>104 ± 27</td>
<td>&lt;0.0001</td>
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<tr>
<td>Body fat, %</td>
<td>31 ± 9</td>
<td>34 ± 8</td>
<td>&lt;0.0001</td>
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<td>Fasting glucose, mM</td>
<td>5.1 ± 0.3</td>
<td>5.4 ± 1.6</td>
<td>0.1</td>
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<td>2-h Glucose, mM</td>
<td>6.3 ± 0.9</td>
<td>7.8 ± 3.6</td>
<td>&lt;0.0001</td>
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<td>Fasting insulin, pM</td>
<td>204 ± 108</td>
<td>268 ± 138</td>
<td>&lt;0.0001</td>
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<tr>
<td>2-h Insulin, pM</td>
<td>882 ± 690</td>
<td>1,290 ± 954</td>
<td>&lt;0.0001</td>
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<td>1,553 ± 1,146</td>
<td>1,446 ± 1,105</td>
<td>0.26</td>
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<td>M, mg·kg⁻¹·EMBS⁻¹·min⁻¹</td>
<td>2.81 ± 1.03</td>
<td>2.42 ± 0.80</td>
<td>&lt;0.0001</td>
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<tr>
<td>NEFA, μM</td>
<td>334 ± 102</td>
<td>377 ± 96</td>
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Values are means ± SD. NTG, normal glucose tolerance; IGT, impaired glucose tolerance; AIR, acute insulin response; M, glucose disposal during hyperinsulinemic euglycemic glucose clamp; EMBS, estimated metabolic body size (fat-free mass + 17.7 kg); NEFA, nonesterified fatty acids. Anthropometrics were adjusted for age and sex in linear models. Glucose, insulin, NEFA, and M were adjusted for age, sex, and percent body fat; AIR was adjusted for age, sex, percent body fat, and M. Statistical significance compared with NTG at the initial assessment: *P < 0.05, †P < 0.01, ‡P < 0.001.

Comparison of visit were not associated with change in AIR (P = 0.52 before and P = 0.88 after adjustment; Fig. 1A and Table 3). High fasting plasma NEFA concentrations at the initial visit were not associated with change in M or change in 2-h glucose (all P > 0.2).

**Individuals with IGT**

As expected, at the initial visit, individuals with IGT had a lower mean AIR than those with NTG before (P = 0.05) and there was a trend after adjustment (P = 0.09). Cross-sectionally, high fasting plasma NEFA concentrations at the initial visit were not associated with AIR before and after adjustment (all P > 0.88; Table 2). Longitudinally, high fasting plasma NEFA concentrations at the initial visit were associated with a decrease in AIR (P < 0.0001 before and P = 0.0006 after adjustment; Fig. 1B and Table 3). High fasting plasma NEFA concentrations at the initial visit were not associated with a decrease in M before (P = 0.13) and after adjustment (P > 0.97). High fasting plasma NEFA concentrations at the initial visit were associated with change in 2-h glucose before (P = 0.07) but not after adjustment in individuals with IGT (P = 0.12).

**Comparisons Between Individuals with High and Low Plasma NEFA Concentrations**

It is possible that high fasting plasma NEFA concentrations in subjects with IGT simply reflect other abnormalities, such as low glucose tolerance, low M, and low AIR, at the initial visit, which could explain the effect on change in AIR. Therefore, we arbitrarily divided the NTG and IGT groups at the median plasma NEFA concentrations of 323 μmol/l in NTG and 368 μmol/l in IGT. Differences in baseline and follow-up anthropometrics and metabolic characteristics in subjects with NTG and IGT with high vs. low plasma NEFA concentrations are shown in Table 4. Clearly, baseline data were not different in individuals with IGT and high NEFA vs. IGT and low NEFA, especially 2-h glucose and AIR. In individuals with IGT, AIR decreased significantly in the high-NEFA group. This decrease in AIR was greatest compared with the other three subgroups (Fig. 2).

Table 2. Determinants of AIR (log₁₀), cross-sectional analyses (linear model)

<table>
<thead>
<tr>
<th></th>
<th>NTG Estimate</th>
<th>P</th>
<th>Estimate</th>
<th>P</th>
<th>Estimate</th>
<th>P</th>
<th>Estimate</th>
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<td>-0.0002</td>
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<td>0.1317</td>
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<tr>
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<tr>
<td>Mlog₁₀</td>
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<td>-0.5445</td>
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<table>
<thead>
<tr>
<th></th>
<th>IGT Estimate</th>
<th>P</th>
<th>Estimate</th>
<th>P</th>
<th>Estimate</th>
<th>P</th>
<th>Estimate</th>
<th>P</th>
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<tr>
<td>NEFA</td>
<td>&lt;−0.0001</td>
<td>0.88</td>
<td>&lt;−0.0001</td>
<td>0.98</td>
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<td>0.83</td>
<td>-0.0090</td>
<td>0.83</td>
<td>-0.0777</td>
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<tr>
<td>%Fat</td>
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Estimate, linear slope; %fat, percent body fat.

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DISCUSSION

We have previously established (19) that high fasting plasma NEFA concentrations are a risk factor for the development of type 2 diabetes in Pima Indians, independent of adiposity and insulin sensitivity. However, this association was not independent of AIR, suggesting that high fasting plasma NEFA concentrations may confer the increased risk of type 2 diabetes through a decrease in AIR. In subjects with NGT, we found no association between high fasting plasma NEFA concentrations and change in AIR independent of change in percent body fat and insulin sensitivity. This makes it unlikely that increased plasma NEFA concentrations represent a primary etiologic factor of β-cell dysfunction in the natural history of type 2 diabetes.

Nevertheless, in subjects with IGT, NEFA concentrations were significantly correlated with change in AIR, indicating that high NEFA concentrations were associated with future decreases in AIR. The different results in subjects with IGT vs. NGT may reflect the fact that subjects with IGT are in a more advanced disease stage characterized by a manifest defect in β-cell function that results in inadequately low insulin secretion for the level of insulin resistance (20). It is possible that, when people reach IGT, the β-cell fails to prevent intracellular NEFA accumulation when plasma levels are elevated. Increased intracellular NEFA concentrations may eventually decrease glucose-stimulated insulin secretion.

This hypothesis is supported by in vitro data showing that incubation of β-cells with fatty acids caused both intracellular fatty acid accumulation and increased basal and decreased glucose-stimulated insulin secretion (24). The interpretation above is also compatible with increased islet lipid contents observed in Zucker diabetic fatty (ZDF) rats immediately following fatty acid incubation.

Table 3. Determinants of change in AIR (log10), longitudinal analyses (linear model)

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>P</th>
<th>Estimate</th>
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<th>Estimate</th>
<th>P</th>
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<td>-0.0002</td>
<td>0.74</td>
<td>-0.0001</td>
<td>0.82</td>
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<td>0.65</td>
<td>0.0040</td>
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Time, time of follow-up; F and L in abbreviations of variables, first and last observation, respectively.
Follow-up time, yr 6.6 ± 3.5 5.7 ± 3.4 0.22 5.7 ± 3.3 4.1 ± 2.8 0.07
Age, yr 25 ± 6 32 ± 6 26 ± 6 32 ± 6 0.48 29 ± 6 35 ± 6 29 ± 4 32 ± 6 0.69
Weight, kg 93 ± 21 105 ± 25† 96 ± 25 103 ± 28‡ 0.93 93 ± 23 96 ± 24* 95 ± 18 99 ± 20‡ 0.73
Body fat, % 29 ± 9 33 ± 8§ 33 ± 9 36 ± 8§ 0.89 33 ± 9 37 ± 8* 40 ± 5 41 ± 5* 0.99
Fasting glucose, mM 5.2 ± 0.3 5.1 ± 0.6 5.1 ± 0.3 5.7 ± 2.1* 0.11 5.5 ± 0.4 5.8 ± 0.8 5.5 ± 0.4 4.0 ± 1.5 0.29
2-h Glucose, mM 6.1 ± 1.0 7.2 ± 1.8§ 6.4 ± 0.9 8.4 ± 4.7‡ 0.26 9.4 ± 1.0 10.2 ± 3.5 9.3 ± 0.9 10.3 ± 2.8 0.78
Fasting insulin, pM 158 ± 106 267 ± 133‡ 223 ± 108 269 ± 137† 0.47 256 ± 109 395 ± 100* 288 ± 83 305 ± 120 0.78
2-h Insulin, pM 728 ± 589 1291 ± 886§ 1042 ± 756 1289 ± 1030 0.23 1688 ± 1122 1842 ± 1332 2046 ± 1221 1504 ± 1088* 0.82
AIR, pM 1732 ± 1377 1611 ± 1246 1371 ± 823 1278 ± 921 0.05 1106 ± 510 978 ± 853 1187 ± 740 642 ± 536‡ 0.65
M, mg·kg EMBS⁻¹·min⁻¹ 3.01 ± 1.1 2.45 ± 0.8§ 2.59 ± 0.90 2.38 ± 0.76* 0.04 2.31 ± 0.77 2.18 ± 0.56 2.12 ± 0.36 2.12 ± 0.43 0.77
NEFA, µM 258 ± 44 420 ± 76 <0.0001 301 ± 50 453 ± 64 <0.0001

Values are means ± SD. NEFA, nonesterified fatty acids. Statistical significance (paired t-test) between initial and follow-up measurements in NGT and IGT: *P < 0.05, †P < 0.01, ‡P < 0.001. For comparisons between basal measurements in each NEFA subgroup (general linear model), anthropometrics were adjusted for age and sex. Insulin, glucose, NEFA concentrations, and M were adjusted for age, sex, and percent body fat. AIR was adjusted for age, sex, percent body fat, and M.

Follow-up visit.

Fig. 2. Relationship between AIR and M during initial and follow-up visits. NGT and IGT subjects were arbitrarily divided at the median plasma NEFA concentrations 325 µmol/l in NGT and 368 µmol/l in IGT, respectively. Regression line represents the association between AIR and M in NGT. Arrows indicate change of AIR and M over time in the 4 subgroups by pointing to the mean values during the follow-up visit. Inset: hyperbolic association between AIR and M in subjects with NGT. The main figure is a magnification of this association in the range of M and AIR values indicated on the horizontal and vertical axes, respectively.

Against the background of our new findings, we reanalyzed the data presented in Ref. 19 by investigating whether fasting plasma NEFA concentrations predict type 2 diabetes specifically in subjects with NGT and therefore would have an etiologic role in the disease. We could include 86 subjects who developed type 2 diabetes over a mean follow-up time of 7.7 yr. Fasting plasma NEFA were predictive of type 2 diabetes when NGT and IGT were combined for the analyses but not in 46 NGT alone (data not shown).

In conclusion, our data did not confirm the hypothesis of an etiologic effect of elevated plasma NEFA on the development of type 2 diabetes, especially not one that was due to a change in AIR. In fact, we propose that chronically elevated plasma NEFA have a delete-
rious effect on insulin-secretory capacity only in sub-
jects with IGT.

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